REVIEW OF LITERATURE
Review of Literature

1. Breast cancer

1.1. Epidemiology

Breast cancer is the most commonly occurring cancer in women. In 2008 approximately 1.4 million women were diagnosed by breast cancer worldwide with resultant deaths of 460000. Of these, approximately 450000 women were diagnosed with the disease in Europe reported 140000 deaths, while 68000 women were diagnosed with the disease in Africa with a corresponding 37000 deaths (Abdulrahman and Rahman 2012). Breast cancer is the second most common cancer overall (1.4 million cases, 10.9%) after lung cancer, but ranks 5th in death causing diseases (458,000, 6.1%) (Ferlay et al. 2010). It is the leading cause of death for American women between the ages of 40 and 55. The lifetime risk of a woman developing invasive breast cancer is 12.6 %. One out of 8 females in the United States will develop breast cancer at some point in her life (Richie and Swanson 2003).

The death rate by breast cancer has been slowly declining over the past decade and the incidence has remained level since 1988 after increasing steadily for nearly 50 years. Twenty five percent to 30% of women with invasive breast cancer will die of their disease (Harris et al. 1992). But this statistics also means that 70% to 75% of women with invasive breast cancer will die of something other than their breast cancer. Mortality rates are highest in the very
young (less than age 35) and in the very old (greater than age 75) (Smith et al. 1996). It appears that the very young have more aggressive disease, and that the very old may not be treated aggressively or may have comorbid disease that increases breast cancer fatality (Richie and Swanson 2003).

1.2. Pathology of breast cancer

Ninety five percent of breast cancers are carcinomas arise from breast epithelial elements (Spiliopoulos et al. 2009). Breast cancers are of two major types, in situ carcinomas and invasive (or infiltrating) carcinomas. The in situ carcinomas may arise in ductal or lobular epithelium. In that case it remains confined there without any invasion of the underlying basement membrane that would constitute extension beyond epithelial boundaries. As would be expected with such localized and confined malignancy, there is negligible potential for metastases. If there is extension of the ductal or lobular malignancy beyond the basement membrane that constitutes the epithelial border, then the malignancy is considered invasive (or infiltrating) ductal or lobular carcinoma. The potential for metastases and ultimately death occurs in invasive disease (Bateman 2006).

1.3. Breast cancer etiology and risk factors

The etiology of human breast cancer remains largely unknown. However, the risk factors associated with breast cancer can be classified into three major classes: family history (hereditary) factors, hormonal (reproductive) factors and environmental (including lifestyle) factors. An epidemiological study by
Lichtenstein et al. (2002) reported that more than 70% of breast cancers are attributable to environmental factors. The common risk factors are as follows.

1.3.1. Gender

Gender is the greatest risk factor for breast cancer. That occurs 100 times more frequently in women than men (Block and Muradali 2013). They also pointed out that women’s breast cells are constantly changing and growing, mainly due to the activity of the female hormones estrogen and progesterone. This activity puts them at much greater risk for breast cancer.

1.3.2. Age

In women, incidence rates of breast cancer rise sharply with age until 45 to 50 years. After that age the incidence rate gets decline that probably reflects the impact of hormonal change (menopause) that occurs about this time. But in the ages of 75 to 80 years, incidence decreases sharply (Cady et al. 1998).

1.3.3. Genetics

Genetic factors contribute a little but important risk for breast cancer. Only 5% to 6% of breast cancers are considered hereditary (Malone et al. 1998). *BRCA1* and *BRCA2* account for an estimated 80% of hereditary breast cancer, but again this only represents 5% to 6% of all breast cancers. *BRCA1* and/or *BRCA2* positive women have a 50% to 85% lifetime risk of developing breast cancer and a risk of 15% to 65% in developing ovarian cancer, beginning at age 25 (Haber 2002). Polymorphisms in genes associated with metabolism of estrogens and/or carcinogens (*CYP1A1, CYP1B1, CYP19, COMT, NAT2*, ...)
**GSTM1** etc.), estrogen, androgen and vitamin D, co-activation of gene transcription (**AIB1**) and DNA damage response pathways (**CHEK2**, **HRAS1**, **XRCC1**, **XRCC3**, **XRCC5** etc.) have also been found to be the risk factors for breast cancer in different populations (Lacroix and Leclercq 2005).

### 1.3.4. Familial history

Familial history of breast and other cancer is considered to be a risk factor if a first degree relative develops a premenopausal breast cancer or if it occurred in conjunction with ovarian cancer (Hoskins *et al.* 1995). There is a twofold relative risk of breast cancer if a woman has a single first degree relative (mother, sister or daughter). There is a fivefold increased risk if 2 first degree relatives had breast cancer (Greene 1997).

### 1.3.5. Hormones

The hormonal environment plays a pivotal role in the breast cancer development. Oophorectomy reduces the risk consecutively, the risk increases with higher levels of endogenous and pharmaceutical estrogen exposure (Henderson and Feigelson 2000). Toniolo *et al.* (2007) suggested that estrogens are promoters of mammary tumors which act over a long period of time by causing cell proliferation and clonal expansion of initiated cells. Depending upon concentration of estrogen and ERs and PRs estrogens bind with high affinity to estrogen receptor alpha (ER-α) and progesterone receptors (PR) (Hasan *et al.* 2011). This binding induces DNA synthesis, cell division and production of growth factors and progesterone receptor proteins. Estrogens and
progesterone are essential for normal mammary gland development and function, but their stimulation of breast cell proliferation may be pro-
carcinogenic (Rogan et al. 2003).

