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# Differential (Chemo) Proteomics in Breast Cancer

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***Abstract*:**

Proteomic technologies have emerged as an important addition to the genomic and antibody-based technologies for the diagnosis of cancer. Important technologies include 2-D gel electrophoresis, MALDI-TOF MS, laser capture microdissection, and the detection of molecular markers of cancer and protein patterns [1]. Proteomic technologies have the potential in developing molecular diagnostics and markers for the early detection of cancer [2]. In addition, better breast cancer prognostication may improve the selection of patients eligible for adjuvant therapy. Hence, new markers for early diagnosis, accurate prognosis, and prediction of response to treatment are warranted to improve breast cancer care. Since proteomics can bridge the gap between the genetic alterations underlying cancer and cellular physiology, much is expected from proteome analyses for the detection of better protein biomarkers. Proteomics has the potential to complement and further enlarge the wealth of information generated by genomics in breast cancer. Proteomics is used to detect protein expression patterns at a given time in response to a specific stimulus, but also to determine functional protein networks that exist at the level of the cell, tissue, or whole organism [3].

***Index terms*:** molecular diagnosis, proteomics, cancer diagnosis, proteodiagnostics, biomarkers

## I. INTRODUCTION:

Cancers of the lung, colorectum, stomach, and female breast are the most common causes of death in higher HDI areas, accounting for over 45% of total cancer mortality [4]. Breast cancer is the most common lethal cause of cancer death in women worldwide. Early detection of breast cancer aids in diagnosis and treatment before metastasis. Despite remarkable progress in new medical discoveries and therapies for breast cancer over the previous decades, no significant treatment methods are available for cancer-affected people with invasive and metastatic breast cancer. Because of the recurrence properties of cancer, patients at this stage have less response to cancer therapy. Mammography is useful in diagnosing breast cancer; however, tumors smaller than 0.5 cm remain undiagnosed. Cancer patients' survival rates are heavily influenced by tumor conditions. Breast cancer detection at an early stage can lead to treatment options such as surgical resection with lymph node removal, radiation therapy, chemotherapy, and hormone therapy. Breast cancers have been found to exhibit several genetic changes. Gains along chromosomes 1q, 8q, 17q, 20q, and 11q are more common than losses along chromosomes 8p, 13q, 16q, 18q, and 11q [5- 10].

Interestingly, many of these chromosomal regions contain recognized proto-oncogenes and/or tumor suppressor genes, such as BRCA1, BRCA2, HER2-neu, C-MYC, and Cyclin D1. Low-grade (grade 1) infiltrating ductal carcinomas contain few chromosomal changes, with the most common aberrations appearing as losses on 16q and gains on 1q [11]. Though there has been some noticeable improvement in breast cancer treatment, the lack of biomarkers in serum/plasma causes delays in the early detection of breast cancer. As a result, the molecular structure, including protein markers, must be determined to determine which diseases are responsible. These increase the rate of advanced-stage disease analysis, therapeutic response, and relapses after therapy, as well as the differences that are typical to disease and person. Human genome sequencing has paved the way for today's tremendous revolution in biology and medicine. The

emergence of new and powerful technologies in functional genomics and proteomics, combined with bioinformatics tools, has accelerated the translation of basic discoveries into clinical practice [12].

Recent improvements in the field of molecular genetics, particularly proteomics, paved the way for improving drug development and clinical trial procedure. Furthermore, proteomics offers tools for investigating abnormal molecular changes in cancer tissues and new insights into developing new reagents for understanding all stages of cancer conditions. Additionally, proteomics provides tools for drug discovery [13].

