2. REVIEW OF LITERATURE

2.1. Historical Perspective

The origin of the word breast cancer is credited to ancient Egyptians who noted the disease more than 3,500 years ago. In 460 B.C., Hippocrates described the breast cancer as humoral disease. He postulated that the body consisted of four humors i.e. blood, phlegm, yellow bile, and black bile. He suggested that breast cancer was caused by excess of black bile in the body. He termed the cancer karkinos, a Greek word for “crab,” because the tumors seemed to have the tentacles like the legs of a crab. The Roman physician, Celsus translated the Greek term into cancer. Galen a Greek physician in 130-200 AD, used the word oncos to describe tumors.

2.2. Breast Cancer

Breast cancer is a malignant tumour which starts in the breast cells. A malignant tumour is a group of cancer cells that can grow into surrounding tissues or can spread to distant areas of the body. The female breast is made up of lobules, ducts and stroma (Figure 1). Most of the breast cancers start from cells lining of ducts known as ductal cancers. Some cancers begin in the cell lining of lobules and known as lobular cancers.

The lymphatic system of breast plays an important role in metastasize the breast cancer and have several parts. Lymph nodes are very small, bean-shaped collections of immune system cells which are connected through lymphatic vessels. Lymphatic vessels are small veins that carry a clear fluid known as lymph (instead of blood) away from the breast.

Breast cancer cells enter to the lymphatic vessels and then start growing in lymph nodes. Most of the lymphatic vessels of breast are connected to lymph nodes under the arm (Figure 2). Some of the lymphatic vessels are connected to lymph nodes inside the chest either above or below the collarbone. If the breast cancer cells have spread to lymph nodes, there is a greater chance that the cells could metastasized to other sites in the body.

2.2.1. Types of breast cancers

Breast cancer can be divided into different types based upon the way that how cancer cells look under the microscope. Breast cancers start in epithelial cells of organs and tissues (Figure 3). Most of the breast carcinomas are adenocarcinoma, which starts
in glandular tissue. Other types of carcinoma are sarcomas that start in the muscle cells, fat or connective tissue. The different combination of types of invasive and in situ cancer can also occur (Assi et al., 2013).

1. **Ductal carcinoma in situ**

Ductal carcinoma in situ is non-invasive type of breast cancer. The difference between DCIS and invasive cancer is that the cancer cells do not spread through the walls of the ducts into the breast tissue. DCIS is considered a pre-cancer and progress to become invasive cancers. About one in six breast cancer cases diagnosed as DCIS.

2. **Invasive ductal carcinoma**

This is the very common type of breast cancer. Invasive ductal carcinoma starts in ducts of the breast, breaks the wall of the duct, and then starts growing into the fatty tissue of the breast. About eight out of ten invasive breast cancers are infiltrating ductal carcinomas.

3. **Lobular carcinoma in situ**

Lobular carcinomas in situ are also known as lobular neoplasia. In this type of breast cancer, the cells start growing in the lobules of the milk-producing glands of breast. In LCIS, cells do not break through the wall of the lobules.

4. **Invasive lobular carcinoma**

Invasive lobular carcinoma starts in the milk producing glands. This type of breast cancer can metastasize to other parts of the body. About one in ten of invasive breast cancers are ILC.

![Figure 1: The normal breast tissue (Massardo et al., 2005)](image-url)
Figure 2: Lymph nodes in relation to breast cancer (Heerdt et al., 2017)

Figure 3: Types of breast cancer (I) Ductal carcinoma in situ (II) Invasive (or infiltrating) ductal carcinoma (III) Lobular carcinoma in situ (IV) Invasive (or infiltrating) lobular carcinoma. Breast profile: A. ducts B. lobules C. dilated section of duct to hold milk D. nipple E. fat F. pectoralis major muscle G. chest wall/rib cage. Enlargements: a. normal duct cells b. ductal cancer cells breaking through the basement membrane c. basement membrane d. duct (Assi et al., 2013).
2.2.2. Other types of breast cancer (Li et al., 2005)

1. Inflammatory breast cancer

This is very rare type of invasive breast cancer and accounts for 1% to 3% of breast cancers. In inflammatory, breast cancer the skin of breast become red and get inflamed. In this type of breast cancer the skin becomes thick, pitted.

2. Paget disease of nipple

This type of breast cancer starts in ducts and then spreads to the skin of the nipple and finally to the areola. It is very rare and accounting only for 1% of breast cancer cases. In this type of breast cancer the skin of the nipple and areola become crusted, scaly and red.

3. Phylloides tumour

This is very rare type of breast cancer. In this type tumour starts growing in the breast stroma in contrast to carcinomas, which develop in the ducts or lobules. These tumours are also known as phylloides tumour and cystosarcoma phylloides.

4. Angiosarcoma

This type of cancer starts in cells lining of blood vessels or lymph vessels. It is uncommon type of breast cancer. This is a complication of breast radiotherapy that develops after 5 to 10 years of treatment.

2.2.3. Breast cancer symptoms

The first symptom of breast cancer includes lump or mass (a painless, hard mass). The lump can be seen under a screening mammogram before it can be felt. The lump can be tender, but it is not painful. Pain is more often a symptom of a non cancerous condition. The symptoms of breast cancer include:

- Swelling of breast skin
- Changes in shape or size of breast
- Skin changes i.e.
  - The skin of the breast may become dimpled or pucked. This condition is usually known as orange peel skin.
  - Redness, increased warmth and swelling of breast (signs are more like an infection).
• Itching of the breast nipple.

Nipple changes
- Some nipples get pointed inward.
- The discharge from the nipples can also occur in non-cancerous.
- The blood discharge from nipple is an important sign of breast cancer
- The crusting, ulcers or scaling on nipple is rare types of symptom of breast cancer.

2.3. Incidence of breast cancer

Breast carcinoma is a major health problem throughout the world. Breast cancer is the most frequent cause of cancer related death in women. Breast cancer was the second most common cancer after cervical cancer in developed regions according to GLOBOCAN, 2012 (Figure 4) (Alghamdi et al., 2013). The breast cancer incidence has ranked number one now a days (Malvia et al., 2017). The incidence rates are rapidly rising in developing countries also.

