2. REVIEW OF LITRETURE
2.1 Breast Cancer (BC)

It is described as the formation of a malignant tumor in the mammary gland. It is the most conjoint cancer in female and major cause of cancer-related deaths worldwide. Centre for Disease Control and Prevention United States accounts it about 32 percent of all female cancers. The National Cancer Institute calculation pronounces that near 1 in 8 females in the United States US (around 13.3%) will develop BC during her life (Bahls and Fogarty 2012, Gibbs 2003).

2.1.1. Breast Morphology and Types of BC

Morphologically each breast has been divided into 15-20 sections and subsections referred as lobes and lobules respectively. The lobes and lobules are associated with thin canals, called ducts (Figure 1a). Ductal breast carcinoma is the most frequent type of BC in females. Cancer that starts in lobes or lobules is called lobular cancer (Muller and Vousden 2013). It is more repeatedly originate in both breasts than other types of BC (Christiansen and Westergaard 1992).

![Figure 1: The structure of the female breast. (Source: modified from https://www.myvmc.com/anatomy/breast/)](https://www.myvmc.com/anatomy/breast/)
Furthermore, BC is classified as non-invasive (in situ) and invasive (infiltrating) (American Cancer Society 2012). These terms are given according to the nature of movement by cancerous tissues or cell. The in situ BC is a condition when BC never suppers past the origin point of its development, whereas invasive BC has a propensity to spread (metastasize) to other tissues of the breast and/or other regions of the system.

IBC- Inflammatory BC is a less common category of BC which is characterized by general irritation of the breast. Another irregular type of BC are medullary carcinoma characterized by the formation of a distinct boundary between tumor tissue and normal tissue, mucinous carcinoma for the mucus-producing cancer cells, and tubular carcinoma ie. Cancer in tubules (Albert et al. 2002, Futreal et al.2004)(Figure 1b).

Figure 2: The Common types of BC
2.2 Historical Overview

In reference to recovery with BC, it is now barely a new condition, with elapsing time the rate of recovery is increasing day by day. The documented proof of BC knowledge can be traced back from the beginning of history (Croce 2008). The information of BC can be drawn within Egyptian culture with cautery as a practice for the handling of diseased tissue. The rare practice of surgical procedures was also documented, but due to its painful results and lack of knowledge about antiseptics and anesthetics the procedure was rare to be followed. Approximately in Egyptian culture description of cancer has been reported near to 1600 BC. (Diamandopoulos 1996).

“There is no treatment,” said in his writings by Edwin Smith Papyrus he described 8 types of tumor or ulcers referring with mammary gland and were cured by the technique of cauterization, “the fire drill” kind of instrument. The “Father of Medicine” Hippocrates coined the term cancer which refers to crab (460-370 B.C.), carcinos and carcinomas were the terms described by him to non-ulcer forming and ulcer-forming tumor respectively. According to the doctrines of the Greek physician Caudius Galen 130-200 AD (Wagner and Eferl 2005), melancholia was the chief factor in the development of BC and the recommended treatments were Special diets were the recommended treatment. However, other treatments included exorcism and the use of topical applications which were not preferred by patients (Harvey 1974). During the revival of European culture, Andreas Vesalius recommended removal of breast tissues to cure the BC and sutures at site of surgery to control bleeding. The BC could spread to the local auxiliary nodes was first recognized by Dr. Le. Dran (1685-1770) he also associated poor prognosis with the spread of BC to the lymph nodes. The Scottish surgeon John Hunter (1728-1793) advised that certain cancers can be cured by surgery and labelled how the surgeon may decide identification symptoms to operate on. According to him “If the tumor had not invaded spread nearby tissue and is
“moveable”, he said, “There is no impropriety in removing it”. The detailed record keeping of BC started from mid-1800 (Zenz et al. 2008, Cohen et al. 2011, Petersona et al. 2000, Lennartsson and Ekwall 2009, Doolittle et al. 1983). After the discovery of X-ray by Waterfield et al. (1894), many diseases were understood more clearly and appropriately than ever before. The phenomenon of microcalcification was discovered by Albert Solomon, a German pathologist in the year 1913 by producing an image of 3000 mastectomy specimens of BC (Downward et al. 1984). The period of 1930-1950 accounts and bags down notable achievements for detection and treatment of BC. During this period stereoscopic system for tumor identification, a classification system for BC stages and progression were developed and self-examination of the breast was advocated. Dr. Robert Egan 1960 adapted the high-resolution industrial grade film for mammography, allowing simple and reproducible mammograms with improved image quality (Sherr et al. 1985). In 1963 the first randomized controlled trial of screening by the Health Insurance Plan of New York found that mammography reduced the 5-year BC mortality rate by 30 percent (Zhang et al. 2010). Major improvements in mammography equipment, such as reduced radiation dosage, digital imaging, and computer-aided diagnosis, improved detection of BC (Brunton et al. 2004, Carlo 2008).

