CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Breast cancer continues to be one of the most common cancers and a major cause of death among women worldwide. Breast cancer was the most common cancer in women in the United States accounting for 32 percent of female cancers. Breast cancers are responsible for 18 percent of cancer deaths in women and is second only to lungs cancer. The National Cancer Institute estimates that about 1 in 8 U.S women will develop invasive breast cancer over the course of women’s life. In 2011, an estimated 2,30,480 new cases of invasive breast cancer are expected to be diagnosed in women in the U.S along with 57,650 new cases of non-invasive breast cancer. For women in the U.S breast cancer death rates are higher than those for any other cancer besides lungs cancer. In 2011 there are more than 2.6million breast cancer survivors in the U.S.A women’s risk of breast cancers approximately relative who have been diagnosed with breast cancer. About 15% of women who gets breast cancer have a family member diagnosed with it. About 85% of breast cancers occurs in women who have no family history of breast cancer. These occurs due to genetic mutations that happen as a result of the aging process and life in general.
1.1.1 Breast Cancer

The body is made up of many types of cells. Normally, cells are grow and divide to produce more cells only when the body needs them. This orderly process are helps to keep the body healthy and sometimes cells keep dividing when new cells were not needed. These cells may form a mass of extra tissue called a growth or tumor. Tumors can be benign or malignant one.

- **Benign tumors** are not cancer one. They can be usually removed, and in most cases, they did not come back. Most important, the cells in benign tumors are not invade other tissues and are not spread to other parts of the body. Benign breast tumors were not a threat to life.

- **Malignant tumors** are cancer one. Cells in these tumors can be invade and damage nearby organs and tissues. Also, cancers cell can be break away from a malignant tumor and enter the blood stream or lymphatic system. That is how breast cancer spreads and forms secondary tumors in other area of the body. These are called metastasis.

Breast cancer is a malignant cell growth in the breast. The cancer spreads to other areas of the body, If left untreated. Breast cancer have become a major cause of death among women in developed countries. The most effective way to reduce breast cancer deaths is detect it as earlier [5]. However, earlier treatment requires
ability to detect breast cancer in the early stages. Early diagnosis needs reliable and accurate diagnosis procedure that allows physicians to distinguish benign breast tumors from malignant ones. Thus, finding an accurate and effective diagnosis methods are very important. Biopsy are the best way to accurately determine whether the tumors are benign or malignant. However, it is expensive, invasive and positive findings at biopsy for cancers are less, between 10% to 31%.

Breast cancer are the second most commonly diagnosed cancer worldwide. In order to find cure, it is necessary to quickly diagnose the disease accurately and treat it based on the kind of symptoms. Breast cancer has several classifications, which may helps to determine the best treatment [4] [6]. The most important of these classifications is binary classification, it is either benign or malignant. If the cancers are in benign stage, less invasive and risk of treatments are used than for malignant stage. The reason being the chances of survival of patient is high; it is not beneficial to increase the speed of recovery at the risk of introducing potentially life threatening side effects caused by aggressive treatment. On the other hand a patient with malignant cancer is not so concerned about the kind of treatment or side effect of the treatment.

The main cause of breast cancer are when a single cell or group of cells escapes from the usual controls, that regulate cellular growth
and begins to multiply and spread. This activity may result in a mass, tumor or neoplasm. Many masses were benign that means the abnormal growth are mainly restricted to a circumscribed, single and expanding mass of cells. Some tumors were malignant that means the abnormal growth invades the surrounding tissues and that may metastasize or spread to remote areas. The benign masses may lead to complications whereas Malignant tumors are serious cancer. The majority of breast tumors will have metastasized before reaching a tangible size. So far, various techniques have been discovered for breast cancer detection in tissue level [8]. The methodology of breast cancer model is shown in Fig. 1.1.

![Fig. 1.1 Methodology of Breast Cancer Model](image-url)
1.1.2 Anatomy of the breast

The breasts are mammary glands that produce milk and are common in all mammals. Embryological tissues are responsible in both men and women to develop the breast, but in women the sex hormones, mainly estrogens, promote breast development and growth. The mammary gland is one of the two half-moon-shaped glands on both sides of the adult female chest is shown in Fig. 1.2. The blood to the breast is supplied by the internal mammary and lateral thoracic arteries. The mammary gland is composed of fatty cells which store fat and in adult lactating women, lobules produce milk. There are complex networks of branching ducts within the mammary gland. These ducts transport milk from the lobules out to the nipple. The size of a normal breast on average is 10-12 cm in diameter and in thickness 5-7 cm.