According to an estimate of international agency for research on cancer, approximately twelve million new breast cancer cases were diagnosed worldwide in year 2008; six million of them were found in developed countries and approximately seven million in the developing countries (Dhillon et al., 2009). In year 2012, approximately 1.7 million breast cancer cases were diagnosed (Alghamdi et al., 2013).

Since the 2008, breast cancer incidence has increased by 40%, whereas mortality has increased by 14%. In year 2015, approximately 231, 840 new cases of breast cancer were diagnosed worldwide which was 14% of all new breast cancer cases and 40, 290 people died from the disease which was 6.8% of all cancer deaths (Sparano et al., 2015).

Breast cancer represents the most common female malignancy in both i.e. developed and developing countries. In general, developed countries have higher breast cancer incidence rates than that of the developing countries. Breast cancer is very common in African-American women as compare to White women (Torre et al., 2015).

The risk of breast cancer development and death is lower in Asian, Hispanic, and Native American women. Since past two decades, incidence rate continue to increase rapidly in developing countries also. Breast cancer incidence rate vary about four fold around the world regions ranging from 27/100,000 in middle Africa and Eastern Asia to 92/100,000 in Northern America (Figure 5).
The rate of breast cancer mortality varies around the world. Breast cancer is the most common cause of cancer related death among women in developing countries. The mortality rates of breast cancer is less than that of incidence in developed countries because of the more favourable survival of breast cancer in developed regions, ranging from 6/100,000 in Eastern Asia to 20/100,000 in Western Africa. However, the breast cancer mortality rate is higher in developing countries (Sparano et al., 2015) (Figure 6).

In India, breast cancer ranks first after cervical cancer and is the most common cancer of women (Figure 7). Over 100,000 new breast cancer cases are estimated to be diagnosed annually in India (Gupta et al., 2015; Singh et al., 2016). The age-standardized incidence and mortality rate (per 100,000) was found 22.9 and 11.1 respectively in India which is less when compared with global (incidence-39.0 and mortality-12.5), less developed region (incidence-27.3 and mortality-10.8), more developed region (incidence-66.4 and mortality-15.3) (Abhijit et al., 2017).

![Figure 4: Age standardized incidence and mortality rates of most common cancers (Ferlay et al., 2015)](image-url)
Figure 5: Age standardized incidence rates (per 100,000) of breast cancer around the world (Ferlay et al., 2015).

Figure 6: Age standardized incidence and mortality rates (per 100,000) of breast cancer around the world (Alghamdi et al., 2013).
Although breast cancer incidence is much higher in well developed countries, the survival rates are also higher. For instance, the five year survival rate for Indian women is about 60%, whereas it is 79-85% in developed countries. The reason being majority of patients undergoes inadequate and inappropriate treatment due to lack of high quality infrastructure, skills and financial resources (Ferlay et al., 2010).

By 2030, the global breast cancer burden is expected to double, growing to 21.4 million cases and 13.2 million deaths. This increase will be the result of demographic changes, adoption of unhealthy lifestyles and behaviors related to economic development, such as smoking, poor diet, and physical inactivity.

2.4. Risk factors

Epidemiologic research has identified several characteristics that are risk factors for breast cancer. The association between genetic polymorphism in ESR1 gene and risk of breast cancer has been the subject of increasing interest now days. As the breast cancer incidence varies in different parts of the world. The fact that breast cancer risk increases when moving from a low risk to a high-risk area indicates that not only genetic factors but also the environment affects breast cancer risk (Ziegler et al., 1993; Lichtenstein et al., 2000). The Breast cancer risk factors are associated with the prognosis of disease in several ways. Different risk factors are discussed below:

2.4.1. Sex

Breast cancer can affects both men and women. The incidence is much higher in women. Overall, women are at hundred fold higher risk of breast cancer as compare to men (Thomas et al., 2004). In the year 2004, the age-standardized incidence rate of
breast cancer was 113 per 100,000 for women and 1 per 100,000 for men (Jemal et al., 2005).

2.4.2. Age

The incidence of breast cancer increases with age. Breast cancer is rarely found before age of 25. After the 50 years of age, breast cancer incidence continues to increase in the older ages. At menopause stage, breast cancer is affected by combination of different hormones (Bakken et al., 2011).

2.4.3. Incidence

Breast cancer incidence and mortality rates vary around the world. Age standardized incidence rates vary around five fold in developed countries as compare to less developed countries (like Africa and parts of Asia). Migrants tend to experience the breast cancer incidence of their adoptive countries in two to three generations (Robsahm et al., 2002). Most of the countries either from higher or lower incidence rate is experiencing increase in breast cancer (Robsahm et al., 2002). Regionally, breast cancer incidence in Australia and New Zealand is second to North America although several European countries have higher breast cancer incidence as compare to Australia (Shin et al., 2005).

2.4.4. Family History

The risk of breast cancer increases if women have a history of breast cancer. The risk is influenced by number of breast cancer affected women in first degree relatives (mother, sister, and daughter). McCredie et al. (1975) reported that 78% validation of breast cancer was possible in first degree relatives and 54% in second degree relatives. According to a meta-analysis, the risk of breast cancer for one or more second degree relatives was lower as compare to first degree relatives (95% CI: 1.4-1.6) (Pharoah et al., 1997). In an Australian study, no association of family history was observed with breast cancer (Mellisa et al., 1998).

A collaborative study has estimated the associated breast cancer risks among first and second degree relatives (Azimi et al., 2009). Women with more than three affected first-degree relatives had high relative breast cancer risks i.e. 1.80 (99% CI: 1.69-1.91), 2.93 (99% CI: 2.36-3.64) and 3.90 (99% CI: 2.03-7.49), respectively.
2.4.5. Reproductive and menstrual history

Most of the established breast cancer risk factors are related with menstrual and reproductive events of women’s life. Polymorphism in ESR1 gene in combination with reproductive factors may alter the risk of breast cancer. It is suggested by many studies that polymorphism may exerts their effect at different age levels of women’s life (Modugno et al., 2005).