1.3.6. Hormone replacement therapy (HRT)

To relieve the symptoms of menopause and osteoporosis in post-
menopausal hormone therapy (PHT) has been used for many years (Miller et al. 2002). There are two main types of HRT. For women who have undergone a hysterectomy, estrogen alone can be prescribed. This is commonly known as estrogen replacement therapy (ERT) (McTiernan et al. 2005). But, generally estrogen and progesterone both are prescribed for women who still have a uterus (womb) (combined HRT). Because estrogen alone can increase the risk of cancer of the uterus, to prevent this progesterone is added (Kohn and Liotta 2006). Use of combined HRT therapy increases the risk of getting breast cancer. This increase in risk can be seen with a use of 2 years (Karnofsky and Burchenal 2001). Combined PHT also increases the likelihood that the cancer may be found at a more advanced stage, because it reduces the effectiveness of mammograms (Ross et al. 2008).

1.3.7. First child birth and full time pregnancy

Women had their first child, full time pregnancy after 30 years of age or have no children are at slightly higher breast cancer risk (Britt et al. 2007). The women having many pregnancies and becoming pregnant at a young age reduce breast cancer risk. Pregnancies reduce total number of lifetime menstrual cycles
and reduce the continuous and prolonged exposure of estrogen and progesterone
which may be the reason for this effect (Clarke et al. 2005).

1.3.8. Breast feeding

Breast feeding reduces a total number of lifetime menstrual cycles in
women (Ross et al. 2008). Especially if breast feeding is continued for 1½ to 2
years it may slightly lower breast cancer risk, (Chavez-MacGregor et al. 2008).
However, this is a difficult area to study particularly in countries like United
States and Europe where breast feeding is uncommon (Fisher et al. 2005).

1.3.9. Other factors

In addition of above reviewed factors for the breast cancer there are some
other factors which may contribute to the incidence of breast cancer. Such
factors are use of contraceptive pills to prevent the child birth (Iatrakis et al.
2011), smoking (McKenzie et al. 2013), higher body weight (Enger et al. 2004)
and lack of physical activities (Huang et al. 1997).

1.4. Types of breast cancer

There are several types of breast cancer, but some of them are quite rare.
In some cases a single breast tumor can be a combination of many types.

1.4.1. Ductal carcinoma in situ

Ductal carcinoma in situ (DCIS; also known as intraductal carcinoma) is
the most common type of non-invasive breast cancer. DCIS means that the
cancer cells are inside the ducts but have not spread through the walls of the
ducts into the surrounding breast tissue. About 1 in 5 new breast cancer cases
will be DCIS. Nearly all women diagnosed at this early stage of breast cancer can be cured. A mammogram is often the best way to find DCIS early (Welch et al. 2008). When DCIS is diagnosed an area of dead or dying cancer cells called tumor necrosis within the tissue sample is found that the tumor is likely to be more aggressive. The term comedocarcinoma is often used to describe DCIS with large areas of necrosis (Virnig et al. 2009).

1.4.2. Lobular carcinoma in situ

Lobular carcinoma in situ (LCIS) is not as common as DCIS, LCIS incidence rates also increased 4-fold during late 1970s to early 2000s. Highest increase observed among women having 50 years and older LCIS was first defined as a separate entity in 1941 to differentiate it from invasive carcinoma involving the lobular structures of the breast. LCIS is generally considered as a risk factor rather than a precursor for invasive lobular and ductal carcinomas (Li et al. 2005).

1.4.3. Invasive (or infiltrating) ductal carcinoma

This is the most common type of breast cancer Invasive (or infiltrating). Ductal carcinoma (IDC) starts in a milk duct of the breast, breaks through the wall of the duct and grows into the fatty tissue of the breast. At this point it may be able to spread (metastasize) to other parts of the body through the lymphatic system and bloodstream. About 8 of 10 invasive breast cancers are infiltrating ductal carcinomas (Eheman et al. 2009).
1.4.4. Invasive (or infiltrating) lobular carcinoma

Invasive lobular carcinoma (ILC) starts in the milk producing glands (lobules). Like IDC, it can spread (metastasize) to other parts of the body. About one invasive breast cancer in ten is an ILC. Invasive lobular carcinoma may be harder to detect by a mammogram than invasive ductal carcinoma (Lopez and Bassett 2009).

1.4.5. Less common types of breast cancer

1.4.5.1. Inflammatory breast cancer

This uncommon type of invasive breast cancer accounts for about 1% to 3% of all breast cancers. Usually there is no single lump or tumor. Inflammatory breast cancer (IBC) makes the skin on the breast look red and feels warm. These changes are not caused by inflammation or infection, rather due to blockage of lymph vessels by cancer cells in skin (Alitalo and Detmar, 2012). This type of breast cancer tends to have a higher chance of spreading and a worse outlook (prognosis) than typical invasive ductal or lobular cancer (Alitalo and Detmar, 2012).