![Anatomy of Breast](image)

**Fig. 1.2 Anatomy of Breast**
1.1.3 Breast Cancer’s Development

Cells in the body grow and divide in a controlled fashion and the cells die after several rounds of replication. New cells are formed from progenitor cells as needed. Breast cancer are a result of growth abnormality in the cells of breast resulting in change in consistency of the breast tissues. This abnormality develops usually in the inner lining of the milk ducts or lobules. Breast cancer appears in the form of tumour when there is uncontrollable proliferation of breast cells. Fig. 1.3 shows the developing stages of tumor.

Fig. 1.3 Developing of Tumor
The tumor is said to be malignant when the proliferating cells invade the neighbouring tissues and organs. These cells grow faster than surrounding cells. Without treatment, malignant breast cancer becomes an irreversible process. If the malignant tumor is not removed, the patient will die in early stages. These early stages of abnormal breast cell growth can be hard to detect by the patient and doctors alike. This is detected usually by a mammogram. The tumor growth varies considerably among individual patients and it grows faster among younger women. The steps of tumor development is shown in Fig. 1.4.

Fig. 1.4 Steps of Breast Cancer Development
1.1.4 Classification of Breast Cancer

Breast cancer classification are based on growth, size and stage of the tumor. Tumors are of two types, benign or malignant. A benign tumor does not spread, but a malignant tumor is invasive and life threatening. Four different approaches were used for the classification of breast cancer.

The short description of these four approaches according to the Wikipedia is as follows:

- Pathology: Tumors are classified by their microscopic anatomy i.e. what the tissue looks like in a microscope.

- Grade of the tumor: The histological grade is determined under a microscope. There are three categories for the grade of tumor.
  a) A well-differentiated/low grade tumor resembles normal tissue.
  b) Poorly differentiated (high grade) tumor consists of disorganized cells and looks different from normal tissue.
  c) Moderately/intermediate tumors were somewhere in between.

- Expression of proteins and genes: Expression of proteins and genes helps us to assess the prognosis of breast cancer. Tumors are tested for expression of the Estrogen Receptor (ER), Progestrogen Receptor (PR) and HER2/neu proteins. These tests can help to determine the best treatment.

- Stages of the tumor: The stages of breast cancer that are most widely used are those stated in the NTM classification (Non
Tuberculous Mycobacterium). These include a) the tumor itself, b) whether it have spread to lymph nodes and c) whether there is any further metastasis.

1.1.5 Sign and Symptoms

- When the disease was discovered early, these were more treatment options and better chance for a cure.
- Most breast lumps were not cancerous. Yet the most common sign of breast cancer for women are a lump or thickening in the breast. Often the lump was painless.
- Spontaneous clear or bloody discharge from the nipple, often associated with a breast lump.
- Retraction or indentation of the nipple.
- Change in size or contours of the breast.
- Any flattening or indentation of the skin over the breasts.
- Redness or pitting of the skin over the breast, like the skin of an orange.
- A number of conditions other than breast cancer can cause the breasts to change in size or feel.
- Breast tissues changes naturally during pregnancy.
- Other possible causes of noncancerous (benign) breast changes include fibroa denomas, infection or injury.
- If the changes had not gone away after a month, have them evaluated promptly.
1.1.6 Causes

- In breast cancer, some of the cells in the breast begin to grow abnormally.

- These cells divide more rapidly than healthy cells do and may spread (metastasize) through the breast, to lymph nodes or to other parts of the body.

- The most common type of breast cancer begins in the milk-producing ducts, but cancer may also begin in the lobules or in other breast tissue.

- In most cases, it was not clear what causes normal breast cells to become cancerous.

- Only 5-10% of breast cancers were inherited.

- Families that do have genetic defects in one of two genes, breast cancer gene 1 (BRCA1) or breast cancer gene 2 (BRCA2), had a much greater risk of developing both breast and ovarian cancer.

