Chapter 1

Introduction
DEFINING CANCER:

Cancer is the term used for a group of diseases that cause cells in the body to change and grow out of control. In normal situation, the cells in our body grow and divide in a controlled way to produce more cells as they are needed to keep the body healthy. However, sometimes the genetic material (DNA) of a cell can become damaged or changed, producing mutations that affect the normal cell growth and division. When this happen cells do not die when they need to and the new cells form when they are not required in the body resulting in lump or mass called a tumor (http://www.medicinenet.com/cancer/article.htm).

Cancers are capable of spreading throughout the body by two mechanisms: invasion and metastasis. Invasion refers to the direct migration and penetration by cancer cells into neighboring tissues. Metastasis refers to the ability of cancer cells to penetrate into lymphatic and blood vessels, circulate through the bloodstream, and then invade normal tissues elsewhere in the body. Depending on whether or not they can spread by invasion and metastasis, tumors are classified as being either benign or malignant. Benign tumors are tumors that cannot spread by invasion or metastasis; hence, they only grow locally. Malignant tumors are tumors that are capable of spreading by invasion and metastasis.

Cancer is a common disease. All of us start with about a 1 in 3 (30%) chance of developing cancer (excluding skin cancer) over the course of our lives. When mutation occur in genes controlling cell growth and division, the cell's normal growth-regulation breaks down, allowing it to progress towards malignancy. An inherited factor, or altered gene, is responsible for the pattern of cancer in the family (Farber, 2001). Cancer has emerged beyond heart disease to become the most common cause of death, making the risk of contracting some type of
malignancy during extended lifetimes more likely. Most cancers are triggered by environmental and lifestyle or unknown causes. Most malignancies are not due to the heredity. In general, about 10% of breast cancer and 5% of ovarian cancer cases are contracted by carriers of defective genes (Itzkovich, 2010).

Cancer is the leading cause of death worldwide including India (Figure 1) and second leading cause of death in developing countries (Jemal et al., 2011). According to Siddhartha Mukherjee, Cancer is the “emperor of all Maladies” (Mukherjee, 2010). In 2008, over 12 million cases of cancer were diagnosed worldwide in both sexes combined. About 4.2 and 3.3 million deaths occurred due to cancer in males and females respectively (Jemal et al., 2011).

Cancer is such a disease that spans time, and is thought to originate from single or several cells that have acquired multiple mutations. Robert Weinberg has mentioned that cancer has six functional capabilities that are acquired throughout a tumor’s lifetime. These capabilities are evading apoptosis, sustained angiogenesis, and insensitivity to anti-growth signals, limitless replicative potential, self-sufficiency in growth signals, and tissue invasion and metastasis.

![Figure 1: Year wise total cancer prevalence in India [ICMR, 2006; ICMR, 2009].](image-url)
Cancer is the general name for over 100 medical conditions involving uncontrolled and dangerous cell growth. The disease generally derives from a single cell that is changed dramatically by a series of genetic alterations. A healthy cell has a well defined shape and fits neatly within the ordered array of cells surrounding it. It responds to the environment, giving rise to daughter cells solely when the balance of stimulatory and inhibitory signals from the outside favors cell division. But the process of replication carries the constant hazard of random genetic mutations which can impair the regulatory circuits of a cell (Tindall et al., 2009).

Genetic abnormalities found in cancer typically affect two general classes of genes. Cancer promoting “oncogenes” are typically activated in cancer cells, giving those cells new properties (gain of function). “Tumor Suppressor genes” are then activated in cancer cells, resulting in the loss of normal functions in those cells.

There are more than 200 different types of cancers which may arise in over 60 different organs in the body. Each organ is made up of several different types of cells. For example, there is usually a surface covering of skin or epithelial tissue. Underneath that, there is often a layer of muscle tissue and so on. Each type of tissue is made up of specific types of cells. Cancer can develop from almost any cell type in the body, although one type of cancer will be much more common than the others. Broadly, cancer can be grouped into following categories:

* **Carcinoma**: Cancer that begins in the skin or in tissues that line or cover internal organs

* **Sarcoma**: Cancer that begins in bone, cartilage, fat, muscle, blood, vessels, or other connective or supportive tissue.

* **Leukemia**: Cancer that starts in blood forming tissue such as the bone marrow and causes large numbers of abnormal blood cells to be produced and enter the blood.
Lymphoma and myeloma: cancer that begin in the cells of the immune system.

Central nervous system: cancers that begin in the tissues of the brain and spinal cord.

Cancer is caused by both external factors (tobacco, infectious organisms, chemicals and radiation) and internal factors (inherited mutations, hormones, immune conditions and mutations that occur from metabolism). These causal factors may act together or in sequence to initiate or promote carcinogenesis. Ten or more years often pass between exposure to external factors and detectable cancer. Knowledge about the causes of cancer, and interventions to prevent and manage the disease is extensive. Cancer can be reduced and controlled by implementing evidence based strategies for cancer prevention, early detection and management. One third of cancers could be cured if detected early and treated adequately, based on the observation that treatment is more effective at early stages. In addition, more than 30% of cancers could be prevented simply by behavioral changes that include abstinence from using tobacco, use of healthy diet, maintaining a healthy weight, being physically active and preventing infections that may cause cancer death (Danaei et al., 2005).

According to the epidemiological studies, 80 to 90% of all cancers are due to environmental factors of which, lifestyle related factors are the most important and preventable. The major risk factors for cancer are tobacco, alcohol consumption, infections, dietary habits and behavioral factors. Dietary practices, reproductive and sexual practices account for 20 to 30% of cancers. Studies have shown that appropriate changes in lifestyle will reduce the mortality and morbidity caused due to cancer. This offers the prospect for initiating primary and secondary prevention measures for control and prevention (Murthy and Mathew, 2004).
1.0 BREAST CANCER:

Definition: Cancer that forms in tissues of the breast. The most common type of breast cancer is ductal carcinoma, which begins in the lining of the milk ducts (thin tubes that carry milk from the lobules of the breast to the nipple). Another type of breast cancer is lobular carcinoma, which begins in the lobules (milk glands) of the breast. Invasive breast cancer is breast cancer that has spread from where it began in the breast ducts or lobules to surrounding normal tissue. Breast cancer occurs in both men and women, although male breast cancer is rare.

Breast cancer was believed to be the result of well defined sequence of biological changes in mammary epithelium. Breast cancer is a neoplastic condition that affects the breast tissue. It was suggested that breast carcinogenesis is a multi-step process which starts with hyperplasia progressing through a typical hyperplasia to in situ carcinoma and finally to massive malignant carcinoma (Kinzler and Vogelstein, 1998). The abundance of data in breast carcinogenesis is the result of advancement of molecular biology and genetics. Thus, nowadays it is believed that breast cancer is a complex multi-factorial, polygenic and multi-step process (Beckmann et al., 1997; Ponder 2001; Antoniou and Easton, 2003).

1.1 History of Breast Cancer:

The recorded history of breast cancer traces back to thousands of years. While the incidence of breast cancer as well as the recovery rate continues to rise, breast cancer is hardly a new affliction (Gallucci, 1985; Kardinal and Yarbo, 1979; Diamondopoulus, 1996; Harvey, 1974; Shinkin, 1976). From the dawn of history doctors have written about cancer. Incidence of breast cancer has been documented back to the early Egyptians when the popular treatment was cautery of the diseased tissue. Surgery was practiced but it was an extremely radical treatment considering there was no anesthesia or antiseptics available.
The oldest description of cancer (although the term cancer was not used) was discovered in Egypt and dates back to approximately 1600 BC. The Edwin Smith Papyrus describes 8 cases of tumors or ulcers of the breast that was treated by cauterization, with a tool called “the fire drill”. The writing says about the disease, “there is no treatment”.

