INTRODUCTION
Although there are good advancements in the diagnosis and treatment, cancer is still a huge threat to the human population. In the world, cancer is the second most common disease after cardiovascular related disorders for highest deaths (Kotnis et al., 2005; Jemal et al., 2007). The word ‘Cancer’ comes from the Latin word ‘Carcinoma’ meaning crab. It is the most frightened disease in which a group of abnormal cells grow uncontrollably by disregarding the normal rules of cell division. Normal cells are constantly subject to signals that prescribe whether the cell should divide, differentiate and proliferate into another cell or die. In cancer, cells are developing into tumors caused by the uncontrolled growth of a body cells or group of cells through self direction and spread to other parts of the body. In point of fact, almost 90% of cancer-related deaths are due to tumor metastases. The initiation and progression of cancer depend on both external factors in the environment (tobacco, chemicals, toxic metals, radiation and infectious organisms etc.) and internal factors within the cell (inherited mutations, hormones, immune conditions, minerals deficiency and mutations that occur from metabolism). These factors can act together or in sequence, resulting in abnormal, excessive proliferation and leading to the lightly aberrant stage. Consecutive rounds of mutation and selective expansion of these cells results in the formation of a tumor mass and leads to tumor growth and progression, which eventually breaks through the basal membrane barrier surrounding tissues and spreads to other parts of the body (metastasis).

Cancers are tremendously diverse, requiring different therapy strategies and prognostic aspects. More than one hundred different types of cancers have been identified and twenty-four different types of cancers occur in the different parts of human bodies (Barhar, 2003). Some cancers like leukemia do not form tumors and these cancer cells involve the blood and blood producing organs and circulate through other tissues of the body. Cancer is an important cause of death in Southeast Asian countries, the available data over the last 10-15 years indicates a rising trend in incidence rates. It accounts for about 23 and 7% deaths in USA and India respectively (Brayand and Moller, 2006). In India, cancer is reported to be the fifth leading cause of death and nearly 6-7 hundred thousand new cases of cancer are estimated to occur every year with an estimated prevalence of 1.5 to 1.8 million (Anonymous, 2001). It is expected to be 7.5 thousand millions by 2020 and approximations predict that about 15.0 million new cancer cases will be diagnosed; with the
deaths of about 12.0 million cancer patients at worldwide (Bray and Moller, 2006). According to International Agency for Research on Cancer (IARC), it is estimated indirectly that about 635000 people died from cancer in 2008, representing about 8% of all estimated global cancer deaths and about 6% of all deaths in India (Ferlay et al., 2010). The number of cancer deaths in India is projected to increase because of population growth and increasing life expectancy. Cancer death rates (80-90%) are expected to rise which may be attributable to environmental and lifestyle factors such as tobacco smoking, alcohol consumption, dietary practices, inadequate physical activity, exposure to radiation, chemicals, infections due to viruses and sexual behaviors (Galvao et al., 2007; Jha, 2009). Particularly, breast cancer is the most frequently diagnosed malignant cancer and the leading cause of cancer death among women in both developed and developing countries (Sasco, 2001; Porter, 2009). It has been estimated that 1 out of every 9 women will develop breast cancer during her lifetime and approximately 30% of them will die of the disease. The incidence of breast cancer is gradually rising in every country of the world especially in developing countries such as India. Therefore attempts are being made throughout the developed and developing countries to reduce and prevent the cancer diseases through proscription and prescription approaches.

**Breast cancer**

Breast cancer is a type of cancer originating from breast tissue, most commonly from the inner lining of the milk ducts or the lobules that supply the ducts with milk. The breast is made up of lobes and ducts. Each breast has 15 to 20 sections called lobes, which have many smaller sections called lobules and lobules end in dozens of tiny bulbs that can produce milk. Anatomically breast had the lobes, lobules, and bulbs are linked by thin tubes called ducts (Fig.1). Each breast also has blood vessels and lymph vessels which carried colorless fluid called lymph. Lymph vessels lead to organs called lymph nodes which are small bean-shaped structures that are found throughout the body which filter substances in a fluid called lymph and help to fight infection and disease. Lymph node clusters are found near the breast in the axilla (under the arm), above the collarbone, and in the chest. During breast development, several biological processes occur in the breast that also takes place during breast cancer development and progression. For instance many of the stromal factors involved in breast gland growth also promote or protect against breast cancer. Epithelial and
stromal cells communicate via the extracellular matrix (ECM). Disruption of this interaction and respective communication can induce breast cancer. Breast tumors are composed of three basic cellular components: epithelial cells, stroma cells and vascular components (Cullen et al., 1991). The epithelial cells become transformed and constitute the malignant portion of the tumor, whereas, stroma cells do not typically become transformed, but they do secrete growth factors and other regulatory elements.

![Side View of Breast](image)

**Fig .1 Anatomical representation of the female breast**

These growth factors are secreted by the malignant cells to promote the growth and proliferation of endothelial cells thereby increasing the vascular component. This neovascularization serves to supply the tumor with the blood circulation needed for growth and metastases (Wellstein, 1994). As a result, metastatic cells acquire ability to migrate and invade into the surrounding tissue, intravasate into a blood vessel or lymphatic system survive in circulation, extravasate and finally proliferate at a distant site.

**Classification of breast cancer**

Breast cancer is a multifactorial disease in terms of clinical course and microscopic pathology. Although they can start in any tissue of the breast, most of them begin in the ducts the milk-passages that connect the lobules to the nipple (ducts) or in the cells of the milk-producing glands (lobules). The World Health Organization (WHO) classifies breast cancer based on its histological appearance, into noninvasive and invasive breast cancers (Vainio and Bianchini, 2002; Li et al., 2005). Breast cancers begin in the cells lining the milk ducts and are called ductal carcinomas, cancer that begins in the lobules is called
lobular carcinoma. If the disease has spread outside of the duct and into the surrounding tissue as called invasive or infiltrating ductal carcinoma while, if the disease has spread outside of the lobule, it is called invasive or infiltrating lobular carcinoma (NBOCC, 2009). A disease that has not spread is called in situ, meaning “in place.” The course of in situ disease, as well as its treatment, depends on whether it is ductal carcinoma in situ (DCIS) or lobular carcinoma in situ (LCIS).

**In situ cancers**

**Ductal carcinoma in situ (DCIS)**

Ductal carcinoma in situ (DCIS), or intraductal carcinoma or non-invasive cancer is the most common type of non-invasive breast cancer. DCIS means that the cancer cells are inside the ducts but have not spread through the walls of the ducts into the surrounding breast tissue. It may progress to invasive cancer if it is untreated. The DCIS incidence rate is 1 in 5 new breast cancer cases. If the breast cancer is diagnosed in the DCIS stage it can be cured.

**Lobular Carcinoma in Situ (LCIS)**

Lobular Carcinoma in situ (LCIS), or lobular neoplasia, or non-invasive lobular breast cancer occurs in the milk-producing glands but does not grow through the wall of the lobules. LCIS is not an invasive cancer very often, but women with this condition do have a higher risk of developing an invasive breast cancer in the same breast or in the opposite breast (Sewell, 2004). For this reason, a woman who has LCIS should make sure they have regular mammograms and doctor visits.

**Invasive cancers**

**Invasive ductal carcinoma (IDC)**

Invasive (or infiltrating) ductal carcinomas, it accounts for about 70% of all female breast cancers (Berg and Hutter, 1995). It is the most common type of breast cancer; it starts in a milk passage (duct) of the breast. It breaks through the wall of the duct and grows into the fatty tissue of the breast (Fig. 2). At this point, it is able to spread (metastasize) to other parts of the body through the lymphatic system and bloodstream. Among various types of breast cancers, the majority (8 out of 10) are the invasive infiltrating ductal carcinomas. Invasive ductal carcinomas are graded into three groups (I, II and III) according to the degree of tumor tubule formation, cell mitotic activity, and nuclear pleomorphism of tumor
cells (Elston and Ellis, 1998). Depending on the stage of tumor spread, it is clinically classified as stage I (cells spread but not detected in lymph nodes), stage II, stage III and stage IV breast cancer. Based on the site of origin, invasive breast cancer may be an invasive ductal carcinoma (IDC) or invasive lobular carcinoma (ILC).