1.4.5.2. Paget disease of the nipple

This type of breast cancer starts in the breast ducts and spreads to the skin of the nipple and then to the areola, the dark circle around the nipple. It is rare accounting only about 1% of all cases of breast cancer. The skin of the nipple and areola often appears crusted, scaly and red with areas of bleeding or oozing (Vani et al. 2013). Paget disease is almost always associated with either ductal
carcinoma \textit{in situ} (DCIS) or infiltrating ductal carcinoma (Spiliopoulos \textit{et al.} 2009).

1.4.5.3. Phyllodes tumor

This is another rare breast tumor develops in the stroma (connective tissue) of the breast in contrast to carcinomas, it develop in the ducts or lobules (Singer \textit{et al.} 2013). Other names for these tumors include phyllodes tumor and cystosarcoma phyllodes. These tumors are usually benign but on rare occasions may be malignant. When a malignant phyllodes tumor has spread, it can be treated with the chemotherapy given for soft tissue sarcomas (Mishra \textit{et al.} 2013)

1.4.5.4. Angiosarcoma

Angiosarcoma starts in cells that line blood vessels or lymph vessels. It rarely occurs in the breasts. It usually develops as a complication of previous radiation treatments (Haroon \textit{et al.} 2013). Angiosarcoma can also occur in the arms of women who develop lymphedema as a result of lymph node surgery or radiation therapy to treat breast cancer (Vuille-Dit-Bille \textit{et al.} 2013). These cancers tend to grow and spread quickly.

1.5. Molecular sub-types of breast cancer

Most of the studies broadly classify breast cancer into four major molecular subtypes. Namely, Luminal A, Luminal B, Triple negative/basal like and Her-2 type. Other less common molecular types have also been described including normal breast like, apocrine molecular type and claudin low type.
Breast cancers that do not fall into any of these subtypes are often listed as unclassified. The complex profile of each subtype is determined using molecular and genetic information from tumor cells. However, some characteristics (including hormone receptor status, HER2/neu status and proliferation rate) can be used roughly to define the four major subtypes (Pusztai et al. 2006).

1.5.1. Luminal A

Most breast cancers are luminal tumors. Luminal tumor cells look like the cells of breast cancers that start in the inner (luminal) cells lining the mammary ducts. Luminal A tumors tend to be estrogen receptor positive (ER$^+$) and/or progesterone receptor positive (PR$^+$), HER2/neu-negative (HER2$^-$). Of the four subtypes luminal A tumors tend to have the best prognosis with fairly high survival rates and fairly low recurrence rates (Carey et al. 2006).

1.5.2. Luminal B

Luminal B tumors tend to be estrogen receptor-positive (ER$^+$) and/or progesterone receptor-positive (PR$^+$), highly positive for Ki67 (have a high number of cancer cells actively dividing) and/or HER2/neu-positive (HER2+). Women with luminal B tumors are often diagnosed at a younger age than those with luminal A tumors. Compared to luminal A tumors, they tend to have factors that lead to a poorer prognosis including Poorer tumor grade, large tumor size, lymph node positive and $p53$ mutation (about 30%) (Dawood et al. 2011).
1.5.3. Triple negative/basal like

Most triple negative tumors are basal like and most basal like tumors are triple negative. However, not all triple negative tumors are basal like and not all basal like tumors are triple negative. Triple negative breast cancers are estrogen receptor negative (ER−), progesterone receptor negative (PR−) and HER2/neu negative (HER2−). Whereas basal like tumors have cells with features similar to those of the outer (basal) cells lining the mammary ducts. Basal like tumors tend to express HER1 and/or cytokeratin 5/6 proteins and most contain p53 mutations (Potemski et al. 2005; Turner and Reis-Filho, 2006).

1.5.4. HER2 type

HER2 type tumors are named for their HER2/neu positive status. They tend to be estrogen receptor negative (ER−), progesterone receptor negative (PR−) and lymph node positive with poorer grade tumor (Carey et al. 2006). HER2 type tumors have a fairly poor prognosis and are prone to early and frequent recurrence and metastases (Hartman et al. 2011). Women with HER2 type tumors appear to be diagnosed at a younger age than those with luminal A and luminal B tumors (Voduc et al. 2010).

1.5.5. Normal basal like

This is a less common type of breast cancer. About 6 to 10 percent of all breast cancers fall into the normal breast like category. These tumors are usually small and tend to have a good prognosis (Carey et al. 2006).
1.5.6. Race/ethnicity and subtypes of breast cancer

The prevalence rates of the four major molecular subtypes of breast cancer are different among races. However, Luminal B and HER2 type tumors do not appear to differ by race (Dawood et al. 2011).

1.6. Breast cancer types on the basis of heredity

Breast cancer may arise due to inheritance or without involvement of heredity. On the basis of inheritance breast cancer can be classified into two broad classes; namely, familial (hereditary) breast cancer and sporadic (spontaneous) breast cancer.