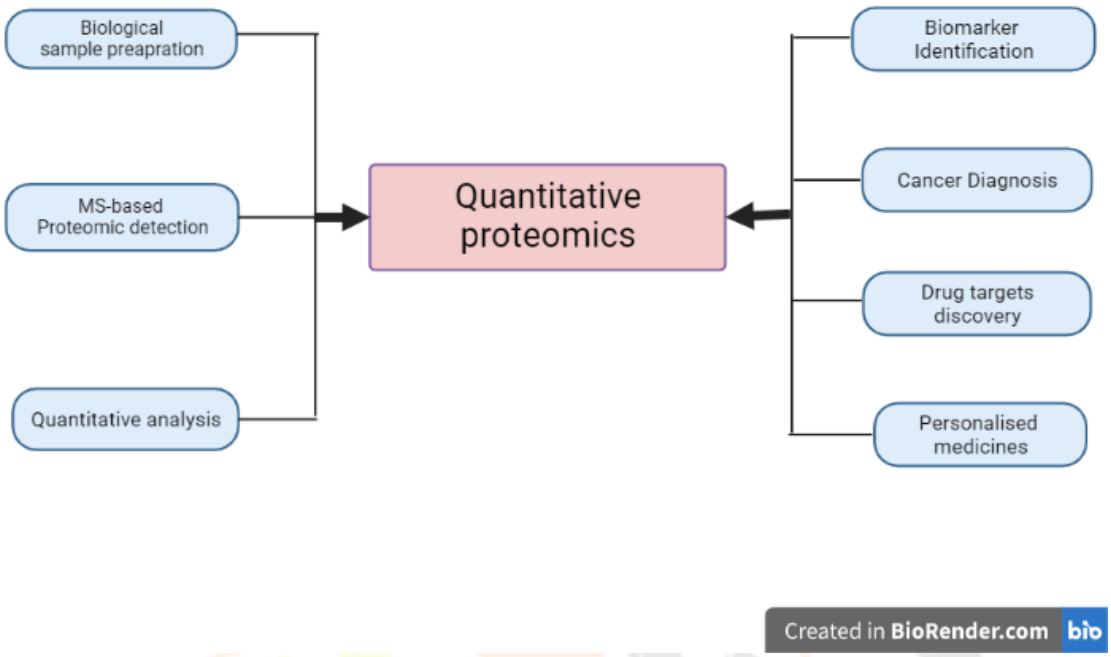


**Fig. 1- Various methods of quantitative proteomics**

Proteomics in breast cancer integrates genomics, transcriptomics, metabolomics, and epigenomics. Breast cancer-integrated proteotranscriptomics decipher new disease characteristics, and reveal the molecular basis of intraoperative radiotherapy-treated tumors, and subtypes of triple-negative breast cancers (TNBC) [14].