2.4.5.1 Age at menarche

Menarche is time of start of menstrual cycles. It is characterized by fluctuations in hormone levels, ovulation and monthly cellular proliferation. The breast begins to develop one to two years before menarche. In this state, the epithelial cells of breast are more vulnerable to many carcinogens. Random errors in the genetic material may occur at menarche that may be passed to next generations (Hsieh et al., 1990).

Epidemiological studies of breast cancer have found that women who had their first menstrual cycle at the age less than 12 years have high breast cancer risk (Bernstein et al., 2002; Willett et al., 2004; Colditz et al., 2005). The involvement of polymorphism as breast cancer risk was studied by many researchers (Modugno et al., 2005; Jakimiuk et al., 2007; Ramalhinho et al., 2013). Cai et al. (2003) proposed significant association of GTn polymorphism with years of menstrual cycle and supports the hypothesis that breast cancer risk is associated with longer estrogen exposure (Cai et al., 2003).

Some studies have reported that menarche occurred six months later in girls with AA genotype of the XbaI polymorphism and TT genotype of the PvuII polymorphism (Modugno et al., 2005; Jakimiuk et al., 2007). The age of menarche was found to be associated with XbaI XX homozygote and homozygous for the PX haplotype (Modugno et al., 2005).

The effect of PvuII polymorphism was not statistically significant. The strong linkage disequilibrium of XbaI polymorphism and PX haplotype with the age of menarche has been shown in some studies (Stavrou et al., 2002; Jakimiuk et al., 2007). Modugno et al. (2005) reported that more than 13 years of age at first menses was associated with -401 C/C, T/C genotype (Modugno et al., 2005). In contrast, some studies did not report any association between polymorphism and age of patient at menarche (Heffler et al., 2005; Ramalhinho et al., 2013).
2.4.5.2 Age at menopause

Many epidemiological studies have suggested that longer exposure to estrogen might be a risk for breast cancer. At the menopause stage, involution process occurs in the breast. In involution process, the decrease of cell proliferation and epithelial cells has been shown. Postmenopausal women have a 15 to 50% lower breast cancer risk as compare to premenopausal women with same age (Colditz et al., 2005).

Many studies found ESR1 gene polymorphism association with breast cancer risk by age and menopausal status (Cai et al., 2003). The PvuII polymorphism was reported to show association with increased breast cancer risk at menopause stage. On the other hand, XbaI polymorphism was confined to postmenopausal women (Zuppan et al., 1989; Shin et al., 2003; Cai et al., 2003). In some studies, breast cancer cases with pp genotype were associated with young age at onset of breast cancer as compare to PP or Pp genotype (Parl et al., 1989; Cai et al., 2003).

Some studies have found the increased PP genotype frequency of PvuII SNP in women with premenopausal status as compare to post-menopausal women (Weel et al., 1999; Surekha et al., 2007). Chattopadhyay et al. (2014) observed the association of T allele of PvuII polymorphism with post-menopausal status of women (Chattopadhyay et al., 2014).

In some studies, a strong correlation of PvuII and XbaI polymorphism with menopausal status was observed. The premenopausal women carriers of pp and xx genotypes of XbaI and PvuII SNPs were found with increased risk of breast cancer (Hu et al., 2007). On the other hand, some studies reported the association of A and T alleles of both polymorphisms with postmenopausal status of breast cancer patients (Kok et al., 2005; Gail et al., 2008). In addition to these, some studies found no relation between ESR1 polymorphism and breast cancer risk among younger or elder women (Ladd et al., 2008; Ramalihnho et al., 2013).

2.4.5.3 Parity

On average, parous women have about 30% lower risk of breast cancer than nulliparous women. In nulliparous women, breast is composed of lobule type 1 with some progression to type 2 and minimum formation of lobule type 3 during sexual maturity. At the menopause, these lobules involute to lobule type 1. But in parous
women, the breast undergoes a complete cycle of development through the formation of lobule type 4 in pregnancy and lactation which later regress (Figure 8).

In parous women, breast cancer risk decreases with the increase in age at first full-term pregnancy and number of children. The association appear to be independent of the effect of breastfeeding (Kelsey et al., 1993). Breast cancer risk is about 40% higher in the women having first full term pregnancy after 29 years of age as compare to women having first child before the age of 25 years (Ebbeling et al., 2002).

Some studies found increased risk of breast cancer in the years following the pregnancies (Kelsey et al., 1993). The elevated breast cancer risk after pregnancies may be effect of the hormones produced during pregnancy (Gadducci et al., 2005). During pregnancy, increased rate of proliferation of epithelial cells takes place. If DNA damage has already been occurred in breast cells, it may persist as cancer. Longer the time period between menarche and a first full-term pregnancy, greater is the chance of occurrence of mutations in epithelial cells of breast.

The significant association of C/C genotype of PvuII polymorphism was observed for parity (Sundarrajan et al., 1999). Shin et al. (2003) observed elevated breast cancer risk among nulliparous women or had first full term pregnancy at the late age, although this association was not statistically significant. In nulliparous women, the xx genotype of XbaI polymorphism was found at four fold breast cancer risk as compared with women with X allele. The P allele of PvuII polymorphism was found to be associated with reduction in breast cancer risk among nulliparous women (Modugno et al., 2005).

Figure 8: (A) Life cycle of breast in nulliparous women. (B) Life cycle of breast in parous women (Wheler et al., 2010).
2.4.5.4 Endogenous and Exogenous hormones

The use of exogenous and endogenous hormones has also been linked to an increased risk of breast cancer. Many researchers have reported the use of hormones in any form is associated with an increase in breast cancer risk in young women (Brinton et al., 1995; Chu et al., 2001).

2.4.5.4.1 Birth control pills

There is considerable debate over whether the use of birth control pills may affect breast cancer risk. The risk of breast cancer development depends on the level of estrogen present in the birth control pill, dose and the time duration of use. The first birth control pills were available in early 1960. At that time, birth control pills had much higher levels of estrogen and the use of birth control pills was found to be associated with increased breast cancer risk. Women using birth control pills were diagnosed with an advanced stage of breast cancer. The effects of oral contraceptive use are seen more among women having longer duration of use (Ebbeling et al., 2002).

However, other study has reported no association among oral contraceptive use and breast cancer in young women (Marchbanks et al., 2002).