1.6.1. Familial breast cancer

A breast cancer is regarded as familial if patient have a family history. In other words if breast cancer has been diagnosed at least once either in last two generation or up to second cousins of the patient, the patient will be regarded as familial breast cancer patient (Hasan et al. 2013). As estimated 15% to 20% of breast cancers are associated with some family history or breast cancer without any evidence of autosomal transmission. Small proportions of all breast cancer (up to 10%) are attributable to germline mutation in single susceptibility genes such as BRCA1 and BRCA2. These cancers result from a strong genetic predisposition and cancer susceptibility and are transmitted in an autosomal dominant fashion in these families (Claus et al. 1996). Familial breast cancers attributable to mutations in BRCA1 exhibit higher grade tumor, elevated mitotic count, presence of a lymphocytic infiltrate and the presence of a smooth non
infiltrative border to the cancer. Familial Breast cancers attributable to \textit{BRCA2} mutations are also of higher overall grade, predominantly as a result of less tubule formation (Lakhani \textit{et al.} 2000). In addition to \textit{BRCA1} and \textit{BRCA2} genes mutations in the \textit{PTEN}, \textit{TP53}, and \textit{ATM} etc. are known or suspected to be associated with an increased risk of breast cancer. But none of these genes fail to explain an important fraction of familial aggregation of breast cancer (Liaw \textit{et al.} 1997; Birch \textit{et al.} 1994; Swift \textit{et al.} 1991).

\textbf{1.6.2. Sporadic breast cancer}

Sporadic breast cancers arise spontaneously/sporadically rather through inheritance of certain genes. This may occurs due to the epigenetic effects (DNA methylation) or the defects of DNA repair mechanisms that allow environmentally triggered mutations. Approximately 80\% of the breast cancers are sporadic without any familial history of the disease. Primary tumors are of high grade. They show lower expression of estrogen and progesterone receptors (mostly ER\textsuperscript{-} and PR\textsuperscript{-}). EGFR is over expressed in sporadic tumors with \textit{BRCA} mutations. However they are mostly \textit{HER2/neu} positive (HER-2\textsuperscript{+})(Van der Groep \textit{et al.} 2006).

\textbf{1.7. Diagnosis of breast cancer}

Sometimes breast cancer found to be appeared after symptoms. But in many cases women with early breast cancer have no symptoms.
1.7.1. Symptoms of breast cancer

Formation of new painless, hard and irregular lump or mass in the breast is the most common symptom of breast cancer. But breast cancers can be tender, soft or rounded. They can even be painful in some cases. Other possible signs of breast cancer include swelling of all or part of a breast (even if no distinct lump is felt), skin irritation or dimpling, breast or nipple pain, nipple retraction (turning inward), redness, scaliness, or thickening of the nipple or breast skin and nipple discharge (other than breast milk) (Barber et al. 2004).

1.8. Staging of breast cancer

The staging systems currently in use for breast cancer are based on the clinical size and extent of invasion of the primary tumor (T), the clinical absence or presence of palpable axillary lymph nodes and evidence of their local invasion (N), together with the clinical and imaging evidence of distant metastases (M). This is then translated into the TNM classification which has been subdivided into stages.

**Stage 0** called carcinoma *in situ* (lobular carcinoma *in situ* (LCIS) and ductal carcinoma *in situ* (DCIS).

Four broad categories are distinguished by the Union International Centre Cancer (UICC). They are the following.

**Stage I** – early stage breast cancer where the tumor is less than 2 cm across and has not spread beyond the breast.
Stage II – early stage breast cancer where the tumor is either less than 2 cm across and has spread to the lymph nodes under the arm; or the tumor is between 2 and 5 cm (with or without spread to the lymph nodes under the arm); or the tumor is greater than 5 cm and has not spread outside the breast.

Stage III – locally advanced breast cancer where the tumor is greater than 5 cm across and has spread to the lymph nodes under the arm; or the cancer is extensive in the under arm lymph nodes; or the cancer has spread to lymph nodes near the breast bone or to other tissues near the breast.

Stage IV – metastatic breast cancer where the cancer has spread outside the breast to other organs in the body (Singletary et al. 2002).

There are some changes and modifications in 7th edition of TNM system of staging. They can be referred in the review article of Sinn et al. (2010)

2. Breast cancer susceptibility genes

Approximately 5–10% of all breast cancers are caused by germ line mutations in well recognized breast cancer susceptibility genes. These genes can be roughly divided into ‘high risk’ and ‘low to moderate risk’ breast cancer susceptibility genes. The high risk breast cancer susceptibility genes include BRCA1, BRCA2, PTEN, TP53, LKB1/STK11 and CDH1 with relative lifetime risks higher than 4. The CHEK2, TGFβ1, CASP8 and ATM genes belong to the ‘low to moderate risk’ breast cancer susceptibility genes (Oldenburg et al. 2007).
2.1. High risk susceptibility genes

2.1.1. **BRCA1 and BRCA2**

The *BRCA1* gene is located on chromosome 17q21 and the *BRCA2* gene is located on chromosome 13q12. *BRCA1* and *BRCA2* do not share any distinct sequence homology; the similarities between them are interesting. Both genes are larger genes: *BRCA1* has 22 exons, spans approximately 100 kb of genomic DNA and encodes an 1863 amino acid protein, while *BRCA2* has 27 exons, spans around 70 kb and encodes a protein of 3418 amino acids (Tavtigian *et al.* 1996). They are both characterized by the presence of an extremely large exon 11. Both genes are the “caretakers” as these genes behave like sensors of DNA damage and participate in the repair process. Their inactivation allows other genetic defects to accumulate and leads to genetic instability (Kinzler and Vogelstein 1997; Breivik 2005).