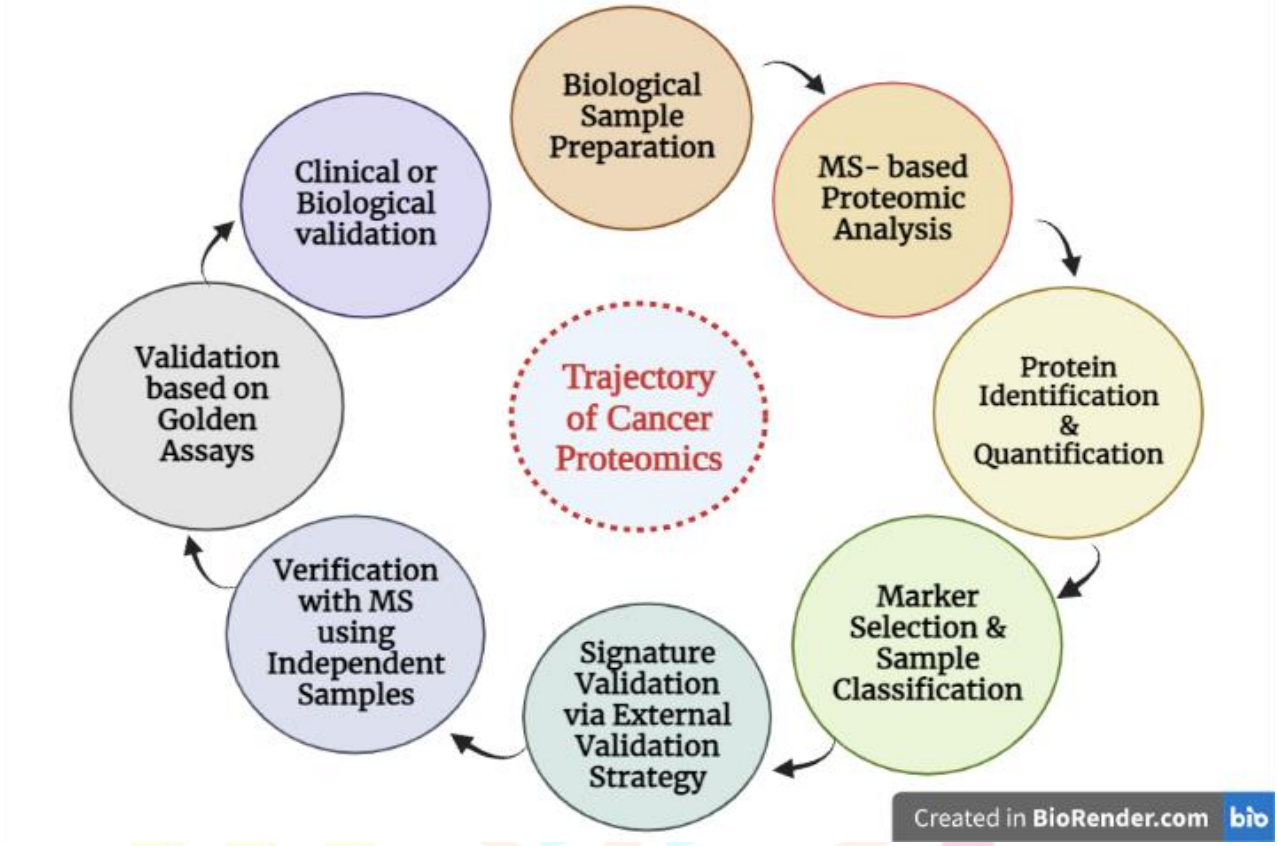


**Fig. 2- Steps in cancer proteomics [9]**

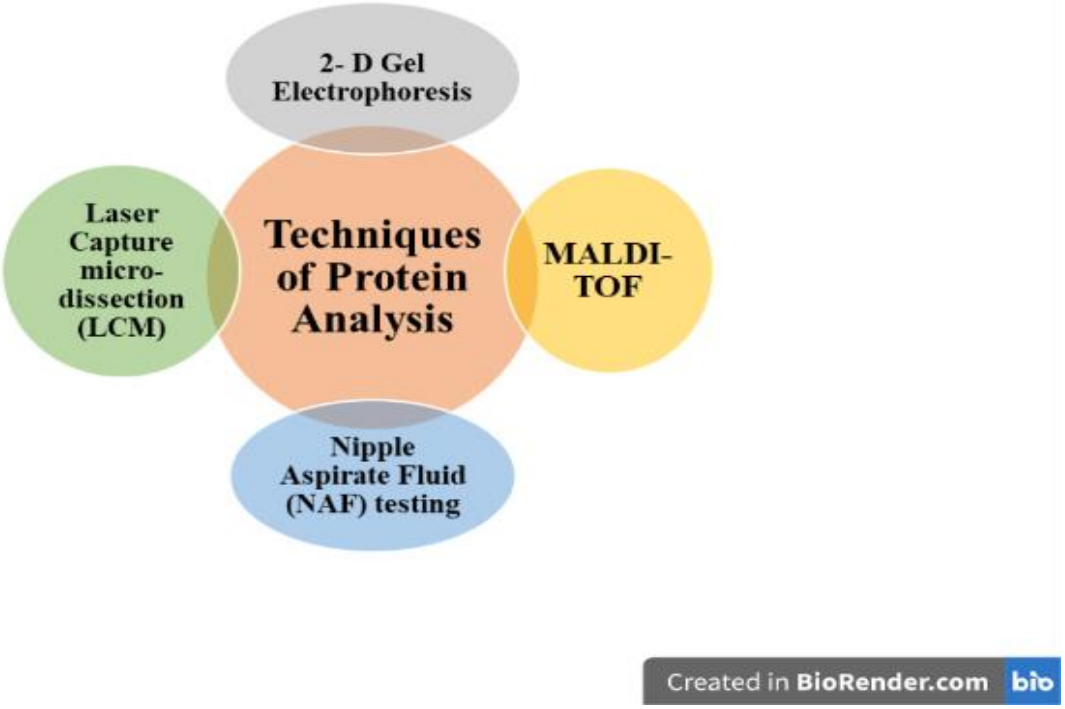


**Fig. 3- Techniques of protein analysis**

**Table 1: Different Techniques Used for Protein Analysis**

| Name of technique | Procedure | Results |
|---|---|---|
| 1) **2-D GEL ELECTROPHORESIS: -**<br><br>Two-dimensional gel electrophoresis (2DGel) is a popular method for detecting and analyzing proteins. It was created as a hybrid of the 2DGel, IEF, and SDS-PAGE methods and is used to analyze complex protein mixtures [15]. | a) In the first step, IEF is used to separate the protein into its charges, and in the second step, the protein is separated based on its mass [15].<br><br>b) SDS treatment negatively charges the separated protein on the gel with IEF, and electrophoresis is performed by inserting the gel horizontally into the SDS-PAGE gel [15]. | Proteins with more than a twofold difference in average quantitative expression between cancer and control tissues were considered statistically regulated [15]. |
| 2) **MALDI-TOF: -**<br><br>MALDI-TOF Mass spectrometry is a versatile analytical technique for detecting and characterizing organic molecule mixtures. It is used in microbiology as a quick, accurate, and low-cost method of identifying microorganisms [9]. | Microbes are identified using either intact cells or cell extracts during the MALDI-TOF MS process. The process is quick, sensitive, and cost-effective in terms of both labor and materials [9]. | The proteins that were found to be differentially expressed were identified using peptide mass fingerprints.<br>tissues in addition to control tissues [9]. |

| | | |
|---|---|---|