2.3 BC Risk Factors:

Women's are at risk for evolving BC (Liu et al. 2013). The “well-known” risk factors for BC are female gender, age, previous BC, benign breast disease, genetic factors (family history of BC), and early age of obesity, less physical activity, and race/ethnicity and high-dose exposure to ionizing radiation early in life (IARC 2011). The Figure1C gives an idea for the incidence of BC worldwide. Although men can do develop BC, the disease is 100 times more likely to occur in a woman than in a man. Also, estrogen plays important role in
advancement of BC. Middle-aged and elderly women are at higher risk for BC than younger one (Farmer et al. 2005).

![Figure 3: Age-standardised incidence rate of BC per 100000](image)
(Source: Modified from, IARC and Inas Ellater professor of Biostatistics and epidemiology, National Cancer Institute Cairo University)

In the United States, more than three-fourths of all BCs occur in women aged 50 or older. Women's with the previous history of BC has three to four-fold higher risk of developing BC in another breast. Women's with benign breast problems are at higher risk but with lesser extent (Halazonetis et al. 2008, 25). Woman’s having a family history of BC having a close relative with BC are at higher risk (Linet et al. 2012). However, approximately 80 percent of the disease has been shown to develop in women without family history of BC (Lee et al. 2010). Genetic influences are considered as the prime reason in woman developing BC along with family history of BC.

The best evidence consists of the pedigree of Broca's family (AICR). He was a famous French surgeon (1824-1880), and in his family tree (comprising over five generations) 10 out of 24 women died of BC. Epidemiological studies have also gave a view that the in females with family history of BC, the risk of BC is amplified two- to three-fold. Studies have also revealed that there are relations in which BC risk is inherited in an
autosomal-dominant manner. In recently reported cases for hereditary BC, the BRCA1 and BRCA2 gene family polymorphism or mutations were found playing a major role in disease progression (Vahteristo et al. 2001).

Histopathological results and cautious autopsy inspections have played a major role in the recognition of many ancestral cancer conditions (Cuendet et al. 2006), there are as yet unidentified genetic defects that predispose to BC development (McLaughlin-Drubin and Munger 2008), and additional studies may help in identifying these genes in the future.

In count to alterations in the BRCA1 and BRCA2 genetic factor, Women who reach menarche at a comparatively early age (12 or younger) and those who reach menopause at a relatively late age (55 or older) are slightly more likely at risk than other women to develop BC (25 or younger). These associations are supposed to be facilitated through estrogen production. During these generative years, a woman’s body makes high levels of estrogen.