During the past two decade many of the cellular and biochemical functions of the BRCA1 and BRCA2 proteins have been discovered. Together these suggest how BRCA1 and BRCA2 might play a role in carcinogenesis. For BRCA1 these roles include DNA repair, protein ubiquitylation, chromatin remodeling and cell cycle checkpoint control. BRCA2 is involved in double strand break DNA repair through homologous recombination (Scully and Livingston 2000; Venkitaraman 2004; Narod and Foulkes 2004). Antoniou *et al.* (2002) estimated that in a Caucasian population the prevalence of heterozygous carriers of high risk mutations for *BRCA1* is one in 1000
whereas for BRCA2 is one in 750. However this frequency may be higher in certain populations due to founder mutations.

Germ line mutations in BRCA1 or BRCA2 have lifetime higher risks of breast cancer and ovarian cancer as well as smaller risks to some other cancer types (Dunning et al. 1999). The cumulative risk of breast cancer at the age of 70 in BRCA1 and BRCA2 mutation carriers had been estimated as 85% and 84%, ovarian cancer 63% and 27% respectively in multiple case families (Ford et al. 1998). Meta-analysis on 22 populations based and hospital based studies showed that the average cumulative risks in BRCA1 and BRCA2 mutation carriers at 70 years were 65% and 45% for breast cancer and 39% and 11% for ovarian cancer. The relative risks of breast cancer declined significantly with age for BRCA1 mutation carriers (Antoniou et al. 2003). There are other genes which may be the risk modifier of breast and ovarian cancer in BRCA1 and BRCA2 mutation carriers. For example a C/G polymorphism in the 5’ untranslated region of RAD51 was found to modify both breast and ovarian cancer risk in carriers of a germ line BRCA2 mutation (OR, 3.2; 95% CL, 1.4–40; p = 0.01) (Wang et al. 2001; Kadouri et al. 2004). A length variation of the poly glutamine repeats in the estrogen receptor co-activator NCO3A influences breast cancer risk in carriers of BRCA1 and BRCA2 (OR, 1.96; 95%CI, 1.25–3.08; p for trend = 0.0036) (Rebbeck et al. 2001).

For both BRCA1 and BRCA2 it has been shown that cancer risks are influenced by the position of the mutation within the gene sequence (Thompson
and Easton 2002). Women with a mutation in the central region of the BRCA1 gene were shown to have a lower breast cancer risk than women with mutations outside this region. The ovarian cancer risk associated with mutations upstream this central region was higher than that associated with mutations downstream this region. For BRCA2, mutations in the central region (OCCR; ovarian cancer cluster region) were associated with a higher risk of ovarian cancer than mutations outside this region, whereas mutations in the OCCR were associated with a lower breast cancer risk than mutations outside the OCCR. In addition to a predominantly high increased risk to female breast cancer and ovarian cancer, BRCA1 or BRCA2 mutation carriers are at increased risk to “other cancers” as well. An increased relative risk to colon cancer, cervix cancer, uterus, pancreas and prostate has been suggested in BRCA1 mutation carriers. In BRCA2 mutation carriers an increased relative risk to male breast cancer, gall bladder and bile ducts cancer, gastric cancer, malignant melanoma, pancreas, prostate, bone and pharynx cancer has been observed (The Breast Cancer Linkage Consortium, 1999; Van Asperen et al. 2005).

2.1.2. TP53: Li-Fraumeni syndrome

The TP53 gene is located on chromosome 17p13.1 which encodes a protein involved in many overlapping cellular pathways that control cell proliferation and homeostasis such as cell cycle, apoptosis and DNA repair. The expression of the TP53 gene is activated in response to various stress signals, including DNA damage. Loss of TP53 function is thought to be as a tumor
suppressor (Pluquet and Hainaut 2001). Germ line mutations in TP53 are very rare: fewer than 400 families with germ line mutations have been reported worldwide. Mutations in the TP53 gene account for roughly 70% of families fulfilling the classical criteria for Li-Fraumeni syndrome (e.g. one patient with a sarcoma diagnosed <45 years with a first degree relative with any cancer diagnosed <45 years and an additional first or second degree relative diagnosed with cancer <45 years or a sarcoma at any age) (Varley et al. 1997). Mutations in TP53 are less common in breast cancer/sarcoma families not fulfilling these classical criteria. Susceptibility to cancer in Li-Fraumeni families follows an autosomal dominant pattern of inheritance. One of the most frequently occurring cancers in Li-Fraumeni families is breast cancer with an estimated penetrance in TP53 mutation carriers of 28–56% at 45 years (Evans et al. 2002).