| 3) **LASER-CAPTURE MICRODISSECTION (LCM): -**<br><br>It is a technique for obtaining subpopulations of tissue cells under direct microscopic observation [16]. | LCM technology can directly harvest cells of interest or isolate specific cells by removing unwanted cells to provide histologically pure enriched cell populations. DNA genotyping and loss-of-heterozygosity (LOH) analysis, RNA transcript profiling, cDNA library generation, proteomics discovery, and signal-pathway profiling are just a few of the downstream applications [16]. | Laser microdissection allowed for the isolation of invadosomes while minimizing contamination from other cellular elements. Manual delineation of invadosomes was required when performing microdissection [16]. |
| 4) **NIPPLE ASPIRATE FLUID (NAF) TESTING: -**<br><br>Nipple aspirate fluid (NAF) is a fluid released by the mammary ductal and lobular epithelial cells that contain a set of particular breast tissue proteins. These proteins are closely associated with the microenvironment of the development and evolution of breast malignant tumors in breast cancer cases [17]. | For all tests, a NAF sample is obtained from a single healthy breast of a woman with unilateral fibroadenoma benign proliferative lesion. NAF collection and protein quantification are carried out. The sample contains no protease inhibitor mixture. The woman has not nursed in the last two years, has not used exogenous hormones in the last six months, and has never had breast surgery [17]. | NAF proteome description is obtained [17]. |

- **DETECTION OF MOLECULAR BIOMARKERS: -**

The rapid development of protein-based molecular characterization of breast tumors has been facilitated by parallel advances in high-throughput protein quantification techniques [18,19]. This method has been used to discover cancer biomarkers such as the -chain of haptoglobin (Hp-) for ovarian cancer and -defensin for bladder cancer [20]. A molecular marker is a trait that can be objectively measured as an indicator of pathogenic or normal biological processes, or as a pharmacological response to a therapeutic intervention [21].