- Other inherited mutations – including the ataxiatelangiectasia mutation gege, the cell-cycle checkpoint kinase 2 (CHEK-2) gene and the p53 tumor suppressor gene – also make it more likely that will develop breast cancer.

- If one of these genes are present in the family, it will have a 50 percent chance of having the gene.

- Yet most genetic mutations related to breast cancer is not inherited.

- These acquired mutations may result from radiation exposure – women treated with chest radiation therapy for lymphoma in childhood or during adolescence when the breasts are developing
had a significantly higher incidence of breast cancer than do women not exposed to radiation.

- Mutations may also develop as a result of exposure to cancer-causing chemicals, such as the polycyclic aromatic hydrocarbons found in the robbaco and charred red meats.

**1.1.7 Breast Cancer Risk Factors**

Every woman are at risk for developing breast cancer. Several relatively strong risk factors for breast cancer that affect large proportions of the general population have been known for some time. However, the vast majority of breast cancer cases occur in women who have no identifiable risk factors other than their gender [9]. The “established” risk factors for breast cancer is female gender, age, previous breast cancer, benign breast disease, hereditary factors (family history of breast cancer), early age at menarche, late age at menopause, late age at first full-term pregnancy, postmenopausal obesity, low physical activity, race/ethnicity and high-dose exposure to ionizing radiation early in life. The “speculated” risk factors for breast cancer include never having pregnant, having only one pregnancy rather than many, not breast feeding after pregnancy, use of postmenopausal estrogen replacement therapy or postmenopausal hormone (estrogen/progestin) replacement therapy, use of oral contraceptives, certain specific dietary practices (high intake of fat and low intakes of fiber, fruits, and vegetables, low intake of phytoestrogens), alcohol consumption, tobacco smoking and abortion.
Although men can and do develop breast cancer, the disease are 100 times more likely to occur in a woman than in a man [10]. Women were at a higher risk of breast cancer because they had much more breast tissue than men do. Also, estrogen promotes the development of breast cancer.

The risk of breast cancer are higher in middle-aged and elderly women than in young women [11]. This risk increases as a woman ages, rising sharply after the age of 40. In the United States, more than three-fourths of all breast cancer occurs in women aged 50 or older. A woman have previously had breast cancer have a three- to four-fold increased risk of developing a new cancer in the other breast. Women who have benign breast problems were also at increased risk but to a lesser extent [12] [13]. The risk of breast cancer is higher among women who had a close blood relative (mother, sister, or daughter) who had the disease. The increase in risk is especially high if the relative developed breast cancer before the age of 50 or in both breasts [14]. However, most women who get breast cancer (approximately 80 percent) has no such family history of the disease [15]. The effect of family history on breast cancer risk are believed to be primarily due to genetic factors. As much as 5–10 percent of all breast cancer cases were attributable to specific inherited single-gene mutations and many other cases had some genetic component. The evidence from individual families in which breast cancer occurs very frequently and from large epidemiological studies have shown that
some women has a familial predisposition to breast cancer. The evidence includes the pedigree of Broca’s family [16]. He is a famous French surgeon (1824-1880) and in his family tree (comprising over five generations) 10 out of 24 women died of breast cancer.

Epidemiological studies have been shown that in women with a family history of breast cancer, the risk of breast cancer was increased two to three fold. Studies has also shown that there is families in which breast cancer risk are inherited in an autosomal-dominant fashion (‘hereditary breast cancer’). Recently, it have been shown that germline mutations in the BRCA1 and BRCA2 genes account for a large proportion of cases of hereditary breast cancer [17]. Histopathological findings and careful autopsy examinations had played a major role in the recognition of many familial cancer syndromes [18]. In addition to mutations in the BRCA1 and BRCA2 genes, there were as yet unidentified genetic defects that predispose to breast cancer development [19] and additional studies may help in who never has children or those who has their first child relatively late in life [13]. The biologic basis for this relationship are not entirely clear. Obesity have been consistently associated with an increased risk of breast cancer among postmenopausal women [20] [21]. This relationship may be mediated again by estrogen production. Fat cells produce some estrogen and obese postmenopausal women, therefore, tend to had higher blood estrogen levels than identifying these genes in the future. Women who reach menarche at a relatively early age (12
or younger) and those who reach menopause at a relatively late age (55 or older) were slightly more likely than other women to develop breast cancer. These relationships were believed to be mediated through estrogen production [20]. During the reproductive years, a woman’s body produces high levels of estrogen. Women who start to menstruate at an early age and/or reach menopause at a late age are exposed to high levels of estrogen for more years than were women who had a late menarche or early menopause. Age at first pregnancy is another aspect of reproductive history that are associated with breast cancer risk. Women who has their first full-term pregnancy at a relatively early age has a lower risk of breast cancer than those lean women.