Age at which the first pregnancy occurred is also considered as a major role in the development of BC. Women's having a first pregnancy at an early age are at lower risk of developing BC compared to those who never had a pregnancy or had their first child at relatively late in life (Reedman and Klein 1973). The biological foundation for this association is not fully clear. The connectivity of obesity and menopause to development of BC are consistently linked. And females with this combination are at higher risk (Renne et al., 1996). A probable connectivity of disease development with estrogen production in females, as in fat menopausal woman the number of fat cells is always higher compared to a normal menopausal woman, which leads to production and secretion of higher levels of estrogen within the body leading to raising conditions favourable for BC (Smith et al. 2002, Clapper 2000). Studies on the cultural/ethnic dependence of BC, say that non-Hispanic white, Hawaiian, and black women have higher levels of BC risk than Asian/Pacific Islander collections and Hispanic women have lesser intensities of risk. Korean and Vietnamese
women show the minimal of risk occurrence of BC (Gonzalez 1995). Women high quantities of radiation contact during puberty are at higher risk of developing BC. This connection has been detected between both i.e. atomic bomb survivors and among women who received high-dose radiation for medical reasons (Bartsh 1995, Goode et al. 2002, Kelsey 1990). Having children and the age of the woman at the birth of her first offspring are other endogenous hormonal factors that influence BC. Women who have never had children (nulliparous) are at larger risk for the incidence of BC than women who have had children (parous). There is also strong evidence that first gestation finished earlier in age 30-35 have lowered risk of BC and that first full-term gestation after age 30-35 increases risk. Women who breastfeed their babies might remain less likely to develop BC than those who have children but do not breastfeed (AICR). The studies for linking the relation in between Oral contraceptive drugs and BC risk have been unsatisfying (Cuendet et al. 2006). A thinkable connection among BC and food has been recommended due to the difference of BC in cultures with different national diets, the high chances in Western industrialized nations and low rates in Asia, Latin America, and Africa (Zhang et al. 2013).

When compared the BC risk in between vegetarian and meat-eating women results were inconclusive. BC risk and dietary total fat, saturated fat, or cholesterol was inconclusive. Plant phytoestrogens have been wondered that these constituents may be defensive against BC (McLaughlin-Drubin and Munger 2008). Alcohol consumption and BC risk show modest association with BC risk in most studies. A positive, but modest association between alcohol use and BC risk is seen in most studies (Reedman and Klein 1973, Atkinson 2003 and Chen 1999). There is also some indication that cigarette smoking may be linked with a small growth in BC risk (Palmer 1993).

However, epidemiological studies have variably shown helpful, contrary, or useless links. Among women who have previously been analysed with BC, smoking may be linked to
an amplified risk that cancer will grow more quickly. In some studies, the untimely ending of pregnancy appears to BC risk (Renne et al. 1996). In unfinished pregnancy, if the breast is exposed to the high estrogen levels of early pregnancy, it may be accountable for the amplified risk seen in such women. However, some studies were un-conclusive for connection with abortions and increased risk of BC (Clapper 2000, Newcomb 1996, Erlandsson 2003).

2.4. Stages of BC

The TNM system commonly and widely used staging system for BC is based on the tumor size and invasive nature of the primary tumor (T), the clinical non-appearance or occurrence of intense axillary lymph nodes and sign of their local invasion (N), together with the clinical and imaging evidence of distant metastases (M). Combining called as TNM classification, which has been divided into Stage 0 called carcinoma in situ (lobular carcinoma in situ (LCIS) and ductal carcinoma in situ (DCIS) and four broad categories by the Union International Centre Cancer (UICC), which are the following. (Gonzalez 1995)

2.4.1. Stage I: Early stage BC where the tumor is smaller than 2 cm across and hasn't spread other than breast. The tumor id confined in the breast.

2.4.2. Stage II: Early stage BC where the tumor is either smaller 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 hasn't spread outside the breast.

2.4.3. Stage III: locally advanced BC where the tumor is larger than 5 cm across and has spread to the lymph nodes under the arm; or the cancer is widespread in the underarm lymph nodes; or cancer has spread to lymph nodes near the breastbone or to other tissues near the breast.
2.4.4. **Stage IV**: Metastatic BC where cancer has spread outside the breast to other organs in the body.