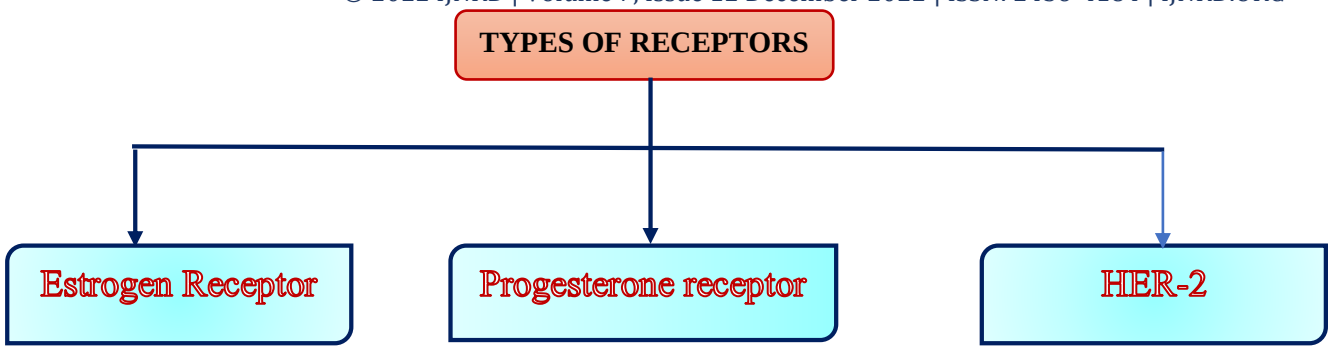


**Fig. 3- Types of Receptors in Breast cancer detection**

- ***Estrogen Receptor (ER)*** –

  Estradiol diffuses across the breast epithelial membrane and binds to its nucleus receptor. This causes a conformational shift, and estradiol bound to its receptor dimerizes with other receptors, activating estrogen-responsive genes [22].

- ***Progesterone Receptor (PR)*** –

  Progesterone receptor (PR) expression is used as a biomarker of estrogen receptor (ER) function and breast cancer prognosis. PR binds to ER and directs ER chromatin binding events within breast cancer cells in the presence of agonist ligands, resulting in a distinct gene expression program associated with a favorable clinical outcome [23].

- ***Human Epidermal Growth Factor Receptor 2 (HER-2)***-

  HER-2 is a tyrosine kinase receptor with a transmembrane domain.
  According to crystallographic analysis, HER-2 belongs to a family of epidermal growth factor receptors and is ready to bind conformation even without a ligand, explaining why this receptor lacks natural ligands [6]. Abnormalities in HER-2 expression at the gene, message, or protein level have been linked to a poor prognosis in both lymph node-negative and lymph node-positive breast cancer [24].

Breast cancer subtypes have traditionally been defined by the expression of three molecules: the estrogen receptor (ER), the progesterone receptor (PR), and the human epidermal growth factor receptor 2. (HER2). Triple-negative breast cancer (TNBC) is one of these subtypes, distinguished by the absence of all three receptors.

- ***TNBC*** –

  Triple-negative breast cancer is a kind of aggressive breast cancer that is frequently resistant to chemotherapy. However, whether this resistance is due to chemotherapy's selection of pre-existing clones or genetic mutations occurring during chemotherapy has not been determined. A more in-depth and detailed analysis of eight patients using single-cell DNA sequencing and single-cell RNA sequencing analysis revealed that drug-resistant cancer cells were present before treatment and were adaptively selected for by neoadjuvant chemotherapy, while gene transcription analysis revealed that drug-resistant cancer cells were present before treatment and were adaptively selected for by [25].

❖ **PROTEOMIC INDICATORS: -**

1) ***Serum*:**

   Serum, which contains a high concentration of proteins, provides a simple and low-cost method for disease detection and has the potential to revolutionize diagnostics. These differentially expressed proteins in serum have played an important role in disease monitoring. The availability of emerging proteomic techniques raises

hopes that serum will one day be used as a bio medium for clinical diagnostics. Biomarker research has advanced to the point where serum is now recognized as an excellent diagnostic medium for disease detection. A comprehensive proteome of human serum fluid with high accuracy and availability has the potential to open up new avenues for disease biomarker discovery and diagnostics, providing insights useful for future research [26].

2) ***Needle Aspiration Fluid (NAF):***

A type of biopsy procedure is fine needle aspiration. A thin needle is inserted into an area of abnormal-appearing tissue or body fluid during fine needle aspiration. Fluid can be aspirated non-invasively from the breast nipple (nipple aspirate fluid, NAF). NAF is available in a variety of colors, including clear, white, yellow, green, and red/brown. In a population of women who presented to a breast cancer evaluation clinic, NAF color was associated with the presence of breast cancer and the progression from precancer to cancer. The color of NAF played a role in developing a highly predictive breast cancer detection model [9].

3) ***Tumor Tissue and interstitial Fluid (TIF):***

Tumor interstitial fluid (TIF) surrounds and perfuses tumorigenic tissues and cells in the body, and it can accumulate tumor and stromal cell byproducts in a relatively local space. Interstitial fluid has several important advantages for the discovery of biomarkers and therapeutic targets, particularly in cancer. TIF has been extensively studied and may be a source of potential biomarkers for cancers such as breast, ovarian, and head and neck [27]. An extensive IHC analysis of 10 proteins is performed to determine whether selected protein candidates with subtype-specific patterns originate from malignant cells, normal cells, or TILs, including anterior gradient protein 3 (AGR3), Lutheran/basal cell-adhesion molecule (BCAM), Cadherin EGF LAG seven-pass G-type receptor 1 (CELSR1), membrane-anchored protein C35 (MIEN1), N-acetyltransferase 1 (NAT1) (ULBP2). These proteins are chosen for investigation based on the availability of highly specific antibodies as well as the quality and specificity demonstrated in a series of control studies [28].