Studies have consistently shown that the risk of breast are cancelower among physically active premenopausal women than among sedentary women [23] [24]. Physical activity during adolescence may be especially protective and the effect of physical activity may be strongest among women who has at least one full-term pregnancy. Studies of racial/ethnic characteristics of breast cancer reveal that non-Hispanic white, Hawaiian and black women has the highest levels of breast cancer risk. Other Asian/Pacific Islander groups and Hispanic women has lower levels of risk. Some of the lowest levels of risk occur among Korean and Vietnamese women [25]. Women who are exposed to high doses of radiation, especially during adolescence, has an increased risk of breast cancer. This association have been observed both among atomic bomb survivors and among women who
received high-dose radiation for medical purposes [26] [27]. Parity (having children) and the age of woman at the birth of her first offspring were other endogenous hormonal factors that influence breast cancer. Women who has never had children (nulliparous) is at greater risk for the development of breast cancer than women who have children (parous). There are also consistent evidence that first pregnancy completed before age 30-35 lowers risk of breast cancer, and that first full-term pregnancy after age 30-35 raises risk. More limited evidence suggests that women who has many pregnancies may be less likely to develop breast cancer than those who has only one pregnancy [22]. Some studies has shown that women who breast-feed their babies may be less likely to develop breast cancer than those who has children but do not breast-feed [28]. Other studies, however, indicate that there may be little or no relationship between breast feeding and breast cancer risk. If breast-feeding does protect against breast cancer, it may do so by delaying the resumption of ovulation (with its accompanying high estrogen levels) after pregnancy. The long-term (more than five years) use of postmenopausal estrogen therapy or combined estrogen/progestin Hormone Replacement Therapy (HRT) may be associated with an increase in breast cancer risk [29]. The associations between the use of oral contraceptives and breast cancer has been studied.

Many studies attempting to link oral contraceptives with increased breast cancer has been inconclusive [30]. But these studies
has shown that oral contraceptives do not have a large effect on breast cancer risk. Whether they have a small effect on risk are less clear. A possible relationship between breast cancer and diet have been suggested due to the variation of breast cancer in societies with different national diets (the high rates in Western industrialized nations and the low rates in Asia, Latin America and Africa). A comparison of vegetarian versus meat-eating women produced inconclusive results. No relation between breast cancer risk and total fat, saturated fat or cholesterol is found.

Some of the effects that are once attributed to dietary fat intake are probably due to obesity rather than to fat intake per se. And the effects of fiber, fruits, and vegetables now appear to be small, at best. Diets high in fruits and vegetables and low in fat and calories were healthful for many reasons and they may indirectly reduce the risk of breast cancer by helping to prevent obesity [113]. Plant substances called isoflavones (sometimes referred to as phytoestrogens) is most commonly found in soy products. It have been speculated that these substances may be 10 protective against breast cancer [31]. They appear to had effects similar to those of estrogen in some tissues while antagonizing the effects of estrogen in other tissues. A positive, but modest association between alcohol use and breast cancer risk is seen in most studies [32] [33]. There are also some evidence that cigarette smoking may be associated with a small increase in breast cancer risk. However, epidemiological studies has variably shown positive,
inverse or null associations [112]. Among women who had already been diagnosed with breast cancer, smoking may be associated with an increased risk that the cancer will progress more rapidly [34]. In some studies, premature termination of pregnancy appears to increase breast cancer risk [35]. In incomplete pregnancy, the breast are exposed only to the high estrogen levels of early pregnancy and thus may be responsible for the increased risk seen in these women. However, some other studies found no association between abortions and increased risk of breast cancer [36].

