IDENTIFICATION OF POTENTIAL BIOMARKERS FOR BREAST CANCER

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DEPARTMENT OF BIOTECHNOLOGY

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CERTIFICATE

This is to certify that the work titled "**IDENTIFICATION OF POTENTIAL BIOMARKERS FOR BREAST CANCER**" submitted by "**ZOYA NADEEM FARUQUI**" in partial fulfillment for the award of degree of **Bachelor of Technology** of Jaypee University of Information Technology, Waknaghat has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

SIGNATURE OF SUPERVISOR.Ajain.....

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Date- 26.05.2013

SUMMARY

The promise of biomarkers as indicators to advance and revolutionize many aspects of medicine has become a reality for researchers in all sectors of biomedical research. While targeted therapies provide molecular biomarkers of efficacy for the mechanisms being targeted. Effective biomarkers may replace or complement traditional clinical and histopathological markers in assessing tumour behaviour and risk.

Keeping in mind the above lines and an increasing demand for potential biomarkers for early detection, treatment, prognosis and reduction in the different types of breast cancer cases, this project is a part of a bigger project that aims to discover and validate effective biomarkers for contributions towards the advances in this field leading to a better and healthier future for cancer patients and individuals at a risk of this disease.

After standardization and optimization of all the protocols, proteins have been carefully extracted and profiled using 1-D of SDS-PAGE from different sources, specifically, breast cancer tissue samples, a breast cancer cell line-MCF-7 and normal human blood. The concentration of proteins was estimated for each specimen source.

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LIST OF FIGURES

LIST OF SYMBOLS AND ACRONYMS

APS- Ammonium Per-Sulphate

BSA- Bovine Serum Albumin

ICMR- Indian Council of Medical Research

PEDF-Pigment-Epithelium Derived Factor

TEMED- Tetramethylethylenediamine

Chapter 1

INTRODUCTION

1.1 What is Breast Cancer?

The female breast is made up mainly of lobules (milk-producing glands), ducts (tiny tubes that carry the milk from the lobules to the nipple), and stroma (fatty tissue and connective tissue surrounding the ducts and lobules, blood vessels, and lymphatic vessels).

Most breast cancers begin in the cells that line the ducts (ductal carcinomas). Some begin in the cells that line the lobules (lobular carcinomas), while a small number start in other tissues.

Breast cancer occurs in humans and other mammals. While the overwhelming majority of human cases occur in women, male breast cancer can also occur.

The primary risk factors that have been identified with breast cancer are gender, heredity, age, nulliparity, hormones, a high-fat diet, alcohol intake, obesity, and environmental factors such as radiations.

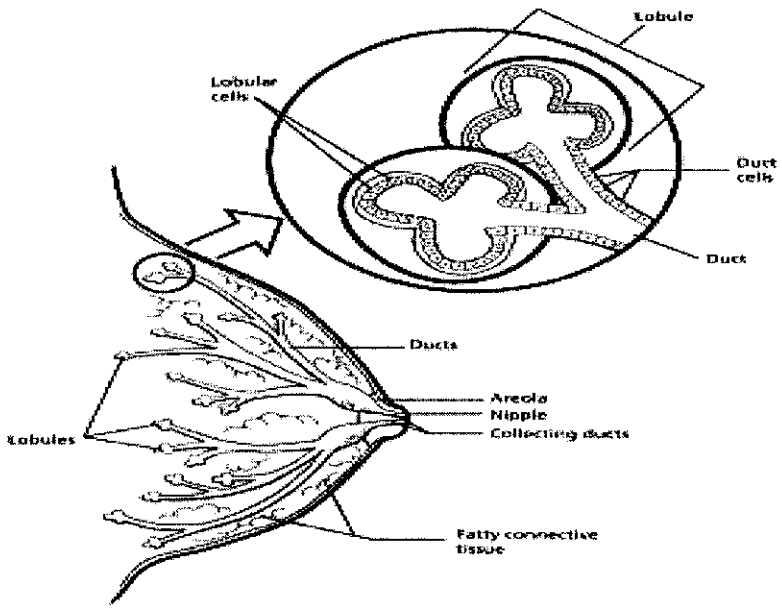
Findings in animal models and supported by epidemiological studies, have shown that prolonged exposure of undifferentiated (immature) breast cells to estrogen or estrogen-mimetic compounds during early development increases breast cancer risk in adult life. This phenomenon is called **estrogen imprinting**. These studies can explain why, in addition to genetic factors, the risk of breast cancer is affected by pregnancy, lifestyle in terms of intake of food and drink, and environment.

Breast cancer may be invasive or noninvasive. Invasive means it has spread from the milk duct or lobule to other tissues in the breast. Noninvasive means it has not yet invaded other breast tissue. Noninvasive breast cancer is called "in situ."

Indications of breast cancer other than a lump may include thickening different from the other breast tissue, one breast becoming larger or lower, a nipple changing position or shape or becoming inverted, skin puckering or dimpling, a rash on or around a nipple, discharge from nipple/s, constant pain in part of the breast or armpit, and swelling beneath the armpit or around the collarbone. Pain ("mastodynia") is an unreliable tool in determining the presence or absence of breast cancer, but may be indicative of other breast health issues.

The size, stage, rate of growth, and other characteristics of a breast cancer determine the kinds of treatment. Treatment may include surgery, drugs (hormonal therapy and chemotherapy), radiation and /or immunotherapy.

Fig 1.1.1 Various parts of the normal human female breast



Prognosis and survival rates for breast cancer vary greatly depending on the cancer type, stage, treatment, and geographical location of the patient. Survival rates in the Western world are high; for example, more than 8 out of 10 women (84%) in England diagnosed with breast cancer survive for at least 5 years. In developing countries, however, survival rates are much poorer.

Another important issue is whether epigenetic regulation plays a role in the development of drug resistance in breast cancer. Fan et al. has performed a genome-wide analysis of gene expression and DNA methylation profiles in breast cancer cells after adapting the cells to the anti-estrogens, tamoxifen and fulvestrant. Their results demonstrated that the development of anti estrogen resistance was associated with the changes in methylation profiles of multiple genes. Their findings imply that aberrantly epigenetic regulation is involved in adaptation of breast cancer cells to anti-estrogens. This proposed concept is further supported by the recent finding that epigenetic silencing of cyclin-dependent kinase 10 (CDK10) is implicated in the development of tamoxifen resistance. These several lines of evidence, taken together, point out the significance of epigenetic regulation in initiating breast carcinogenesis, promoting tumorigenic phenotypes, and assisting in the development of drug resistance.

1.2 Global Scenario of Breast Cancer

Breast cancer is the first reported cancer as in Papyrus writing in Egypt during 1600 BC. It is also the most invasive cancer in women. Breast cancer is the most common of all cancers and is the second leading cause of cancer deaths in women worldwide, accounting for 23% (1.38 million) of the total new cancer cases and 14% (458,400) of the total cancer deaths and case fatality rates are highest in low-resource countries (Dubey,2012). Despite the high incidence rates, in Western countries, 89% of women diagnosed with breast cancer are still alive 5 years after their diagnosis, which is due to