2.1.3. PTEN

The chromosomal region containing the Cowden syndrome gene was known to contain a tumor suppressor gene (PTEN) that had been found to be mutated in sporadic brain, breast and prostate cancer. In a study germline mutations in the PTEN gene were found in four of five families with Cowden syndrome (CS). About 80% of CS families Mutations in the PTEN gene are present (Liaw et al. 1997). Women carrying a PTEN mutation have 25–50% (2–4 folds) lifetime breast cancer risk. The majority of Cowden syndrome related breast cancers occur after the age of 30–35 years (Eng 1998). Breast cancer at young age has been observed in male carriers of a germ line PTEN mutation.
with the classical CS phenotype suggesting an increased risk for males as well (Fackenthal et al. 2001). However no mutations in the PTEN gene have been detected in breast cancer families without features of CS (Chen et al. 1998). Also in sporadic breast cancer patients germ line and somatic mutations in the PTEN gene are rare (Freihoff et al. 1999). Although LOH at the PTEN locus is found in 11–41% of sporadic breast cancers, no somatic mutations have been observed in the remaining allele (Feilotter et al. 1999).

2.1.4. LKB1/STK11

The LKB1/STK11 gene is located on chromosome 19p13.3 and encodes a transcript of \( \sim 1.3 \) kb, which acts as a tumor suppressor. Germ line mutations in the serine/threonine kinase gene (LKB1/STK11) causes Peutz-Jeghers syndrome (PJS). An elevated risk of gastrointestinal malignancies, breast cancer, pancreas, ovary, uterus, cervix, lung and testicular cancers is recognized in patients with PJS (Boardman et al. 1998). It is suggested that LKB1/STK11 can play the role of a tumor suppressor gene in sporadic breast cancer and low expression of the LKB1/STK11 protein is significantly associated with a shorter survival (Shen et al. 2002).

2.1.5. CDH1/E-cadherin

The E-cadherin gene (CDH1) is located on chromosome 16q22.1. The mature protein product belongs to the family of cell–cell adhesion molecules and plays a fundamental role in the maintenance of cell differentiation and the normal architecture of epithelial tissues. Germ line CDH1 truncating mutations
are associated with hereditary diffuse gastric cancer syndrome (HDGC syndrome). The lifetime risk of developing breast cancer was estimated at 20–40% (Graziano et al. 2003). Somatic CDH1 mutations are frequently found in infiltrating lobular breast cancer and in situ lobular breast cancer (LCIS) (Mastracci et al. 2005).

2.2. Low to moderate risk breast cancer susceptibility genes

2.2.1 ATM

The ATM gene is located on chromosome 11q22–23. The ATM protein plays a central role in sensing and signaling the presence of DNA double strand breaks. In the unirradiated cell nucleus, ATM is held inactive which is dissociated by rapid intermolecular autophosphorylation after irradiation (Bakkenist and Kastan 2003). This initiates cellular ATM kinase activity which has many substrates including the protein products of TP53, BRCA1 and CHEK2. Carriers of homozygous or compound heterozygous mutations in the ATM gene suffer from the rare recessive disorder ataxia-telangiectasia. Female heterozygous carriers AT patients are at increased risk of breast cancer (Easton, 1994). The estimated relative risk of breast cancer in obligate AT heterozygotes range between 1.3 and 13 in the different studies conducted (Hall, 2005). Studies of sporadic and familial breast cancer have failed to demonstrate an elevated prevalence of germ line ATM gene variants among breast cancer cases relative to controls (FitzGerald et al. 1997).

2.2.2 TGFβ1
The TGFβ gene is located on chromosome 19q13.1 which contains seven exons and very large introns. TGFβ is a multifunctional peptide that controls proliferation, differentiation and other functions in many cell types. Dysregulation of TGFβ activation and signalling may result in apoptosis. For most normal cell types, TGFβ acts as a potent inhibitor of proliferation, migration and promotes apoptosis, properties associated with tumor suppression (Janda et al. 2002). However studies support a model in which TGFβ inhibits the development of early benign lesions but promotes invasion and metastasis when the tumor suppressor activity is overridden by oncogenic mutations in other pathways (Derynck et al. 2001). To date several somatic mutations that disrupt the TGFβ-signalling pathway have been reported in human breast tumors (Xie et al. 2002).

2.2.3. Caspase 8 (CASP8)

The CASP8 gene is located on chromosome 2q33–34 that contains 13 exons and spans 51.2 kb. Caspases are important mediators of the apoptotic process. Death receptor mediated apoptosis provokes the formation of death inducing signaling complex (DISC)which comprises the death receptors, adaptor proteins , initiator caspase 10 (CASP10) and caspase 8 (CASP8). Because of the involvement in initiation of apoptosis, it was hypothesized that CASP8 and CASP10 might act as low penetrance familial breast cancer susceptibility genes. Combined analysis of two different studies surprisingly
showed that one missense variant (D302H) in CASP8 was associated with a reduced risk of breast cancer in a dose dependent manner (Frank et al. 2006).

3. Single Nucleotide Polymorphism

A single nucleotide polymorphism is a DNA sequence variation occurring when a single nucleotide — A, T, C or G — in the genome (or other shared sequence) differs between members of a biological species or paired chromosomes in a human. The genomic distribution of SNPs is not homogenous. SNPs usually occur more frequently in non-coding regions in comparison of coding regions. However natural selection is acting and fixating the allele of the SNP that constitutes the most favorable genetic adaptation (Barreiro et al. 2008). Other factors like genetic recombination and mutation rate can also determine SNP density (Nachman 2001). SNP density can be predicted by the presence of microsatellites: AT microsatellites in particular are potent predictors of SNP density with long \((AT)_{(n)}\) repeat tracts tending to be found in regions of significantly reduced SNP density and low GC content (Varela and Amos 2010).