1.1.8 Stages of Breast Cancer

There are 4 stages of breast cancer. In stage1 breast cancer are split into 2 Stages; Stage1A: Tumor are 2 cm or smaller and have spread outside the breast. Stage1B: Small areas of breast Cancer cells were found in the lymph node closed to the breast and either the tumor are 2 cm or smaller. Stage 2 breast cancer, stage 2A: Tumor 2cm or smaller in the breast and cancer cells were found in 1 to 3 lymph Node in the armpit or in the lymph Node near the breast bone. Stage 2B: The tumor are larger than 2 cm but not larger than 5 cm and small areas of cancer cells were in the lymph node 2 to 5 cm. It spreads up to 1 to 3 lymph nodes in the armpit or near the breastbones or the tumor are larger than 5cm and have not spread to the lymph node. Stage 3 as, Stage 3A: No tumor are seen in the breast or the tumor may be any size and cancer are found in 4 to 9 lymph glands under the arm or in the lymph glands near the breast bone.
The tumor are more than 5 cm and have spread into up to 3 lymph nodes near the breast bone. Stage 3B: The tumor have spread to the skin of the breast or to the chest wall and made the skin break down or cancer swelling. The cancer by then may has spread to the up 9 lymph Node in the armpit or to the lymph glands near the breast bone. 

Stage 4 breast cancer: The tumor can be any size. The lymph Node may or may not contain cancer cells. The cancer have spread to the other parts of the body such as bone, lungs, liver and brain. The TNM (Tumor, Node and Metastasis) system are specified for each type of cancer. Once a patient’s T, N and M categories has been determined and this information is combined in a process called stage grouping to determine a women disease stage from Stage0 (the least advantage stage) to Stage4 (the most advantage stage).

### 1.1.9 Detection of Breast Cancer

Increased breast cancer awareness with breast self-examinations and major improvements in routine breast cancer screening have a paramount effect on early detection of breast cancer. Improvements in conventional mammography (an x-ray technique to visualize the internal structure of the breast) such as the low radiation dosage, enhanced image quality, development of statistical techniques for computer-assisted interpretation of images, long-distance, electronic image transmission technologies (telemammography /teleradiology) for clinical consultations and improved image-guided techniques to assist with breast biopsies (the removal of cells or
tissues for examination under a microscope) continue to lower the morbidity and mortality of breast cancer. The support of research on technologies that do not use x-rays and were not used for routine breast cancer screening, such as Magnetic Resonance Imaging (MRI), ultrasound and breast-specific Positron Emission Tomography (PET) may play a considerable role in further improvements of breast cancer early detection. In most cases, the earlier breast cancer are detected was the better than survival rate. Today mammography are the best available method to detect breast cancer in its earliest, most treatable stage - an average of 1.7 years before the woman can feel the lump [64]. Generally, treatment are most effective before the disease spreads. When breast cancer are diagnosed at a local stage, the 5-year survival rate are greater than 90%. This rate decreases to less than 50% when the disease have spread to the lymph nodes and less than 20% when it have spread to distant organ sites.

Despite recent progress in early detection and surgical therapy, the mortality due to breast cancer have changed little over the past decades, primarily because the occult dissemination of cancer cells can occur at an early stage of carcinogenesis [65]. Occult dissemination of tumor cells in patients with operable cancer can subsequently lead to formation of metastasis, yet it was usually missed by conventional tumor staging. The success of routine mammography screening for breast cancer are that it involves increasingly more patients with small primary tumors formerly
thought to has an overall excellent prognosis. Yet, only approximately two thirds of these patients actually has this favorable prognosis, while the remaining third develops metastatic disease. Thus, there are emerging evidence that tumor cells can disseminate into secondary organs at an earlier stage of primary tumor development than appreciated by current risk classifications.