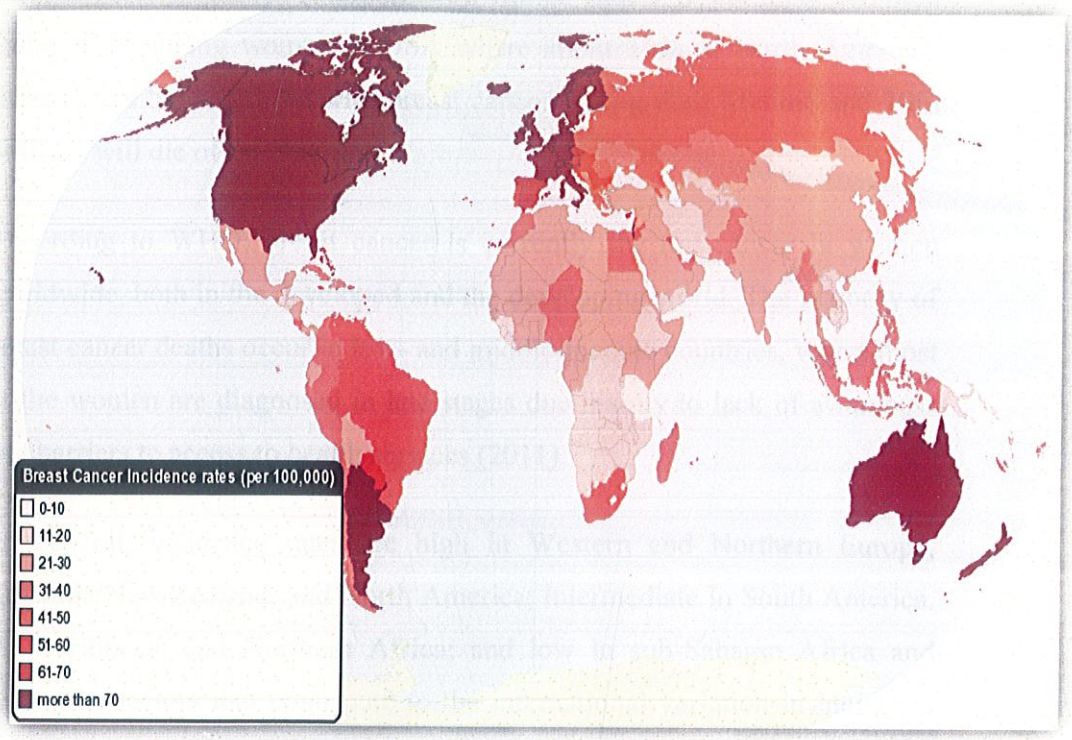


Fig 1.2.1 Breast Cancer Incidence Rates Globally

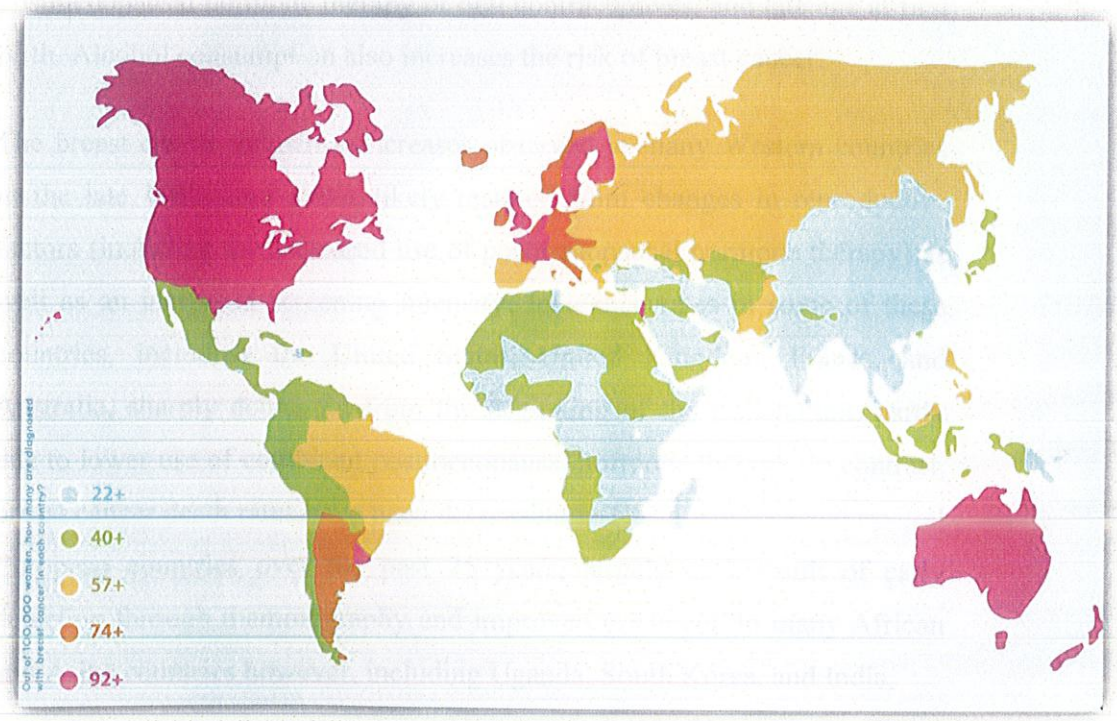


Fig 1.2.2 Breast Cancer Detection Rates Globally

detection and treatment (Parkin,2008). Breast cancer is the most common malignancy among women in USA where about 10% of North American women will be diagnosed with breast cancer during their lifetime and 20% of those will die of the disease.

According to WHO, breast cancer is currently the top cancer in women worldwide, both in the developed and the developing world. The majority of breast cancer deaths occur in low- and middle-income countries, where most of the women are diagnosed in late stages due mainly to lack of awareness and barriers to access to health services (2011).

In general, incidence rates are high in Western and Northern Europe, Australia/New Zealand, and North America; intermediate in South America, the Caribbean, and Northern Africa; and low in sub-Saharan Africa and Asia. The factors that contribute to the international variation in incidence rates largely stem from differences in reproductive and hormonal factors and the availability of early detection services. Reproductive factors that increase risk include a long menstrual history, nulliparity, recent use of postmenopausal hormone therapy or oral contraceptives, and late age at first birth. Alcohol consumption also increases the risk of breast cancer.

The breast cancer incidence increases observed in many Western countries in the late 1980s and 1990s likely resulted from changes in reproductive factors (including the increased use of postmenopausal hormone therapy) as well as an increased screening intensity. Incidence rates in some of these countries, including the United States, United Kingdom, France, and Australia, sharply decreased from the beginning of the millennium, partly due to lower use of combined postmenopausal hormone therapy. In contrast, breast cancer death rates have been decreasing in North America and several European countries over the past 25 years, largely as a result of early detection through mammography and improved treatment. In many African and Asian countries however, including Uganda, South Korea, and India,

incidence and mortality rates have been rising, with changes in reproductive patterns, physical inactivity, and obesity being the main contributory factors; increases in breast cancer awareness and screening activity may be partially responsible for the rising incidence in these populations.

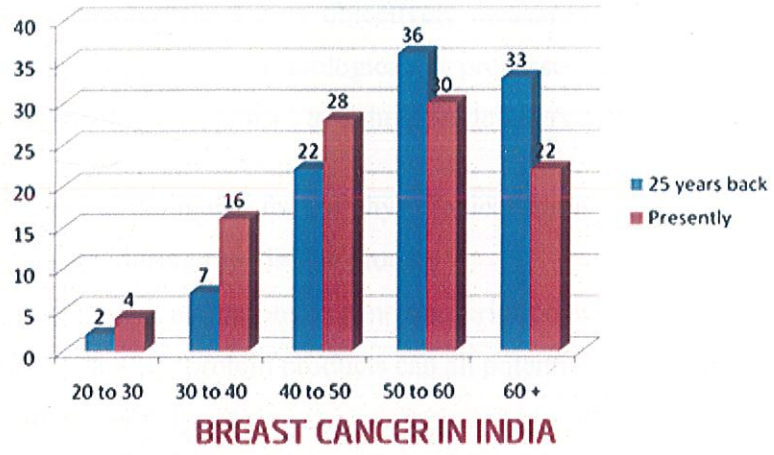
Maintaining a healthy body weight, increasing physical activity, and minimizing alcohol intake are the best available strategies to reduce the risk of developing breast cancer. Early detection through mammography has been shown to increase treatment options and save lives, although this approach is cost prohibitive and not feasible in most economically developing countries. Recommended early detection strategies in these countries include the promotion of awareness of early signs and symptoms and screening by clinical breast examination. However, while mammography is highly sensitive, its lack of specificity has created the need for other highly sensitive diagnostic methods for early detection of breast cancer.

1.3 Breast Cancer Incidence in India

Breast cancer is now the most common cancer in most cities in India, and 2nd most common in the rural areas. A recent study of breast cancer risk in India revealed that 1 in 28 women develop breast cancer during her lifetime. This is higher in urban areas being 1 in 22 in a lifetime compared to rural areas where this risk is relatively much lower being 1 in 60 women developing breast cancer in their lifetime. In India the average age of the high risk group in India is 43-46 years unlike in the west where women aged 53-57 years are more prone to breast cancer. Breast cancer accounts for about 25% to 33% of all cancers in Indian women. If these percentages are converted into actual numbers, the numbers are very high!

Based on National Cancer Registry Programme (ICMR), report of (2001-03), about 25% of the total cancer cases among Indian women constitutes of Breast cancer. The crude incidence rate of Breast cancer at India level is about 85 per 100,000 women per year. It is estimated that about 85000 new cases of Breast cancer are occurring in India, every year. Over the years, the incidences of breast cancer in India have steadily increased and as many as 100,000 new patients are being detected every year (Yip et al, 2006; Michael et al, 2003). A 12% increase has been registered by cancer registries from 1985 to 2001, which represented 57% rise of cancer burden in India (Yip et al, 2006; Hadjiiski et al, 2006).

Fig 1.3.1. Breast Cancer Incidence in India



The horizontal line lower down represents the age groups: 20 to 30 years, 30 to 40 yrs and so on. And the vertical line represents the percentage of cases. The blue colour represents the incidence 25 years back, and maroon colour represents the situation today. An increasing numbers of patients are in the 25 to 40 years of age, and this definitely is a very disturbing trend.