The origin of the word cancer is credited to the Greek physician Hippocrates (460 – 370 BC), the “Father of Medicine”. Hippocrates used the terms carcinos and carcinoma to describe the non-ulcer forming and ulcer-forming tumors. In Greek these words refer to crab, most likely applied to the disease because the finger-like spreading projections from a cancer to remind the shape of a crab. Carcinoma is the most common type of cancer.

According to doctrines of the Greek physician Caudius Galen (130 – 200 AD), whose works on physiology and anatomy dominated medical thought until, the Middle Ages; melancholia was the chief factor in the development of breast cancer. Special diets were the recommended treatment. However, other treatments included exorcism and the use of tropical applications, which were seldom preferred by patients. During the Renaissance, Andreas Vesalius recommended mastectomy as well as ligatures (sutures) to control the bleeding rather than cautery. Recognition that breast cancer could and did spread to the regional auxiliary nodes was first recognized by the Physician Le Dran (1685 – 1770). Dr. Le Dran was likely the first to associate poor prognosis with the spread of breast cancer to the lymph nodes.

The famous Scottish surgeon John Hunter (1728 – 1793) suggested that some cancers might be cured by surgery and described how the surgeon might decide which cancers to operate on. If the tumor had not invaded nearby tissue and was “moveable”, he said “there is no impropriety in removing it”.

During the mid 1800’s, surgeons first began to keep detailed records of breast cancer. Those statistics indicate that even those treated by mastectomy had a high rate of recurrence
within eight years—especially when the glands or lymph nodes were affected. Nevertheless, the common treatment was to remove the breast and the surrounding glands in an effort to stave off any further tumor development.

Figure 2: Saint Agatha, the patron saint of breast disease, was martyred for her Christian beliefs. Her torture included amputation of the breasts shown here in a painting.

In 1960 Dr. Robert Egan (Houston) adapted high-resolution industrial film for mammography, allowing simple and reproducible mammograms with improved image detail. And in 1963 the first randomized controlled trial of screening by the Health Insurance Plan of New York found that mammography reduced the 5-year breast cancer mortality rate by 30 percent. Major improvements in mammography equipment, such as reduced radiation dosage, digital imaging, and computer-aided diagnosis, improved detection of breast cancer.

1.2 Prevalence of Breast Cancer:
Breast cancer is the most common cancer diagnosed in women worldwide with over 1.3 million new cases per year. There is a wide variation in the geographical burden of the disease with the
highest incidences seen in the more developed regions of the world and the lowest incidences observed in the least developed regions. More recently the incidence of breast cancer has been observed to be increasing in low income countries and data suggests that over the next twenty years the majority of the increase in the worldwide burden of the disease will be due to rising incidences in these countries.

Breast cancer is the most frequent cause of cancer related death for women in both developed and developing countries. The estimated number of deaths globally due to breast cancer in 2008 was 458367. Mortality rates show less geographical variation compared to incidence rates because of the more favorable survival of breast cancer in high-incidence developed regions. The world standardized mortality rate for breast cancer is 12.5 per 100000 and ranges from 17.5 per 100000 in Western Europe to 6.3 per 100000 in Eastern Asia.

Figure 3: Age standardised incidence and mortality rates for breast cancer worldwide per 100000 in 2008
The incidence of breast cancer is rising in India and is now the second most commonly cancer diagnosed in women after cervical cancer. It is estimated that in 2008 there were 115251 new cases of breast cancer with an age standardised incidence rate of 22.9 per 100000. It is estimated that by 2030 the number of new cases of breast cancer in India will reach just fewer than 200000 per year (Ferlay, 2010).

Data from National Cancer Registry Programme shows that in all urban areas of India breast cancer has now surpassed cervical cancer as the most frequently diagnosed cancer in women. The most recent data available from the National Cancer Registry Programme show a wide variation in age standardised incidence rates observed between rural and urban populations ranging from 36.1 in Bangalore to 7.2 in Sikkim state.

![Figure 4: Age standardized incidence rates for breast cancer in women NCRP 2006-8](image)

The age standardized mortality rate for breast cancer in India is 11.1 per 100000 (12.5 per 100000 globally). In common with other developing regions mortality rates for breast cancer...
in India are high in comparison to incidence rates. Poor survival may be largely explained by lack of or limited access to early detection services and treatment.

1.2 Symptoms:

The following are the symptoms associated with breast cancer:

- The first noticeable symptom of breast cancer is typically a lump that feels different from the rest of the breast tissue. More than 80% of breast cancer cases are discovered when the woman feels a lump. The earliest breast cancers are detected by a mammogram. Lumps found in lymph nodes located in the armpits can also indicate breast cancer.

- Indications of breast cancer other than a lump may include changes in breast size or shape, skin dimpling, nipple inversion, or spontaneous single-nipple discharge. Pain (mastodynia) is an unreliable tool in determining the presence or absence of breast cancer, but may be indicative of other breast health issues.

- Unexplained weight loss can occasionally herald an occult breast cancer, as can symptoms of fevers or chills.

- Symptoms of inflammatory breast cancer include pain, swelling, warmth and redness throughout the breast, as well as an orange-peel texture to the skin referred to as peau d’orange (Green et al., 2002; Taylor & Meltzer, 1938).

- In Paget's disease of the breast, the syndrome presents as eczematoid skin changes such as redness and mild flaking of the nipple skin (Kaelin, 2004). As Paget's advances, symptoms may include tingling, itching, increased sensitivity, burning, and pain (Kaelin, 2004). There may also be discharge from the nipple. Approximately half of women diagnosed with Paget's also have a lump in the breast (Kaelin, 2004).
Bone or joint pains can sometimes be manifestations of metastatic breast cancer, as can jaundice or neurological symptoms. These symptoms are "non-specific", meaning they can also be manifestations of many other illnesses. Most symptoms of breast disorder do not turn out to represent underlying breast cancer. Benign breast diseases such as mastitis and fibroadenoma of the breast are more common causes of breast disorder symptoms (Shaaban et al., 2002). The appearance of a new symptom should be taken seriously by both patients and their doctors, because of the possibility of an underlying breast cancer at almost any age.

The most common breast cancers have an epithelial origin.

1.3 Types of Breast Cancer:

Breast cancer is considered as highly heterogeneous group of cancers arising from different cell types and each having its own clinical implications. Breast cancer can begin in different areas of the breast – the ducts, the lobules, or in some cases, the tissue in between. Depending on from where the cancer arises, breast cancer is divided into different types.
1. Non-invasive breast carcinoma – when breast tumors are discovered at an early stage, they are still small and confined. In such cases, cancer cells have not grown into the surrounding tissues and remain within the borders of a duct or lobule. These tumors are known as non-invasive or in situ tumors. In situ tumors are too tiny to form a "lump", and so they usually are not felt or detected during a physical exam and are diagnosed by mammography. Non invasive carcinoma include –

   a. *Ductal Carcinoma in situ (DCIS)* – DCIS also known as intraductal carcinoma or non-invasive ductal carcinoma starts inside the milk ducts and is the most common type of non-invasive breast cancer. DCIS isn’t life-threatening, but having DCIS can increase the risk of developing an invasive breast cancer later on (Bellamy *et al.*, 1993; Page *et al.*, 1982). Each year, about 19% of women with high grade DCIS develop invasive breast cancer after lumpectomy (Page *et al.*, 1982). Thus DCIS is a potential marker for invasive carcinoma.