![Stages of Breast Cancer](http://advocates4breastcancer.org/index.php/about-breast-cancer/stages)

**Figure 4**: Showing stages of BC.

2.5. Techniques for BC Detection

2.5.1. Self-Examination

Self-Examination The self-awareness with breast self-examinations and major improvements in routine BC screening had a supreme effect on early detection of BC. Performing breast self-examination (BSE) is debatable as the advantage in terms of fallen mortality has not been proven (Bartsch1995). Most clinicians advise women to perform monthly BSE to become aware of their standard anatomy and empower them with respect to their own healthcare (Goode et al.2002).

2013 NCCN guidelines suggest yearly Clinical Breast Examination CBE for females with average risk lesser than 40 years of age side by side NCCN also focused to develop awareness programme for Self breast examination and health awareness in females (Gallucci 1985). Enhancements in conventional mammography such as the low radiation dosage, enhanced image quality, development of statistical methods with computer assisted analysis of images long distance electronic image transmission technologies for clinical sessions, and
better-quality image-guided methods to assist with breast biopsies continue to lower the indisposition and death from BC. The Use of screening facilities such as magnetic resonance imaging MRI ultrasound and breast specific positron emission tomography PET may play a significant role in further perfections of BC early detection. The earlier BC is identified, better persistence rate in maximum cases.

2.5.2. Mammography

At present Mammography is the best available technique to diagnose BC in its initial stage. The most treatable stage which is an average of 1.7 years before of woman starting feeling of the lump in the breast (Kardinal and Yarbo 1979). One of the most important achievements in the treatment of BC is early detection of non-palpable tissue masses. However, there is still controversy regarding mortality from BC in the subset of women aged 40-49 years (Rubenstein et al. 2007, Minami et al. 1999, Rautalathi et al. 1983). Current randomized control trials have confirmed the profits from screening mammography in women aged 40 to 70 years (Claus et al. 2003, Collaborative Group 2001, Huard Huard and Pozzy 1961). A Cochrane Review (2013) proposes that death is an outcome partial to screening, tedious mammography leads to excessive pressure and doubt in the face of false-positive results which increases the total numbers of lumpectomies and mastectomies but no decrease in mortality (Ford and Easton 1995). Disagreement for mammography is related to the inborn lead time and length time biases in screening for disease. Lead time bias is an overestimation of persistence among screen-detected cases related to clinically detected cases when true survival time actually remains unchanged. Length bias is an overestimation of survival time among screening-detected cases, which is caused by those slowly succeeding cases that may never be clinically relevant. The NCCN guidelines 2013 recommends annual screening mammography in women ≥ 40 years of average risk and annual mammography at
age 25 or individualized based on onset of cancer in pro-band in patients who are high risk by prediction models, known history or genetic predisposition syndrome as well as the advising and education of risks and benefits related to participating in cancer screening (Jass 1997).

2.5.3. Magnetic resonance imaging (MRI)

Mammography is the gold standard for BC imaging but MRI has become a significant modal in the finding, evaluation, staging, and managing of BC in particular patients. Screening with MRI is more sensitive but high risk women are less specific to cancer detection 0.33-0.39 MME sensitivity is 0.77-0.79 Compared to the MMI compared to 0.95 0.89-0.95 MMI and the combined sensitivity and 0.94 and 0.77, respectively. MRI is highly appreciated in patients of high risk patients enhancing the nature of effective screening methods in breast cancer patients or patients with similar imbalances on other imaging methods (Dite et al. 2003, Henderson et al. 2002)

2.5.4. Ultrasound

Studies in favor of adjunctive screening with ultrasound in high-risk patients with dense breast tissue, which conveys a large but putative number of false positive results (Hirose et al. 2001). Full breast ultrasound allows the doctor to screen BC by traditional conventions especially in thick breasts where the magnetic sensitivity is low (Brentstand et al. 1994). Single center study shows that BC growing identity by ultrasound only gives some extra benefits to women after screening with mammogram (Friedenreich et al. 2001).