The only credible data on a large proportion of population is available from the population-based cancer registries – both urban and rural – and the various hospital-based cancer registries which work under the national cancer registries program of the Indian Council of Medical Research (ICMR). There are numerous other non-ICMR cancer registries organized and run by hospitals and institutions. One major hindrance in collation of data from these diverse registries is the lack of uniform methods of data collection and storage. This makes any meaningful interpretation of nationwide data an arduous task, and any efforts at this are often viewed with suspicion.

1.4 What are Biomarkers?

A **biomarker**, or **biological marker**, is an indicator of a biological state. It is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.

- They serve as hallmarks for the physiological status of the cells at a given time and change during the disease process
- Gene mutations, alterations in gene transcription and translation, and alterations in their protein products can all potentially serve as specific biomarkers for a disease

Effective identification and deployment of biomarkers is essential to achieving a new era of predictive, preventive and personalized medicine. Biomarkers promise to accelerate basic and translational research, speed the development of safe and effective medicines and treatments for a wide range of diseases, and help guide clinical practice.

Indeed, in the last decade, microarray technology has arguably offered the most promising tool for discovery-driven, patient-based analyses and, consequently, for biomarker discovery.

1.4 Biomarkers in Cancer

Biomarkers are the foundation of cancer detection and monitoring. Cancer biomarkers are present in tumor tissues, blood or serum and encompass a wide variety of molecules, including DNA, mRNA, transcription factors, cell surface receptors, and secreted proteins. Effective biomarker identification depends on multiple levels of study design, each of which must be optimized to ensure the utility of a biomarker or panel of biomarkers for this specific clinical application, early detection. The success of biomarkers for the early detection of cancer is measured by the fact they should not only detect disease early but also reduce the mortality from that cancer. Cancer biomarkers can be used for prognosis: to predict the natural course of a tumor, indicating whether the outcome for the patient is likely to be good or poor (prognosis). They can also help doctors to decide which patients are likely to respond to a given drug (prediction) and at what dose it might be most effective (pharmacodynamics). In the definition of cancer indicators, the proteomic technologies are producing very valuable data, differentiating and defining functional and regulatory pathways, determining the structure of disease causing molecules in tissue and biologic fluids, and manifest the disease stages or differences specific to the disease or to the individual.

Referring to work from Pepe et al, five phases of biomarker development have been described:

1) pre-clinical exploratory phase that identifies promising directions;
2) clinical validation in which an assay can detect and characterize a disease;

3) retrospective longitudinal validation (i.e. a biomarker can detect disease at an early stage before it becomes clinically detectable or has other predictive value);

4) prospective validation of the biomarker accuracy and

5) testing its usefulness in clinical applications to predict clinically relevant parameters.

An emerging theme in biomarker research is the expectation that panels of biomarker analytes rather than single markers will be needed to have sufficient sensitivity and specificity for the presymptomatic detection of cancer. Biomarkers may provide prognostic information of disease enabling interventions using targeted therapeutic agents as well as course-corrections in cancer treatment. Novel genomic, proteomic and metabolomic technologies are being used to discover and validate tumor biomarkers individually and in panels.

1.5 Importance of Protein Biomarkers in Breast Cancer

Breast cancer is a heterogeneous disease and biomarkers able to correctly classify patients into prognostic groups are needed to better tailor treatment options and improve outcomes. One powerful method used for biomarker discovery is sample screening with mass spectrometry, as it allows direct comparison of protein expression between normal and pathological states.

Differential proteomics can be used to differentiate between two physiologic states using tissue samples that represent underlying biology and pathology. Isotopic labelling and label-free mass spectrometry proteomics enable the quantification of proteins and thus allow direct comparison of protein expression between two sample sets.

The protein composition may be associated with disease processes in the organism and thus have potential utility as diagnostic markers since-

- Proteins are closer to the actual disease process, in most cases, than parent genes
- Proteins are ultimate regulators of cellular function
- Most cancer markers are proteins
- The vast majority of drug targets are proteins

Palacios et al. have confirmed this molecular classification with a study (CCND1, hormone receptors, p53, ERBB2, cell cycle regulators, apoptosis and basal cell indicator proteins) at the protein level covering 37 proteins. **BRCA2 cancers** have been found to be related to cycle regulators, D type cyclines (D1, D3), and CDK4. However, it has also been reported that in BRCA1 cancers, the ER/ERBB2 negativity, rapid proliferation, and basal phenotype is widespread. In the recognition of the molecules of lobular **and ductal cancers**, the use of the proteins **EMP1, DVL1, DDR1** and **PRKC1** is recommended together with **E-Cadherin**.

Medullar breast carcinoma is a rare cancer but is known to have a poor prognosis. The persistence of the difficulties with morphological diagnosis has required the molecular definition. A definition using a series containing 18 proteins has been confirmed by Bertucci et al. with their study using a DNA microarray. At the protein level, an increase in p-cadherin, M1B1/Ki67, negative ERBB2, and positive p53 have been linked with medullar breast carcinoma.

In the studies which bring clarity to advances in breast oncogenesis, it has been observed that the role of the protein 14-3-3σ, described as a tumour suppressor in previous publications, is less than had been supposed, and the lymph node metastasis has been linked to ERBB2 status. Another cancer, whose diagnosis involves difficulty, is the **inflammatory breast cancer**.

Very few things are known concerning the molecular structure of this cancer which is rare but can frequently be fatal. In proteomic studies, with a protein signature defined by an increase in E-cadherin, ER(-), MIB1(+), MUC1(cytoplasmic staining) and ERBB2(+), 91% of the cancer can been defined.

An example of exploratory studies is the identification of a distinct phenotype of functional T cell responses and cytokine profiles that distinguish immune responses to tumor antigens in breast cancer patients. Tumor antigen-specific immune responses in cancer patients were observed to differ from responses to common viruses. In particular, a reduced frequency of **IFN-γ-producing CD4 T cells** was observed.

Following the steps of validation, a retrospective analysis has suggested that survival is associated with development of memory immune responses or changes in serum transforming growth factor (TGF)-β values are prognostic in breast cancer.

Together with the pathologic characteristics of breast cancer, in studies which are conducted on large sample series defining the molecular sub-types, as many as 97 proteins have been determined, including **ER, PR, ERBB2, p53, CK5/6, CK8/18, cyclin E, Ki67, BCL2, cyclin D1, and E-Cadherin.**

Tyrosine kinases are among the most important oncogenes yet known. Receptor tyrosine kinases are found on the plasmalemma and bind to peptides, such as epidermal growth factor, platelet-derived growth factor, fibroblast growth factor, and insulin and insulin-like growth factor. Various new breast cancer drugs and drug candidates are aimed at protein kinase targets, such as epidermal growth factor receptor (EGFR)/HER-1 and HER-2.

To establish and define protein patterns in the clinical meaning, elegant studies have been conducted, mostly with the MALDI/SELDI methods in numerous biological sources such as serum, plasma, tissue interstitial fluid, thin needle aspiration fluid, ductal lavage fluid, and saliva.

Despite being a less commonly chosen source in clinical studies due to the difficulty in tissue sampling, because of the diagnostic biomarkers peculiar to cancer, tumour tissue is a very good source. Umar et al. have reported 9 tryptic peptides, which was determined as specific to breast cancer but was not defined structurally, as a diagnostic biomarker. Sanders et al. defined S100-A6 protein, as a growth factor in breast tumour, for being an increasing marker, and the proteins S100-A8 and ubiquitin as a reducing marker.

A matrix has been constructed for breast cancer biomarkers in saliva secretions. It was found that soluble c-erbB-2 and CA 15.3, and 5 proteins of high molecular weight (18, 113, 170, 228, and 287 km/z), as yet without a structural definition, increased in cancer cases.

1.7 CURRENTLY AVAILABLE MARKERS FOR BREAST CANCER

Extracts secreted by or extracted from breast cancer cell lines, proximal fluid, and breast tumor biopsies have highly complex proteomes that must be extensively and reproducibly fractionated in order to identify candidate cancer biomarkers with clinical relevance. At present several tumor markers of breast cancer are being used for diagnosis such as-

- **CA-15.3** - Cancer Antigen 15-3 is a tumor marker used to monitor certain cancers, especially breast cancer. It is found on the surface of many types of cancer cells and shed into the blood stream. It is used to monitor advanced or metastatic cancer and the progress of treatment.

Elevated CA15-3, in conjunction with alkaline phosphatase (ALP), was found to be associated with an increased chance of early recurrence in breast cancer. CA15-3 and associated CA27-29 are different epitopes on the same protein antigen product of the breast cancer-associated *MUC1* gene.