2.4.5.4.2 Postmenopausal hormone treatment

After menopause, ovaries can no longer produce estrogen. The loss of estrogen has been found to be associated with increase in risk of heart and blood vessel disease. To counteract these effects, the estrogen hormone is used as treatment, which relieves the discomforting symptoms of menopause.

Recent studies have examined that postmenopausal treatment with estrogen and progesterone might leads to breast cancer risk (Chen et al., 2002). These studies have found the worse effects of combined (formulations containing estrogen and progesterone) hormone therapy greater than other formulations (Chen et al., 2002). Some studies have found that hormone replacement therapy has been associated with increased breast cancer risk up to five years after cessation (Jernstrom et al, 2003; Rosenberg et al., 2006).
2.4.5.5. Breastfeeding

Breastfeeding is associated with a decreased risk of breast cancer. Henderson et al. (1985) postulated the mechanism related with delay of onset of menses during lactation (Henderson et al., 1985). This could reduce breast cancer risk as it may be positively correlated with the ovulatory cycles because mitotic activity is increased in the luteal phase of the menstrual cycle (Henderson et al., 1985).

It has also been suggested that lactation might reduce breast cancer risk by temporarily draining the potential chemical carcinogens and oxytocin hormone (Murrell et al., 1991; Cassoni et al., 2001). In a study, protective effect of having more than three children and breastfeeding for more than three years have been shown to reduce breast cancer risk upto 10–20%. In contrast some researchers found no association of breast feeding and risk of breast cancer (Javed et al., 2011).

2.4.5.6. Lifestyle

A number of personal behaviours have been established as risk factors for breast cancer. These behaviours include physical activities, diet, drinking alcoholic beverages, smoking and body size. Even though some women have high risk lifestyle factors but they may not develop breast cancer. This could be explained on the basis that genetic factors modify the effect of lifestyle on breast cancer risk (Lichtenstein et al., 2000).

Dietary risk factors include alcohol and related beverage consumption. Daily alcohol consumption of 3 to 4 drinks has been associated with an approximately 30% higher risk than with non-consumption (Hamajima et al., 2002). The risk increases to almost 50% with consumption of more than 4 drinks. Many studies proposed the association between PvuII, XbaI polymorphism and breast cancer risk including smoking, alcohol consumption, etc. (Madigan et al., 2000; Cai et al., 2003; Shin et al., 2003).

Some studies found strong correlation between alcohol consumption and XbaI polymorphism (Shin et al., 2003). The effect of smoking on breast cancer risk may be difficult to evaluate, as it may be confounded by alcohol (Hamajima et al., 2002; Jernstrom et al., 2003). Smoking has been associated with an increased risk in premenopausal women in some studies (Jernstrom et al., 2003). In a Swedish cohort of postmenopausal women, no association between tobacco smoking and risk of breast cancer was found (Magnusson et al., 2007).
Anthropometric factors are associated with estrogens levels and thus breast cancer risk. The estrogen production is proportional to the amount of body fat (McTiernan et al., 2013). Overweight women have a higher body mass index (BMI), i.e. weight/length$^2$ (kg/m$^2$), and may therefore at higher postmenopausal breast cancer risk (Boyapati et al., 2004). Breast volume has also been associated with an increased risk of premenopausal breast cancer in lean women (Kusano et al., 2006).

The association between ESR1 polymorphism and height, body mass index in premenopausal as well as postmenopausal women is the well-studied in intron 1 SNPs (Hsieh et al., 1990; Galanis et al., 1998). Deng et al. (2000) found that 454-397C → T, TT genotype was associated with high BMI and against a tendency to gain weight with age, which were associated with an increased breast cancer risk (Deng et al., 2000).

Madigan et al. (2000) found high estrogen level in women having high BMI, despite this they did not observed any effect of BMI in association with breast cancer risk and (GT)n polymorphism (Madigan et al., 2000). No association of PvuII polymorphism was noted with breast cancer risk factors (Cai et al., 2003). The XbaI polymorphism has been found to be significantly associated with body weight and greater BMI (Okura et al., 2003).

In an Indian study, overweight and obese patients were found at elevated risk of breast cancer. The proposed mechanism was that fatty tissue can absorb and accumulate the end products of xenobiotic. The distribution of adipose tissue is mediated by activation of ER through endocrine and paracrine effects. Hence estrogen exposure increases the breast cancer risk (Surekha et al., 2007). The P allele and PP genotype frequencies of PvuII SNP tended to increase with increase in BMI, while Pp genotype frequency was increased only in heavy weight patients (Surekha et al., 2007).

2.5. Diagnosis and Prognosis of breast cancer

2.5.1 Diagnosis

Majority of breast cancer cases are diagnosed at advance stages i.e. stage II, III and stage IV (Meshram et al., 2009). Breast cancer is diagnosed through a triple diagnostic procedure including clinical examination, mammography, and fine needle aspirations or tissue biopsy. The national guidelines state that mammography screening should be offered to all women aged 40 to 74 years. Breast cancer in young women
may have a higher proliferation rate than in postmenopausal women (Smith et al., 2011).

2.5.2 Prognostic Factors

2.5.2.1 Clinicopathological Factors

Investigators made association between ER phenotypes and clinicopathological factors such as tumor size, grade, tumor stage and lymph node status. A study has found that large tumor size increases the incidence of lymph node metastasis (Carter et al., 1989). Lymph node metastasis is related with disease progression, recurrence and plays an important role in making decision whether chemotherapy should be given (Goldhirsh et al., 1995; Canavese et al., 1998). Many studies of lymph node metastasis have considered various genetic factors including cell motility, vascular invasion. As tumor size and stage of disease were considered, polymorphic genotype frequency increases in patients having large tumor size and later stage of disease (Surekha et al., 2007). In order to predict outcomes after the primary operation the prognostic factors includes:

1. Size of invasive tumor
2. Lymph node involvement
3. Histological grade
4. Age at diagnosis
5. HER2
6. Estrogen Receptor
7. Progesterone Receptor

2.5.2.1.1 TNM Classification

Tumors are classified according to invasive size of the tumor (pT), lymph node involvement (pN) and distant metastases (M), where ‘p’ refers to a pathological examination. pT0 represents no sign of primary tumor. pT stands for carcinoma in situ, which is a pre invasive type of cancer where the cancer cells are proliferating in an uncontrolled manner, but have not invaded through the basal membrane into the surrounding normal tissue. pT 1-3 represents different sizes of the tumor and T4 represents a tumor that has grown into the chest wall or involves the skin, independent of its size. Increased tumor size is associated with an elevated risk of lymph
involvement and a decreased chance of survival (Carter et al., 1989). pN0 represents no spread to the lymph nodes. pN1 tumors have spread to the axillary lymph nodes.