2.5.5. Polymerase chain reaction (PCR)

Polymerase Chain Reaction PCR molecular Technique PCR detects circulation of tumor cells and small envasion at molecular level PCR based assays are used to detect tumor
cells in the lymph nodes, resection margins, bone marrow, and blood (Miller 1997). An ideal prognostic marker is one that undoubtedly defines a precise prognostic group, is 100% specific, extremely sensitive, low-cost and easy to conduct on a minor volume of fresh or fixed tissue. No such marker exists but a number of possible prognostic markers have been extensively examined. Multiple proteins have been seen to be precisely overexpressed in certain types of tumor (i.e. Her2neu, PSA, p53, pRB, melanoma antigens, etc) (Preston et al. 2002). 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 BC patients.

### 2.5.6. Microarrays

The recovery rates are increasing as an outcome of early identification better staging and better treatment. It is believed that the reappearance and death frequency was high only because of nonspecific early diagnostic techniques. According to the American Cancer Society, death rates of BC deteriorated thru past 10 years with great fall in younger women (Taneja et al. 2010). These slightly exciting trends are mainly linked with value-added screening methods and the increase in analysis at an early stage when most cancers are more effectively treated. Unfortunately, most current therapies have limited efficacy in curing late-stage disease. Therefore, there continues to be a need to develop new approaches to diagnose cancer early in its clinical course, more efficiently treat its advanced stages, predict tumor response to therapy, and finally stop cancer A better view of how certain genes, their expressed proteins take part in the beginning of disease and tumor development and how they respond to treatment in patients would be the only way to accomplish these goals. Our era of genetic, biological, and biochemical innovations gives prominent opportunities to address these questions uncovering the molecular basis of cancer. DNA Microarray become an
important tool in the biomedical area in this area and one of the most optimistic and powerful
techniques in reinforcing local biology Statistics of partial settings have been created at mass
scale for thousands of genes with the efforts of human genome project (Holbro et al. 2003,
Zhou and Hung 2003). The roles these genes play in various biological procedures are yet to
be clarified.

2.5.7. Tumor-based Markers

A tumor marker is a protein or material which is derived, secreted or overexpressed
on the tumor specifically, which may utilized as a differentiating parameter for distinguishing
parameter between neoplastic and benign nature of the tumor.

Tumor markers are proteins, receptors which are found in cells, tissues, and bodily
fluids such as cerebrospinal fluid, serum, plasma, sweat, saliva, and milk. A perfect marker
would be useful in analysis, staging, and prediction of cancer. It also provides knowledge of
disease burden, serves as a monitoring parameter for efficacy of treatment, detection of
recurrence, localization of tumors and screening of disease in general population (Dershaw et
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tumor markers are always elevated in specific cancers; maximum are less expectable. These
markers are not completely related to cancer, it may present in circulations of healthy
individuals in very low concentrations too. These may also associate with the non-neoplastic condition as well as Cancer.

The vast colonization of tumor marker includes enzymes, iso enzymes, hormones, specific cell membrane proteins, and cell-specific antigens, carbohydrate epitopes, oncogene products, genetic changes etc. (Waterfield et al., 1983).

2.5.7.1. AFP and CEA

These two proteins oncofetal antigens, alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) are especially expressed during embryonic development and decrease soon after birth. Oncofetal antigens are very generic and produced by approximately in a wide variety of cancer. These are used for monitoring the efficacy of therapy during the course of treatment. CEA is a cell surface glycoprotein and it is a marker for colorectal, gastrointestinal, lung, and breast carcinomas. High levels of CEA in BC have no relation with tumor burden but are very beneficial for monitoring treatment and detecting relapse (Monila et al. 2005).

2.5.7.2. Cancer antigen

Cancer antigens are the proteins which are found to be produced by cancerous cells in the high level during the disease prognosis. The circulatory levels of these proteins determine the efficacy of therapy. (CA) 27.29 is elevated in breast carcinoma, ovarian and lung cancer, 1st trimester of normal pregnancy, benign breast disease, cirrhosis and hepatitis (Harris et al. 2007). For recurrent breast carcinoma, CA 27.29 has a sensitivity of ~57% and a specificity of ~87% (Shin et al. 1991). It lacks the obligatory sensitivity and specificity for a monotonous finding of BC and does not distinguish patients with early carcinoma with those
having benign breast disease. CA 27.29 is associated with the early recognition of recurrent BC.