➢ **BRCA-1** – *BRCA-1* or breast cancer 1, early onset, is a human gene that belongs to a class of genes known as tumor suppressor genes. Its cytogenetic location is 17q21. It inhibits the growth of cells that line the milk ducts in the breast. The protein made by the *BRCA1* gene is directly involved in the repair of damaged DNA. Researchers have identified more than 1,000 mutations in the *BRCA1* gene, many of which are associated with an increased risk of cancer (particularly breast cancer in women). Most of these mutations lead to the production of an abnormally short version of the BRCA1 protein, or prevent any protein from being made from one copy of the gene. Other *BRCA1* mutations change single protein building blocks (amino acids) in the protein or delete large segments of DNA from the*BRCA1* gene. Researchers believe that a defective or missing BRCA1 protein is unable to help repair damaged DNA or fix mutations that occur in other genes. As these defects accumulate, they can allow cells to grow and divide uncontrollably and form a tumor.

➢ **BRCA-2** - *BRCA2* is a human gene that is involved in the repair of chromosomal damage and belongs to a class of genes known as tumor suppressor genes. Its cytogentic location is 13q12.3. Although the structures of the *BRCA1* and *BRCA2* genes are very different, their functions appear to be similar. The BRCA2 protein binds to and regulates the protein produced by the RAD51 gene to fix breaks in DNA. Researchers have identified more than 800 mutations in the *BRCA2* gene, many of which are associated with an increased risk of breast cancer. Many *BRCA2* mutations insert or delete a small number of DNA building blocks (nucleotides) in the gene. Most of these genetic changes disrupt protein production from one copy of the gene

in each cell, resulting in an abnormally small, nonfunctional version of the BRCA2 protein. Researchers believe that the defective BRCA2 protein is unable to help repair damaged DNA or fix mutations that occur in other genes. As these defects accumulate, they can allow cells to grow and divide uncontrollably and form a tumor.

- **Human Epidermal Growth Factor Receptor-2 (HER-2)** – It is a proto-oncogene of the EGF-2 receptor family of receptor tyrosine kinases. Several large clinical studies have shown a relationship between the frequency of HER-2 over expression/amplification and breast cancer. HER2 is over expressed in 25–30% of breast cancers and its over expression is associated with a high risk of relapse and death. In about 1 of every 5 breast cancers, the cancer cells make an excess of HER2 due to a gene mutation. HER2-positive breast cancers tend to be more aggressive than other types of breast cancer. They are also less responsive to hormone treatment. However, treatments that specifically target HER2 are very effective. For example, Trastuzumab (Herceptin) and Lapitinib (Tykerb).

- **Estrogen Receptor (ER)** - A cancer is called estrogen-receptor-positive (or ER+) if it has receptors for estrogen. This suggests that the cancer cells, like normal breast cells, may receive signals from estrogen that could promote their proliferation. Breast cancer tumors that are ER/PR-positive are 60% likely to respond to endocrine therapy. There are two subtypes of estrogen receptor (ER)- ERalpha and ERbeta, that are normally present in the mammary gland. The role of ERalpha as a prognostic marker in breast cancer is well established due to the beneficial effect of providing tamoxifen as adjuvant therapy. The role of ERbeta, however, is less clear. ERbeta expression provides independent prognostic information for breast cancers with ERalpha/PR-positive status, a feature typical among screen-detected breast cancers.

➢ **Progesterone Receptor (PR)** - The cancer is progesterone-receptor-positive (PR+) if it has progesterone receptors. Again, this means that the cancer cells may receive signals from progesterone that could promote their growth. Hormone receptor-positive breast cancers tend to grow more slowly and may have a better outlook than cancers without these receptors. Cancers that have these receptors can be treated with hormone therapy such as tamoxifen or aromatase inhibitors. In a study, the presence of progesterone receptors has been found to be associated with a favorable prognosis in 98 patients with primary breast cancer. The occurrence of metastases was seen to be 3.6 times less probable in patients with progesterone receptor-positive tumors than in patients with progesterone receptor-negative tumors.

➢ **Ki-67** – It is a cell cycle regulation protein with molecular weight of 358,694 Da that may be a marker of proliferating cells, involved in chromatin compaction. It is a cancer antigen that is found in growing, dividing cells but is absent in the resting phase of cell growth. This characteristic makes Ki-67 a good tumor marker. High levels of Ki-67 indicate an aggressive tumor and predict a poor prognosis. A British study has found that breast cancers could vary in regards to hormone-sensitivity, lymph node status (positive or negative), but if the tumor tested positive with high levels of Ki-67, the risk of recurrence was higher than average.

➢ **Decorin (DCN)**- The *DCN* gene belongs to a family of genes called proteoglycans. It also belongs to a family of genes called small leucine-rich repeat family. Its cytogenetic location is 12q21.33. Its molecular weight is 90-140 kDa. Decorin is involved in the organization of proteins called collagens. This protein likely helps regulate cell growth and division, the attachment of cells to one another (cell adhesion), and the self-destruction of cells (apoptosis). Studies suggest that decorin plays a role in the formation of new blood vessels (angiogenesis), wound healing, bone development, inflammation, and preventing the growth of cancerous tumors.

Decorin also regulates the activity of several growth factors, including transforming growth factor-beta (TGFβ) and epidermal growth factor receptor (EGFR). These growth factors control a diverse range of processes important for cell growth. It is also capable of suppressing the growth of various tumor cell lines.

- **Endoplasmin (HSP90 B1)** – It is a 90 kDa protein belonging to the hsp90 family of heat shock proteins. It is also called 94kDa glucose-regulated protein or GRP94. It is a constituent of the endoplasmic reticulum. It is calcium (Ca2+) binding and functions as a molecular chaperone. High expression of HSP90B1 is associated with distant metastasis ($p<0.0001$) and decreased overall survival ($p<0.0001$) with patients also appearing to benefit significantly from hormonal treatment.

- **Carcinoembryonic Antigen (CEA)** - Carcinoembryonic antigen (CEA) is a glycoprotein involved in cell adhesion. It is normally produced during fetal development, but the production of CEA stops before birth. Therefore, it is not usually present in the blood of healthy adults, although levels are raised in heavy smokers. CEA measurement is mainly used as a tumor marker to identify recurrences after surgical resection, metastasis to breast and other organs or localize cancer spread through dosage of biological fluids. It is mainly used to test colorectal cancer.

Chapter 2

REVIEW OF LITERATURE

Breast cancer affects more than 1.6 million women worldwide and takes more than 400,000 lives every year. Mortality due to breast cancer is high and the diagnosis of this disease is being carried out later than 30 years (National Institute of Cancer, 2008). What this means in clinical terms is that under today's therapeutic conditions, the early diagnosed cases have approximately a nine times greater expectation of remaining healthy for 10 years compared to those of advanced periods.

In cancer researches, the protemoic technologies produce very valuable data, differentiating and describing functional and organisational medical pathways, determining the molecular structure which is the cause of the disease in tissue and biological liquids, the stages of the disease or the differences peculiar to the disease and the patient. In recent years, the proteome studies that are done in various biological samples have increased.

Patients with local disease have a significantly better 5 year overall survival (98%) than patients with lymph node metastasis (83.6%) or distant metastasis (23%). Lymph Node and distant metastases are therefore strong predictors of poor prognosis. The clinical challenge is to correctly identify those patients that have or will develop Lymph Node or distant metastasis and that therefore will behave poorly, and use this information to offer supplemental treatment after local therapy. A sensitive and specific biomarker able to accurately predict disease recurrence and LN or distant metastasis is lacking and markers of disease progression continue to be

needed to improve patient classification. Biomarkers such as CA 15-3 and CEA are employed to monitor the progress of the disease as an indirect measurement of tumor burden but with somewhat limited success and are not currently widely used in clinical practice (Thomas R. Cawthorn, 2012).

Numerous preliminary studies are being performed on tumour tissue and biological fluids (serum, needle aspiration fluid, ductal lavage fluid and tumour intercellular fluid) for the purpose of understanding the disease processes better in the breast cancer, too. However, the limiting aspect in all these array methods is that, the analyses are limited to those performed with known antibodies. Autoantibodies to MUC1 have been found in the sera of women with benign breast disease, as well as invasive breast cancer at early and advanced stage.

The diagnosis of cancer at an early phase will help to increase the living span of patients affected with breast cancer. A sensitive assay to identify biomarkers using non-invasively collected clinical specimens is ideal for breast cancer detection (Lei Zhang et al, 2010). At present several tumor markers of breast cancer such as CA-15.3, BRCA-1, BRCA-2, human epidermal growth factor receptor-2 (HER-2), estrogen receptor (ER) & progesterone receptor (PR) are being used for diagnosis (Robertson *et al.*, 1994). Although all genes that are associated with an increased risk of breast cancer have not yet been identified, intensive research into the genetic basis of familial breast cancer led in the 1990s to the identification of the 'high risk' breast cancer susceptibility genes BRCA-1 and BRCA-2 (Miki, 1994 & Wooster, 1995). Cancer antigen 15.3 (CA 15.3) is produced by cells in the breast; increased levels are associated with breast cancer (Hayes et al., 1986). Tumor marker is a substance released into the circulation by tumor tissue, whose detection in the serum / plasma / tissue indicates the presence of a specific type of tumor (Dubey et al, 2012). Pigment epithelium-derived factor (PEDF) expression was found to be dramatically decreased in breast cancer.