   b. *Lobular Carcinoma In Situ (LCIS)* – LCIS is also known as non-invasive lobular carcinoma usually occurs in women who have not undergone menopause. Lobular means that the abnormal cells start growing in the lobules, the milk producing glands at the end of breast ducts. LCIS is a multifocal (located in more than one area) disease that typically affects both breasts in contrast to DCIS, which generally is unifocal or at least limited to one region of the breast. Because of the multifocal character of LCIS, women with this disease should receive careful examinations of both breasts. However, over 99% people with LCIS do not develop invasive breast cancer (Frykberg *et al.*, 1987; Singletary *et al.*, 1994).
2. Invasive Carcinoma – If breast cancer penetrates the membrane that surrounds the lobules or ducts, it is called an infiltrating or invasive carcinoma. Invasive carcinoma can grow into the supporting tissue between the ducts, blood vessels, lymph nodes, and other structures within the breasts. Therefore, there is a greater chance that invasive carcinoma will metastasize throughout the body. Invasive carcinoma include –

a. Ductal Invasive Carcinomas: About 75% of all invasive breast cancers are ductal carcinoma (Li et al., 2003). Under the microscope, ductal carcinoma looks like a mass with poorly defined edges that have begun to extend into the surrounding tissue (Mai et al., 2000). As the cancer invades the fatty tissue around a duct, it causes the formation of fibrous, scar-like tissue (Harris et al., 1984). Such scar formation makes ductal carcinoma appear larger than it actually is. Depending upon the location of the tumor, the symptoms of invasive ductal carcinoma may include retraction (drawing inward) of the nipple or nipple discharge and skin changes such as wrinkling or dimpling.

b. Lobular Invasive Carcinoma: these cancers make up approximately 5% to 10% of all invasive breast cancers (Borst and Ingold, 1993; Li et al., 2003; Martinez and Azzopardi, 1999). ILC is characterized by small round cells that are bland in appearance and have scant cytoplasm, which infiltrate the stroma in single file and surround benign breast tissues in a targeted manner (Fisher 1975; Martinez and Azzopardi, 1979). Lobular breast cancer is more difficult to detect by mammography because it may not occur as a distinct lump (Krecke and Gisvold 1993, Yeatman et al., 1995). Instead lobular carcinoma may appear as an irregular thickening in the breast. A small proportion of women (~5%) may
develop lobular carcinoma in both breasts (Dixon *et al*., 1983; Du toit *et al*., 1989; Lesser *et al*., 1982).

In addition to ductal and lobular carcinoma, three well recognized types of invasive breast cancer are: tubular cancers (slow-growing, tube-shaped cancer), medullary cancers (cancers that look like the medulla [gray matter] of the brain), and mucinous cancers (cancers that contain a mucous protein) (Diab *et al*., 1999; Li *et al*., 2003).

3. Inflammatory carcinoma – It is a very serious, rapidly spreading type of tumor that accounts for about 1% of all breast cancers (Anderson *et al*., 2004; Wingo *et al*., 2004). It produces symptoms like swelling, redness, and skin warmth, which result from blockage of the skin’s lymphatic vessels by cancer cells (Green *et al*., 2002; Taylor *et al*., 1938). Because of such symptoms, inflammatory carcinoma sometimes is confused with mastitis – a breast infection that may or may not be associated with breastfeeding and can be cured by antibiotics.

4. Paget’s disease – Paget’s disease of the nipple is a rare form of the breast cancer in which cancer cells collect in or around the nipple. The cancer usually affects the ducts of the nipple first (small milk carrying tubes), then spreads to the nipple surface and the areola (the dark circle of skin around the nipple). The nipple and areola often become scaly, red, itchy, and irritated (Kaelin, 2004). It usually strikes middle-aged women and may occur in association with an underlying in situ or invasive ductal carcinoma of the breast (Kaelin, 2004).

### 1.4 Stages of Breast Cancer:

Breast cancer staging is based on the TNM system, defined by the American Joint Committee on Cancer, which takes into account tumor size (T), the extent of regional lymph
node (L) involvement, and the presence or absence of metastasis (M) beyond the regional lymph nodes. Using the system, stage 0 implies in situ cancer, while stages I to IV indicate invasive cancer, with IV implying metastatic spread to distant organs.

<table>
<thead>
<tr>
<th>Primary Tumor (T)</th>
<th>Regional Lymph Node Status (L)</th>
<th>Distant Metastasis (M)</th>
<th>Stage Groupings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma in situ</td>
<td>No evidence of cancer in nearby nodes</td>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>Tumor ≤ 2 cm</td>
<td>No evidence of cancer in nearby nodes</td>
<td>No</td>
<td>I</td>
</tr>
<tr>
<td>No evidence of primary tumor Tumor ≤ 2 cm</td>
<td>Metastasis to 1 – 3 nodes</td>
<td>No</td>
<td>IIA</td>
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<tr>
<td>Tumor &gt; 2 cm but ≤ 5 cm</td>
<td>Metastasis to 1 – 3 nodes</td>
<td>No</td>
<td>IIB</td>
</tr>
<tr>
<td>Tumor &gt; 2cm but ≤ 5cm Tumor &gt; 5cm</td>
<td>Metastasis to 1 – 3 nodes</td>
<td>No</td>
<td>IIIA</td>
</tr>
<tr>
<td>No evidence of primary tumor Tumor ≤ 2cm Tumor &gt; 2 cm but ≤ 5 cm Tumor &lt; 5cm Tumor &gt; 5 cm</td>
<td>Metastasis to 4 – 10 nodes</td>
<td>No</td>
<td>IIIB</td>
</tr>
<tr>
<td>Tumor of any size with direct extension to chest wall or skin Tumor of any size with direct extension to chest wall or skin Tumor of any size with direct extension to chest wall or skin</td>
<td>No evidence of cancer in nearby nodes</td>
<td>No</td>
<td>IIIC</td>
</tr>
<tr>
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<td>Metastasis to &gt;10 nodes</td>
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<td>IV</td>
</tr>
<tr>
<td>Any tumor designation</td>
<td>Any lymph node designation</td>
<td>Yes</td>
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</tbody>
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*Size measurements are for the tumor’s greatest dimension

**Includes micro invasion of 0.1 cm or less in greatest dimension.

*Table 1: Criteria for staging breast tumors according to the American Joint Committee on Cancer’s TNM classification (Green et al., 2002)*
1.5 Morphology of Breast:

The structure of a female breast is remarkable and complex. Mammary gland consists of around twenty lobes each of which has a branching duct system ending in terminal ducts. For
the most part, a woman’s breast consists of fat and connective tissue. But there are other, less conspicuous parts in the female breast, including milk ducts, lobules, arteries and lymph nodes. Each breast has 15 to 20 sections (lobes). Each lobe is made up of many smaller structures (lobules), cluster of epithelial cells which radiate from tiny bulbs that can produce milk (alveoli). At the beginning of menarche, and with the influence of estrogen and progesterone, lobules are formed which replace pre-existing terminal ducts. They are bound together by fairly dense connective tissue septa. The contour of breast is filled out by fat issue, as illustrated in fig 1. Complete morphologic maturation of the breast tissue only occurs following pregnancy (Boron Boulpaep, 2003).

Figure 7: Schematic representation of branching duct to lobule structure during lactation.

1.6 Breast Cancer Risk Factors:

Over the decades, there are a number of well-established risk factors listed to have increased breast cancer risk. These included age, female reproductive factors, exogenous
estrogen exposure, radiation exposure, and other non-genetic factors. The Surveillance Epidemiology and End Results (SEER) cancer statistics review from NCI have suggested that breast cancer rate is increasing with age.

