

Novel Tumor Markers of Breast Cancer Patients

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Abstract: ***Objective:** The objective of the present study is to look for some Novel Tumor Markers for the diagnosis of Breast Cancer and screening females having high risk of developing Breast Cancer by exploring the Anthropometric, Cytogenetic and Biochemical Technique. **Method:** The study consists of three parameters for which informed consent were taken from each individual. First for the Anthropometric Analysis, "angle of triradius" (atd) angle of around 55 Breast Cancer patients (age group 18-70 years) along with age matched 55 Healthy Control females were taken (Harold Cummins, 1960). For the Cytogenetic Analysis, peripheral blood of the same group of individuals were subjected to lymphocyte culture by standard protocol of Moorehead et. al., (1964) and chromosomal aberrations were observed by karyotype preparation. For the Biochemical Analysis, plasma samples of both the groups were studied by running them in cellulose acetate membrane in an Electrophoretic chamber (Robyt, John F, 1990) and any altered plasma protein patterns that were specific for Breast Cancer patients were observed. **Result:** In the Anthropometric Analysis using dermatoglyphic tool "atd" angle, Breast Cancer patients were found to be having wider angles (>42 °) than their healthy counterparts. In the Cytogenetic Analysis, Breast Cancer patients were showing a much higher frequency of numerical as well as structural aberrations than the healthy females and in the Biochemical Analysis, Breast Cancer patients were found to be having a raised beta globulin proteins while healthy females showed a normal range. In addition, most breast cancer females showed a gamma globulin pattern of polyclonal origin. **Conclusion:** These parameters in combination have a potential to be used as Tumor Markers for the diagnosis of breast cancer and these are not only cost effective but also non-invasive which makes them more accessible especially for screening females at high risk of developing Breast Cancer.*

Keywords: Anthropometry, Breast Cancer, Cytogenetic study, Dermatoglyphics, Plasma Protein.

1. Introduction

Breast cancer is one of the most common types of cancer in women and accounts for nearly 22.9% of all cancer type worldwide and it is increasing at an alarming speed. Therefore, there is a need to look for new tumor markers for its early diagnosis and efficient treatment plan.

The cornerstone for early detection so far has been mammography but it has also resulted in over-diagnosis and a number of false negative cases thus increasing the anxiety of women suspecting breast cancer.

Hence, there is a need to look for newer avenues which are not only efficient but also cost effective and non-invasive to establish new high-risk categories so that only the high risk group should go for screening.

In this regard, the present research work aims to look for novel tumor markers by cytogenetic analysis, biochemical analysis and anthropometric analysis of Breast Cancer patients compared to that of Healthy Individuals.

1.1 Chromosomal Aberration Assay

Chromosomal instability is a known feature of cancer and a number of aberrations, both numerical and structural are found in all types of cancer. The majority of cancer cells represent dynamic karyotypic changes, including chromosomal rearrangements (Guleria K et. al., 2005). A positive association between the frequency of chromosomal aberrations in peripheral blood lymphocytes (PBLs) and the risk of cancer at different sites has been supported by numerous clinical observations [Mathur R et. al., 2000]. Different case - control studies have also reported a significant increase in the frequency of aberrant cells in PBLs of cancer patients (Barios L et. al., 1991). The

discovery of Philadelphia chromosome (9;22 translocation, Nowell and Hungerford, 1960). was a breakthrough discovery in the field of cytogenetics which led to the development of cytogenetic analysis as a powerful tool for the diagnosis of some type of cancer. In case of Breast Cancer also chromosomal aberrations play a very important role and hence cytogenetic marker of breast cancer can be a very powerful diagnostic tool for this dreaded disease.

In this regard, it can also be useful in the study of Breast Cancer and if any specific chromosomal aberration pattern is indeed found, it can be used for screening females who are at high risk to develop this deadly disease and also for monitoring the prognosis and treatment response of Breast Cancer patients. Therefore, it is essential to assign, role of cytogenetic endpoints such as chromosomal aberrations for the early detection and further management of breast cancer.