2.5.7.3. HER-2/neu

It is an oncogene-encoded growth factor receptor, also known as c-erbB-2 overexpression of HER-2/neu has been observed during BC. It is estimated from tissues for biopsy either by PCR or by immunological methods. Its presence confirms aggressive tumor growth and poor recovery during BC and ovarian cancer (Keshgegian et al. 1995). The presence of this receptor also determines the choices for treatment and forecast's a greater persistence advantage from the Her 2-targeted therapy. It may also forecast for resistance against some conventional therapies.

2.5.7.4. Estrogen receptor (ER) and progesterone receptor (PR)

The presence of ER and PR receptors on tumor cells can predict the efficacy of hormonal therapy for treatment of BC in both pre and post-menopausal women. In both pre and postmenopausal women, levels of steroid receptors ER and PR can predict which women are likely to benefit from hormone treatment (Su et al. 2000).

The presence of ER and PR are suggested to use in the diagnosis, prognosis, and treatment planning for women with BC. ER gives an indication of responsiveness to therapy. Tissue from a biopsy is used to measure the estrogen receptor.

Most BCs in post-menopausal women are ER-positive, meaning that they require estrogen to grow (Palmieri et al. 2002). These ER-positive BCs are less aggressive than ER-negative BCs, which are found generally in premenopausal women.
2.5.7.5. P53 tumor suppressor gene polymorphism

It is a tumor suppressor gene which is mutated or transformed in more than 50 percent of the tumor. Analysis of p53 as a tumor marker supports researchers to know how tumor form, but detecting p53 mutations in cancer patients has not been shown to predict changes in persistence or quality of life. P53 was indicated as responsible for tamoxifen tolerance in BC signifies that it can affect in action response of drug (Humphreys et al. 1997, Muller, P.A. and Vousden, K.H., 2013).

2.5.7.6. Cathepsin-D

These are the lysosomal enzymes high levels of this enzyme may indicate BC. There is not enough info to acclaim using cathepsin-D levels to make treatment choices for patients with primary or metastatic BC and especially to analyze the disease but studies have revealed its link with decreased disease-free and overall survival of BC patients (Taneja et al. 2010). Researchers continue working on specific molecular pathways involved in oncogenesis, tumor response, tumor development, etc. to discover new molecular markers that can have a possible to be regularly used in medical practices of BC.

Laboratory techniques for the study of potential prognostic markers are rapidly developing at both the gene and protein level. Most methods now permit the examination of fresh or archival tissue. Some of the newly revealed markers are markers involved in cell cycle regulation (cyclin D1, p16INK4a, p14ARF) (Donegan 1997), tumor invasiveness (VEGF, factor VIII related antigen, Cox-2) (Donegan 1997), stromal-breast epithelium interactions (uPA and related proteins, E-cadherin, b1 integrin) (Holmes et al. 1990).
CA 15-3 belongs to a large family of glycoproteins encoded by the MUC-1 gene that are heterogeneously expressed (Singh et al., 2018). The expressed proteins are found on the apical surface of normal epithelial cell types, including breast (Brayman et al., 2004). It has been identified on the apical side of alveoli, ducts of mammary glands and as a circulating antigen. It is also known as polymorphic epithelial mucin (PEM) (Gendler et al., 1990), sialomucines, episialin (Wesseling et al., 1996). The precise function of CA15-3 is still unclear, although in general, the physiological function of mucins is in lubrication and hydration of cell surfaces, protection of proteins and cells from proteolysis and in the protection of tissues from microbial attack (Duffy 1999). However, other evidence suggests CA15-3 appears to play a role in cell-adhesion, where it modulates cell-cell and cell-extracellular matrix (ECM) interactions (Wesseling et al., 1996). In malignant conditions, PEM is expressed on the entire cell surface and shred down into the circulation. PEM may also interfere with cellular adhesion of immune cells to aberrant cell and thus it may play a role in the escape from immune cells and metastatic process (Wesseling et al., 1996). CA15-3 is an excellent indicator of bone metastases in particular (Brein et al., 1992). Numerous studies have indicated that CA15-3 is a reliable marker in breast carcinoma and correlates well with the disease stage (Kerin et al., 1989). CA15-3 level has been related with tumor size, primary untreated tumor and biological character of tumor such as estrogen or progesterone receptor status (Viale et al., 2009). The level of CA15-3 is observed much higher in medullary carcinoma than in ductal carcinoma (Gion, et al., 1991). CA15-3 marker has been also used to screen preliminary on patient who needs radiological investigation (Nicolini et al., 2006). In reference of MUC series, the immuno dominant epitope of MUC1 is an extracellular portion and membrane bounded and corresponds to tumor marker antigen CA15-3 (Klee et al., 2004). Due to shredding in serum it increases in circulation of BC.
patients. Which are estimated by immunological assays during testing BC. The levels of CA15-3 that have measured value greater than 35 U/mL is an indication of the progression and recurrence of BC (Uehara et al., 2008).