A risk factor is defined as ranging from a lifestyle choice an inherent characteristic that influences a person's chances of developing a disease (NBOCC, 2009). The definite cause for breast cancer development is far from being unraveled; some factors have been associated with increased risk of developing the disease.

1.6.1 Non-modifiable Risk Factors:

The non-modifiable risk factors constitute characteristics that cannot be changed, and are usually present independent of the individual's actions.

1.6.1.1 Gender: breast cancer is predominantly a female disease, with only 1% of the cases occurring in male (Fentiman et al., 2006). Women have an increased risk of developing the disease during their lifetimes compared to men. The reason women develop breast cancer more is not merely because of more breast cells than men, but lies in the fact that their cells are constantly exposed to the growth-promoting effects of the female hormones estrogen and progesterone (Wu et al., 2002).

1.6.1.2 Age: increasing age is the single strongest risk factor of breast cancer, apart from the female gender. It is a rare disease before the age of 30 years, after which the incidence rises steeply with increasing age up to about the age of 50 years (Greenle et al., 2000). Thereafter incidence still increases with age, but at a slower rate. The strong dependence of breast cancer on age, apart from the accumulating genetic damage that occurs during the lifespan, has long been shown to correlate with the duration of hormonal exposure (Pike et al., 1983). The chance
of getting breast cancer goes up as a woman gets older (Edwards et al., 2002; Ries et al., 2000; Wu et al., 2002). About 1 out of 8 invasive breast cancers are found in women younger than 45, while about 2 out of 3 invasive breast cancers are found in women at an 55 or older.

1.6.1.3 Family History: Family history of breast cancer is an established risk factor of the disease. A woman’s risk of developing breast cancer is higher if her mother, sister or daughter had breast cancer. The risk is higher if one of her family member got the disease before the age of 40 years (Claus et al., 2003). About 18% of breast cancers occur in women who have a history of the disease in a first degree relative such as daughters, mother or sisters (Slattery and Kerber 1993, Collaborative Group on Hormonal Factors in Breast Cancer, 2001). Genetic studies have shown that a greater proportion of the familial breast cancer is due to specific germ line mutations within a family pedigree. Depending on the probability of disease occurrence, the genes associated with increased breast cancer may be low penetrance or high penetrance genes. An example of high penetrance genes are the BRCA1 and BRCA2 genes whose mutation accounts for about 20% of the familial aggregation of breast cancer, and have been associated with inheritable susceptibility to breast and ovarian cancer (Brody and Biesecker 1998). Heritable breast cancers account for 5 – 10 % of all breast cancers, half of which are related to BRCA1/2 gene mutations (Newman et al. 1998, Hemminki et al. 2002).

1.6.1.4 Genetic Alterations: Inactivating germline mutations in tumor suppressors and/or oncogenes are the best understood causal mechanism for Genetic susceptibility to cancer, which is triggered in several ways (Levine, 1998; Cooper, 1990). The genetic events affecting oncogenes often result in increased stimulatory function, whereas those affecting tumor suppressor genes may cause loss of inhibitory function.
Menstrual History: Women who have had more menstrual cycles because they started menstruating at an early age (before age 12) and/or went through menopause at a later age (after age 55) have a slightly higher risk of breast cancer (Helmrich et al., 1983; Winer et al., 2000). This may be related to a higher lifetime exposure to the hormones like estrogen and progesterone (Henderson et al., 1988). Women who are exposed to endogenous sex hormones over a longer period of their lifetime turn to have increased risk of developing breast cancer. The concept of hormonal carcinogenesis is consistent with epidemiological observations that late menarche and early menopause have a protective effect against breast cancer (Shin et al., 2010). This is consistent with the fact that late menarche (onset of menstruation) and/or early menopause (before age of 55 years) have fewer numbers of menstrual cycles and therefore shorter exposure to ovarian hormones during the reproductive years compared to women who have early menarche and/or late menopause.

1.6.2 Modifiable Risk Factors:

1.6.2.1 Parity, Reproductive History and Breast Feeding: Parity and an early first full-term pregnancy (FFTP) both have been shown to decrease the long-term breast cancer risk (Lambe et al., 1996; Sinha et al., 1988). Nulliparous women are also at increased risk of acquiring breast cancer (Huo et al., 2008). Before pregnancy, mammary gland cells are in a vulnerable undifferentiated state but differentiate to functioning milk-producing structures during pregnancy which are refractory to carcinogenesis (Russo et al., 1994, 1997, 2001). It is thought that pregnancies and FFTP in particular, consecutively decrease the pool of vulnerable breast cells (Russo et al., 1994, 1997, 2001). The undifferentiated cells found in the breast of nulliparous women never undergoes through the process of differentiation, retaining a high concentration of epithelial cells that are targets for carcinogens and are therefore susceptible to
undergo neoplastic transformation (Boulanger et al., 2005; Henry et al., 2004; Wagner et al., 2002). Breast feeding also reduces BC risk and is thought to exert its effects through hormonal mechanisms (Huo et al., 2008; Lord et al., 2008; Zeng et al., 2010).

1.6.2.2 Post-menopausal hormone therapy (PHT): This therapy also known as hormone replacement therapy (HRT) and menopausal hormone therapy (MHT), is used by many older women to relieve the symptoms of menopause and to help prevent osteoporosis (thinning of the bones). 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; Beral, 2004).

1.6.2.3 Abortion/miscarriage history: Some studies have reported an increased risk of breast cancer among women who have had induced abortions (Newcomb et al., 1996; Zeng et al., 2010). In incomplete pregnancy, the breast is exposed only to the high estrogen levels of early pregnancy and thus may be responsible for the increased risk seen in these women. However, some other studies have found no association between abortions and increased risk of breast cancer (Erlandsson et al., 2003).

1.6.2.4 Physical Activity: Studies have consistently shown that the risk of breast cancer is lower among physically active premenopausal women than among sedentary women (Bernstein et al., 1994; Friedenreich et al., 2001). Physical activity during adolescence may be especially protective, and the effect of physical activity may be strongest among women who have at least one full-term pregnancy. A woman's exposure to estrogen is lowered by exercise, which affects the menstrual cycle and can inhibit ovulation. Research suggests that the less a woman ovulates (that is, the fewer ovulation cycles she has), the lower her risk of breast cancer. So exercise with its apparent ability to affect estrogen and, likewise, ovulation - may indirectly lower the risk of breast cancer (Bray et al., 2004).
The preventive effect of physical activity may be due to the non-specific immune stimulation and decreased estrogen levels during recovery (Hardman, 2001) as well as delayed onset of menarche (Hankinson et al., 2004).

1.6.2.5 Obesity: Obesity has been consistently associated with an increased risk of breast cancer among postmenopausal women (Brown and Allen, 2002; Calle et al., 2003; Hirose et al., 2001). This relationship may be mediated again by estrogen production. Fat cells produce some estrogen and obese postmenopausal women, therefore, tend to have higher blood estrogen levels than lean women.