2. Material and Method

2.1 Collection of Sample

Peripheral blood samples were collected from 55 registered Breast Cancer patients of age 18-70 years from the ward and Pathology Department of Jawaharlal Nehru Cancer Hospital & Research Centre after obtaining patient informed consent other details such as Medical History, Family History, Ob/Gyn History and the sample were processed for cytogenetic analysis. Blood samples were also be collected and analyzed from age-matched healthy females for control study. For cytogenetic analysis, blood sample were cultured by following standard Lymphocyte Culture protocol (Moorehead et. al., 1964) and Karyotype were prepared to look for any chromosomal aberrations that might be identified in breast cancer patients.

2.2 Lymphocyte Culture

Lymphocyte cultures were set up by Moorhead *et. al.*, protocol. Heparinized whole blood (1 ml) was added to a mixture containing 5 ml of culture medium RPMI 1640 and 300 ul of phytohemagglutinin (PHA). Then the culture vials were kept in HERA cell CO₂ incubator for 72 hrs, at 37 °C with 5 % CO₂. Then 0.02% of Colchicine solution was added at 70th hour of incubation period to arrest cells at metaphase. The cells were collected by centrifugation, resuspended in a prewarmed hypotonic solution (KCl, 0.075 M) for 17 minutes and fixed in chilled methanol/acetic acid (3:1 v/v) solution (Carnoy's fixative). Then drops of cell suspension were allowed to fall from at least 6 inches height on pre chilled and chemically cleaned slides. These slides were air dried on a hot plate at 50-60 °C. All slides were labelled and stained with Giemsa stain and observed under microscope to look for well spread chromosome. The slides showing best metaphase plates were then selected for GTG Banding to prepare karyotype and study chromosomal aberrations. Slides were GTG banded according to the Benn and Perle method. Banded slides were scanned for numerical aberrations. For each subject, 50 clear metaphases were assessed for Chromosomal Aberrations. Of these, 10 metaphases were karyotyped.

2.3 Plasma Protein Electrophoresis

2.3.1 Material and Method

a) Sample Collection

The study was conducted after taking ethical approval from Institutional Human Ethical Committee (IEC No/01/06/18.5.13). Peripheral blood sample were collected in heparinized vacutainer tubes (VAKU-8, HMD Healthcare, UK) from the registered patients through Department Of Pathology, Jawaharlal Nehru Cancer Hospital & Research Centre, Bhopal. Plasma Protein Electrophoresis was performed within two hours after plasma separation.

b) Method

Plasma were electrophoresed after mixing the sample with Bromophenol Blue on cellulose acetate paper (Sartorius,

Germany) Electrophoresis unit (Bangalore Genei, Bangalore) at 70 V with Tris — Borate — EDTA (pH 8.3) buffer conditions by standard protocol. The bands were separated till the dye front reached the end of cellulose acetate paper and then stained with Ponceau S stain (SD Fine Chemicals, Mumbai) and destained with 7% acetic acid for 30 min. Plasma protein bands for each fraction were quantified for their absolute values in densitometer (Systronics, India).

2.4 Dermatoglyphics

2.4.1 Material and Method

a) Selection of subjects

Fifty five breast cancers female were selected from the hospital registry of JLNCH & RC at random. All the cases belonged to age group of age group 18-70 years. Fifty five age-matched healthy controls were also enrolled for comparison studies.

b) Dermatoglyphics

The method adopted for printing palm was modified ink method by **Purvis Smith** (1969). The materials used were printers, duplicating ink from, Cardboard roller, gauze pads and sheets of paper.