More than four positive lymph node involvements have been associated with decreased survival irrespective of tumor size (Carter et al., 1989). Distant metastases are denoted by M, where M0 represents no distant metastases and M1 distant metastases. Breast cancer can be divided in four main stages based on the TNM classification, but the use of this classification varies from country to country and the cancers are sometimes referred to as early and later-stage breast cancer, or simply node-negative or node positive.

2.5.2.1.2 Histological grade

The histological grade of the tumor is based on the evaluation of tubular differentiation, nuclear pleomorphism, and mitotic count (Elston et al., 1991). Each of these three morphologic features is given a score between 1 and 3. The overall histological grade is obtained by adding the score of each characteristic. The total score is given between 3 and 9. A score between 3 and 5 denotes Grade 1 tumor, score between 6 and 7 denotes Grade 2 tumor and score between 8 and 9 denotes Grade 3 tumor.

2.5.2.1.3 HER2

HER2 (HER2/neu, c-erbB-2) is a tyrosine kinase receptor that is over expressed and/or amplified in approximately 15-25% of all breast cancers (Paik et al., 2004; Owens et al., 2004). HER2 is not only a prognostic but also a treatment predictive factor for the response to the monoclonal antibody trastuzumab and the tyrosine kinase inhibitor (Mass et al., 2005; Rasmussen et al., 2008).

2.5.2.1.4 ER and PR status

ER and PR expression is related to the degree of tumor differentiation. ER is expressed in approximately 80% of all newly diagnosed breast cancers in Sweden (Subik et al., 2010). In sporadic breast cancer, postmenopausal women tend to have higher ER concentrations than premenopausal women (Caldarella et al., 2011). ER and PR are mainly used as endocrine treatment predictive factors (Hill et al., 1989).
2.5.2.1.5 Tumor classification and intrinsic subtypes of breast cancer

Breast cancer subtypes act as important prognostic factor. Hierarchical clustering revealed four major clusters, or intrinsic subtypes, of breast tumors. The ER positive/luminal epithelial like tumors were characterized by expression of genes commonly expressed in luminal epithelial cells like estrogen receptor, cytokeratins 8 and 18. The basal like tumors was characterized by expression of cytokeratins 5/6 and the absence of estrogen receptor expression. The ErbB2 group was characterized by high HER2 levels and lower levels of the estrogen receptor.

Based on previous gene clustering analyses, the breast cancer subtypes were divided into: luminal A (ER+ and PR+, HER2-); luminal B (ER+ and PR+, HER2+); HER2+/ER- (ER-, PR-, HER2+); and basal-like (ER-, PR-, HER2-, CK5/6+ and HER2+). Tumors that did not fit these definitions were called as unclassified. Intrinsic subtypes have been described in several populations worldwide (Kim et al., 2006; Adebamowo et al., 2007; Nalwoga et al., 2007).

A standard clinical practice guideline prefers measurement of ER, PR, and HER2 expression in all primary invasive breast tumors to determine treatment course (Harris et al., 2007). Hormone receptor expression is predictive of response to endocrine therapies, such as selective estrogen receptor modulators, aromatase inhibitors (Harris et al., 2007). The tumor that does not express ER, PR or HER2 is termed as triple negative and is routinely use in therapy determination.

2.6. Estrogen and Breast cancer

Estrogen stimulates the development and maintenance of female secondary sexual characters. The natural estrogens produced by women are steroid molecules, derived from a particular type of molecular skeleton containing four rings of carbon atoms (Figure 9). The most prevalent forms of human estrogen are estradiol and estrone which are produced by the ovaries. Estrone is also made in the adrenal glands and other organs.

During each menstrual cycle, estrogen triggers the cells proliferation that forms the lining of milk glands in the breast. If pregnancy does not occur, estrogen levels falls dramatically. In the absence of high estrogen levels, milk gland cells that have proliferated every month, deteriorate and die (Figure 10). For the average woman, hundreds of cycles of breast cell division and cell death repeated from puberty to
menopause. How these estrogens induced cycles increase the risk of developing breast cancer is still unknown (Figtree et al., 2009; Nyante et al., 2015).

There are different ways by which estrogen can induce cellular changes. Estrogen act on target tissues by binding to estrogen receptors. Estrogen receptors is the protein molecule and are the main targets for estrogen action. Estrogen receptors contain a specific site to which only estrogen can bind. Estrogen molecules circulate in the bloodstream and exert effects on cells that contain estrogen receptors (Simonini et al., 2010).

In the absence of estrogen molecules, estrogen receptors remain inactive and have no influence on DNA. The estrogen receptor exists in two forms i.e. ERα and ERβ. Both subtypes of estrogen receptors act as transcription factors to initiate target gene expression (Heldring et al., 2007). When estrogen molecule enters into the cell and passes through the nucleus, binds to its receptor and causes a change in shape of receptor.

The estrogen receptor complex then binds to specific DNA sites, known as estrogen response elements. These are located nearby genes that are controlled by estrogen. After the attachment of estrogen response elements in DNA, estrogen-receptor complex binds with coactivator proteins to activate nearby genes. The activated genes produce the mRNA that guide the proteins synthesis. These proteins can then influence cell behaviour in many ways, based on the cell type which are involved (Figure 11). Estradiol is considered to be the more biologically potent estrogen because it has strongest binding affinity for ERα (60%) and ERβ (40%) (Wedren et al., 2004).