In post menopausal women obesity has little effect on the serum concentration of estrogen probably due to reduced ovarian estrogen by a negative feedback, hence contributes little changes to the risk of breast cancer. Interestingly, obesity in this group of women has been associated with a decrease of breast cancer risk before menopause, yet the mechanism remains unclear (Freidenreich, 2001; Huang et al., 1997; Lahmann et al., 2004). On the other hand, large studies have recorded that obesity and weight gain increase breast cancer risk in postmenopausal women. The risk is evident among obese women who don’t use hormone replacement (HRT) with the relative risk upto 2 (Freidenreich 2001; Harris et al., 1992; Huang et al., 1997; Lahmann et al., 2004). Every 5kg of weight gain since the lowest adult weight increase the breast cancer risk by 8% (Trenthan-Dietz et al., 2000). In another study, women older than 55 with an increase in body mass of 10kg have been associated with 7% increase in breast cancer risk (Tryggvadottir et al., 2002). The mechanism by which post menopausal obesity increases the risk for developing breast cancer may due to the unregulated estrogen level by negative feedback, and obesity instigates an increase in the serum concentration of bio available estradiol (Clemons and Goss, 2001; Favero et al., 1998; Key et al., 2001; Mc.Tierman
et al., 2003). Also, sex hormone binding globulin decreases with increase in body mass index (BMI) and might contribute to the increased breast cancer risk (Verkasalo et al., 2001).

1.6.2.5 Alcohol Use and smoking: The risk of breast cancer is increased among women who consume alcohol or smoke (Atkinson, 2003; Chen et al., 2002; Hamajima et al., 2002; Palmer & Rosenberg, 1993). Studies have shown that consumption of more than two drinks a day leads to an increased level of estrogen in the blood leading to increased risk of breast cancer.

1.7 Genetics of Breast Cancer:

Mutations targeting Oncogenes result in the hyper-activation of these genes in cancer cells, giving those cells new properties, such as hyperactive growth and division, recalcitrance against programmed cell death, loss of respect for normal tissue boundaries and the ability to become established in diverse tissue environments (Bishop, 1989; Varmus, 1989). This can be achieved by a number of simple molecular mechanisms, including point mutations that constitutively activate an enzyme, deletions that remove negative regulatory regions from proteins (Bishop, 1991), or increased expression resulting from promoter deregulation or from multiplication of the number of copies of the gene by a mechanism called amplification (Savelyeva and Schwab, 2001). Activation of an oncogene is a dominant mechanism, since alteration of a single allele is sufficient to confer a gain of function for onset of cancer progression.

Mutations targeting Tumor suppressor genes (TSGs) results in the loss of normal functions essential for the maintenance of normal proliferation, accurate DNA replication, control over the orientation and adhesion within tissues, and interaction with protective cells of the immune system. Loss of function of a tumor suppressor gene, is typically recessive (Fearon, 1998) and can happen due to gene inactivation mechanisms like mutations, methylation or loss
of alleles (most often through the loss of entire chromosomal sections encompassing several dozen genes), small deletions or insertions that scramble the reading frame of the gene, transcriptional silencing by alteration of the promoter region, or point mutations that change the nature of the residues that are crucial for the activity of the corresponding protein. TSGs are classified into two major groups; first group genes are nicknamed “gatekeepers” which are negative regulators of the cell-cycle, acting as brakes to control cell division. The genes of the second group are called “care-takers” as their primary aim is not to control the speed or timing of cell division but rather its accuracy. Care-taker genes are usually involved in the DNA repair and in controlling genomic instability. Their inactivation does not enhance cell proliferation per se but primes the cell for rapid acquisition of further genetic changes (Kinzler and Vogelstein, 1997).

![Figure 8: Pathway for Tumor Suppressor Genes in Breast Cancer](Image)
1.7.1 \textit{BRCA1} and \textit{BRCA2} genes:

The \textit{BRCA1} gene covers 81 kilo bases (kb) of genomic DNA on chromosome 17q21 and has 24 exons, 22 of which are coding (Miki \textit{et al.}, 1994; Smith \textit{et al.}, 1996). The \textit{BRCA2} gene is distributed over roughly 70kb of genomic DNA on chromosome 13q12, and of its 27 exons, 26 are encoding (Wooster \textit{et al.}, 1995; Tavtigian \textit{et al.}, 1996). Both genes have a large exon 11 (comprising 61\% and 48\% of the whole coding sequences of \textit{BRCA1} and \textit{BRCA2}, respectively) and have translational start sites in exon 2 (Miki \textit{et al.}, 1994; Tavtigian \textit{et al.}, 1996). In \textit{BRCA1} exon 4 is not translated (Miki \textit{et al.}, 1994; Smith \textit{et al.}, 1996). The genomic regions of \textit{BRCA1} and \textit{BRCA2} have unusually high (47\%) densities of repetitive DNA elements (Smith \textit{et al.}, 1996; Welch and King, 2001).

The 7.8kb \textit{BRCA1} mRNA encodes a protein with 1863 amino acids and a predicted molecular weight of 208 kilodaltons (kDa). The \textit{BRCA2} transcript is 12kb long and encodes a protein with 3418 amino acids and a predicted molecular weight of 384 kDa. The \textit{BRCA1} and \textit{BRCA2} proteins bear little resemblance to one another or to other known proteins (Venktaraman, 2002). Nevertheless, there are striking similarities in their expression patterns, and they both appear to be involved in the process of proliferation and differentiation in multiple tissues, notably in the mammary glands in response to ovarian hormones (Marquis \textit{et al.}, 1995; Rajan \textit{et al.}, 1996, 1997; Spillman and Bowcock, 1996; Marks \textit{et al.}, 1997). Several functional domains and structural motifs have been identified in \textit{BRCA1} and \textit{BRCA2}, and they have been found to interact with each other and with various other proteins, including transcription factors and proteins involved in DNA double-strand break repair (Zheng \textit{et al.}, 2000; Welch and King 2001; Venkitaraman, 2002).
Cells deficient in \textit{BRCA1} or \textit{BRCA2} accumulate chromosomal abnormalities (Tirkkonen \textit{et al.}, 1997; Abbott \textit{et al.}, 1998; Lee \textit{et al.}, 1999; Xu \textit{et al.}, 1999; Moynahan \textit{et al.}, 2001) and are hypersensitive to genotoxic agents (Sharan \textit{et al.}, 1999; Gowen \textit{et al.}, 1998; Scully \textit{et al.}, 1999; Moynahan \textit{et al.}, 2001). This suggests that \textit{BRCA1} and \textit{BRCA2} may function as caretakers whose loss leads to genetic instability and increases the probability that inactivation of caretaker tumor suppressor genes and activation of proto-oncogenes will occur, eventually leading to tumor formation (Kinzler and Vogelstein, 1997).

\textit{BRCA1} and \textit{BRCA2} have been shown to suppress proliferation of breast cancer cell line, suggesting that they act directly to suppress tumor growth, hence possessing gatekeeper tumor suppressor functions as well (Thompson \textit{et al.}, 1995; Holt \textit{et al.}, 1996; Somasundaram \textit{et al.}, 1997; Randrianarison \textit{et al.}, 2001; Wang \textit{et al.}, 2002). Although the precise functions of \textit{BRCA1} and \textit{BRCA2} remain unclear, there is strong evidence that they are involved in the DNA damage response pathway, and they have been proposed to play roles in transcriptional regulation, cell cycle checkpoint control, DNA damage repair and recombination (Zheng \textit{et al.}, 2000; Welch and King, 2001; Venkitaraman, 2002).

The tissue specificity of \textit{BRCA1} and \textit{BRCA2} mutation associated carcinogenesis has been proposed to be related to their estrogen responsiveness (Hilakivi-Clarke, 2000; Welch and King, 2001). Estrogens induce cell proliferation and stimulate development of tissues involved in reproduction. However, they may also predispose cells to DNA damage during periods of rapid cellular proliferation. Furthermore, estrogens have been reported to be able to induce direct and indirect free radical mediated DNA damage (Cavalieri \textit{et al.}, 2000; Liehr, 2000). \textit{BRCA1} and \textit{BRCA2} have been suggested to function in protecting breasts and ovaries.
from genetic instability during estrogen induced periods of rapid cellular proliferation (Fan et al., 1999; Hilakivi-Clarke, 2000).