Yet now, there are 62,676,337 SNPs in human genome were reported and Out of them 44,278,189 have validated. This data base reports for 27,608,151 SNPs in coding regions (dbSNP, 2013). There are variations between human populations, so a SNP allele that is common in one geographical or ethnic group may be much rarer in another (Dobrovic and Simpfendorfer 1997).
3.1 Types of SNPs

Single nucleotide polymorphisms may fall within coding sequences of genes, non-coding regions of genes (3’ and 5’ untranslated regions) or in the intergenic regions (regions between genes). Due to degeneracy of the genetic code SNPs within a coding sequence do not necessarily change the amino acid sequence of the protein produced. SNPs in the coding region are of two types, synonymous and non-synonymous SNPs. Synonymous SNPs do not affect the protein sequence while non-synonymous SNPs change the amino acid sequence of protein. SNPs that are not in protein coding regions may still affect gene splicing, transcription factor binding, messenger RNA degradation or the sequence of non-coding RNA. Gene expression affected by this type of SNP is referred as an eSNP (expression SNP) and may be upstream or downstream from the gene (Alshatwi et al. 2012b).

3.2 SNPs in BRCA1 and breast cancer

Mutations in the cancer susceptibility gene BRCA1 greatly increase the risk of breast and ovarian cancer (Miki et al. 1994). At present most mutations in the BRCA1 gene have been identified to be point mutations or small insertions and deletions. Literature survey showed that there is a wide choice of literature on the BRCA1 gene related to breast cancer. However a computational study revealed that a total of 477 SNPs in the BRCA1 gene, 65 were found to be unsynonymous and 4 and 10 SNPs were found to be in the 5’ and 3’ untranslated regions (UTR). Of 65 nsSNPs, 12 nsSNPs were found to be deleterious. Two
SNPs in the 5′ UTR and 3 SNPs in the 3′ UTR were found to be of functional significance. It was found that the major mutation in the native protein of the \textit{BRCA1} gene was from proline to serine. It concluded that rs1800751 with a mutation of proline to serine at position 1812 in the native protein could be the main target mutation for the breast cancer caused by the \textit{BRCA1} gene (Rajasekaran \textit{et al.} 2007).

4. DNA Methylation

Modern aspects of epigenetics refer to the modification of DNA and related proteins without variation in nucleotide sequences which passes the contained information to next generation. DNA methylation is the most studied and the best understood epigenetic modification. On the basis of many studies on a battery of housekeeping genes and growth regulator genes, DNA methylation is established as an additional mechanism for gene inactivation in different cell types including cancer cells (Lehmann \textit{et al.} 2002). DNA methylation plays an essential role in maintaining cellular function. Its role in carcinogenesis has been a topic of considerable interest in recent years. Changes in DNA methylation patterns may contribute to the development of cancer. Aberrant global methylation of DNA is frequently found in tumor cells. Global hypomethylation can result in chromosome instability. Hypermethylation has been associated with the inactivation of tumor suppressor genes (Whitman \textit{et al.} 2008).
The methylation of DNA is an outcome of the activities in a family of enzymes known as DNA methyl transferases (DNMTs) which take up the methyl group from co-factor SAM (Jeltsch et al. 2007) and mediate the transfer to a nucleotide particularly cytosine (Bujnicki and Radlinska 1999). Three families of mammalian DNMTs have been found and are DNMT1, DNMT2, and DNMT3 (Hermann et al. 2004). Among these DNMT2 has little involvement in methyl group transfer to DNA. Recently it has been found to be involved in the methylation of a cytosine residue in the anticodon loop of tRNA-Asp (Goll et al. 2006).

In normal cells actively transcribing chromatin is hypomethylated whereas non transcribing chromatin is methylated in such a way that it makes compact structures which hinder RNA Pol II activities (Lorincz et al. 2004). The methylation of DNA is found to be involved in the stabilization of chromatin structure in an inactive conformation and inhibits gene transcription (Keshet et al. 1986). Therefore it is believed that the mechanism of gene regulation through DNA methylation involve essential genetic events including differentiation, genomic imprinting and X-chromosome inactivation (Bernardino-Sgherri et al. 2002; Richardson and Yung 2002).

4.1. Types of DNA methylation

Methylation or demethylation of DNA is an essential feature of a normal genome. But whenever DNA becomes abnormally methylated it leads to many abnormalities. Abnormal DNA methylation accounts for (a) the excess of
methylation (hypermethylation) of genes that must be transcriptionally active in normal conditions and (b) the excess of genome wide demethylation (global hypomethylation). A major abnormality associated with hypermethylation of the promoter regions of tumor suppressor genes and DNA repair genes (p15, p16, p57, p53, SLC5A8 and BRCA1) is an onset of different kind of cancers, e.g. skin, blood, breast and ovary (Hayslip and Montero 2006; Chanda et al. 2006). Hypomethylation of DNA causes instability of the genome and is also related with certain cancers. For example, a case control study from Spain reports that leukocyte genomic DNA hypomethylation is associated with increased risk of developing bladder cancer (Moore et al. 2008). Hypermethylation takes place mostly at CpG islands of promoter region of a gene, but hypomethylation usually is concerned with repeated DNA sequences such as Long Interspersed Nuclear Elements (Ehrlich 2002). Global hypomethylation also activates transposable elements (TE) to transcribe in both sense and antisense directions (Roman-Gomez et al. 2005).