There were several challenges that must be addressed in an effort to continue to lower the mortality associated with this disease. Molecular oncology is currently one of the most promising fields, which may address the major problems with early detection and accurate staging of women with breast cancer. The advent of highly sensitive, molecular techniques, such as the Polymerase Chain Reaction (PCR), enables the detection of circulating tumor cells and small metastasis at the molecular level. PCR-based assays is used for the detection of tumor cells in lymph nodes, resection margins, bone marrow and blood. Methods to detect metastatic disease and circulating tumor cells at a molecular level were of two types: those that detect somatic events such as point mutations or chromosomal rearrangements and those that detect expression of tumor specific mRNAs. Both methods has been applied for the detection of many different tumor types. The main limitation of the first method are that not all tumors contain mutations suitable for PCR amplification. For the second method to work, the molecular marker must be transcriptionally elevated in malignant cells and not in the
surrounding cells or tissue. Gene amplification/over expression are a common event in the progression of human cancers and amplified genes has been shown to serve as molecular markers and have diagnostic, prognostic and therapeutic relevance. Currently, molecular markers offer the unique opportunity to identify occult metastases in early stage cancer patients not otherwise detected with conventional staging techniques. The completion of the human genome as well as an enormous amount of information on the transcriptional activities in cancer cells enables the selection of specific markers for the detection of cancer cells. The ideal prognostic marker are one that clearly delineates a particular prognostic group are 100% specific, highly sensitive, inexpensive and easy to perform on a small quantity of fresh or fixed tissue. No such marker exists but a number of potential prognostic markers has been extensively investigated. Multiple proteins has been found to be specifically overexpressed in certain types of tumors (i.e. Her2neu, PSA, p53, pRB, melanoma antigens, etc.).

Detection and quantification of potential tumor markers using sensitive molecular methods could assist in the early diagnosis of cancer disease as well as in the efficacy of anti-cancer therapy. The clinical application of molecular markers in the diagnosis, staging and management of breast cancer continues to expand. The molecular detection of circulating tumor cells and micrometastases may help to develop new prognostic markers. Extensive work by various groups
have been done on Minimal Residual Disease (MRD) detection in blood, bone marrow and lymph nodes in cancer patients with different types of cancer. In the past 10 years, numerous investigators has attempted the detection of occult tumor cells in malignancies using the highly sensitive Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) technique. This was a particularly sensitive technique for the purpose of detecting occult breast cancer cells in the blood, bone marrow and lymph nodes of breast cancer patients. That are simple, rapid and semi-automatic and are an alternative method to fluorescence in situ hybridization and immunochemistry. RTPCR have produced sensitivity levels of 1 tumor cell in 10,00,000 normal cells. It can detect micrometastases based on amplification of mRNA expressed exclusively in the cancer cells of interest or in significantly larger amounts in cancer versus non-cancer cells localized to the lymph node, other distant organs or circulating in the blood. RT-PCR may be a powerful tool for large randomized, prospective cooperative group trials and support future tumor-marker based biological and gene-therapy approaches. The labor intensive and time consuming pathological investigations can be minimized or substantially assisted using automated RT-PCR assays. These assays, in the vast majority, has been directed against tissue specific markers. In most studies on prostatic carcinoma, RT-PCR is able to specifically detect prostatic tissue specific markers in the peripheral blood, bone marrow and lymph nodes of patients with localized and metastatic disease.
Melanoma related transcripts are detected by RT-PCR in the peripheral blood, bone marrow and lymph nodes of patients with localized and advanced tumors. Many author has shown a statistically significant correlation between RT-PCR positivity and a poorer outcome in both melanoma and prostatic carcinoma.

In breast carcinoma, all markers that has been extensively tested are shown to be non-specific. The presence or absence of axillary lymph node metastasis are still the single most reliable predictor of the final outcome in breast cancer and the primary determinant for the use of systemic therapy in patients with newly diagnosed cancer confined to the breast. Although these patients were considered potentially curable, a substantial number of them develop recurrent carcinoma and die of their disease in 5 to 10 years, including nearly 30% of patients with negative axillary lymph nodes. As mentioned earlier, occult dissemination of tumor cells in the patients with operable cancer can subsequently lead to formation of metastases, yet it was missed by conventional tumor staging with histopathology and immunohistochemistry in the lymph nodes. The blood and bone marrow were not currently routinely examined for tumor cells in women with breast cancer. However, it is likely, that if these sites were routinely assessed, tumor cells would be identified. Ultimately, knowledge of tumor cells presence in the blood or bone marrow may impact the survival of breast cancer patients via earlier detection and initiation of adjuvant therapy. A reliable RT-PCR assay
have not been developed for breast cancer. The targeted tumor markers for detection by RT-PCR in breast cancer patients has not been identified as in melanoma. In addition, because of the extreme sensitivity of RT-PCR technique, an accepted cut-off value that defines tumor presence or absence have not been established. However, it was widely accepted that the detection of mRNA tumor markers and 16 well-defined experimental protocols can greatly improve the sensitivity, specificity and reliability of the RT-PCR assay system. Breast tumors were composed of a heterogeneous collection of cells with differing levels of individual gene expression.