1.7.2 p53 Gene:

The p53 gene is localized on the short arm of the chromosome 17 (17q13.1), contains 11 exons and encodes a protein of 393 amino acids with central region harboring in DNA binding domain. The molecule appears to play a major role in the maintenance of the genomic integrity (Lane, 1992). Following DNA damage, p53 can either arrest the cells at the G1 phase of the cell cycle, thus providing time for the damage to be repaired (El-Deiry et al., 1993; El-Deiry et al., 1994; Nelson and Kastan, 1994), or induce apoptosis (Lane, 1993). Both pathways prevent replication of damaged DNA and further accumulation of mutations. Cells containing biologically inactive p53 protein are devoid of such protective mechanisms and they are genetically unstable. Genetic mutation is the most common pathway for p53 inactivation. Being mutated in approximately 50% of all tumors, p53 is currently considered the most frequently altered gene in human tumorigenesis.

Since the discovery of this gene, more than 4000 abnormalities have been reported, the majority of them being missense point mutations that result in single amino acid substitutions. These mutations cluster between exons 5 – 9, which correspond to the highly conserved domains of the protein (Caron and Soussi, 1992).

Many mutants have a different conformation and a longer lifetime compared to the wildtype protein. Increased lifetime causes mutant p53 protein accumulation in the tumor cells, which is detectable by conventional immunohistochemical or other immunologic methods.
1.7.3 Inheritance and Penetrance:

*BRCA1* and *BRCA2* are tumor suppressor genes, and therefore mutation in both alleles is required within the normal cell for neoplastic transformation to occur (Knudson *et al.*, 1975). It is therefore often observed that the wild type allele of the gene is lost in tumors of heterozygous carriers. Consequently, such cancer predisposition is inherited in a dominant fashion, while the predisposing allele behaves as a recessive allele in somatic cells. The mutation inherited through the germ line is generally small and restricted to the gene, while the second "hit" usually occurs somatically and involves large stretches of DNA. Knudson (1993) predicted that genes which confer a risk of cancer due to germ line mutations are expected to be somatically mutated in sporadic cancers of the same type. This theory has been found to hold true for a number of cancers, such as retinoblastoma, colon cancer and Von Hippel Lindau syndrome, but not for breast cancer. Given that *BRCA1* and *BRCA2* play an important role in inherited susceptibility to breast cancer, it is rather surprising that it is very infrequently mutated in sporadic breast cancer. It has however been shown that *BRCA1* and *BRCA2* or its resultant product may be inactivated by other mechanisms besides somatic mutation (Rice *et al.*, 2000).

While *BRCA1* mutation is primarily associated with breast and ovarian cancers, *BRCA2* mutations have been linked to a wide spectrum of cancers, including prostate cancer, pancreatic cancer, fallopian tube cancer, male breast cancer and skin cancer.

The average age specific cumulative cancer risks associated with mutations in *BRCA1* and *BRCA2* have been estimated by a number of studies. After the initial diagnosis of breast cancer in a *BRCA1* or *BRCA2* carrier, the risk of cancer in the contra lateral breast (a new
primary cancer) increases approximately 3% per year (Metcalfe et al., 2004; Verhoog et al., 2000).

A broad range of associated risks is consistent with the hypothesis that risks in BRCA1 and BRCA2 mutation carriers can vary substantially due to the presence of additional risk factors for breast cancer, including genetic modifiers. Common genetic modifiers of breast cancer risk for carriers of mutations in BRCA1 and BRCA2 have been identified in essentially three ways: studies of single nucleotide polymorphisms (SNPs) in candidate genes (Cox et al., 2007; Antoniou et al., 2007; Engel et al., 2010), studies (GWAS) to be associated with a small increased breast cancer risk (odds ratio <1.30) in the general population (reviewed in Milne and Antoniou, 2011) and GWAS carried out in mutation carriers (Antoniou et al., 2010). BRCA1 and BRCA2 mutation carriers could potentially be among the first groups of individuals for whom clinically applicable risk profiling could be developed using the common breast cancer susceptibility variants identified through GWAS.

It has been estimated that, around 5 – 10% of breast cancer cases are caused by germ line mutations in breast cancer susceptibility genes. BRCA1 and BRCA2 are so called high penetrance genes, leading to a high probability of developing breast cancer among carriers of the variant alleles of these genes. The proteins encoded by these genes, which are suggested to act as tumor suppressors, are involved in the maintenance of genomic integrity. They participate in repairing DNA damage by homologous recombination and are also proposed to regulate in transcription. It is possible that other factors, like genes at other loci, endogenous or exogenous factors can interact with BRCA1 and BRCA2 mutations to modify the risk. This might explain the inter-individual variability in cancer risk. The frequency of risk alleles of BRCA1 and BRCA2 varies considerably in different populations.
1.7.4 Spectrum of Mutations:

*BRCA1* and *BRCA2* s the most frequently sequenced genes to date pathogenic mutations are identified throughout the complete coding and splice site region of both genes, without evidence of hot spot regions. Since the identification of the *BRCA1* and *BRCA2* genes (Miki *et al*., 1994; Wooster *et al*., 1995; Tavtigian *et al*., 1996), more than 1000 distinct sequence variants have been described in each gene (BIC database).

The most prevalent type of mutations in *BRCA1/2* are truncating mutations, these include nonsense, frame shift (due to small insertions and/or deletions) and splice site mutations. Over 670 distance truncating mutations have been reported for *BRCA1* and 780 for *BRCA2* in the Breast Cancer Information Core (BIC) (data retrieved in February, 2011, BIC). Missense variants have been identified as well, but their effect on carcinogenesis is not as easy to determine in the case of protein-truncating mutations, which are considered to be functionally deleterious (Shattuck-Eidens *et al*., 1997; Spain *et al*., 1999). However, in *BRCA1* exon 27, four sequence variants that result in a stop codon have been proposed to be non-disease associated (Mazoyer *et al*., 1996; Wagner *et al*., 1999; The BIC database). Significance of some common missense variants has been studied by comparing the frequencies of the variants in large series of Breast cancer cases and matched controls, and most of them do not appear to confer an increased risk of breast cancer (Durocher *et al*., 1996; Dunning *et al*., 1997; Healey *et al*., 2000; Deffenbaugh *et al*., 2002).

Functional studies of *BRCA1* have given further support for the hypothesis that missense alterations located within functional domains play a role in disease predisposition (Scully *et al*., 1999; Vallon-Christersson *et al*., 2001). Only a small number of missense variants in either gene have been described as deleterious mutations (Gorski *et al*., 2000;
Sekine et al., 2001a; Vallon-Christersson et al., 2001; de La Hoya et al., 2002; Meindl and German Consortium for hereditary Breast and Ovarian Cancer, 2002; BIC database) and the clinical significance of a number of amino acid substitutions in BRCA1 and BRCA2 remains still to be resolved (The BIC database).