**Fig. 2** Enlargement shows the arrangement of the infiltrating ductal cells in an infiltrating ductal cell carcinoma.

**Invasive lobular carcinoma (ILC)**

Lobular invasive carcinoma is the second most common breast carcinoma, accounting for about 8-14% of all female invasive carcinomas (Singletary et al., 2005). This type of cancer starts in the milk-producing glands (lobules). Like IDC, it can spread (metastasize) to other parts of the body. Invasive lobular carcinoma tissue is very harder to detect by the mammogram than invasive ductal carcinoma (Li et al., 2003). Among invasive ductal carcinomas medullary, mucinous, papillary and tubular ductal carcinomas are less accountable invasive ductal carcinomas.

**Medullary Ductal Carcinoma**

Medullary Ductal Carcinoma is a type of cancer occurs in only 1 to 7 percent of cases and these tumors may feel more like a spongy change of breast tissue rather than a standard lump. This kind of tumor usually shows up on a mammogram. This type of breast cancer is found in only 3 to 5% of all breast cancers diagnosed. It is quite difficult to distinguish from invasive ductal carcinoma and is usually treated as the same as IDC.

**Mucinous Ductal Carcinoma**

This type of cancer occurs two percent, when cancer cells within the breast produce mucous, which also contains breast cancer cells. The cells and mucous combined to form a tissue mass or tumor. This mucus contains breast cancer cells that are easily distinguished from normal cells under a microscope. In concert, the cancer cells and mucous form a jelly -
like tumor. Most of the breast mucinous carcinomas are estrogen-receptor positive and HER2/neu negative. This type of breast cancer rarely spreads to lymph nodes.

**Papillary Ductal Carcinoma**

Papillary Ductal Carcinoma looks like tiny fingers under the microscope. It occurs very rare and incidence will be 1-2% of the breast cancers and it is invasive. This form is common among women aged 50 and older. Treatment for this kind of cancer is similar to the treatment for ductal carcinoma in situ, a less aggressive form of cancer.

**Tubular Ductal Carcinoma**

Tubular ductal carcinoma occurs in only about two percent of cases. These cancers are very less estrogen-receptor positive cancers, which mean they respond to hormones. The cancer looks like hundreds of tiny tubes under the microscope. It is more common in women older than 50.

**Inflammatory breast cancer**

In inflammatory breast cancer is another type of rare and aggressive breast carcinoma in nature with unknown etiology and generally poor prognosis (Anderson et al., 2005). The breast looks red and swollen and feels warm. The redness and warmth occur because the cancer cells block the lymph vessels in the skin. The skin of the breast may also show the pitted appearance called peaud’orange (like the skin of an orange).

**Staging and grading**

**Staging**

Proper staging of breast cancer patients is of decisive and most significant diagnostic process. It is not only useful for exact prognosis but also useful in therapeutic strategy planning in many cases. The TNM staging system is contrived to be a useful diagnostic tool in determining the prognosis of cancer patients and in planning their therapy (Singletary et al., 2002). The TNM system derived from Tumor size (T), Lymph node status (N) and distant metastases (M). This clinical stage based on all information including physical examination and imaging before surgery. Then pathological staging (pTNM) adds additional information gained by examination of the tumor microscopically. The kind of treatment is decided for each patient and the prognosis is closely linked to the stage of the tumor. After the tumor examination parameters such as tumor size, axillary lymph node status and metastasis, the pathologist can then assess the overall stage of the cancer as 0, I, II, III or IV (Escobar et al., 2007).
**Stage 0:** In Situ ("in place") disease in which the cancerous cells are in their original location within normal breast tissue, sometimes referred to as a “non invasive carcinoma” there are abnormal cells present that might suggest that one is at higher risk for cancer.

**Stage I:** Tumor that is less than 2 cm in diameter and has not spread into the other parts of the areas.

**Stage II:** Tumor that is 2 to 5 cm in size and has spread into the surrounding areas including the lymph nodes (which must also be removed to prevent the further spread of the cancer).

**Stage III:** Tumor that is smaller than 5 cm in size with spread to auxiliary lymph nodes which are attached to each other or to other structures, this stage of cancer is near to advanced stage and more than 2cm across and has spread to the more lymph nodes.

**Stage IV:** This stage of cancer has spread past the breast and the lymph nodes and needs immediate treatment of chemotherapy and hormonal therapy to keep it under control. In this stage, a tumor that is of any size, which spread beyond the region of the breast (metastasize) and chest wall, such as liver, bone, or lungs. The staging indices provide useful information about the present status of cancer detection, management and the success of implementing new aspects (Singletary and Connolly, 2006).

**Tumor grading**

Once cancer is confirmed, it is given a grade assessment based quantititative measures, and also the size and shape of nuclei, similar to the grading for DCIS or LCIS carcinomas. The following features are considered in the grading of breast cancer (Bloom and Richardson, 1957). The features are 1) Tubule formation 2) Nuclear pleomorphism and 3) Mitotic rate are used and scored 3-5 points (Grade I), 6-7 points (Grade II) and 8-9 points (Grade III) the tumors.

- **Well-differentiated tumors (low grade)**
  
  This type of tumors resembles with normal tissue and known as Grade I tumors.

- **Poorly differentiated tumors (high grade)**
  
  Tumors are composed of disorganized cells, does not resemble normal tissue, these tumors are known as Grade III tumors.

- **Moderately differentiated tumors (intermediate grade)**
  
  Grade II tumor characteristics resemble both of the above.
**Epidemiology of breast cancer**

Breast cancer is the most common malignant cancer and it is leading cause of death among women in the worldwide (Sasco, 2001; Wonghongkul *et al*., 2006; Porter, 2009). It has been rated one of the most common cancer in women. Its incidence and mortality rate is modified by innumerable of environmental, reproductive, hereditary and dietary influences. This makes female breast cancer the second most common site of malignant neoplasm after the lung cancer (Parkin *et al*., 2005) (**Fig. 3**). The breast cancer incidence rates are highest in the economically developed countries in North America, western and northern Europe, Australia, New Zealand and Israel. Low rates are found in Africa and Asia. In India, during the past, the incidences of breast cancer have steadily increased and as many as 100,000 new patients are being detected every year (Michael and Jernal, 2003; Yip *et al*., 2006). In view of that, a 12 percent increase has been registered by various cancer registries from 1985 to 2001 (Yip *et al*., 2006; Hadjiiski *et al*., 2006). More than 10% of these cases arise in the breast. Over 70% of India's population currently derives their livelihood from land resources, including 84% of economically active women (Rambabu, 2004). The International Agency for Research on Cancer estimated that the breast cancer patients may be 250,000 approximately in India by 2015. Ferlay *et al*. (2010) reported that the incidence of breast cancer is rising in India, it is now the most prevalent malignant tumor among the women and its mortality is second only to that of cervical cancer. The Indian women average age risk is 40-46 years unlike the Western countries where women aged 52 years and above are more prone to breast cancer.

![Fig. 3 Incidence of new cancer cases in thousands adapted from Parkin *et al*., (2005).](image-url)
Although historical data is limited, the incidence of breast cancer is thought to have increased during the 20th century in most developing countries due to changes in the environment, lifestyle and reproductive pattern (Colditz et al., 2006). Thus, it appears, the breast cancer is a threatening problem among the women and has become a global burden. As early detection gives the best chance of survival, the International breast cancer Organizations and health activists should educate the Indian women who are unable to avail breast cancer education, the screening and treatment facilities that can reduce the gravity of the breast cancer.