Therefore, the predominant cell type or its metastasis may not express a particular marker [42], [43]. Therefore, it was believed that multimarker approaches with a panel of tumor-specific mRNA markers may improve the sensitivity and specificity for the detection of tumor cells over single marker assays in breast cancer patients. Furthermore, tumors continuously evolve genetically over time in response to host pressures and treatment interventions, which suggests that single marker testing may not be able to effectively monitor cancer progression. Simultaneous detection of such markers by newly developed methodologies such as real-time quantitative RT-PCR will enable the accurate monitoring of the level of mRNA markers as well as the precise comparison to known internal mRNA standards. Lymph node metastatic involvement are arbitrarily subdivided into micro and macrometastases, usually according to the size of the tumor deposits.
with the cut-off point ranging from 0.2 to 2.0 mm[44], [45]. Serial sectioning and immunohistochemistry appeared to increase the detection rate by 9-33%. A definite survival disadvantage is noted for patients with such occult metastases [68]. The use of extensive serial sectioning and immunohistochemistry on all axillary lymph nodes are too expensive and labor-intensive to be practical. Sentinel Lymph Node (SLN) are the first lymph node in the axillary basin to receive metastases from the primary breast cancer if they had occurred. The concept of SLN is introduced by Cabanas. The SLN accurately predicts the pathology of the remaining axillary basin allowing a focused pathologic analysis of it. If the SLN does not contain tumor, the chance of tumor in the remaining axillary lymph nodes are less than 1%. Therefore, patients without tumor in the SLN can avoid unnecessary Axillary Lymph Node Dissections (ALND). A SLN biopsy in comparison to an ALND have significantly less morbidity in terms of lymphedema, numbness to the arm and decreased range of motion. The assessment of the SLN at the time of initial diagnosis of breast cancer may improve upon the current staging molecular method - multiple mRNA marker RT-PCR analysis, which would identify the sub-group of patients with metastases in the SLN but thought to be free of disease by conventional pathologic examinations. Therefore, the analysis of micrometastatic cells opens a new avenue by which to assess the molecular determinants of both early tumor cell dissemination and subsequent outgrowth into overt metastases.
Tumor cell detection in the bone marrow are being regarded increasingly as a clinically relevant prognostic factor for breast cancer [69]. Many studies suggest that tumor-cell shedding already occurs during the early stages of breast cancer and had demonstrated a significant correlation between tumor cell detection in the bone marrow and decreased disease-free and overall survival studies has shown that breast cancer patient may harbor bone marrow metastases alone or in conjunction with axillary metastases. These studies suggest that the tumor status of the bone marrow may be a better prognostic indicator than the axillary lymph node status [51]. Since the primary breast tumor can spread by both the lymphatic and hematogenous route, it was possible that patients may have metastases to the bone marrow and not the axillary lymph nodes. It was now clear that bone marrow are one of the most prominent secondary organs to receive disseminated tumor cells and are an important determinant for micrometastatic organ involvement due to its ease of accessibility and normal physiological absence of epithelial cells [67]. Bone marrow aspirates can be easily obtained from breast cancer patients at the time of surgery.