CA15-3 is a valuable tool for monitoring the course of disease in BC patients. Assays of CA15-3 are based on the use of two monoclonal antibodies MAbs to polymorphic epithelial mucin (PEM) (Price et al.,1990). The human MUC1 gene codes for a mucin glycoprotein that is expressed on the ductal cell surface of most glandular epithelia. Various MAbs have been raised against epitopes present on this heavily glycosylated protein. These MAbs form the basis for the development of serum assays valuable in the management of BC patients. A single-determinant assay is based on MAb B27.29, which recognizes the 8-amino-acid sequence (SAPDTRPA) within the 20-amino-acid tandem repetitive sequence of the mucin core (Ben et al., 1997). This epitope overlaps in part with that recognized by MAb DF-3 incorporated in the CA15-3 assays (Figure 5).

![Figure 5: Amino acid sequence in the epitope region to which MAb B27.29 and MAb DF3 are directed, present on the peptide tandem repeat as part of the MUC1 gene-derived mucin molecule.](image)

CA15-3 is a circulating human breast tumor associated antigen defined and assayed by two monoclonal antibodies DF3 & 115D8 with specificity about 95% and 65% respectively. The ASCO panel evaluated multiple serum markers for BC, including assays for MUC1 protein cancer antigen CA15-3 and CA 27.29 (Ben et al., 1997).

CA15-3 is elevated in a proportion of BC patients with distant metastases. Pre-operative levels of CA15-3 have a significant and independent relation to outcome in patients with early BC. Patients with high concentrations have a significantly worse prognosis than
those with low concentrations (Ebeling et al., 2002). It is biologically plausible, since in many adenocarcinomas mucins are aberrantly over expressed throughout the cytoplasm and on the cell surface in an under glycosylated form, and then shed into the circulation (Kufe 2002).

This high molecular weight (300-450 kD) Mucin-like glycoprotein have distinct epitopes. The structure CA15-3 consists of a cytoplasmic tail, which may have potential role in cell signal transduction and has been reported to contribute to metastases (Rachagani et al., 2009). The extracellular domain mainly consists of a region of nearly identical repeats encoding 20 amino acids. The number of repeats is highly variable in the human population, leading to substantial differences in molecular weights of the episialin molecules from different individuals. The repeats together with adjacent degenerated repeats contain many serine and threonines which are potential attachment sites for O-linked glycans and constitute the mucin-like domain which comprises more than half of the polypeptide backbone, even in the smallest allele detected. The number of tandem repeat sequences in each allele can vary from approximately 30 to 90 (Engelmann et al., 2001).