Some mechanisms can modify the genes expression without directly binding with DNA (Figure 12). One example is the interaction between ERα and c-rel subunit of the NFkB complex. The interaction prevents NFkB from binding and stimulating expression from the interleukin-6 (IL-6) promoter (Galien et al., 1997). Afterward, E2 inhibits the expression of the cytokine IL-6 (Ray et al., 1994; Galien et al., 1997).
Another example of indirect action on DNA is the physical interaction of ERα with the Sp1 transcription factor (Porter et al., 1997; Qin et al., 1999). ERα enhancement of Sp1 DNA binding is hormone independent. ERα and ERβ subtypes activate the transcription of the retinoic acid receptor gene (Porter et al., 1997; Zou et al., 1999). In one study, ERβ subtype activated RAR-1 promoter reporter in the presence of the estrogen antagonist 4-OH-tamoxifen, raloxifen (Zou et al., 1999).
Both ERα and ERβ subtypes can interact with the fos/jun transcription factor complex on AP1 sites (Webb et al., 1999). In the presence of ERα, typical agonists such as E2 and DES as well as the antiestrogen tamoxifen function as agonists in the AP1 pathway. In contrast, in the presence of ERβ, tamoxifen and raloxifene behave as fully competent agonists in the AP1 pathway, while E2 inhibit the activity of both tamoxifen and raloxifene (Figure 13).

Biological and epidemiological evidence suggests that the estrogen receptor is a major factor in breast tumor formation and survival. Estrogen receptor expression has been considered to be involved in two thirds of the total breast cancer cases but many studies suggested its incidence might be closer to 80% (Nadji et al., 2005).

The primary focus of ERα gene in breast cancer is for predicting the response to hormonal treatment. Breast cancers patients which expressing ERα are approximately seven to eight times more likely to get benefited from endocrine therapy than ERα-negative patients. For the initial three to five years after primary diagnosis, ERα positive breast cancer patients generally have a better outcome than ERα-negative patients.

2.7. ERα (ESR 1) Polymorphism

Each individual is unique and comparison of genome of any two individuals shows ~0.1% difference. Single nucleotide polymorphism (SNPs) explains 95% of all variant DNA sites (Meyer et al., 2004). According to data obtained from the dbSNP, there are 14,110,048 registered SNPs in the database (Sherry et al., 1999), which can be compared with the approximately 3.2 billion bases in the human haploid genome. The four different nucleotides (Adenine; A, Cytosine; C, Guanine; G, Thymine; T) that constitute the building blocks of DNA, can be altered in different ways. These alterations arise somatically at a high rate, particularly in cancer cells but they may also occur in germ cells, and can be transmitted to next generations.

Various DNA repair mechanisms normally act to preserve high genome integrity, but never with complete fidelity. From evolutionary perspective, a level of continuous germ line mutagenesis can be necessary to allow individuals to adjust with environmental. Sequence alterations or mutation can take place in the genome. The vast majority may end up in non-coding sequences which have no or little effect on cell function. Repetitive DNA sequences (e.g. microsatellites) are susceptible to alterations (Nyante et al., 2015).
Figure 11: The target and non-target cell of estrogen and its mechanism of gene activation 
(Ricke et al., 2008)

Figure 12: Different models representing the modes of action of estrogen receptors in 
transcriptional modulation of genes (Sasano et al., 2009)

Figure 13: Comparison of the structures and homology between types of estrogen 
receptors (ER α & ER β) (Li et al., 2013)
2.7.1. Single nucleotide polymorphism (SNP)

It involves the variation at a single nucleotide that occurs in at least one percent of a population (Risch et al., 2000). SNPs basically arise from mutation but there are several factors which keeps them in the population viz. founder effect, genetic drift and natural selection. There may be natural variations in a gene in which DNA sequence have no adverse effects on the individual and occur with high frequency in the general population. On the other hand, there are certain variations in the DNA sequences which can affect human health.

Many SNPs may be present in genes and in the surrounding region of the genome that control their expression. The occurrence of SNPs is about once every 1000 base pairs in the genome. The SNPs build up the bulk of the 3 million variations in the genome. The frequency of a particular polymorphism tends to remain stable in the population. SNPs can be either transitions or transversion. A transition is an exchange of a purine for a purine or a pyrimidine for a pyrimidine, whereas a transversion is a replacement of a purine by a pyrimidine or vice versa (Figure 14).

Each registered SNP has rs number, to facilitate nomenclature. SNP may have either a dominant or a co-dominant effect (Minelli et al., 2005). Although the effect of a SNP on a gene may not be large, yet subtle effects can influence susceptibility to particular diseases. When the combination of two or more SNPs occurs in a population, they are considered to be in linkage disequilibrium (LD).

Haplotypes can be defined as SNPs that are located close together on the same chromosome, that are less likely to be disrupted by meiotic crossing-over and that are thus inherited together. Certain regions in genome are protected against such recombination and are referred to as Haplotype blocks. Therefore a number of SNPs may capture most of the genetic diversity across that specific region (Johnson et al., 2001). SNPs can be divided into silent, harmless, harmful and latent SNPs (Gerhard et al., 2004). Silent SNPs are variants in non-coding or coding regions and are thought to be non-functional (Sauna et al., 2007). Silent SNPs may not be functional, rather as markers, i.e. in linkage disequilibrium with the functional SNPs. Harmless SNPs are located in coding or regulatory regions, but mostly have a less impact on genetic and cellular function.
Harmless SNPs may change the phenotype and appearance without causing disease. Harmful SNPs are responsible for the increased risk of diseases such as cancer. Latent SNPs may be harmless unless a certain lifestyle factor or exposure is present, e.g. hormones and breast cancer medications.

Previous studies have reported that ER-α polymorphisms are associated with coronary artery and cardiovascular disease risk. ER-α polymorphisms may modify the effect of estrogen on cholesterol level, changes in bone mineral density and cause many diseases (Shearman et al., 2003; Herrington et al., 2002). As far as breast cancer is concern, there are several known polymorphisms in both exons and introns of the ESR1 gene, some of which alter the function of the receptor (Hall et al., 2001) (Figure 15).
Some of the known single nucleotide variants are 454-397T>C PvuII (rs2234693), 454-351A>G XbaI (rs9340799) polymorphism in intron 1, Exon1/codon10 (TCT-TCC), exon4/codon325 (CCC-CCG), exon8/594 (ACG-ACA), +2464 C/T (rs3020314), -4576 A/C (rs1514348), ESR1- 104062C>T (rs851982), SNP rs3798577(T/C) (located at 3’UTR), -104062 C/T, SNPs, rs3020407, and rs3020401 (Siddig et al., 2008; Mavaddat et al., 2009; Anghel et al., 2010; Madeira et al., 2014).