**Pathobiology and breast cancer**

The pathogenesis of breast carcinoma is still unknown. As is the case with most malignancies, age has been recognized as a more important risk factor for breast cancer. Since, over 70 percent of breast cancers occur in women over 50 years old. About 14 % (1 in 7) of women will develop breast cancer during their lifetime (Jemal et al., 2011). Several well-known risk factors contributing to increased risk of breast cancer are early age at menarche, nulli-parity or first birth after the age of 35, breast feeding, late menopause, a first degree relative with breast cancer (Turbull and Rahman, 2008), exposure to female hormones (e.g. post-menopausal hormone replacement therapy), oral contraceptives at the time of the use, and exposure to radiation (Key et al., 2002, Sasco et al., 2003). Experimental data strongly suggest that estrogens are potent mitogen and have a role in the development and growth of breast cancer (Clemons and Goss, 2001). Increasing the number of menstrual cycles could predispose women to greater DNA damage in the proliferating breast ductal tissue and thus could increase the risk of mutations that directly leads to breast cancer. Furthermore, increasing evidence implicates smoking, high alcohol consumption, exposure to environmental pollutants (Toxic metals, DDT, PCB and other chemicals), low vegetable intake, physical inactivity, as well as adult and post-menopausal obesity in developing breast cancer (Key et al., 2001). Likewise, dietary fatty acids have been associated with increased risk of breast cancer (Rose, 1997; Escrich et al., 2011). High body mass index (BMI) is another risk factor which is related to increased endogenous estrogen production, which may explain the association between obesity and breast cancer (Berclaz et al., 2004).
Genetic predisposition

Genetic predisposition also accounts for 5-10% of all breast cancers (Lacroix and Leclercq, 2005). The most common cause of hereditary breast cancer is an inherited mutation in the DNA repair gene(s) BRCA1 and/or BRCA2. Both of these genes act as tumor suppressors and their loss-of-function promotes malignancy in breast and ovarian cancers. BRCA1 and BRCA2 mutations increase the risk of breast cancer by 10-20 fold compared to the general population and the patients often suffer earlier cancer onset (Chen and Parmigiani, 2007; Allain, 2008). Compared to sporadic cancers BRCA1 associated carcinomas are more frequently poorly differentiated high grade tumors (Gage et al., 2012). BRCA2 related cancers do not show a distinctive phenotype although displaying often higher grade tumors (Allain, 2008). Less than 10% of hereditary breast cancers are due to mutations in other genes such as CHEK2, P53, PTEN, LKB1, STK11, BRIP1, PALB2, and ATM (Key et al., 2001; De jong et al., 2002; Gage et al., 2012). The genetic testing of patients for these mutations is at present not well established than in the case of BRCAs and further research is desired for more detailed profiling of patients with hereditary breast cancer (Dite et al., 2003). However, a number of variables, or personal characteristics, may predict plausibility for breast cancer.

Risk factors of breast cancer

Every woman is at the risk of breast cancer. Relatively, strong risk factors for breast cancer that affect large proportions of the general population have been known for some time. However, the enormous breast cancer cases occur in women who have no identifiable risk factors other than their gender (Kelsey and Gammon, 1990). The well-known risk factors for breast cancer are female gender, age, previous breast cancer, virus infectious diseases, benign breast disease, hereditary factors (family history of breast cancer), early age at menarche, late age at menopause, late age at first full-term pregnancy, postmenopausal obesity, low physical activity, race/ethnicity and high-dose exposure to ionizing radiation early in life. Several epidemiological studies suggested that risk factors can be considered in terms of those which relate to anthropometrical risk factors includes Height, weight, Body mass index (vanden Brandt et al., 2000) and lifestyle factors (Diet, alcohol consumption, smoking, exercise and physical activity). The higher incidence of breast cancer observed in the developed country as compared to the developing country which
reflects the long-standing high prevalence of other risk factors associated with an increased risk of breast cancer.

**Anthropometrical risk factors**

**Age**

Since, cancer can occur in persons of every age, it is common among the aging population (Fig. 4). Sixty percent of new cancer cases and two thirds of cancer deaths occur in persons > 65 years. The incidence of common cancers (e.g., breast, colorectal, prostate, lung and cervical cancers) increases with age. About 80 percent of breast cancer occurs among women 50 years of age and older (Sasieni *et al.*, 2011) (after menopause); at age 50, a woman’s chance of developing breast cancer is about 1 in 50; and by age 60, her chance is closer to 1 in 24. Approximately 77 percent of women with new diagnoses of breast cancer are over age 50.

![Age-related cancers](image)

**Fig. 4** Age-related incidence and mortality for all types of cancers (Adapted from SEER Cancer Statistics Review, NCI, USA)

Therefore, age is the strongest risk factor for development of breast cancer, like most other cancers. An increasing trend in the incidence rates of the breast cancer has been reported from the various registries of National Cancer Registry Project (Siddiqui *et al.*, 2001). It is constituted 18.5 percent of the total new cancer cases in Indian women today.

**Height and weight**

Height also a risk factor for developing breast cancer but it is controversial. Several prospective cohort studies have shown that greater height is associated with a greater risk of
breast cancer in both pre-and postmenopausal women (van den Brandt et al., 2000). An 11% increase in breast cancer risk is found for every 5 cm increase in height at 14 years. Recent studies have suggested that adult weight gain, especially just before and after menopause, increases breast cancer risk. After menopause a woman's ovaries stop producing estrogen and the primary source of estrogen is a woman's body fat. Therefore, a woman with a higher level of body fat during the post-menopausal years would be expected to have a higher level of body estrogens than a relatively lean woman.

**Body mass index (BMI)**

Overweight and obesity is measured by a body mass index (BMI). The association between body mass index (BMI) and risk of breast cancer has been analyzed in various studies. BMI is calculated by dividing weight in Kg by height in meters square. A BMI under 18.5 is classified as underweight, 18.5-24.9 as healthy weight, 25-29.9 as overweight and 30 or over as obese) Compared to lean (BMI 22.5-24.9) women, overweight post-menopausal women have a 10-20% increased risk of breast cancer, and obese post-menopausal women a 30% increase in risk (Franceschi et al., 1996; Shu et al., 2001). Women with a BMI under 22.5 have a 15% reduction in risk compared to women with a BMI of 22.5-24.9. In contrast, lower breast cancer is associated with high body mass index is reported among premenopausal women (London et al., 1989).

**Lifestyle factors**

As with other types of cancer, studies continue to show that various lifestyle factors may contribute to the development of breast cancer.

**Alcohol consumption**

Alcohol abuse is clearly linked to an increased risk of developing breast cancer. The risk increases with the amount of alcohol consumed. Compared with non-drinkers, women who consume 1 alcoholic drink a day have a very small increase in risk. Those who have 2 to 5 drinks daily have about 1½ times the risk of women who drink no alcohol. Excessive alcohol use is also known to increase the risk of developing other cancers like mouth, throat, esophagus, and liver.
**Exercise**

Studies have shown that women who exercise regularly have a lower risk of breast cancer. Some evidence suggests that circulating levels of estrogen are lower in women who exercise regularly. Body fat is often reduced in women who exercise and body estrogen levels may also be reduced thereby exercise may extend the length of a woman's menstrual cycle (Fact sheet, 1998). Longer menstrual cycles correspond with fewer cycles over a lifetime, and fewer menstrual cycles can result in less lifetime exposure to estrogen. Therefore, it is especially important for young girls to establish a pattern of regular exercise, since girls may carry these patterns through their adult years.

**Physical activity**

A number of studies have assessed the association between physical activity and breast cancer risk. Many studies show an approximately 25% decrease in breast cancer risk among the most physically active women compared with the least active women in 47 of 62 studies included in the review of Friedenreich and Cust, 2008. A case-control study suggested that a 6% reduction in risk for each additional hour of physical activity per week assuming that the level of activity would be sustained (Monninkhof et al., 2007). So far, the critical time periods in life with respect to physical activity and breast carcinogenicity are unknown and it is not clear what combination of frequency, duration, and intensity of physical activity is optimal to reduce the risk of breast cancer (Monninkhof et al., 2007).