❖ **PROTEOMICS METHODS-**

1) ***Tissue Microarrays***-

Tissue microarray is a relatively new advancement in pathology. A microarray is a single histologic slide that comprises numerous small representative tissue samples from hundreds of distinct cases, allowing for a high throughput study of multiple specimens at the same time.
Molecular data is collected in situ in the context of cell morphology and tissue architecture, and specimens are generated from FFPE archival tissues (although TMAs can also be performed on frozen tissues) (cryo-TMAs). TMA has been used to screen different tumor types for the expression of a protein of interest (multi-tumor TMAs).

**Advantages:**

a) Less time consuming
b) Not labor intensive
c) Economically feasible

**Disadvantage:**

A disadvantage of the tissue microarray method is that the potential expression heterogeneity of a biomarker may not be represented adequately in small-volume samples. However, this can be avoided if several punches from different tumor regions are included in the array and a larger biopsy diameter is chosen [29].

2) ***Protein Microarrays***-

A protein microarray (or protein chip) is a high-throughput method for tracking the interactions and activities of proteins and determining their function on a large scale. Its main advantage is that it can track a large number of

proteins at the same time. These approaches tend to replicate mRNA expression profiling studies at the protein level by arraying various protein probes onto specific surfaces and then measuring interactions with specific proteins in complex samples. The antibody microarray is the most advanced format in this setting, with the bait proteins being specific antibodies printed on solid surfaces [30]. Protein and antibody arrays may also provide information on targeted proteins' post-translational modifications [31]. This has been used on breast cancer cell lysates to assess the activation of ERBB receptors in response to ligand binding and after specific perturbation with receptor inhibitors [32].

**Advantages:**

a) Protein arrays allow the monitoring of several proteins in the same assay (HT technology).
b) Wide range of applications: Serum screening, biomarker discovery, and functional proteomic studies.
c) Easy control of experimental conditions.

**Disadvantages:**

a) Protein arrays require validation, and experiments because false positives can be detected.
b) The highest array reported to date included only 9000 different proteins.
c) Whole eukaryotic protein arrays still have not been reported.
d) Difficulty to control post-transcriptional modifications.
e) Arrayed proteins may not be functional on the surface [33].

3) ***SELDI-TOF-***

SELDI is typically used with time-of-flight (TOF) mass spectrometers to detect proteins in tissue samples, blood, urine, or other clinical samples; however, by simply modifying the sample surface, SELDI technology can potentially be used in any application. SELDI-TOF MS is the most widely used and advertised non-gel-based method at the moment. The method directly connects protein separation to presentation to a mass spectrometer. Surface-enhanced laser desorption ionization-time of flight mass spectrometry (SELDI-TOF MS) combines retention chromatography and mass spectrometry principles to provide a fast, high-throughput, and relatively sensitive screening approach capable of evaluating complicated protein samples. This method can also separate, identify, and analyze proteins at the femtomole level straight from biological materials. It is a tool in and of itself that helps the analysis of vast numbers of distinct samples while simultaneously studying many biological characteristics. SELDI-TOF MS combines retention-based "on-array" separation of complicated protein mixtures with mass spectrometry detection.

This technique allows for protein profiling from a variety of biological samples, including cancer cell lines, serum, nipple aspirate, plasma, urine, and tissue extracts, and has the advantage of high sample throughput, which allows for the generation of enough data to adequately power statistical tests.

SELDI principles and clinical applications have been studied, and the technique has been effectively applied in biomarker identification on a variety of tissues and samples. Specific molecular recognition mechanisms, such as antibody-antigen, enzyme-substrate, receptor-ligand, and protein-DNA interactions, can also be exploited using special biochemically active surfaces.

This is a distinct advantage of SELDI over other types of MS, where crude samples cannot be used for protein analysis reliably. Mass spectrometry and protein peak comparison can reveal considerable changes in protein abundance between samples. As a result, SELDI-TOF MS enables powerful protein analysis from a wide range of sample types with minimal sample consumption and processing [34].