2.7.2. Microsatellites

Microsatellite DNA is a short repeat unit of 1-15 nucleotides, such as CAGCAGCAG etc, repeated many times. Many human diseases are caused by triplet repeat expression mutations. Such repeated units accumulate during rare mistakes of DNA synthesis. The daughter strand slips backward along the template strand to insert additional bases into the daughter strand for the formation of microsatellite (Figure 1).

There are tandem repeats of mono-, di-, tri- or tetra-nucleotide units or more that form clusters <10 to >100 base pairs in length. These sequences affect DNA replication machinery and polymerase slippage that can result in unrepaired deletions or duplications of single or multiple repeat units. This occurs in germ cells and may results in high level of variability in number of repeats in population.

The variability may give rise to non Mendelian inheritance within a generation. The length of these repetitive fragments affects transcription of genes and hence the protein levels that can influence the coding sequences to alter protein function (Sand et al., 2002; Lundin et al., 2007).

The microsatellites vary in repeat lengths that may have different effect on the gene compared with both longer and shorter repeat (Lundin et al., 2007). Microsatellites are the markers to study genetic linkage as they have high heterozygosity as compare to SNPs. They are mutable markers with 15 or more alleles in a particular population.

Many researchers studied (GT)n dinucleotide repeat polymorphism in promoter region located at 6.6 kb upstream of transcription start site. Some studies have found the association with breast cancer (Cai et al., 2003; Boyapati et al., 2004; Anghel et al., 2006).
Figure 16: Formation of microsatellite DNA (Ellegren et al., 2004)

2.8. ERα (ESR1) polymorphisms and breast cancer susceptibility

There have been several association studies of ESR1 polymorphisms and breast cancer but results have been somewhat inconsistent. Hill et al. (1989) reported the association of 454-397T>C PvuII polymorphism in ESR1 gene in 188 breast cancer patients (Hill et al., 1989). Some studies have reported that XbaI not PvuII polymorphism was associated with risk of breast cancer (Andersen et al., 1994; Shin et al., 2003). Most of the studies have found increased breast cancer risk for XbaI and PvuII polymorphism (Franzel et al., 2005; Shen et al., 2006; Jakimiuk et al., 2007).

Many studies have been reported the association between ESR1 polymorphism and breast cancer. A Korean population based study reported the decreased breast cancer risk in women having G allele of XbaI polymorphism as compare to A allele (Wedren et al., 2004; Gail et al., 2008). They found G allele as protective and A allele as a risk in c454-351A>G XbaI polymorphism. No association of T allele was found with breast cancer risk in c454-397T>C PvuII polymorphism, similar to many other studies (Andersen et al., 1994; Shin et al., 2002; Cai et al., 2003).

In a Caucasian study, the association of breast cancer risk was found with A allele of XbaI polymorphism as compared to G allele. They also reported no association between risk of breast cancer and PvuII polymorphism (Gail et al.,
In contrast, Saad et al. (2008) found PvuII polymorphism associated with breast cancer risk however they found no association of XbaI polymorphism with risk of breast cancer (Saad et al., 2008). Some studies have also found both PvuII and XbaI polymorphism associated with elevated risk of breast cancer (Shen et al., 2006; Jakimiuk et al., 2007; Javed et al., 2011). In addition, some studies found no association of breast cancer risk with ESR1 polymorphism (Shin et al., 2003; Li et al., 2012; Alsheyab et al., 2012).

Many studies have examined the association of (GT)$_n$ dinucleotide repeats with breast cancer risks (Cai et al., 2003; Boyapati et al., 2004; Anghel et al., 2006; Tsezou et al., 2008). They observed (GT)$_n$ dinucleotide as highly polymorphic and significantly associated with breast cancer risk. Patients having (GT)$_{17}$ or (GT)$_{18}$ alleles have been associated with decrease risk of breast cancer (Cai et al., 2003). However, Boyapati et al. (2005) observed increased risk of death among breast cancer patients carrying one (GT)$_{18}$ allele (Boyapati et al., 2005).

2.8.1. Genotypic/allelic and Haplotype frequencies

Genotypic frequencies are the indication of the genotypes which are the most or least prevalent in the population. The genotype and allele frequencies are almost related, the only difference is that allele frequencies refer to the frequency of a single allele in a population, while genotype frequency refers to the frequency of the different combination of those alleles in population. Gene or allele frequency may be defined as the percentage of all alleles in a given population (King et al., 2006).

Upon investigating, the genotype distribution of associated SNPs in ESR1 gene and prevalence of their haplotypes have been shown to play an important role in determining the frequency of sporadic breast cancer (Cai et al., 2003; Franzel et al., 2005; Shen et al., 2006). These studies have found that TT, TC genotype of PvuII SNP and AA, AG genotype of XbaI SNP was significantly associated with breast cancer. In some studies, presence of AA genotype of XbaI SNP appears to be more prevalent in breast cancer cases (Shin et al., 2003; Jakimiuk et al., 2007). Whereas in some studies, prevalence of heterozygous genotype TC, GA has been found in breast cancer cases than in control groups (Araujo et al., 2011; Ramalihinho et al., 2013; Madeira et al., 2014) (Table 1, 2).
Further, some studies have focused on haplotypes to identify patterns of genetic variation that are associated with disease (Table 3). Haplotype is a set of single-nucleotide polymorphisms on a single chromatid of a chromosome pair that are associated statistically. These groups of genes inherited together due to genetic linkage.

In some studies, haplotype prevalence was examined in PvuII and XbaI polymorphism to show the effect on increased breast cancer risk (Stavrou et al., 2002). The information on distribution of ESR1 PvuII and XbaI genotype frequency and their haplotypes is shown in table 3. The Haplotype distribution varies among different ethnic groups (Bergink et al., 2003). The Asian population showed an increased frequency of Px haplotype and a reduced frequency of PX haplotype with respect to Caucasian population of European ancestry. While in African population px haplotype was found at a lower frequency (Van et al., 2003).