**Non reproductive risk factors**

**Gender**

The risk of development of breast cancer is increased in females compared to males at a ratio 100:1, whereas, men can also develop breast cancer, although the incidence is very low. So, gender is therefore a marker for events and exposures that happen more often or more strongly to women than to men. Men who are carriers of BRCA2 mutation have higher risk of development of breast cancer. The gene BRCA2 mutation has about 6 percent chance of developing breast cancer during his lifetime. Of the 4,418 breast cancer cases reported to the WCRS in 1998, only 46 (1 percent) occurred among males. Breast cancer reported only 0.4 percent of total male cancer cases (Wisconsin Cancer Reporting System, 2000).
**Personal history and family history**

Women who have had a breast cancer cause an increased risk of getting breast cancer in their other breast. A woman with cancer in one breast has a 3-4 fold increased risk of developing a new cancer in the other breast or in another part of the same breast. Women who have had benign breast problems are also at increased risk but to a lesser extent (Minami *et al.*, 1999). Besides, family history of breast cancer is one of the most well-established breast cancer risk factors. Women with a family history of breast cancer, especially in a first-degree relative (mother, sister, or daughter), are at increased risk of developing breast cancer (Turnbull and Rahman, 2008). However, over 85% of women who have a close relative with breast cancer will never develop the disease, and more than 85% of women with breast cancer have no family history (FBC, 2001). The risk of family history of breast cancer risk is believed to be due primarily to genetic factors. As many as 5–10 percent of all breast cancer cases are attributable to specific inherited single-gene mutations and many other cases have some genetic component. The evidence from individual families in which breast cancer occurs very frequently and from large epidemiological studies has shown that some women have a familial predisposition to breast cancer. Recently, it has been shown that germ line mutations in the BRCA1 and BRCA2 genes account for a large proportion of cases of hereditary breast cancer (Ford *et al.*, 1998).

**Abode and Diet**

The incidence of breast cancer is higher among women living in urban areas than in rural areas. Studies reported that, the incidence rate of breast and cervical cancers are high in an urban environment with a high standard of living. The role of diet in the etiology of breast cancer remains controversial. Interestingly, low incidence of breast cancer was observed in Asian people that intake of animal foods is lower than the western population. Various studies have shown that diets high in fat, particularly polyunsaturated, have enhanced the production of tumors in animals challenged with chemical carcinogens (Rohan *et al.*, 1988; Boyd *et al.*, 2003). Studies suggest that, exploring the relationship of diet and breast cancer incidence and mortality have not demonstrated any difference between vegetarians compared to non-vegetarians. Additionally some dietary factors may increase breast cancer risk directly by increasing levels of estrogen in the blood, and indirectly by affecting obesity. Obesity is thought to increase the risk of postmenopausal breast cancer.
Religion

Religion is also one of the risk factors in developing breast cancer. India is a vast country with broadly varying social, cultural, and religious; and each of these factors differs depending on the religion. In India, the major religion is Hinduism followed by Islam, Christianity and other religions like Jains, Buddhists, Sindhis, Sikhs and Parsi etc.) Such kinds of religions probably cannot be found in any other countries of the world. Studies suggest that the risk of developing breast cancer is high in Parsi women as compared to Christian, Muslim and Hindu women (Jussawalla et al., 1981) and this fact may be due to the higher proportion of Parsi women remaining unmarried, their higher age at marriage, late age at first pregnancy, a small number of pregnancies and in addition to better socioeconomic conditions (Paymaster and Gangadharam, 1972) and leading a more westernized lifestyle (Jayant, 1986).

Reproductive risk factors (RFs)

Reproductive and hormonal factors are contributing to the development of breast cancer. Age at menarche, age at marriage, age at first pregnancy, the number of miscarriages, parity, nulli-parity, breastfeeding and years since menopause are major reproductive risk factors of breast cancer.

Early menarche and late menopause

Age at menarche is an established risk factor for breast cancer. However, 10-20% increase in breast cancer risk is observed in women who reached menarche at less than 12 years of age compared to those with more than 14 years of age, likely because of an increase in lifetime exposure to ovarian hormones due to the earlier onset of regular ovulatory menstrual cycles. Breast cancer risk is doubled for women who experience natural menopause at age 55 years or later, compared to those whose natural menopause occurs at age 45 or younger (Bernstein, 2002). Delayed menopause is thought to increase breast cancer risk by maximizing cumulative ovarian hormone exposure (Bernstein, 2002). Women who started their menstrual periods at an early age (before age 12) or go through menopause late (after age 55) are at higher risk for developing breast cancer. These relationships are believed to be mediated through estrogen production (Henderson et al., 1988). During the reproductive years, a woman’s body produces high levels of estrogen. Women who start to menstruate at an early age and/or reach menopause at a late age are
exposed to high levels of estrogen for more years than are women who have a late menarche or early menopause.

**Marital status and age at marriage**

The risk of breast cancer is higher among never-married than ever-married women (Talamini et al., 1984). Several epidemiological studies have reported that the risk of breast cancer is higher among single women than married women (Brignone et al., 1987; Ewertz 1988b). In addition women who have late marriage (after about age 30) have a greater chance of developing breast cancer than women who have a child at a younger age.

**Age at first pregnancy or age at first live birth**

Women who have their first full-term pregnancy at an early age have a decreased risk of developing breast cancer later in life. For example, in women who have a first full-term pregnancy before age 20, the risk of developing breast cancer is about half that of women whose first full-term pregnancy occurs after the age of 30 (Bernstein, 2002). In addition lower risk is limited to hormone receptor-positive cases than hormone receptor-negative breast cancers (Ma et al., 2006; Lord et al., 2008). The biologic basis for this relationship is still unknown.

**Parity and Nulli-parity**

On average, women who have had children (i.e. parous women) have 30% lower risk of breast cancer than women who have had no children i.e. nulliparous women (Willett et al., 2004; Ma et al., 2006). In parous women, breast cancer risk decreases with the number of children. But it increases with the increase of age at first full-term pregnancy (30 years or above). Both the factors appear to be independent of the effect of breastfeeding (Kelsey et al., 1993). Nulliparous women had a higher risk for breast cancer compared to parous women (Lokman et al., 2001; Tavani et al., 1999). The protective effect of parity is thought to be due to permanent changes that occur in the breast epithelial cells during the third trimester in preparation for lactation. These more mature (i.e. differentiated) cells are thought to be less vulnerable to DNA damage hence, breast cancer risk during the years following a full-term pregnancy is reduced (Gadducci et al., 2005).

**Breast feeding**

Breast feeding is a point of research in developing the breast cancer. However, many case control studies have reported a significant protective effect of increasing duration
of lactation against breast cancer in premenopausal women (Yoo et al., 1992; Romieu et al., 1996). Several studies have shown that women who breast-feed their babies may be less likely to develop breast cancer than those who have children but do not breast-feed (Katsouyanni et al., 1996).

**Menopausal status**

Post-menopausal women had about 40% higher risk of breast cancer compared with premenopausal women. Women who attained menopause naturally at 50 years of age and above are at slightly elevated risk of breast cancer compared with women who attained menopause at less than 50 years of age. In addition, positive family history of breast cancer is associated with an increase in risk both premenopausal and post-menopausal women (Romieu et al., 1996).

**Other exposures:**

**Oral Contraceptives (birth control pills) and radiotherapy**

Oral contraceptives may slightly increase the risk for breast cancer, depending on age, length of use, and other factors (Marchbanks et al., 2002). Additionally, estrogen pills women who have been consuming diethylstilbestrol (DES) to prevent miscarriage may have an increased risk of breast cancer. The long-term (more than five years) use of postmenopausal estrogen therapy (ERT) or combined estrogen/progestin hormone replacement therapy (HRT) may be associated with an increase in breast cancer risk (Porch et al., 2002). Women whose breasts are exposed to radiation before age 30, especially those who are treated with radiation for Hodgkin's disease, are at an increased risk for developing breast cancer (Preston et al., 2002). Studies show that the younger a woman is, while receiving radiation treatment, the higher her risk for developing breast cancer later in life. Factors such as ionizing radiations and virus are believed to play a major role in bringing about these mutations.