The patient and controls were asked to wash their hands with soap and water to remove grease and dirt and dried. Then ink was applied over the palm and fingers with a gauze piece and smeared thoroughly in light strokes. A sheet of paper was kept at the edge of the table. The finger ridges were printed starting from thumb to little finger in the same order. The fingertips were rolled manually to ensure the full prints of the ridges, then the palm was rolled on cardboard roller with paper taking care that the cupped regions of the palm were printed properly. A line was drawn from axial triradius 't' to the digital triradii 'a' and 'd' and all the three angles in the triangle were measured using a protractor.

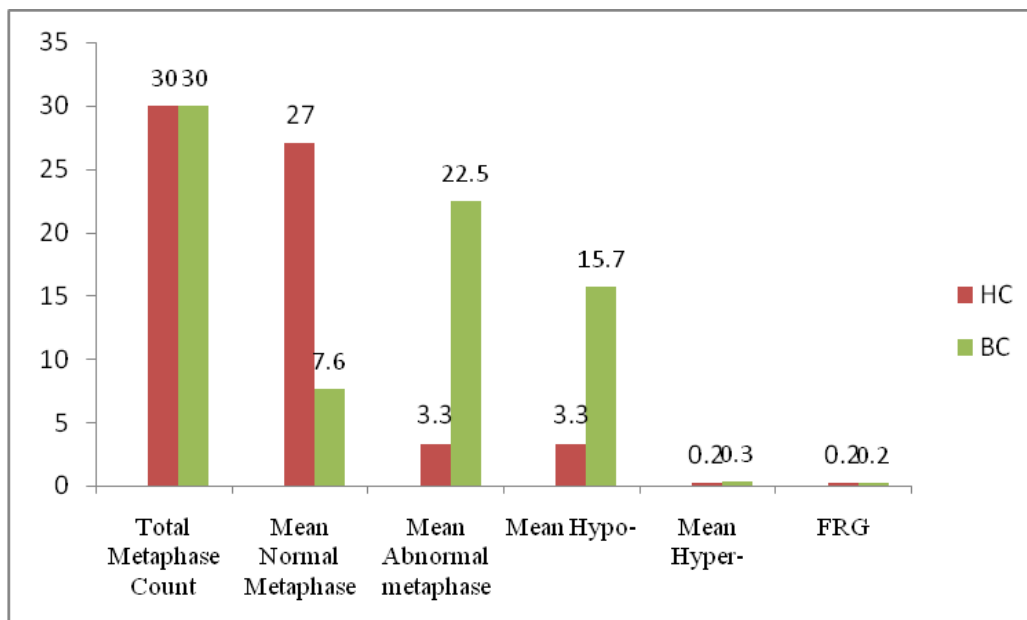
3. Result and Observations

A. Chromosomal Aberration Assay

Table 1: Mean Numerical Chromosomal Aberrations in Healthy Females and Breast Cancer patients

Code No.	Total Metaphase Count	Mean Normal Metaphase	Mean Abnormal metaphase	Mean Hypo-aneuploidy	Mean Hyper-Aneuploidy	FRG
HC (N=55)	30	27	3.3	3.3	0.2	0.2
BC (N=55)	30	7.6	22.5	15.7	0.3	0.2

HC: Healthy Control, BC: Breast Cancer patients, N=No. of Cases



Graph 1: Graph showing comparative numerical chromosomal aberrations in Breast Cancer Females and Healthy Females

Figures

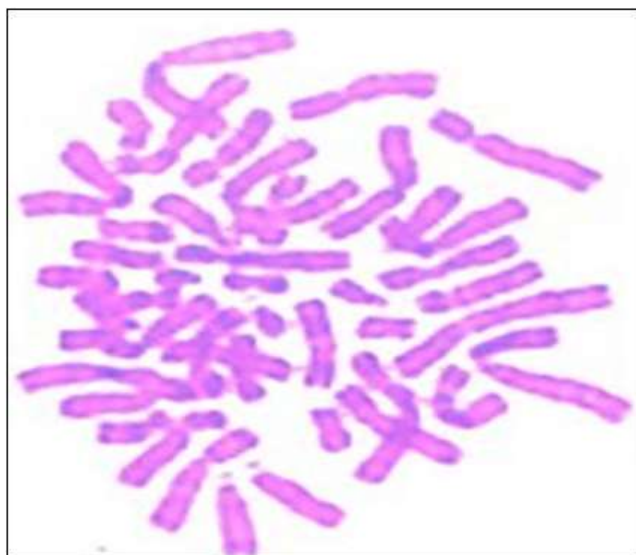


Figure 1: Normal Metaphase in Healthy Control Female (N=46)