PEDF is one of the most potent inhibitors of angiogenesis and is a candidate tumor suppressor in a variety of cancers.

Explorative studies have evaluated the potential use of salivary proteins such as c-erbB-2, VEGF, EGF, and CEA in the initial detection and/or follow-up screening for the recurrence of breast cancer. However, these investigations were not based on biomarker discoveries from saliva specimens, rather they were testing blood biomarkers in saliva.

The American Society of Clinical Oncology (ASCO) has updated its recommendation for use of tumor markers in prevention, screening, treatment and surveillance of breast tumor markers are recently considered and 6 were new to the guidelines. The categories recommended for use in practice, based on evidence of clinical use, were CA15.3, CA27.29, carcinoembryonic antigen (CEA), estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER-2), urokinase plasminogen activator (uPA), plasminogen activator inhibitor 1 (PAI-1) and certain multi parameter assays for gene expression. However, not all applications for these markers were supported (Harris et al., 2007).

In breast cancer, ER/PgR, HER2, and Ki-67 are important biological markers for predicting prognosis and making effective treatment decisions (Reiki Nishimura et al, 2011).

Clinicians can use biomarkers to guide therapeutic decisions in estrogen receptor positive (ER+) breast cancer. One such biomarker is cellular proliferation as evaluated by Ki-67. This biomarker has been extensively studied and is easily assayed by histopathologists but it is not currently accepted as a standard (Elisabeth Luporsi et al, 2011). Previous studies have shown that expression of p16 in tissue biopsies of patients with ductal carcinoma in situ (DCIS) is associated with increased risk of breast cancer, particularly when considered in combination with other markers such as

Ki67 and COX2 (Derek Radisky, 2011).

Stressors that can alter protein structure include low glucose, hypoxia and acidic conditions, which are commonly seen in tumour microenvironments. One of many responses that take place when cells face stressful microenvironments is a rapid and transient increase in the expression of heat shock genes. Heat shock proteins, Heat shock protein 90 kDa beta (Grp94) member 1 (HSP90B1) being one of them, facilitate cell survival by stabilizing and refolding denatured proteins after stress. HSP90B1 also helps cells escape apoptosis and preserves the function of various proto-oncogenes important for breast cancer growth. Using quantitative proteomic profiling of primary breast cancers, two new promising prognostic and predictive markers were found to identify patients with worse survival. (Thomas R. Cawthorn, 2012).

For the determination of biomarkers which will provide prediction of therapeutic response and follow up of therapy, in the studies performed on drug sensitive and drug resistant (doxorubicin and paclitaxel) breast cancer cells with SELDI-TOF-MS, a large number of structurally undefined protein peaks have been proposed. Dowling *et al*, proposed the use of transferrin fragments, linked with poor clinical progress, for the prediction of paclitaxel resistance. In treatments stimulating apoptosis, a decrease in ubiquitin and S100-A6 and abnormal expression in breast cancer tissue was found.

Biomarker proteins, either overly abundant or variant proteins can be detectable in the circulation as the free, shed proteins or as novel autoantibodies to such proteins; the latter indicating that the host immune system can be exploited as biosensor of the disease. In addition, tumor-specific biochemical changes result in post-translational modification of proteins via glycosylation or phosphorylation providing a variety of biomarker molecules.

Cancer-related biochemical changes often affect measurable metabolic variations within a cell or organism which may be powerful biomarkers.

Cancer biomarkers are discovered and utilized with a specific purpose in mind such as the (a) early detection of cancer, (b) diagnosis, (c) prognosis, (d) response to anticancer therapies or (e) cancer recurrence. Cancer cells provide the biomarker material that can lead to their own detection, which then provides the opportunity for their non-invasive detection in body fluids and tissues so as to reveal the presence of tumors or the level of tumor burden.

Thus, it is proposed through this project to identify new biomarkers for breast cancer for the early detection and progress of the disease.

Chapter 3

METHODOLOGY AND THE WORK DONE

3.1 Sample Collection

The cancer samples were collected from the pathology laboratory of Dr Asha Agarwal at Kanpur. There were 10 different cancer tissue samples in totality.

3.2 Homogenization of cancer tissue sample

In cancer tissue lysates, an increase in ubiquitin and a decrease in ferritin light chain have been linked with good clinical progress, and have been confirmed in independent groups and cell series.

Protocol

1. 1ml whole cell lysis buffer (WCL)/ extraction buffer per 100mg of tissue was added.
2. Homogenization of tissue sample was done using blade and homogenizer.
3. The homogenized tissue was transferred to 1.2ml eppendorf tubes.
4. The sample was centrifuged at 13,000 rpm for 10 minutes at 4°C.
5. The supernatant was pipette out and saved in another tube.
6. The supernatant was centrifuged again due to requirement.
7. Protein concentration was estimated by Lowry's method.

Table-3.2.1 Constituents of Whole Cell/Tissue Lysis Buffer

Reagents	Stock	100ml	Final conc.	Vol. for 10 ml
Tris HCl	0.5 M	10 ml	50 mM	1 ml
EDTA	0.5 M	400 µl	2 mM	0.04 ml
EGTA	0.5 M	200 µl	1 mM	0.02 ml
Glycerol	-	10 ml	10%	1 ml
Tween 80	-	100 µl	0.1%	0.01 ml
DTT	0.5 M	200 µl	1 mM	0.02 ml
PMSF	0.1 M	1 ml	1 mM	0.1 ml
PIC	-	200 µl	-	20 µl
NaCl	-	3.522 g	500 mM	0.2922 g

3.3 Lowry's Method

Protein biomarkers are often identified in basic science studies of cancer cells as overexpressed proteins. Cancer-specific alterations in proteins may occur at the level of protein abundance or post-translational protein modification such as glycosylation or phosphorylation.

Reagents used in Lowry's Method-

A- 2% Sodium Carbonate in 0.1N NaOH

B- 1% Sodium-Potassium Tartrate in distilled water

C- 0.5% Copper(II) Sulphate in distilled water

D- 48ml of A, 1ml of B, 1ml of C

E- Phenol reagent: 1 part of Foline-Ciocalteau's (2N) : 1 part of distilled water

F- Bovine Serum Albumin (BSA) standard- 1mg/ml

Procedure

1. Different dilutions of BSA solutions were prepared by mixing stock BSA solution (1 mg/ ml) and water in the test tube as given. The final volume in each of the test tubes was 5 ml. The BSA range was 0.05 to 1 mg/ ml.
2. From these different dilutions, 0.2 ml protein solution was pipetted out to different test tubes and 2ml of alkaline copper sulphate reagent (analytical reagent) was added. The solutions were mixed well by vortexing.
3. This solution was incubated at room temperature for 10 minutes.
4. Then 0.2 ml of reagent Folin-Ciocalteau's phenol reagent was added to each tube and incubated for 30 minutes. The spectrophotometer was auto zeroed with blank and the optical density (absorbance) was taken at 750 nm.

3.4 Protein Estimation by Bradford Assay

Procedure

1) The protein of varying concentrations was added to different wells of the microtitre plate and the volume was raised to a final equal volume with distilled/miliQ water in each well. The concentration was made as per the following table-

Table 3.4.1 Standardization of Bradford Assay using BSA

Protien amount(µg)	1	2	4	6	8	10	12	14	16	18	20
BSA std.(2mg/ml) in µl	0	1	2	3	4	5	6	7	8	9	10
dH_2O(µl)	10	9	8	7	6	5	4	3	2	1	0

2) To each well, 200µl of Bradford reagent was added using a micropipette.
3) The plate was allowed to stand in an incubator at 37°C for 30 minutes.
4) The readings were then recorded in a colorimeter at 595nm and a standard curve was plotted.

3.5 Protien precipitation by TCA assay

Procedure

1. 1 volume of TCA was added to 4 volume of protien sample in vials.
2. The vials were incubated at 4°C for 10 minutes.
3. The samples were spinned at 14,000 rpm for 5 minutes.
4. Then the supernatant was removed and whitish fluffy pellet was observed.
5. The pellet was washed with 200µl cold acetone.
6. Again the tubes were spinned at 14000 rpm for 14 minutes.
7. Steps 4-6 were repeated for total 2 acetone washes.
8. The pellet was dried at 95°C in heat block for 5-10 minutes.
9. Then 2X or 4X sample buffer (lysis) was added.