**Biomarkers and breast cancer**

A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. In cancer, a biomarker refers to a substance or process that is indicative of the presence of cancer in the body. A biomarker might be either a molecule secreted by a tumor or it can be a specific response of the body to the presence of cancer.
Genetic, epigenetic, proteomic, glycomic, and imaging biomarkers can be used for cancer diagnosis, prognosis and epidemiology (Hinestrosa et al., 2007). These are observed in hairs, nails, tissues, and body fluids. However, cancer biomarkers are used in the detection, the chance of recurrence, accurate evaluation and management of the disease in different stages. These are helpful in predicting throughout the course of the disease which includes early detection, prognosis of the disease, prediction of how the disease will respond to drug treatment and detection of recurrence and screening of the disease. However, few cancer biomarkers are highly sensitive and specific for cancer detection at the present time. In the recent past, the immunological molecular markers such as estrogen and progesterone receptors (ER/PR) and HER2/neu oncoprotein proteins are useful in the management of breast cancer (Jordan and Brodie, 2007). It make-out to predict the eventual prognosis and can assist in the determination of the most appropriate treatment for the individual. Besides, other new emerging biomarkers such as p53, ki-67 and Bcl2 could be considered for prognosis determination and prediction of response to treatment. As well, during recent past the analysis of trace metals, pro-and anti-oxidant status and metallothioneins expression in breast cancer tissues has gained great concern due to their role in tumorigenesis.

**Immunohistochemical markers**

**Estrogen receptors (ER)**

Estrogens have long been distinguished as being important for stimulating the growth of a large proportion of breast cancers (Ali and Coombes, 2002). The discovery of the estrogen receptor (ER) provided us not only with a powerful predictive and prognostic marker, but also an efficient target for the treatment of hormone-dependent breast cancer with anti-estrogens (Sommer and Fuqua, 2001). Estrogen by interacting with estrogen receptor (ER) plays a central role in regulating the proliferation and differentiation of normal breast epithelium and a subset of breast carcinomas that express ER. It is widely recognized that estrogen promotes pre-neoplastic and malignant growth via interaction with estrogen receptors alpha and beta (ERα and ERβ). This interaction then can lead to transcription either by directly binding to estrogen response elements (ERE), or through non-genomic pathways. The non-genomic action of estrogen very often includes ligand-binding to the ER at the plasma membrane, and leads to the activation of signaling pathways such as MAPK, protein kinases A and C, and calcium pathways (Coleman and smith, 2001), initiating a cell signaling sequence that leads to proliferation. Another non-classical action of the ER is its
direct binding to AP-1. Measurement of ER is urged to use in the diagnosis, prognosis, and treatment planning for women with breast cancer. ER gives an indication of response to therapy.

The estrogen receptors are highly expressed around 70% of breast cancer cases, and are referred to as “ER-positive” tumors. The estrogen binds to ER and stimulates proliferation of mammary cells, with the resulting increase in cell division and DNA replication and increases mutation rate. This causes disruption of the cell cycle, apoptosis and DNA repair processes eventually leading to tumor formation. The estrogen receptor expression is undoubtedly the most important biomarker in breast cancer, because it provides the index for sensitivity to endocrine treatment.

**Progesterone receptor (PR)**

The progesterone receptor (PR) is an intracellular steroid receptor (nuclear receptor subfamily 3, group C, member 3) that binds progesterone. PR is encoded by the PR gene which lies on chromosome 11 (11q22) (Law et al., 1987). PR is expressed in reproductive tissue and has a key role in folliculogenesis, ovulation, implantation and pregnancy (Gadkar-Sable et al., 2005). It has been regulated by estrogen receptor (ER) with many of the same prognostic implications as ER in breast cancer (Clark, 1996). Hence, it is usually measured in addition to the ER because it indicates whether or not the central estrogen/ER regulated pathways are in fact providing enhanced predictive specificity when the two tests are combined (McGuire, 1991).

**HER-2 oncogene**

HER2/neu also known as “Human Epidermal growth factor Receptor 2” (ErbB-2, ERBB2) family protein, it implies higher aggressiveness breast cancers (Quenel et al., 1995). HER2/neu belongs to a family of four transmembrane receptor tyrosine kinases involved in signal transduction pathways that regulate cell growth and proliferation (Zhou and Hung 2003). Over expression and amplification of HER2 can be detected in about 15% of all primary breast cancers, and this group of patient’s benefit significantly from anti-HER2 therapies. HER2 status should be assessed in every diagnosed case of breast cancer (Romond et al., 2005, Smith et al., 2007b). Clinically, HER2/neu is important as the target of the monoclonal antibody trastuzumab (marketed as Herceptin). Trastuzumab is only effective in breast cancer where the HER2/neu receptor is over-expressed. Thus, the
amplification status of HER2 may be a highly predictive biomarker for the assessment of cancer diseases.

**Tumor suppressor gene (p53)**

The p53 gene normally functions as a tumor suppressor by regulating transcription, cell cycle, and apoptosis. Mutations of p53 detected in breast cancers are mainly point mutations that often lead to loss of function of wild type p53 and over-expression of mutant p53 in malignant cells (Ravaioli et al., 1998, Lacroix et al., 2006). The p53 mutations are the most frequent genetic events in human cancer and found in most types of tumors, with frequencies ranging from 5% (cervix) to 50% (lung). Between 20-35% of breast tumors have been shown to express a mutant p53 and are relatively common in intraductal breast carcinoma, but they are not observed in the adjacent normal breast lobules or ducts. These mutations found within the DNA-binding domain of p53 disrupt its proper conformation and thus the mutant p53 is defective in the sequence-specific transcriptional activation dependent on the wild-type p53-binding consensus element. Furthermore, mutant p53 displays a dominant-negative behavior toward wild-type p53 through the formation of hetero-tetramer with wild-type p53 and has oncogenic potential (Herskowitz, 1989; Chen et al., 1990). It is evident that certain cancer-derived mutant forms of p53 transactivate various target genes such as the multiple drug resistance gene 1 (MDR1), c-myc, proliferating cell nuclear antigen (PCNA), interleukin-6 (IL-6), insulin-like growth factor 1 (IGF-1), fibroblast growth factor (FGF) and epidermal growth factor receptor (EGFR) (Cadwell and Zambetti, 2001). Studies have been reported that cancer-derived mutant p53 transactivates asparagine synthetase (ASNS) and telomerase reverse transcriptase (TERT) (Scian et al., 2004). Therefore, it is likely that certain cancer-derived p53 mutants transactivate growth-promoting and oncogenic genes, thereby leading to the progression of the aggressive cancers such as breast cancer. In addition, mutations of P53 gene have been reported in human breast carcinoma, especially in more advanced and/or more aggressive tumors (Tsuda and Hirohashi, 1994).

**Bcl-2 oncoprotein**

The B cell lymphoma-2 (Bcl-2) proto-oncogene has been considered to be a cell death suppressor gene that regulates the programmed cell death called apoptosis (Hockenbery, 1994). It is normally expressed in the cells in a proliferation state, including hematopoietic progenitor, breast, and ovarian cells and also expressed in ductal epithelia of
normal breast (Binder, et al., 1995). The Bcl-2 gene is implicated in a number of cancers, including melanoma, breast, prostate, and lung carcinomas (Bargou, et al., 1995). The family of Bcl2-related proteins constitutes one of the most biologically relevant classes of apoptosis regulators acting at the effector stage (Kroemer, 1997) of apoptosis, with some members functioning as suppressors of apoptosis and others as promoters of cell death. The ultimate vulnerability of cells to diverse apoptotic stimuli is determined by the relative ratio of various pro-apoptotic and anti-apoptotic members of the Bcl2 family (Oltvai et al., 1993; Yang and Korsmeyer, 1996). The Bcl2-interacting protein, Bax is a pro-apoptotic member of the Bcl2 family and its expression is induced by γ-radiation, chemotherapeutic drugs, and other forms of genotoxic stress (Kitada et al., 1996). In addition to the Bcl2 family members, the tumor suppressor gene p53 is required for checkpoint control during cell cycle progression, as well as induction of apoptosis (Meikrantz and Schlegel, 1995). Thus, the expression of Bcl-2 protein could help to assess the malignant tissues progression and it could also be used as a prognostic biomarker.