The observed mutation spectrum is surely influenced by techniques used in mutation screening. Most studies searching for germline mutations of BRCA1 and BRCA2 have analysed the coding regions and splice sites of the genes, and used techniques based on polymerase chain reaction (PCR), e.g., direct sequencing, single strand conformation polymorphism (SSCP) analysis, conformation sensitive gel electrophoresis (CSGE), denaturing gradient gel electrophoresis (DGGE) and protein truncation test (PTT). Although the majority of germ line alterations identified in BRCA1 and BRCA2 (57% and 63% respectively) are unique (The BIC database), several recurrent mutations have been described in a number of ethnic groups and populations (the BIC database), e.g., in Ashkenazi Jews (Roa et al., 1996; Struewing et al., 1997; Fodor et al., 1998), French Canadians (Sinard et al., 1994; Tonin et al., 1998), Icelanders (Johannesdotir et al., 1996; Thorlacius et al., 1997), Finns (Vehmanen et al., 1997a; Huusko et al., 1998), Swedes (Hakansson et al., 1997; Bergman et al., 2001), the Dutch (Peelen et al., 1997; Petrij-Bosch et al., 1997; Verhoog et al., 2001), Belgians (Peelen et al., 1997; Goelen et al., 1999), Russians (Gayther et al., 1997a), Polish (Gorski et al., 2000), and Hungarians (Ramus et al., 1997; Van der Looij et al., 2000). The proportion of recurrent mutations to unique mutations varies in different populations and subpopulations, reflecting historical influences of migration, population structure, and geographical and cultural isolation (Szabo and King, 1997).
1.8 Breast Cancer Detection:

Increased breast cancer awareness with breast self-examinations and major improvements in routine breast cancer screening had a paramount effect on early detection of breast cancer. Improvements in routine breast cancer screening had a paramount effect on early detection of breast cancer. Improvements in conventional mammography (an x-ray technique to visualize the internal structure of the breast) such as the low radiation dosage, enhanced image quality, development of statistical techniques for computer-assisted interpretation of images, long distance, electronic image transmission technologies (telemammography/teleradiology) for clinical consultations, and improved image-guided techniques to assist with breast biopsies (the removal of cells or tissues for examination under a microscope) continue to lower the morbidity and mortality of breast cancer. The support of research on technologies that do not use X-rays and are not used for routine breast cancer screening, such as magnetic resonance imaging (MRI), ultrasound, and breast-specific positron emission tomography (PET) may play a considerable role in further improvements of breast cancer early detection. In most cases, the earlier breast cancer is detected the better the survival rate. Today mammography is the best
available method to detect breast cancer in its earliest, most treatable stage - an average of 1.7
years before the women can feel the lump. Generally, treatment is most effective before the
disease spreads. When breast cancer is diagnosed at a local stage, the five years survival rate is
greater than 90%. This rate decreases to less than 5% when the disease has spread to the lymph
nodes and less than 20% when it has spread to distant organ sites.

Despite recent progress in early detection and surgical therapy, the mortality due to
breast cancer has changed little over the past decades, primarily because the occult
dissemination of cancer cells can occur at an early stage of carcinogenesis. Occult
dissemination of tumor cells in patients with operable cancer can subsequently lead to
formation of metastasis, yet it is usually missed by conventional tumor staging. The success of
routine mammography screening for breast cancer is that it involves increasingly more patients
with small primary tumors formerly thought to have an overall excellent prognosis. Yet, only
approximately two thirds of these patients actually have this favorable prognosis, while the
remaining third develops metastatic disease. Thus, there is emerging evidence that tumor cells
can disseminate into secondary organs at an earlier stage of primary tumor development than
appreciated by current risk classification. There are several challenges that must be addressed
in an effort to continue to lower the mortality associated with this disease.

Molecular oncology is currently one of the most promising fields, which may address
the major problems with early detection and accurate staging of women with breast cancer. The
advent of highly sensitive, molecular techniques, such as the polymerase chain reaction (PCR),
enables the detection of circulating tumor cells and small metastasis at the molecular level.
PCR based assays are used for the detection of tumor cells in lymph nodes, resection margins,
bone marrow and blood. Methods to detect metastatic disease and circulating tumor cells at a
molecular level are of two types: those that detect somatic events such as point mutations or
chromosomal rearrangements, and those that detect expression of tumor specific mRNAs. Both methods have been applied for the detection of many different tumor types. The main limitation of the first method is that not all tumors contain mutations suitable for PCR amplification.

Gene amplification/over expression is a common event in the progression of human cancers, and amplified genes have been shown to serve as molecular markers and have diagnostic, prognostic and therapeutic relevance. Currently, molecular markers offer the unique opportunity to identify occult metastases in early stage cancer patients not otherwise detected with conventional staging techniques. The completion of the human genome as well as an enormous amount of information on the transcriptional activities in cancer cells enable the selection of specific markers for the detection of cancer cells. The ideal prognostic marker is one that clearly delineates a particular prognostic group, is 10 specific, highly sensitive, inexpensive and easy to perform on a small quantity of fresh or fixed tissue. No such marker exists but a number of potential prognostic markers have been extensively investigated. Multiple proteins have been found to be specifically over expressed in certain types of tumors. Detection and quantification of potential tumor markers using sensitive molecular methods could assist in the early diagnosis of cancer disease as well as in the efficacy of anti-cancer therapy. The clinical application of molecular markers in the diagnosis, staging, and management of breast cancer continues to expand. In the past 10 years, numerous investigators have attempted the detection of occult tumor cells in malignancies using the highly sensitive reverse transcriptase polymerase chain reaction (RT-PCR) technique.

Increased accuracy in staging breast cancer patient disease and initiation of earlier therapeutic interventions unequivocally are beneficial consequences of technological advancements that identify high-risk patients early in their disease curse. Blood testing
provides a minimally invasive method to evaluate the presence of circulating tumor cells that may serve as indicators for assessing risk or recurrence.

1.9 Breast Cancer in India:

Breast cancer is the most common form of cancer and the second most common cause of death from a neoplastic disease affecting women. Breast cancer is a disease in which breast cells become abnormal and multiply to form a malignant tumor. One in eight women will develop breast cancer in her lifetime (Ferlay et al., 2010; Laloo and Evans, 2012). Breast cancer is now the most common cancer in both developed and developing countries. The disease ranks as the fifth cause of death from cancer, but it is still the most frequent cause of cancer death in women in both developing countries and developed regions (Yanhua et al., 2012).

Breast cancer refers to the cancers originating from breast tissue, most commonly from the epithelial cells that line the milk ducts or the lobules that supply the ducts with milk (Jensen, 1976).

Although there are significant similarities across region of the world, striking differences exist in the peak age of breast cancer in Asian countries which is 40 to 50 years, whereas the peak age in the Western countries is between 60 and 70 years. The incidence of breast cancer in Asia is rising and is associated with increased mortality, in the West, although the incidence of increasing, the mortality rate is definitely decreasing (IARC, 2008). There were over a million new cases of breast cancer in the world in 2000, making it the second most common in the world and the most common among women with 47% in developing countries. Although rates are 5 times higher in industrialized countries, the burden of disease is more in poorer countries because breast cancer is highly curable if detected early and unfortunately,
about 80% of the cases are detected at advanced stages in developing countries (Boutayeb and Boutayeb, 2005).

Breast cancer accounts for 19 to 34 percent of all cancers causes among women in India. According to the National Cancer registries and regional Cancer Centers, it is the commonest cancer amongst women in Delhi, Mumbai, Ahmedabad, Kolkata and Trivandrum (ICMR, 2001).

In India, breast cancer is the second most common cancer (after cervical cancer) with an estimated 115,251 new diagnoses and second most common cause of cancer-related deaths with 53,592 breast cancer deaths (Ferlay, 2001). Breast cancer accounts for 22.2% of all new cancer diagnosis and 17.2% of all cancer deaths among women in India. Breast cancer in Urban areas of India is three times higher than in rural parts of the country. Although breast cancer is the second most common cancer in all Indian women, recent data from the Atlas of Cancer in India project- a study to assess nationwide patterns of cancer incidence across urban and rural parts of the country (http://canceratlasindia.org) suggest that breast cancer is the most common cancer in metropolitan cities and is predicted to be the most common type of cancer in the coming decade (Programme NCR, 2009). In the metropolitan cities, breast cancer is the leading cancer diagnosis in women with rates nearly twice as common as cervical cancer. In Bangalore, Chennai, delhi, Mumbai and Kolkata, the age adjusted incidence rates are 30.9, 33.0, 31.4, 29.3 and 20.6 per 100,000 (Incidence and Distribution of Cancer, 2008).