3.6 SDS-PAGE

Materials-

Reagents used:

5x Sample Buffer

10%(w/v)	SDS
10 mM	Beta-mercapto-ethanol

20%(v/v)	Glycerol
0.2 M	Tris-HCl (pH 6.8)
0.01%(w/v)	Bromophenol Blue

1x Running Buffer:

25mM	Tris-HCl
200mM	Glycine
0.1%(w/v)	SDS

5X Stock Tris-Glycine Electrophoresis Buffer:

(Total Volume=1 litre):

15.1 g	Tris Base
94g	Glycine
50ml	10% Electrophoresis-Grade SDS
900ml	Distilled Water

Table 3.6.1 - Resolving gel (12%)

Acylamide percentage	6%	8%	10%	12%	15%
H_2O	5.2ml	4.6ml	3.8ml	3.2ml	2.2ml
Acrylamide/Bis-acrylamide (30%/0.8% w/v)	2ml	2.6ml	3.4ml	4ml	5ml
1.5M Tris(pH=8.8)	2.6ml	2.6ml	2.6ml	2.6ml	2.6ml
10% (w/v)SDS	0.1ml	0.1ml	0.1ml	0.1ml	0.1ml
10% (w/v) ammonium persulfate (AP)	100µl	100µl	100µl	100µl	100µl
TEMED	10µl	10µl	10µl	10µl	10µl

Table 3.6.2: Stacking gel (5%)

Reagents	15 ml	**4 ml**	5 ml	10 ml
H_2O	10.2 ml	**2.66 ml**	3.4 ml	6.8 ml
30% acrylamide	24.9 ml	**0.67 ml**	0.83 ml	1.66 ml
1 M Tris(6.8)	1.89 ml	**0.5 ml**	0.63 ml	1.26 ml
SDS(10%)	150 µl	**0.04 ml**	0.05 ml	100 ml
APS(10%)	150 µl	**0.04 ml**	0.05 ml	100 ml
TEMED	15 µl	**0.004 ml**	0.005 ml	10 µl

Protocol

1. **Assembling the glass plates:**

   The glass plates were cleaned with detergent (tween-20) and water then with ethanol. They were then assembled with spacers. For the mini-gel, the glass plate assembly was attached to the apparatus and the whole assembly was then placed in a small, flat Tupperware container.

2. **Casting of the gel:**

   The gels were cast beginning with the resolving gel followed by the stacking gel.

   The degassed resolving gel was transferred to a beaker followed by addition of APS/TEMED and then mixed. Quickly the resolving gel solution was added with the help of pipette to the centre of the plates to a height of 4 cm from the top of the large plate. Iso-butanol is quickly added to the top till the plate's level and allowed to stand till the resolving gel polymerized. This prevents the gel's contact with air/oxygen that will react with the free radicals' generated by APS in the process and delay polymerization.

   The stacking gel reagents were added to the flask and degassed for 10 minutes. Meanwhile, the iso-butanol was poured off and water was used several times to rinse it within the gel plate space. The space was then air-dried in an incubator at 37°C. To polymerize the stacking gel, APS/TEMED was added and mixed followed by pouring on top of the polymerized resolving gel. The comb was inserted straight down into the stacking gel while it was allowed to polymerize undisturbed for 20-30 minutes. Any bubbles in the gel must be removed carefully.

3. Loading the gel

Tris-glycine electrophoresis buffer was poured into the upper and lower chambers. The wells were flushed with syringe just before loading to get rid of any unpolymerized polyacrylamide that may have seeped in. When loading the gel, 1X loading buffer was loaded in every lane so the dye front will migrate more evenly.

4. Sample Preparation

The samples were prepared with BSA as standard. 15µl of BSA with 6µl of loading buffer was used as marker. 27µl of each sample alongwith 6µl of loading buffer was prepared. The samples and marker were heated at 100°C for 10-12 minutes in a water bath then vortexed for 10 seconds. The samples were then loaded onto the gel which was run at 50V till the stacking gel was crossed followed by 100V till the end.

5. Staining the gel

The gel was stained using Coomassie blue. 12mg of Commassie blue was used from the stock of 60mg/litre and added to the staining solution. Staining solution was prepared using 10% acetic acid and 40% methanol in a total volume of 200 ml. The volume was raised using distilled water. The gel was then submerged in staining solution for 30 minutes.

6. Destaining the gel

The gel was then destained using destaining solution and the bands were observed. The destaining solution constituted of 50% Methanol, 10% Acetic Acid and the rest was distilled water in a total volume of 100ml. The gel was immersed in destaining solution till the solution turned blue. The step was repeated till clear bands became visible and the gel was washed with distilled water and stored.

3.7 1-D Profiling of proteins by Silver staining

Proteomic changes in cancer can be discovered by a combination of two-dimensional gel electrophoresis for separation of the proteins with a variety of potential methods for their visualization such as direct radioactive labeling, covalent attachment of fluorescent tags, and silver staining.

Reagents used in Silver Staining:

Farmer's reagent: 0.3g Potassium ferricyanide

0.7g Sodium thiosulphate

0.1g Sodium carbonate

In 250 ml of distilled water.

$AgNO_3$ solution: 0.2g in 200ml distilled water

Developer: 2.5% Sodium Carbonate in 270 µl formaldehyde

Stop solution: 2% Acetic acid and 10% Methanol then raise the volume with distilled water till 200 ml.

Protocol

1. For direct silver staining after SDS- PAGE fix in 90% (100ml) methanol and 10% (25ml) acetic acid with gentle stirring (250ml) for 2 hours.
2. The gel was washed with 10% methanol for 10 minutes. The washing was repeated for 5 minutes.

3. The gel was washed with distilled water for 30 minutes changing the distilled water after every 5 minutes. This was done to wash off traces of ethanol.

4. The gel was then dipped in farmer's reagent for 50-60 seconds.

5. It was rinsed with distilled water for 5 minutes (thrice).

6. Silver Nitrate solution was used to immerse the gel for 1-5 minutes until it became black and then the solution was discarded. Light was prevented during weighing silver nitrate and while the reaction was going on.

7. The gel was rinsed with Sodium Carbonate for 5 minutes.

8. It was dipped in developer for 30 seconds to 2 minutes. As soon as bands appear the reaction was stopped with stop solution. 2% acetic acid can also be used for stopping the reaction.

9. Farmer's reagent was again used to dip the gel in order to remove background.

10. Farmer's reagent was removed by washing the gel thrice with distilled water.

3.8 Extraction and 1-D profiling of proteins from MCF-7 Cell Line

Cell lines are widely used in many aspects of laboratory research and particularly as *in vitro* models in cancer research. They have a number of advantages, including being easy to access and offering "clean" results with statistically significant signals. However, human systems are quite complex, and many candidate biomarkers discovered in cell lines do not readily transfer to human tissues or blood, in which clinical testing will be eventually performed. Therefore, profiling human plasma using proteomics techniques offers an appealing alternative to cell lines or tissue bio-specimens in developing protein biomarkers. For example, an angiogenesis inhibitor protein, thrombospondin-1, has been detected in MCF-7 cells.

Keratin 8 has been abundantly found in MCF-7 cell line hence it is a candidate biomarker for that particular cell type. A total of 3085 protein biomarkers have been identified from the five breast cell lines, MCF-10A, BT474, MDA-MB-468, MD-MB-468, and T47D/MCF7.

Protocol

1. Cultured cells of MCF-7 cell line were transferred to an eppendorf.
2. Cells were centrifuged at 2000 rpm for 7-8 minutes at 4℃.
3. Lysis buffer(50 µl) was poured into the eppendorf.
4. Then 2 µl of protease inhibitor and 2 µl of PMSF were added.
5. The eppendorf was left for incubation at room temperature and vortexing was done after every 10 minutes.
6. After vortexing the vial was stored at -20℃.
7. Next day, the samples were taken and run on SDS-PAGE to observe the bands of its protein profile.

3.8 Protien profiling of human blood (as control)

Molecular biomarkers have become increasingly important clinical tools for cancer screening, diagnosis, treatment customizations. . A patient's blood plasma/serum is considered to be the most desirable clinical specimen for biomarker research because it is attainable non-invasively, extraction is simple and affordable, and it is likely to contain tumor markers, albeit in significantly low abundance. It is well documented that not only do tumors leak or secrete proteins into the circulation, but also the surrounding stroma releases proteases and other mediators of cancer growth. There has been an increasing number of research reports on developing breast cancer biomarkers, especially in blood.

Many molecular biomarkers with expression level changes have been identified in breast cancer tissue samples or blood, for example, HER2, PNCA, Lipofilin B, Cyclin D1, CEACAM6, Osteopontin-c, RCP, and FOXA1.