The divergences in haplotype distribution were found in several studies. The haplotype Px was detected in very low frequency (<24%) in most of the studies (Onland-Moret et al., 2005; Araujo et al., 2011; Ramalihno et al., 2013). Commonly pX haplotype was not found or found at a very lower frequency in several populations. This frequency distribution might results from incomplete linkage disequilibrium.

2.9. Treatment strategies: Local, Adjuvant and Neoadjuvant

Breast cancer is primarily treated with surgery, either by modified radical mastectomy, in which the complete breast is removed, or by means of breast-conservative surgery. During surgery, axillary lymph nodes are removed in many cases. In developing countries, majority of breast cancer patients are managed by general surgeons.

The surgical management of breast cancer is inappropriate at the community level as it may suffer from indiscriminate diagnostic lumpectomy, incomplete mastectomy and omission of axillary lymph node clearance. All these factors may affect the prognosis of breast cancer cases (Deo et al., 2010). A large proportion of patients get suffered with improper initial surgical procedures before they managed for advanced treatment strategies.
Adjuvant therapy improves the prognosis for many patients, but this advantage should be considered to have certain side effects. Adjuvant therapies for breast cancer patients include radiation therapy (RT), chemotherapy (CT), endocrine therapy and antibodies, which are chosen based on prognostic and treatment predictive factors. Combinations of these adjuvant regimes are often used in treatment of the disease. Adjuvant endocrine therapy includes the anti-estrogen tamoxifen, aromatase inhibitors, luteinizing hormone releasing hormone (LHRH) analogues, and oophorectomy or radiation of the ovaries in premenopausal women (Howell et al., 2005). Although different drug combinations are used to treat early breast cancer and advanced cancers is often treated with chemotherapy. Some combinations of carboplatin or cisplatin plus gemcitabine are also used to treat the advanced breast cancer.
<table>
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<th>Genotyping method</th>
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Table 3: The comparison of percentage frequency of PvuII and XbaI haplotypes of the ESR1 gene in different ethnic groups

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</tbody>
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Trastuzumab is an antibody-based therapy directed against the growth factor receptor HER2 (Romond et al., 2005; Smith et al., 2007). Approximately two thirds of all breast cancer patients are cured by surgical treatment and only one third of the patients need to be selected for additional therapy. Adjuvant therapy is delivered to around 80-90% of patients. Further, patients who suffer recurrence after adjuvant therapy need other treatment regimes. New techniques for sub classification of tumors in order to predict better treatment response are currently being evaluated.

2.10. Pharmacogenetics and Pharmacoepidemiology

It is estimated that approximately 20% of drug therapies are influenced by genetic polymorphisms in drug-metabolizing genes (Kalow et al., 1998; Ingelman et al., 2004). Pharmacogenomics refers to the general study of different genes that determine drug behavior, whereas pharmacogenetics refers to the study of inherited differences or variations in drug metabolism and response (National Center for Biotechnology Information). Some researchers refer to the study of selected genes or polymorphisms to genetics and genomics referring to the whole genome wide scans.
Pharmacoepidemiology is the study of the use of drugs, their effects and side effects in real life. An important example of pharmacogenetics is the attempt to individualize treatment with the anti-estrogen tamoxifen (Goetz et al., 2008).

Many clinical studies suggested, ESR1 positive status was correlated with the improved prognosis. Metastatic breast cancer with ER positive status gives good response to endocrine therapy (McGuire et al., 1975). Estrogen receptor positive tumor type have an improved prognosis and give good responses to antiestrogen therapy and leads to longer disease-free survival in many studies (Knight et al., 1977; Maynard et al., 1978; Hihnel et al., 1979; Gapinski et al., 1980; Manni et al., 1980). The expression rate of estrogen receptor (ER) positive tumors to hormonal therapy has been reported as 50% to 75%. On the other hand, ER-negative tumors have a less than 10% chance of response in some studies (Osborne et al., 1980; Wittliff et al., 1984).

The role of estrogen receptors as prognostic and therapeutic tools has widespread acceptance in the management of breast cancer. Approximately half of all ER positive patients fail to respond to anti-estrogen therapy has been observed. The breast cancer patients with lower expression of ESR1 are not controlled by endocrine therapy resulting in more tumor aggressiveness and hence poor prognosis. Some researchers suggested the role of genetic polymorphism for such effects (Ingelman et al., 2004; Giacinti et al., 2006). Particular variant genotypes of ESR1 polymorphism might be associated with specific responses to the therapy.

In addition, Pharmacoepidemiology may also limit the number of adverse drug reactions, since patients with particular genotype who cannot respond to the drug administered may be offered with alternative treatment. Moreover, the development of new therapy panels in clinical trials would be more effective in the response evaluation in patients. Pharmacogenetics may therefore have a major impact, not only on the quality of life but also socioeconomic status of the patients.

2.11. Scenario of current research

Research in association between common genetic variation and cancer risk is advancing rapidly. Technological advances have enabled the efficient multi-marker genotyping that has resulted in a large number of studies analysing associations between SNPs on breast cancer. Current research has many positive aspects. The low cost of genotyping has allowed for analyses to be conducted in large population-based
studies (Easton et al., 2007; Stacey et al., 2007). As a result, these studies have facilitated the identification of additional low penetrance alleles associated with breast cancer. Still, there are several areas in which different approaches might improve ability to identify breast cancer susceptibility of alleles.

A large number of studies are stratifying the breast cancer by estrogen-receptor status, menopausal status, or clinical markers it is rare that SNP associations are evaluated when stratified on the joint status of more than two characteristics. The researchers must recognize that breast cancer is a heterogeneous disease and outcome of single characteristic may mask the effect of other. Kristensen et al. (2008) noted that use of tumor subtype is an efficient way of detecting breast cancer-associated SNPs (Kristensen et al., 2008).

Previous SNP studies did not analyse the effect of haplotypes on breast cancer risk and associated characteristics. Haplotypes composed of closely spaced SNPs are likely to represent a meaningful biological unit and alleles in cis position can have synergistic effects. Furthermore, haplotype analysis can reduce the number of independent statistical tests performed, improving statistical significance.