Age adjusted rates in India suggest that the disease peaks at a younger age (e.g., 40 – 50 years) than in Western countries, as a result, the majority of new diagnosis occur in pre-menopausal women. According to the National Cancer Registry Programme projections, the number of breast cancer deaths in India will climb to 106,124 in 2015 and to 123,634 in 2020.
The majority of new cases are advanced stage—locally advanced or high stage—at the time of diagnosis (Advani 2004; Chopra, 2001). The increasing burden of disease may be associated with lifestyle factors such as later age at marriage, age at first birth, reduced breast feeding and westernization of diet and physical activity patterns (Moore et al., 2011; Dhillon et al., 2005).

1.10 Breast Cancer Prevention and Early Detection:

The risk of breast cancer may be lowered to the extent that one can make lifestyle changes consistent with modifiable risk factors and may be modified by menopausal and/or hormone replacement therapy status. In addition, healthy lifestyle choices such as limiting alcohol intake, maintaining a healthy body weight, high soy intake and engaging in regular physical activity may help to lower one's risk. There is some evidence to suggest that environmental factors with estrogenic properties may play a role in the etiology of the disease, however there is no consistent epidemiological evidence of long term data in human.

The best way to protect one's self is through early detection. Breast cancer screening includes three methods of early detection: 1) breast self examination 2) clinical breast examination 3) mammographic screening.

1. A Clinical Breast Exam – it is performed by the clinician or other health professional and involves a systematic examination of the breast tissue or skin. The health professionals look for signs and symbols or if any changes occur, including development of a lump or swelling, skin irritation or dimpling, nipple pain or retraction (turning inward), redness or scaliness of the nipple or breast skin, or a discharge other than breast milk. In countries where mammography is widely practiced, the CBE
doesn't provide additional efficacy in mortality reduction and in resource-poor countries.

2. A Breast Self Examination – it is performed by the woman herself and involves a similar examination as the CBE of the breast skin and tissue based on palpations by her hands. The woman is examining the look and feel of her breasts as well as any signs, symptoms or changes to the breasts.

3. A mammogram is an X-ray of the breast that uses very low levels of radiation (0.1-0.2 rads per picture). The images capture calcifications (benign) and masses, which include benign cysts that are fluid-filled, benign solid tumors and cancer.

1.11 Breast Cancer Treatment:

There are several treatment options for women diagnosed with breast cancer that include surgery, chemotherapy, radiation therapy, hormonal therapy and targeted therapies. The most appropriate treatment depends on the woman’s risk profile and stage of disease, which can range from I – IV and is based on the tumor size, location, involvement of lymph nodes and whether or not tumor has spread to surrounding tissue or distant organs.

Treatment includes:

a. Surgery includes lumpectomy (removal of a lump and surrounding tissue), which is also called breast conserving survey, mastectomy (removal of all breast tissue although muscles underneath breast are no longer removed), lymph node removal (or axillary lymph node dissection) which takes place during time of lumpectomy or mastectomy if biopsy shows that breast cancer has spread. Other options include preventive surgeries such as prophylactic mastectomy for women at high-risk and prophylactic ovary removal to lower estrogen production in the body.
b. **Radiation Therapy** includes external beam and internal (implantation of radioactive seeds) radiation, and is usually given after surgery to destroy any remaining cancerous cells left behind. While the former is a well-tested, long standing treatment option, the latter has recently been developed and is still being studied for its efficacy and adverse effect profile.

c. **Chemotherapy** is a systematic therapy that can be administered either before surgery (to shrink the tumor) or afterwards (to reduce the risk of recurrence). For early stage disease, it is usually administered to help remove cancer cells from the body and reduce the risk of recurrence. For advanced stage disease, it is given to destroy as many cancer cells as possible.

d. **Hormonal Therapy** is a treatment option for hormone receptor-positive cancers. It can be given for early-stage disease to either reduce the amount of estrogen or block its action to reduce the risk of recurrence. It can also be given for advanced-stage or metastatic disease to shrink or slow the growth of existing tumors. Hormone therapy includes aromatase inhibitors, selective estrogen receptor modulators and estrogen receptor down-regulators as well as surgical treatments such as removal of ovaries and fallopian tubes.

e. **Targeted therapies** target cancer cell properties specifically as opposed to chemotherapy which also destroys normal, healthy cells and includes treatments such as herceptin and tykerb, which both block cancer cell growth in HER2-positive breast cancers, and avastin, which blocks growth of new blood vessels depending on cancer cell growth.
1.12 Why Breast Cancer Research Is Important?

Carcinoma of the breast is a threat to a woman, because, it affects the perception of body image and sexuality to a degree greater than any other carcinomas. It not only damages the tissues to the household. Studies from different parts of India has shown that breast cancer has become the number one cancer and the incidence rate of the disease has been faster in younger women, however the causes remain unknown (Rao DN, 1991).

One of the commonest causes of death in middle-aged women in many developed countries is breast cancer and it is becoming frequent in developing countries as well. Mortality rates from breast cancer have increased during past 60 years in every country (Park K, 2001). Breast cancer is not just a physical disease; it compels the clients and the health care professionals to face the meaning of their lives. As there is significant role of the breast in a woman's beauty and sexuality, the responses may include fear of disfigurement, loss of attractiveness, abandonment and death. These fears too may cause some women to delay seeking health care (Wagner Frederick B, Martin Richard G, 2001).

According to the study of International Agency for Cancer Research, a part of World Health Organization, there was 79,000 breast cancer patients in India in 2001 and the number crossed 80,000 in 2002. The age-standardized rate of breast cancer in India in 2001 and the number crossed 80,000 in 2002. The age standardized rate of the breast cancer in India in 1.75 per 1,00,00 and accounts for 60,600 new cases (19.3% of total female cancers). The estimated cases according to the states in India showed an excess over cervical cancer in 7 states in India (Rao DN, Ganesh B, 1998).

Early detection of breast cancer is best done by increasing the awareness of risk factors of breast cancer, improving the skill in breast self-examination, screening with mammography and prevention is made possible by the identification of breast cancer genes \textit{BRCA1} and \textit{BRCA2}. 

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*BRCA2* with prophylactic mastectomies. Breast cancer may develop at any time after puberty and the 5-year survival rate has been increasing because of earlier diagnosis and better treatment (Asian Marry Ann., *et al.*, 1998).

All the urban and rural, literate and non-literate women of all ages need more information on breast cancer as the knowledge levels are low, which needs to be imparted to the needy. There is no easy solution to curtail the gigantic cancer system. However, some of the predisposing factors could be avoided through education, which would improve the lifestyle of women and add greatly to curtail the disease (Measagopu Wilson D, Min. 2003). Males can also develop breast cancer because the breast is composed of identical tissues in males and females. Male breast cancer incidence is less than 1% of all breast cancer cases (Agrawal, 2007; Ayantunde *et al.*, 2007).
1.13 Objectives:

On the background of the foregoing presentation the objectives of the present study are

- To understand the molecular heterogeneity of BRCA1 and BRCA2 genes among the Bengalee Hindu Caste population of West Bengal.
- To study the gene expression from the tissues of Breast Cancer patients.
- To study the association of genotype and other lifestyle variables for the Breast Cancer.