Reagents: RBC Lysis Buffer

Tris HCL	2.42 g
Ammonium Chloride (NH_4Cl)	7.5 g
Deionised water	1 litre

Protocol

1. 4ml of human blood was collected in EDTA coated vials and kept in ice.
2. 500µl of blood was distributed in each vial.
3. 1.5ml of RBC lysis buffer was added and the vials were inverted for 15 minutes.
4. The vials were centrifuged at 13000 rpm for 1 minute.
5. Supernatant was discarded and the pellet was retained.
6. Pellets were rinsed with 1ml PBS to wash off supernatant blood.
7. Then 40µl cell lysis buffer was added to the pellet.
8. And 40µl PMSF was added to each vial.
9. Vortexing was done every 10 minutes for half an hour till the pellet got suspended.
10. 5µl of loading dye was added to each vial.
11. Vials were kept at 95°C for 12 minutes.
12. Then vials were centrifuged for 14 seconds at 2000 rpm to proceed for protein profiling using 1D PAGE.

Chapter 4

RESULTS AND DISCUSSIONS

4.1 Protein Estimation by Lowry's Method

The absorbance against protein concentration was plotted as follows to get a standard calibration.

Concentration of BSA(1mg/ml)	O.D @750nm
25	0.0799
50	0.1781
100	0.3478
200	0.5231
400	0.8887
800	1.432

Table 4.1.1 Standardization of Lowry's Protocol

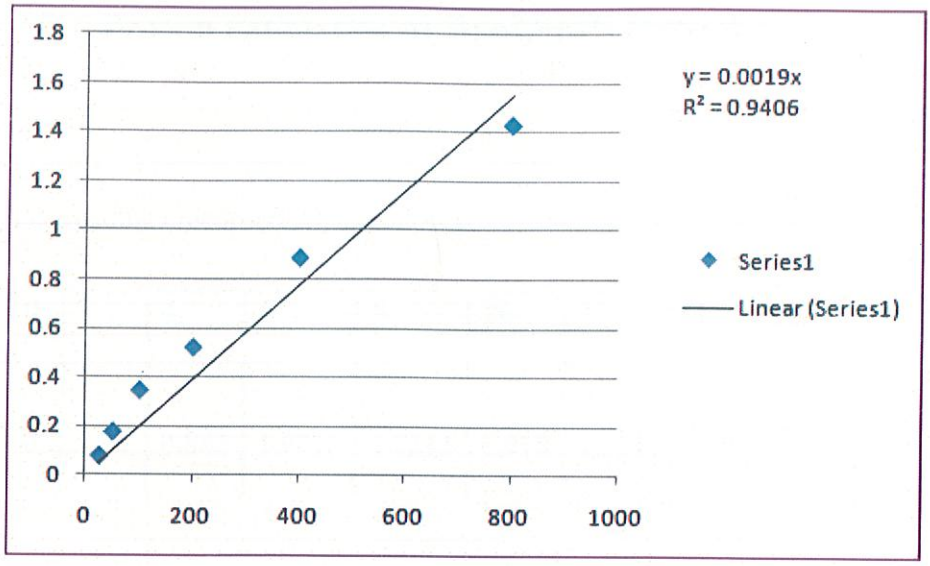


Table-4.1.2 Standard Curve for Lowry's Assay

Concentration of the samples

From the standard curve, the absorbance readings were obtained and the respective concentrations of proteins for breast cancer samples came out to be as follows-

1. S1- 1.398mg/ml

2. S2- 1.858mg/ml

The concentrations of proteins in the samples were very low probably because the samples were formalin-fixed tissues. Although formalin-fixed samples stored for as long as fourteen years have yielded whole amount of proteins as freshly obtained samples, there might be a possibility of degradation of proteins. Therefore, we aimed for fresh cancer samples particularly snap-frozen in liquid nitrogen.

4.2 Protein Estimation by Bradford's Method:

The readings recorded are as follows-

Protien amount(µg)	0	2	4	6	8	10	12	14	16	18	20
Od	0	0.34	0.865	1.0215	1.1215	1.018	1.321	1.231	1.404	1.54	1.55

Table-4.2.1 Average Absorbance for Bradford's Standard Plot

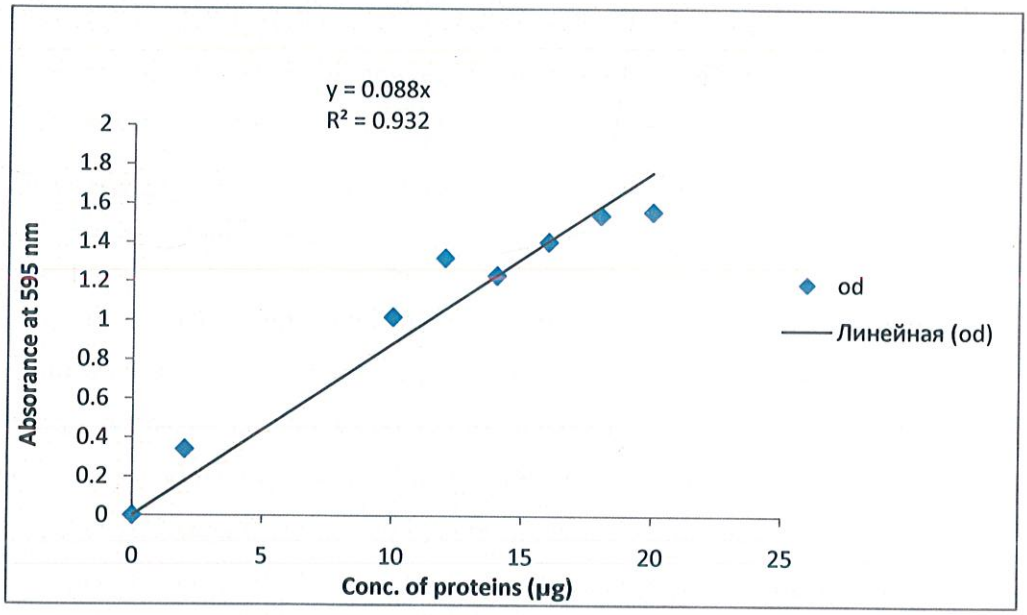


Table-4.2.2 Standard Plot for Bradford's Assay

Recommended Standard proteins for Bradford Assay include bovine gamma globulin (IgG), lysozyme and ovalbumin because they have closer to the average or typical number of amino acids that bind the dye.

Although BSA is often recommended in literature, but it is not a good choice for a standard for the Bradford assay, as it has a high content of reacting amino acid residues. The 595nm absorbance of BSA is about 2.1 times that of IgG.

The colour development in Bradford assay is complete within 2 minutes and the colour remains stable for upto an hour. With the micro-assay procedure, Bradford assay can be used to determine proteins in the range of 1 to 20μg. Bradford assay has few interfering components known to be Triton X-100 and Sodium Dodecyl Sulphate.

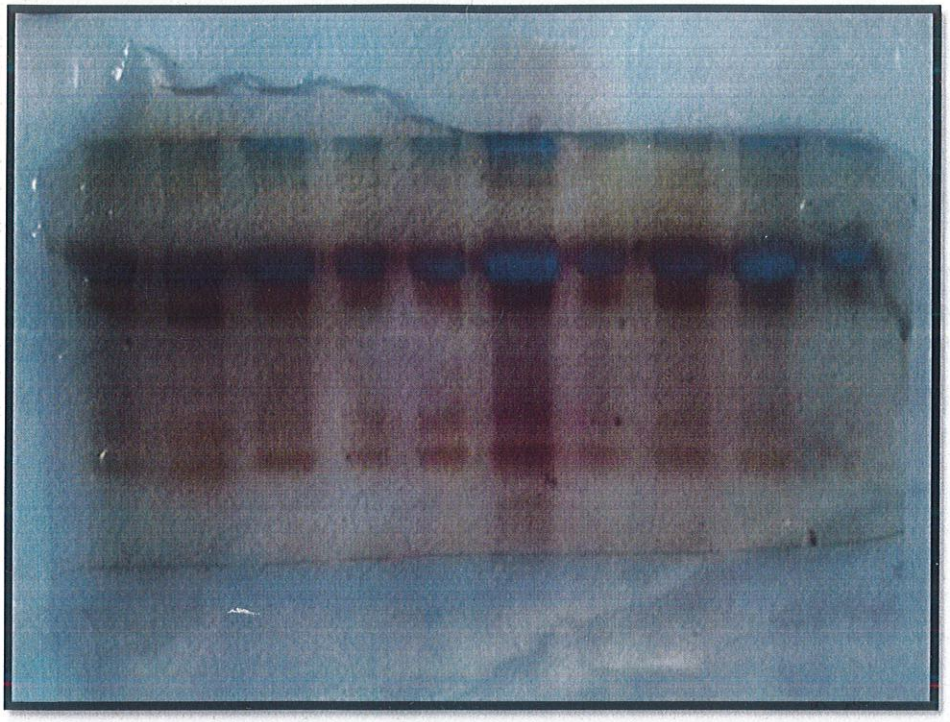
4.3 SDS PAGE:

Initially, in SDS-PAGE no desired bands were observed due to low concentration of protein in samples. There was also a possibility that the protein was degraded or there might have been some problem with the reagents used. Therefore, standardization and optimization procedures were carried out for optimum results.