**Ki-67**

The marker of proliferation Ki-67 is first identified by Gerdes et al. (1983) in the 1980s using a mouse monoclonal antibody against a nuclear antigen from a Hodgkin’s lymphoma cell line. Ki-67 is a nonhistone nuclear protein that is tightly linked to the cell cycle. It is expressed in proliferating cells during the mid G1 phase, increasing in level through S and G2, and to reach the maximum in the M phase of the cell cycle. It is rapidly catabolized at the end of the M phase, and is undetectable in resting (G0 and early G1) cells (Fitzgibbons et al., 2000). Ki-67 expression shows a good relationship with growth fraction in several model systems, (Scott et al., 1991; McCormick et al., 1993), and does not appear to be expressed during DNA repair processes. Hence, it is regarded as an immunohistochemical biomarker of cell proliferation, and in invasive breast cancer (Fitzgibbons et al., 2000).

**Trace metals and breast cancer**

In the recent past, diagnosis and curative approach of the breast cancer is based on predictive and prognostic biomarkers which are involved disease progression. Several reports has been suggested that trace metals play a distinct role in the carcinogenicity (Kuo et al., 2002; Siddiqui et al., 2006; Raju et al., 2006). During industrialization, various metals are used in industry, agriculture, and medicine and that lead to increased exposure to
metal-related occupational workers (Juracek and Ziegler, 2006). Metals are naturally occurring in the environment and human beings are exposed to a variety of sources including air, drinking water and food. Many attempts have been made to investigate trace metals concentrations in different human fluids, and tissues to find the correlation between metals concentration and clinical stages of the tumor (Schrauzer 2000; Zowczak et al., 2001; Kuo et al., 2002; Kucharzewski et al., 2003a; 2003b; Lipinski, 2005; Kriegel et al., 2006). Thus, it has been a growing interest in understanding whether exposure to various toxic and cancer-causing (carcinogenic) metals contribute to the increasing number of breast cancer cases worldwide.

Trace metals are known to play important roles in biological processes including the activation or inhibition of enzymatic reactions, competition among metals and metalloproteinase for binding sites, and modification of the permeability of cell membranes (Nielsen, 1991; Snow, 1992). Many trace metals have been shown to directly modify the DNA, forming DNA adducts as a result DNA damage which induces the chromosomal breaks (Chakrabarti, 2001; De Bont and van Larebeke, 2004). This DNA damage is thought to result from decreased repair capacity and/or by the direct carcinogenic interaction of metallic ions with DNA, as measured by DNA adducts (Hartwig et al., 2002). For example cadmium, lead, nickel and other heavy metals increase oxidative damage through the formation of free radicals such as H\(_2\)O\(_2\), O\(_2^-\), and OH Radical, and directly damaging antioxidant enzymes which reduce oxidative stress such as catalase (CAT), glutathione reductase (GR) thereby modifying the cellular antioxidant, glutathione level (Ercal et al., 2001; Hei and Filipic, 2004; Kawata et al., 2007). Hence, it is evident that the higher levels of trace metals might influence the carcinogenic processes (Kuo et al., 2002; Siddiqui et al., 2006; Raju et al., 2006; Schrauzer, 2006; Silvera and Rohan, 2007). A wide variety of trace metals including lead (Pb), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), nickel (Ni), iron (Fe), selenium (Se), manganese (Mn), zinc (Zn) and vanadium (V) have been pointed as cancer causing agents in both animals and humans, but the basic mechanism of trace metal carcinogenicity was still not clear. Hence, the understanding of breast cancer disease has prompted active area of research to focus on the role of trace metals concentrations in breast cancer.
Trace metals and oxidative stress

Oxidative stress reveals that the balance between oxidative damage in a cell, tissue, or organ caused by reactive oxygen species (ROS) and antioxidant defense system. Oxidative stress occurs when the generation of ROS in a system exceeds that system’s ability to neutralize and eliminate them. It is apparent that ROS plays an important role in the etiology of different human diseases such as carcinogenesis (Frenkel, 1992), irradiation injury (Girotti and Thomas 1984), and tumor promotion (Girotti and Thomas, 1984), in addition to the normal aging process (Jiang et al., 2001). The ability of ROS to damage cellular components, including DNA, is well documented (Halliwell and Auroma, 1991). Metals like lead, cadmium, chromium, arsenic, nickel, copper, iron and others are a major source of oxidative stress (Leonard et al., 1998; Wang et al., 2004; Hei and Filipic, 2004). Studies have been reported that oxidative stress is involved in the development of breast cancer (Gammon et al., 2002; Wu, 2004b). Although, the studies on breast cancer and metals are limited, the levels of trace metals may be used as tumor biomarkers at different levels and also to know the association with breast cancer.

Oxidative Stress

Oxidative stress is a condition in which, the imbalance between the production of free radicals and the efficiency of the antioxidant defense system (Danilova, 2006). The free radicals (ROS & NOS) are formed due to environmental and behavioral stress. These free radicals can alter the structure of biomolecules, such as nucleic acids (DNA), proteins, lipids and lipoproteins (Guyton and Kensler, 1993). Primarily, the target of free radicals on polyunsaturated fatty acids within cell membranes and their interaction results in lipid peroxidation. It is evident that reactive oxygen species play an important role in the etiology of different human diseases, which may lead to carcinogenicity (Frenkel, 1992), irradiation injury, and tumor promotion (Girotti and Thomas, 1984).

Reactive oxygen species (ROS)

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are highly reactive molecules which have an unpaired electron on their outermost orbital. They can act as either beneficial or harmful to living systems (Valko et al., 2004). The beneficial effects like host defense, hormone biosynthesis, fertilization and redox signaling that regulate apoptosis and oxygen sensing (Sumimoto et al., 2005; Nauseef, 2008). In contrast, excess ROS can be important agents for cell damage including, membrane lipids, proteins and
nucleic acids (oxidative stress). Moreover, reactive oxygen spices are regulatory molecules in several disease processes including aging and cancer (Bedard and Krause, 2007). Hydrogen peroxide ($H_2O_2$), hypochloride ($HOCl$), the superoxide anion ($O_2^−$), the hydroxyl radical ($OH•$), peroxyl radical ($ROO•$), alkoxyl radical ($RO•$), thioyl radical ($RS•$) and nitric oxide (NO) are the major free radicals which are derived from the metabolism through various biochemical reactions. The formation of hydroxyl radical occurs in the presence of metals like iron and copper by the Fenton reaction or Haber-Weiss reaction (Kehrer, 2000). Peroxides are the main source for the production of $HOCl$ from $H_2O_2$ (Fu et al., 2003).

Cellular generation of ROS starts with the production of superoxide. Superoxide ions are rapidly dismutases to $H_2O_2$ either spontaneously or through catalysis by superoxide dismutase (SOD). Catalase has the ability to detoxify $H_2O_2$, as does glutathione peroxidase as well as converting lipid hydroperoxides into non-toxic alcohols (Guemouri et al., 1991).

**Oxidative stress and cancer**

Oxidative stress plays a crucial role in various clinical conditions such as cancer, respiratory, cardiovascular, liver, kidney diseases, central nervous system disorders, autoimmune diseases, diabetes mellitus, atherosclerosis, chronic inflammation, viral infection, and ischemia-reperfusion injury (Behrend et al., 2003; Willner, 2004; Galli et al., 2005). It is responsible for DNA, lipid and protein damage and plays a role in the development and progression of many human diseases, including breast cancer (Aghvami et al., 2006; Gago-Dominguez et al., 2007). Oxidative stress is not only known to cause DNA damage and mutations of tumor suppressor genes, which are initial events in carcinogenesis (Kang, 2002), but can also involves in the promotion of multistep carcinogenesis (Ahmed et al., 1999).