The mucin domain of episialin contains many pralines and other helix-breaking amino acids, resulting in a molecule with an extended structure and many β-turns. The extended structure is very rigid as a result of the numerous O-linked glycans attached to the molecule (Ligtenberg et al., 1990). Consequently, the mucin domain of episialin reaches an extreme length. An extensively O-linked glycosylated polypeptide of 20 amino acids is approximately 5 nm long. This means that the mucin-like domain of episialin extends 200 to 500 nm above the cell membrane (Hilkens et al., 1992). The structure of the extracellular subunit includes a region of nearly identical tandem repeats of 20 amino acids, with variable number of 20 – 125 repeats (Singh, and Bandyopadhyay 2007)
2.5.8. Cell line derived from BC patients

As discussed in previous section CA15-3 is a product of gene MUC-1. Human MUC 1–4 is a large, type I transmembrane protein normally expressed on the apical surface of ductal epithelia. MUC1 is synthesized as a single polypeptide chain but exists on the cell surface as a heterodimer. Proteolytic cleavage of the full-length protein results in 2 associated fragments: a large extracellular polypeptide containing the tandem repeat domain that can be released from the cell surface and a polypeptide consisting of the short extracellular domain, the transmembrane domain and the cytoplasmic tail that exists as an integral membrane protein. The tandem repetition domain of MUC1 is rich in serine and threonine and is highly glycosylated with the oligosaccharide O-linked complex. The form of MUC1 which is entirely different when produced by different epithelial for example the pancreas mammary gland kidneys and lungs is partially different due to variations in glycosyl binding. The level of expression and the type of subsequent modification after translation of MUC1 often varies in different lineage of cells. Table-1 gives information of BC derived cell lines.

The appearance of novel oligosaccharide structures on MUC1 expressed by tumor (compared with normal epithelia) or differential glycosylation at different positions on the MUC1 core protein may confer new properties of adhesion that contribute to the ability of tumor cells to metastasize. For example, certain glycoform of MUC1 have been shown to associate with intracellular adhesion molecule 1 (ICAM-1), and it is predicted that certain oligosaccharide structures present on MUC1, such as sialyl Lewis A (sLea) or sialyl Lewis X (sLex) interact with different lectin-like molecules that influence general properties of cell adhesion.
Table 1: Showing the cell-line derived from different breast cancer.

<table>
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<th>Sr. No</th>
<th>Designation</th>
<th>Histologic Type</th>
<th>Sr. No</th>
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In conclusion, mucins are large (well over 106 Daltons) glycoproteins composed of ~75% carbohydrate and 25% amino acids linked via O-glycosidic bonds between N-acetylgalactosamine and serine/threonine/proline (Ser-Thr-Pro) residues. The hallmark of the mucin family is the large and polymorphic central domain, which is composed of a variable number of tandem repeats (VNTR) rich in Ser-Thr-Pro residues (Table 1) that can be modified with a large number of O-linked oligosaccharides and a few N-glycan chains. In this review, we discuss the current status of mucins derived CA15-3 for cancer diagnosis and therapy.
### Table 2: Enlisted Ca15-3 commercial immunoassay kit.

<table>
<thead>
<tr>
<th>Sr. No</th>
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<th>Make</th>
</tr>
</thead>
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<tr>
<td>1</td>
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<td>CELL BIOLABS, INC.</td>
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<td>2</td>
<td>HUMAN CA 15-3 ELISA KIT (SANDWICH ELISA)</td>
<td>LIFESPAN BIOSCIENCES, Inc.</td>
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<td>CA 15-3 ELISA KIT</td>
<td>AVIVA SYSTEMS BIOLOGY</td>
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<td>RAYBIOTECH. INC.</td>
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<td>CALBIOTECH INC.</td>
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<td>HUMAN MUCINI (MUC1) ELISA KIT</td>
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<td>MY BIOSOURCE.COM</td>
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</table>

#### 2.6. Commercially available Immunoassays for CA15-3

The below Table-2 enlists shows commercially available immunoassay for CA15-3 antigen. These kits are available commercially which are needed to get imported leading to bearing of high cost for the end user.