Since this classical approach is indispensible for the field of proteomics study, it allows comparison of multiple protein profiles as illustrated on the successive pages. Despite its popularity, it has some drawbacks such as low sensitivity and poor reproducibility that may pose a problem for determination of potential biomarkers. However, current research using this technique has not hindered the outcome of the experiments in this project that may influence the results in the long run. This is because other aspects for result validation will be carried out.

4.3.1 SDS- PAGE using BSA protein (2mg/ml)

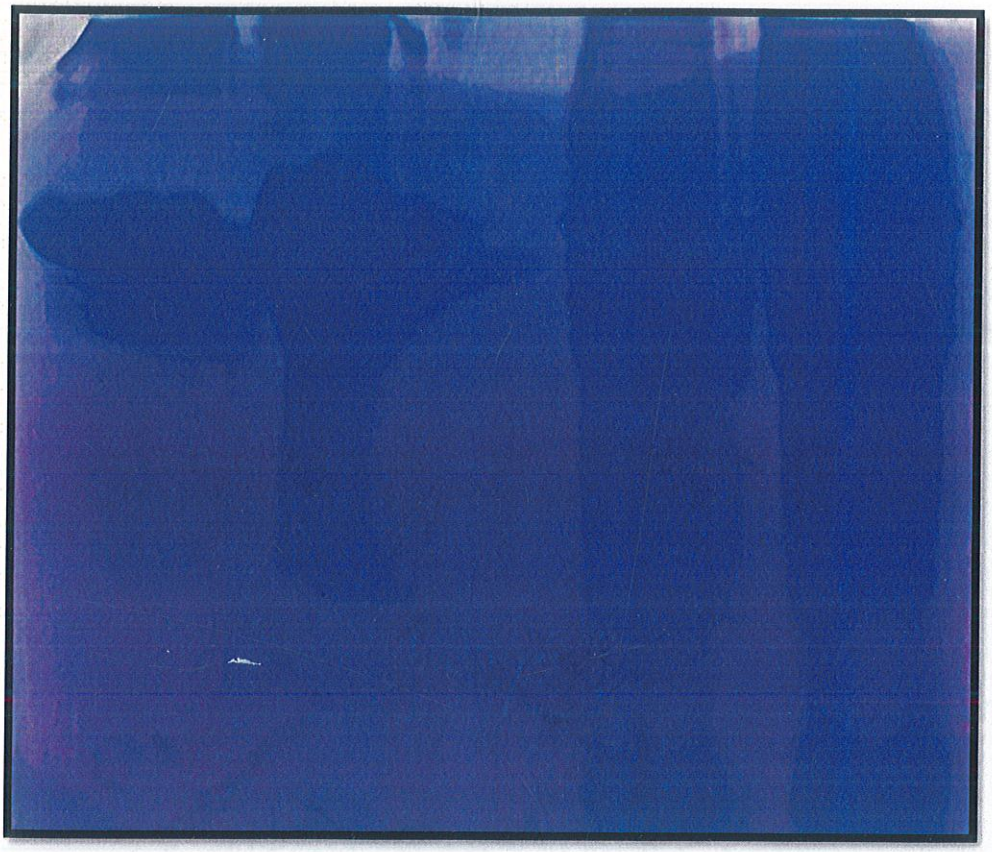
Lane 1 2 3 4 5 6 7 8 9 10



20 micrograms of BSA was loaded in each well. Bands can be clearly seen after commassie blue staining followed by silver staining. Extra bands in a gel containing BSA indicates impurity in the BSA source.

4.4 SDS-PAGE gel picture of proteins extracted from MCF-7 cell line

Lanes 1 2 3 4 5



Lane 1- 25 µg of BSA

2- 50 µg of BSA

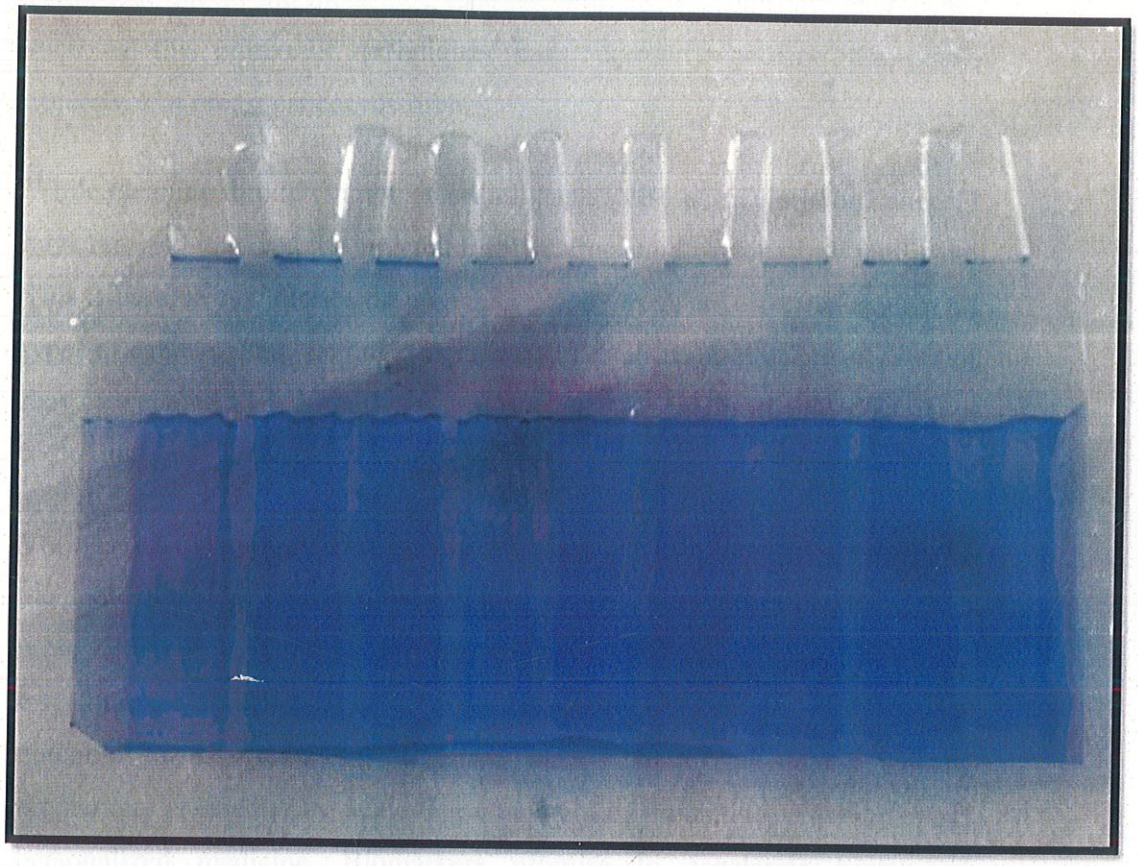
4- 25µg of protein extracted from MCF7

5- 50 µg of protein extracted from MCF7

Several protein bands can be observed in whole cell lysate of MCF-7 cell line after staining with Commassie blue.

4.5 1D profiling of proteins extracted from human blood(control)

Lanes 1 2 3 4 5 6 7 8 9



The most abundant protein present in serum is albumin and, in fact, 95% of the protein content of serum is made up of around 20 highly abundant proteins, which must be removed prior to proteomic analysis in order to prevent them from masking the expression of low abundant putative biomarkers.

Normal human blood serum after lysing RBCs was run on a 1-D gel and several distinct bands could be observed under white light. This was done with the purpose of comparison as matched control.

Appendix

The reagents used were obtained from the following manufacturers:

Sr. No	Reagents	Manufacturers
1.	SDS-PAGE assembly	Biorad
2.	Bradford Assembly	Biorad
3.	TEMED	Biorad
4.	Sodium Carbonate	Sisco Research Labs Pvt. Ltd
5.	Potassium Ferricyanide	S.D.Fine chemical Ltd
6.	Potassium Thiosulphate	Merck
7.	Formaldehyde	Merck
8.	Ethanol	Changshu Yangyuan Chemicals, China
9.	Acetic Acid	Fisher Scientific
10.	Methanol	Merck
11.	Commassie RT50	Biorad
12.	Loading buffer	Biorad

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Brief Resume of the Student

Zoya Nadeem Faruqui will be completing her final year of B.Tech in Biotechnology in May 2013 from Jaypee University of Information Technology. She was appointed office bearer and served as the Coordinator of the University's Environment Club from 2011-2012. She has been granted admission to post-graduation at University College London in UK. Her interests are in Molecular Biology, Genetics and Biochemistry. She aspires to be a worthy researcher in the field of personalized medicine and gene therapy in the long run.