**Lipid peroxidation (LPx)**

Lipid damage can occur as a result of oxidative stress or a disruption in the balance between prooxidant and antioxidant factors. Reactive oxygen species are known to extract hydrogen atoms from unsaturated bonds thereby altering lipid structure or function. This extraction process is easier in poly-unsaturated fatty acid (PUFAs) due to the close proximity of the unsaturated bonds, which allows for an easier abstraction of hydrogen atoms from a methylene group. Lipid peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage. Malondialdehyde
(MDA) is a well characterized, and it is an end product of lipid peroxidation (Pryor and Stanley, 1975), which is a process where reactive oxygen species degrade polyunsaturated lipids. The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism (Moore and Roberts, 1998). Using TBARS (thiobarbituric acid reactive substances) method of quantifying lipid peroxidation (MDA levels) in sample measures this end product. Therefore, it would be of great concern to determine the concentration of lipid peroxidation as a marker of tumor progression in breast cancer.

**Antioxidant system**

Antioxidants are molecules or compounds that act as free radical scavengers in biological system. Several antioxidants are electron donors and react with the free radicals to form harmful end products such as water. These antioxidants bind and inactivate the free radicals such as peroxides and superoxides. Therefore, antioxidants protect against oxidative stress and prevent damage to cells or tissues (Halliwell, 2001). In general antioxidant systems either prevent these reactive oxygen species from being formed or remove them before they can damage vital components of the cell (Davies, 1995; Sies, 1997). These can be divided into three main groups: antioxidant enzymes, chain breaking antioxidants and transition metal binding proteins (metallothioneins) (Young and Woodside, 2001).

**Antioxidant enzymes**

**Superoxide dismutase (SOD)**

Superoxide dismutase (EC 1.15.1.1) is an enzyme that catalyses the conversion of superoxide into hydrogen peroxide and oxygen. SOD exists in several isoforms, differing in the nature of the active metal center and amino acid constituency, as well as their number of subunits, cofactors and other features.

$$2O_2^- + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

In humans there are three forms of SODs; cytosolic Cu and Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD (Landis and Tower, 2005). In addition, FeSOD is present in many aerobic bacteria (Ray and Husain, 2002). The presence of SOD has been shown to protect many types of cells from the free radical damage that is important in aging, senescence, and ischemic tissue damage. SOD also protects the cells from the DNA
damage, lipid peroxidation, ionizing radiation damage, protein denaturation, and many other forms of progressive cell degradation (Campana et al., 2004). In SOD, each subunit contains the active site, a binuclear metal cluster constituted by copper and zinc ions. In addition, Mn-SOD is one of the most effective antioxidant enzymes that have anti-tumor activity.

**Catalase (CAT)**

Catalase (EC 1.11.1.6) is an enzyme present in the cells of plants, animals and aerobic bacteria (Mates et al., 1999). It catalyzes the conversion of hydrogen peroxide to water and molecular oxygen, without production of free radicals. In addition, Catalase can oxidize different toxins, such as formaldehyde, formic acid, and alcohols.

\[
2\text{H}_2\text{O}_2 \xrightarrow{\text{CAT}} 2\text{H}_2\text{O} + \text{O}_2
\]

Catalase also detoxifies various phenols and alcohols (Nordberg and Arner, 2001), which is implicated in the following reaction.

\[
\text{H}_2\text{O}_2 + \text{H}_2\text{R} \xrightarrow{\text{CAT}} \text{H}_2\text{O} + \text{R}
\]

**Glutathione peroxidase (GPX)**

Glutathione peroxidase (EC 1.11.1.9) is a selenium dependent enzyme. GPx is catalyzes the reduced glutathione GSH to its oxidized form of glutathione disulfide (GSSG), while \(\text{H}_2\text{O}_2\) simultaneously decomposes to water. Oxidized form of glutathione is again reduced by the enzyme, glutathione reductase.

\[
2\text{GSH} + \text{H}_2\text{O}_2 \xrightarrow{\text{GPx}} \text{GSSG} + 2\text{H}_2\text{O} \\
\text{GSSG} + \text{NADPH} + \text{H}^+ \xrightarrow{\text{GR}} 2\text{GSH} + \text{NADP}^+
\]

Humans have four different Se-dependent glutathione peroxidases (Mates et al., 1999) and are known to add two electrons to reduce peroxides by forming selenoles (Se-OH). The antioxidant properties of enzymes allow them to eliminate peroxides as potential substrates for the Fenton reaction. The substrate for the catalytic reaction of GPx is \(\text{H}_2\text{O}_2\) or organic peroxide ROOH. GPx decomposes peroxides to water (or alcohol) while simultaneously oxidizing GSH. Notably, GPx competes with catalase for \(\text{H}_2\text{O}_2\) as a substrate and is the major source of protection against low levels of oxidative stress.

\[
2\text{GSH} + \text{ROOH} \xrightarrow{\text{GPx}} \text{GSSG} + \text{ROH} + \text{H}_2\text{O}
\]
Glutathione reductase (GR)

Glutathione reductase is an enzyme (EC 1.8.1.7) that reduces the oxidized glutathione disulfide (GSSG) to the sulfhydryl form reduced Glutathione (GSH), which is an important cellular antioxidant (Mannervik, 1987; Meister, 1988). For every mole of oxidized glutathione (GSSG), one mole of NADPH is required to reduce GSSG to GSH. For every GSSG reduced with NADPH and consequently, two reduced GSH molecules are gained, which can again act as antioxidants scavenging reactive oxygen species in the cell.

$$\text{GSSG} + \text{NADPH} + \text{H}^+ \xrightarrow{\text{GR}} 2\text{GSH} + \text{NADP}^+$$

Glutathione-S-transferase (GST)

Glutathione S-transferase (EC 2.5.1.18) is a group of multifunctional, a selenium independent enzyme which plays a central role in detoxification of free radicals, peroxides and a wide range of xenobiotics and carcinogens (Datta et al., 2000; Abou Ghalia and Fouad, 2000). It shows high activity with lipid peroxides (Sharma et al., 2004), detoxifies endogenous compounds such as pre-oxidized lipids (Leaver and George, 1998), particularly high levels in the liver and serves in detoxification metabolism.

Glutathione reduced (GSH)

Reduced glutathione (GSH) is a cysteine containing peptide. It is synthesized in cell from its constituent amino acids. It has direct antioxidant property, acts as an essential cofactor for glutathione peroxidase (Sastre et al., 1996), participates in leukotriene synthesis and regenerates the major aqueous and lipid phase antioxidants (Atalay and Laaksonen, 2002). GSH is most abundant in the cytosol (85-90%), remaining of GSH is found in mitochondria, nuclear matrix and peroxisomes (Wu et al., 2004a; Masella et al., 2005). It is preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides (Pompella et al., 2003). GSH exists in two forms, the reduced form (GSH) and the oxidized form (GSSG). Under normal redox conditions it exits mostly in the reduced form. In the process, glutathione is converted to its oxidized form glutathione disulfide (GSSG), also called L-(−)-glutathione. The oxidized, glutathione can be reduced back by glutathione reductase, using NADPH as an electron donor. The ratio of reduced glutathione to oxidized glutathione within cells is often used as a measure of cellular toxicity (Pastore et al., 2003).
**Metallothioneins (MTs)**

Metallothioneins (MTs) are low molecular weight (6-7 kDa) and cysteine-rich intracellular proteins (Cherian et al., 2003). MTs are bind a number of trace metals including zinc, cadmium, mercury and silver, and also protect cells and tissues against heavy metal toxicity due to their rich thiol content (Coyle et al., 2002; Theocharis et al., 2003; Smirnov et al., 2005). MT can be activated by a variety of stimuli, including metal ions, inflammatory agents, free radicals, glucocorticoids, pharmacological agents, cytokines and growth factors (Cherian et al., 2003; Florianczyk et al., 2003). In point of fact, the synthesis of MT was shown to be increased by several-fold during oxidative stress (Sato and Bremner I, 1993) to protect the cells against cytotoxicity (Aschner et al., 1998), DNA damage (Cai et al., 1995) and lipid peroxidation (Mendez-Armenta et al., 2003). The induction of MT by various stimulators and the downstream effects of MT over expression are summarized in Figure. 5. MT can alleviate tumor cell growth or drug resistance by two possible mechanisms. 1), MT may play an essential role in development and growth through the maintenance of zinc metabolism. The translocation of MT into the nucleus during the proliferative phase (G1–S) of the cell cycle in human tumors also supports a zinc donor role for MT during tumor growth (Cherian et al., 1994).