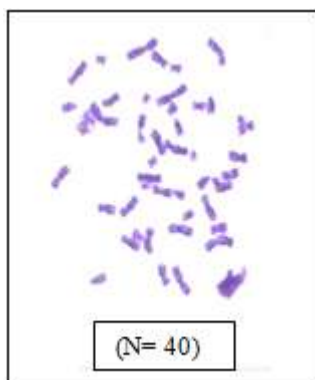


Figure 2: Hypodiploidy in Breast Cancer Females (N=40)

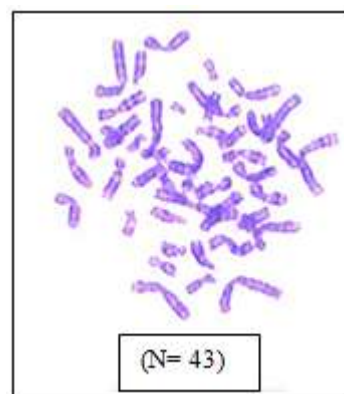


Figure 3: Hypodiploidy in Breast Cancer Females (n=43)

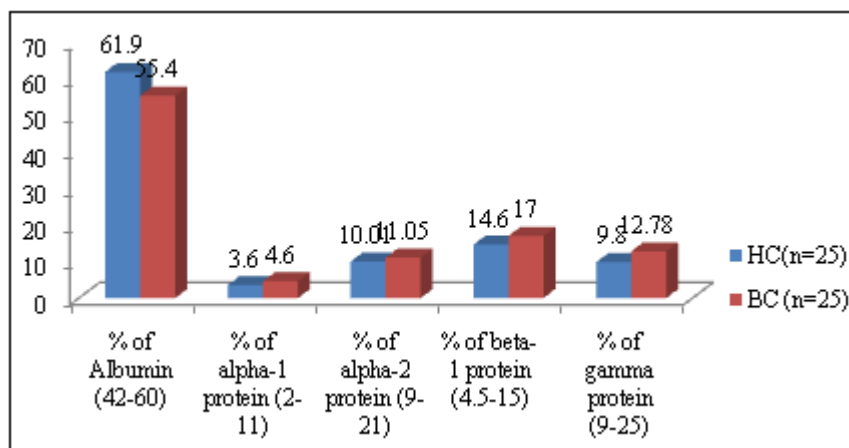
B. Plasma Protein Analysis

Table 2: Mean Plasma Protein Percentage found in Healthy Females and Breast Cancer patients

Group	Mean % of Albumin (NR:42-60%)	Mean % of α1 Globulin (NR:2-11%)	Mean % of α2 Globulin (NR:9-21%)	Mean % of β1 Globulin (NR:4.5-15%)	Mean % of γ Globulin (NR:9-25%)
HC(n=25)	61.9	3.6	10.01	14.6	9.8
BC (n=25)	55.4	4.6	11.05	17	12.78

NR: Normal Range, HC: Healthy Control, BC: Breast Cancer, N=No. of Cases

Graphs



Graph 2: Comparison of Plasma Protein status in Breast Cancer Patients and Healthy Control Females