**Advantages:**

a) Easy to use
b) High throughput
c) Relatively affordable [35]

4) ***Immunohistochemistry-***

Immunohistochemistry, or IHC, is a special staining procedure used on fresh or frozen breast cancer tissue obtained during a biopsy. IHC is used to see if cancer cells have HER2 receptors or hormone receptors on their surface. This knowledge is crucial when it comes to treatment planning.

The 26 proteins tested were chosen based on their known or potential importance as a prognostic/predictive marker in breast cancer, as well as the availability and suitability of a corresponding antibody for paraffin-

embedded tissues. They included hormone receptors [estrogen receptor (ER)], subclass markers (CK5/6, CK8/18), oncogenes, and proliferation proteins. Tumor suppressors (P53, FHIT), adhesion molecules (CDH1, CDH3, CTNNA1, CTNNB1, Afadin/AF-6), proteins from amplified genomic regions (ERBB2, CCND1, STK6), and markers identified in previous studies (EGFR, ERBB2, ERBB3, ERBB4, BCL2, CCND1, CCNE, Ki-67, FGFR1, Aurora A/STK6, TACC1, TACC2, T (GATA3, MUC1). Twelve of these proteins were found to be common among the discriminator genes identified in breast cancer prognosis studies using RNA expression profiling. [7]

**Advantages:**

a) It is possible to use fresh or frozen tissue samples for IHC.
b) IHC is well-established and readily available.

**Disadvantages:**

a) HC stains are not standardized worldwide.
b) While the cost of the procedure is relatively inexpensive, the equipment needed to perform IHC is costly.
c) Quantifying results is difficult.
d) IHC is subject to human error. Well-trained personnel is paramount [36].

## II. CONCLUSION:

Proteomic analysis of cancer sheds significant light on the underlying mechanism that leads to cancer development. The importance of using high throughput systematic approaches to rapidly identify more metastasis-related factors that can be considered in an integrated manner. Current research data demonstrate the value of proteomic analysis in understanding the molecular mechanism involved in metastasis activity. The development of mechanistic studies is expected to be based on a combined approach. They are excellent targets when proteins are mutated, truncated, or expressed as specific isoforms or in large amounts. Further evaluation and characterization of genomic and proteomic variations may lead to the identification of biomarkers that can be specifically applied to metastatic assay and diagnosis in the clinic [37]. Tumors and patient fluids may contain specific protein isoforms that can act as early detection and/or follow-up markers. Proteomics can easily be combined with functional tests. Proteomics can be applied to cell components that deserve special attention or are more convenient to study in some cases. Proteomics has the potential to be used in clinical practice in the future. For example, protein arrays that combine quantitative and functional information, by detecting the activation of kinases or signaling pathways to be targeted by specific antitumor therapies [38]. Proteomics remains under development, and despite technical barriers that precede the use of proteomics analysis in clinical practice and breast cancer complexity MALDI-TOF and SELDI-TOF MS proteomic platforms with their innovations are powerful analytical tools for decoding alterations in the protein expression concerning disease progression [39]. Based on this research outcome we propose that differentially expressed proteins in cancerous conditions could be fundamental steps for developing the markers and proteomic database for breast cancer diagnosis [12].

## III. FUTURE PROSPECTS:

Established classifications, such as IHC-hormone evaluations have become more clinically inexpensive in routine processes. Protein biomarkers' therapeutic potential is also influenced by a variety of parameters such as mRNA transcription rate, protein stability, post-translational modifications, and random mutations [40].

Only four biomarkers have been approved by the FDA for measuring BC in body fluids: cancer antigens (CA), CA 15-3, CA 27-29, Human epidermal growth factor receptor 2 (HER2), and circulating tumor cells (CTC) [41].

The FDA has approved neratinib (Nerlynx) for patients with early-stage HER2-positive breast cancer who have completed at least one year of trastuzumab adjuvant therapy (Herceptin) [42]. Tukysa is a kinase inhibitor, which inhibits cancer cell growth by blocking a specific enzyme (kinase). Tukysa is licensed for use after patients have been treated with one or more anti-HER2-based regimens for metastatic disease [43].

## IV. ACKNOWLEDGMENT:

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