The technical feasibility and the potential prognostic significance of bone marrow metastases makes assessing this site for tumor spread clinically important. Therefore, the multiple-marker RT-PCR assays may be used to molecularly stage the bone marrow detecting micrometastases not identified with conventional pathology
Increased accuracy in staging breast cancer patient disease and initiation of earlier therapeutic interventions unequivocally were beneficial consequences of technological advancements that identify high-risk patients early in their disease course [71]. Blood testing provides a minimally invasive method to evaluate the presence of circulating tumor cells that may serve as indicators for assessing risk of recurrence. Current imaging techniques used to identify breast cancer metastases often require a significant tumor burden for detection. Furthermore, the procurement of sufficient tissue to confirm the diagnosis can be associated with significant morbidity and cost depending on the size and location of the lesion as mentioned previously. Therefore, the utility of detecting tumor cells in the blood potentially offers a practical, safe and cost-effective alternative to traditional methods of diagnosing disease recurrence and/or systemic spreading. In prostate and colon cancer, Prostate Specific Antigen (PSA) and Carcino Embryonic Antigen (CEA), which is measured in the blood, has served as a tremendous tool in the management of these cancer types respectively. To date, well-characterized molecular tumor markers to detect occult breast cancer cells in the blood is limited. There is no breast cancer tumor marker that can be measured in the blood, used for screening, serve as prognostic indicator, measure response to therapy or signal early recurrence of the disease.

The advantage of tumor marker detection in the blood is that it can be serially measured throughout the course of the disease, unlike
lymph node and bone marrow biopsies. The development of a multiplemarker RT-PCR assay to detect micrometastases in the blood would considerably improve tagging at the time of diagnosis of breast cancer patients enabling the early institution of the therapy that could lead to improved survival in patients that had disease relapse. A reliable breast cancer tumor marker that could be measured in the blood would potentially detect the disease before it becomes clinically visible on screening mammography or palpable on clinical exam. The increased levels of the tumor marker could lead to more frequent monitoring, further testing of patients or earlier biopsy of suspicious lesions seen on mammography. It could also be used for postoperative monitoring, for determining the response to chemotherapy and for prolonged post-treatment monitoring. The development of a multiplemarker RT-PCR assay that would be able to detect micrometastases in the blood identifying circulating tumor markers would have the potential to be used in the above mentioned situations. New prognostic markers can be tested in this very efficient way. If the study proves successful, the markers can be adopted for routine use either alone or more probably, in combination with standard clinical assessment. It is believed that a number of molecular markers will make the transition from the laboratory to the clinic over the coming decades with the ultimate benefit being better prognostication and therapy of breast cancer patients.
1.2 OBJECTIVE OF THE THESIS

The main objective of the thesis is for classification of “benign” that are noncancerous or a “malignant”, that is cancerous using various data mining techniques:

- Collection of WBC (Wisconsin Breast Cancer) from UCI machine learning.
- Developing various machine learning techniques to classify breast cancer and noncancerous data from Wisconsin Breast Cancer (WBC) database.
- Comparison of the performance of various classifiers used under this technique in terms of sensitivity, specificity and accuracy to determine the best classifier for the breast cancer classification.

1.3 THESIS OUTLINE

This thesis comprises seven chapters. Chapter 1 provides introduction to breast cancer. Chapter 2 provides the review of existing literature related to the breast cancer. Chapter 3 describes the k nearest neighbors algorithm. Chapter 4 discusses about the Support Vector Machine. Chapter 5 focuses on the Auto Associative Neural Network technique. In Chapter 6, results of the experiments with comparison of performance of three techniques are shown and discussed. Conclusion of this work is discussed in Chapter 7.
1.4 PROPOSED SOLUTIONS

Early diagnosis needs an accurate and reliable diagnosis procedure that can be used by physicians to distinguish benign breast tumors from malignant ones without going for surgical biopsy. The objective of these predictions are to assign patients to one of the two groups either a “benign” that are noncancerous or a “malignant” that are cancerous. The thesis discusses three data mining techniques such as k-Nearest Neighbor, Support Vector Machine and Auto Associative Neural Network.

WBC (Wisconsin Breast Cancer) database was created by William H. Wolberge at the University of Wisconsin. This database are used to distinguish malignant (cancerous) from benign (non-cancerous) samples. The process of defining a set of features will most efficiently or more meaningfully represent the information that are important for analysis and classification. After feature extraction, the extracted features of data are given as inputs to classifiers. classifiers are used to classify the group of data as either affected or normal depending on the feature values.