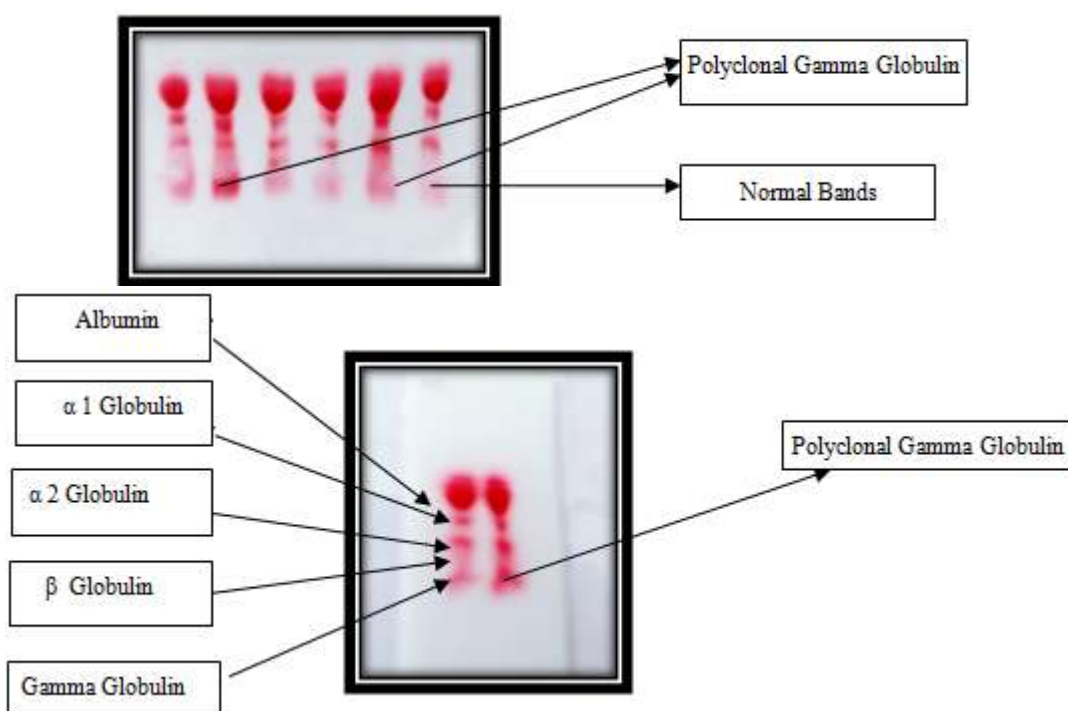


Figure 4: Electrophoretic Bands of Plasma Proteins in Healthy Control and Breast Cancer Females

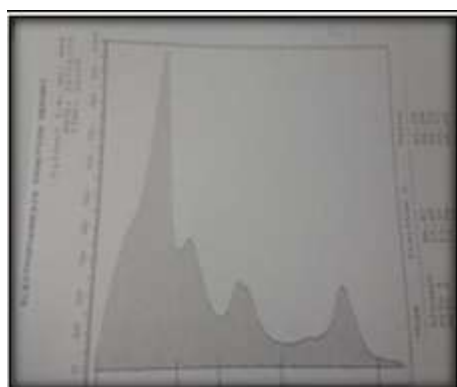


Figure 5: Normal Plasma Protein Pattern



Figure 6: Diffuse Plasma Protein Pattern

C. Anthropometric Analysis

Table 3: Mean "atd" angle of Healthy females and Breast Cancer Patients

Code no	Mean 'atd' angle
HC (N=55)	39°
BC (N=55)	48°

'atd' = Angle of Triradius, HC: Healthy Control, BC: Breast Cancer patients, N=No. of Cases

Figures



Figure 7: 'atd' angle of 55° in BC patient



Figure 8: 'atd' angle of 75° in BC patient

4. Results

Chromosomal Assay showed higher frequency of abnormal metaphases with numerical chromosomal aberrations observed in Breast Cancer patients as compared to Healthy Controls with most of the Breast Cancer patients showing hypoaneuploidy and fragments as shown in **Table 1** and **Figure 2-3**. Healthy females showed normal karyotype; however, very few subjects were presented with minimal numerical chromosomal aberration pattern (**Fig 1**).