The induction of MT is an essential for supplying zinc or other metals to target molecules, including enzymes, zinc-finger transcription factors and tumor suppressor gene products such as p53 (Maret et al., 1999; Meplan et al., 2000). Studies have been reported that zinc incorporation is required for the stabilization of wild-type recombinant p53 in a form capable of binding specifically to DNA (Meplan et al., 2000). MT can protect the cells against radiation and chemotherapeutic agents by asset of its free radical scavenging property (Lazo et al., 1998; Shibuya et al., 2008). The antioxidant properties of MT contribute to its anti-apoptotic function (Greenstock et al., 1987). Nuclear factor (NF)-κB is a transcription factor that also plays a role in the anti-apoptotic function of MT. NF-κB activity is regulated by the intracellular redox status, and reactive oxygen species may be involved in the NF-κB activation cascade. Although, the role of MT for NF-κB activation remains controversial, it indicates that MT expression is play a key role in the growth and survival of tumor cells.
There are ten functional isoforms of MTs identified in mammals, includes MT-1A, 1B, 1E, 1F, 1G, 1H, 1X, 2A, 3 and 4 which encode for 4 MT proteins, MT-I, II, III and IV. MT-I, II and III are found in the central nervous system. The expression of MT-I/II is mainly localized in glia and is induced by exposure to metals including ROS, cytokines and heavy metals (Hg, Cd, Cu and Zn). MT-3 isoform is specifically located in the brain and has involvement in Alzheimer’s disease (Uchida et al., 1991; Palmiter et al., 1992) while, MT-4 is found to be concerned with the differentiation of certain stratified epithelia (Quaife et al., 1994; Theocharis et al., 2004; Vasak, 2005). In addition, MT-1 and MT-2 isoforms are expressed coordinately in most organs but the precise role of these MT isoforms has not been well clarified. The MT-III isoform is mainly present in neurons and is not easily induced by exposure to various agents (Cherian et al., 2003). The role of MT isoforms in breast cancer may not only be useful in expounding the clinical significance of this family of proteins but also will help in developing novel approaches in the ongoing battle against breast cancer disease. Expression of MT-1F isoform has also been found to influence histological differentiation in invasive breast cancer since estrogen is known to play...
important role in breast cancer tumorigenesis, the MT-1E isoform has been hypothesized to participate in alternative processes that replace the function of estrogen (Cherian et al., 2003). MT-2A has been found to be positively related to the cell proliferation in livers (Nagamine et al., 2005). In addition, the MT-2A isoform has been reported to be the most abundant MT isoform in breast cancer cell lines and tissues (Jin et al., 2002). Hence, it would be of great concern to examine the roles of MT-1E, MT-1F and MT-2A in breast cancer progression.

**Metallothionein structure and gene regulation**

Metallothioneins bind to a total of seven bivalent metal ions through thiolate coordination in two separate clusters (Kagi and Hunziker, 1989). Structural studies have shown that this unusual protein with 61 amino acids (mammalian MT) can bind with both essential metals (zinc and copper) and toxic metals (cadmium and mercury) in two distinct cluster structures within the molecule. The tertiary structure of MT consists of two domains (α and β) in which the cysteine residues are configured to form thiolate clusters. In that, one cluster is closer to the N-terminal domain (β-domain containing 1-30 amino acid residues) and three metal atoms are bound to nine cysteines with three bridging sulfur atoms, while in the second cluster closer to the C-terminal domain (α-domain containing 31-61 amino acid residues) and four metal atoms are bound to 11 cysteines with five bridging sulfur atoms (Miles et al., 2000; Schicht and Eva, 2009). MT can bind 7 atoms of cadmium (Cd), zinc (Zn) or mercury (Hg) or 12 atoms of copper (Cu) or silver (Ag) (Uchida et al., 1991; Tsuji et al., 1992; Palmiter et al., 1992). The protein’s affinity for each of these ions is different in the order of Hg > Ag > Cu > Cd > Zn.

**Metallothioneins and oxidative stress**

MT protein contains multiple cysteine residues thereby it is reasonable to expect that sulfhydryl-rich and it may function in similar to glutathione (GSH) as an antioxidant. It has the ability to trap the electrophiles, alkylating agents, and free radicals (Sato and Bremner, 1993; Lazo et al., 1998). The multiple cysteine residues of MT can be oxidized during oxidative stress, and subsequently released the Zn^{2+} ions; thus it has been proposed to be an important protective agent against oxidative damage (Maret, and Valle, 1998). Several studies have reported that scavenging the free radicals and show the antioxidant activity of MT by in vivo and in vitro experiments (Sato and Bremner, 1993). MT has been described
to inhibit hydroxyl-radical induced DNA damage (Abel and de Ruiter, 1989). MT is also found to be involved in the protection of lysosomal destabilization as a result of oxidative stress (Baird et al., 2006).

**MTs expression in breast carcinogenesis**

In the recent past, MTs play a distinct role in carcinogenesis. Its expression is elucidated in a variety of human tumors, in relation to different stages of tumor development and progression (Jasani and Schmid, 1997). MT expression in many fetal tissues of mammals is higher than that seen in adults. In human colonic cancer cells, it has been demonstrated that metallothionein expression is increased 2-3 fold in proliferating cell compartments compared to growth inhibited cells, and peak expression occurs during late G1, or S transition phases (Nagel and Valle, 1995). Earlier, it has also been reported that MTs expression may influence both proliferation and apoptosis in breast cancer (Huang et al., 2003). The MTs positive expression appears to be predominantly associated with more advanced, highly malignant tumors. Studies have recognized a relationship between higher aggressiveness and poor prognosis of breast cancer with increased MT protein expression (Oyama et al., 1996; Sens et al., 2001; Jin et al., 2002; Bay et al., 2006). The exact mechanism by which MT has an impact on breast cancer has not been completely elucidated.

**Metallothioneins are biomarkers**

MTs can be induced by the exposure of various metal ions such as Cd, Hg, Zn, Cu and Ag. In the recent past, the use of MTs as biomarkers of environmental metal pollution has been proposed (Amiard et al., 2006). The induction of MTs is affected by several factors such as concentration, duration of exposure and type of trace metal. In mammals, MTs can be induced by hormones, cytokines, oxidants and stress signals (Haq et al., 2003). It is evident that the MT is a potential prognostic marker in invasive ductal carcinoma of the breast (Goulding et al., 1995; Oyama et al., 1996), skin cancer (Zelger et al., 1994), cervical cancer (Lim et al., 1996) and pancreatic carcinoma (Ohshio et al., 1996). Hence, the evaluation of MTs synthesis in the presence of factors other than metals is of essential importance to understand their correct use as biomarkers.

In view of the above, the curative approach in breast cancer patients has changed over the last few years with the preamble of several biomarkers in daily practice. At
present, a better understanding on the application of some markers and the identification of new biomarkers is essential as they improve the patient’s survival rate and quality of life, while making it possible to establish a suitable individual therapy for each type of tumor and also avoiding undesirable treatment in breast cancer patients.

**Plan of work**

In the view of the above, the present study is designed to understand the relation between the various prognostic risk factors and breast cancer with respect to clinical stages and to understand the association between conventional prognostic markers and immunohistological biomarkers. Further, an attempt has also been made to demonstrate that whether the trace metals, antioxidant enzymes status and metallothionein isoforms might also be used as biomarkers of breast cancer.

**Objectives**

The present study has been undertaken to establish relation between risk factors and to elucidate the selected biomarkers in the development, and diagnosis of breast cancer with the following objectives so as to formulate the best suitable therapeutic approach for individual patients.

- To explore the relation between prognostic risk factors and breast cancer.
- To evaluate the association of immunohistological markers and breast cancer.
- To determine the trace metals content.
- To study the changes in the levels of lipid peroxidation and activity levels of antioxidant enzymes.
- To analyze the expression levels of metallothionein isoforms in breast cancer.