4.2. CpG island in gene promoters and whole genome

The main target of DNA methylation is cytosine. The majority of 5’methylcytosine in mammalian DNA is present in cytosine–guanine (CpG) dinucleotides which are present in promoter regions of many genes. Among all human promoters 72% contain high CpG content and 28% of promoters with low CpG content (Saxonov et al. 2006). As far as the whole human genome is concerned, CpGs are present approximately once per 80 dinucleotides in 98% of
the genome; however, 1–2% of the human genome, the frequency is five times higher (Bird 1986).

4.3 Methylation of cancer related genes in breast cancer

A large body of evidence has demonstrated that CpG island hypermethylation is implicated in loss of expression of a variety of critical genes in breast cancer. Such genes fall into several broad categories including cell cycle regulating, steroid receptor, tumor susceptibility, carcinogen detoxification, cell adhesion and inhibitors of matrix metalloproteinases. $p16$ gene encodes for a cyclin dependent kinase inhibitor, $p16^{INK4A}$. Methylation of the 5’ promoter and exon 1 regions of $p16^{INK4A}$ is observed in many human breast cancer cell lines and 20–30% of primary breast cancers (Woodcock et al. 1999).

The 14–3–3σ gene (also known as $HME1$) is a member of a gene family responsible for instituting the G$_2$ cell cycle checkpoint in response to DNA damage in human (Chan et al. 1999). Studies of the molecular mechanisms responsible for the reduced expression have implicated hypermethylation of the CpG rich exon 1 region of the gene instead of genetic alterations such as loss of heterozygosity (LOH) and intragenic mutations in breast cancer (Ferguson et al. 2000). Epigenetic alteration appears to play a role in inactivation of estrogen receptor ($ER$) gene. $RAR$ genes are the members of the nuclear receptor superfamily. The $RAR\beta$ gene, located at chromosome 3p24, appears to play an important role in limiting the growth of certain tumor types including breast,
lung and others. DNA methylation of the RARβ promoter is believed to be one of the factors linked to RARβ down regulation in breast cancer (Widschwendter et al. 2000).

Recently hypermethylation of the promoter region in CpG islands of tissue inhibitor of metalloproteinase 3 (TIMP3) gene has been found to be the main mechanism for gene expression in gastric cancer (Guan et al. 2013). IGFBP7 belongs to a family of insulin like growth factor-1 regulatory binding proteins. IGFBP7 hypermethylation is associated with its down regulation in various carcinomas and particularly in prostate cancer (Sullivan et al. 2012)

4.4 Promoter methylation of BRCA1 and decreased gene expression

The BRCA1 gene located at chromosome 17q21 is a well-known breast cancer susceptibility gene. Inherited mutations in the BRCA1 gene account for one half of inherited breast carcinomas. In contrast to other tumor suppressor genes somatic mutations in this gene are rare even with the high degree of LOH at the BRCA1 locus in sporadic breast and ovarian cancer (Merajver et al. 1995). However, Janatova et al. (2005) and Zhang et al. (2012) have recently reported the somatic mutation in BRCA1 with frequencies of 0.05 and 0.032 in sporadic cancer patients. Since BRCA1 transcript and protein are either absent or reduced in sporadic breast cancer. DNA methylation has been proposed as an alternative mechanism to inactivate BRCA1 by truncating the gene expression (Dobrovic and Simpfendorfer 1997). By Southern analysis of the BRCA1 promoter region methylation was detected in 11% of sporadic breast cancer cases and was
inversely correlated with expression of both $ER$ and $PR$ (Catteau et al. 1999). A study with 194 primary breast carcinomas demonstrated that the $BRCA1$ promoter is methylated in 13% of unselected primary breast tumors (Esteller et al. 2000).

$BRCA1$ was unmethylated in all normal tissues examined as well as in 21 breast cancer cell lines. Hypermethylation of the $BRCA1$ promoter has been reported for inactivation of $BRCA1$. That occurs in 7–31% of breast and ovarian cancer patients with no familial history (Catteau and Morris 2002). $BRCA1$ methylated sporadic tumors display pathologic features similar to those of $BRCA1$ mutated hereditary breast cancers (Esteller et al. 2001; Hedenfalk et al. 2001). Sporadic tumors with aberrant methylation of the $BRCA1$ promoter can be clustered with tumors derived from women with inherited $BRCA1$ germ line mutations because of similarities in their global gene expression profiles (Hedenfalk et al. 2001; Van 't Veer et al. 2002).

$BRCA1$ methylation is only observed in breast and ovary cancers but not in tumors of colon or liver or leukemia, supporting a tissue specific event for the process. Using chromatin immunoprecipitation and endonuclease chromatin accessibility assays, transcriptional repression of $BRCA1$ by cytosine methylation is also mechanistically linked to histone deactivation and inactive chromatin structure (Yang et al. 2001).