Plasma protein electrophoresis (SPE) levels were studied in 25 patients with Breast Carcinoma and compared to that of Healthy Control females. It was found that beta globulin was raised in most of the patient (**Table 2**) while it was normal in healthy females (**Table 1**). Gamma globulin was found to be of polyclonal nature in Breast Cancer patients as shown in **Figure 4** and it can be used as an important indicator of the diseased condition.

Dermatoglyphic patterns of breast cancer patients enrolled in the present study were found to be a little on the higher side of $>42^\circ$ in case of breast cancer patients (**Fig 7 and 8**). The mean 'atd' angle of breast cancer patient was found to be 48° and that of Healthy Control was found to be 39° as shown in **Table 3**. Around 67.27% of Breast Cancer patients had angle $>40^\circ$ while only 16.36% of Healthy Control female had angle $>40^\circ$.

5. Discussion and Conclusion

Genetic instability is a defining feature of human cancer. In the present study, breast cancer patients had a significantly higher percentage of aberrant metaphases as compared with controls. There was a moderate frequency of numerical abnormalities in the cultured lymphocytes of patients. Thus, determination of the genomic instability level by chromosomal analysis can be used as a diagnostic tool for identifying females with high risk of developing Breast Cancer and also in monitoring disease progression and prognosis. Our study is supported by a similar study done by **Hinglaj Saha et al., 2013** in which they found a variety of Chromosomal Aberrations including aneuploidy, polyploidy, terminal deletions, acrocentric associations, chromosomal breaks and gaps, cluster of cells formation were seen in peripheral blood lymphocytes of Breast Cancer patients of West Bengal.

Plasma protein electrophoresis shows that each of the five serum protein groups moves at a different rate in an electrical field and together forms a specific pattern. This pattern helps identify diseases as diseased condition tends to alter these protein fractions considerably and is used clinically to diagnose diseases including Multiple Myeloma. Thus, it has the potential to be used to screen females prone towards breast cancer as the specific serum protein profile can be an indication towards developing breast cancer. It was found in our result that beta globulin was raised in 60% of Breast Cancer patient while it was within normal range in their healthy counter parts. This result is supported by a work done by **Beneditti Panici et al., 1986** in which they found Beta globulin raised in 73% of Breast Cancer patients. Alpha 1 protein was also raised in some breast cancer females while it was within normal range in the control. Similar result was obtained in a study done by **Lee YT et al., 1976**. Besides this, Breast Cancer females had gamma globulin of polyclonal origin which is generally the pattern in some inflammatory conditions, connective tissue disorders, ovarian cancer and liver diseases. Thus it can be said that Plasma Protein electrophoresis is an easy and cost effective method of diagnosing as well as monitoring Breast Cancer by analyzing the altered protein profile.

The anthropometric marker generated by this work by using dermatoglyphic tool of "atd" angle measurement has huge potential as a very easy, cost effective and non-invasive parameter to screen high risk females of developing Breast Cancer as shown by the result. Our work is supported by a similar work done by **P.E. Natekar et al., 2006** and **Shivaji B. Sukre et al., 2012** in which they performed different parameters of dermatoglyphics including "atd" angle on Breast Cancer patients and came to the same conclusion as ours of Breast Cancer patients having wider "atd" angle.

Future Scope: There is a need to look for novel tumor markers in the field of breast cancer research and the advancement of newer advanced techniques have opened a flood of opportunities to analyze different aspects of this disease to generate novel markers. Sensitive and specific and preferably cost effective tumor markers are the need of the hour to combat this deadly disease and the present work is a huge step in that direction as it suggests cost-effective and non-invasive or minimally invasive method of diagnostic screening of breast cancer. The easy methodologies used are not only cost effective but also non-invasive as in the case of Dermatoglyphic and minimally invasive in case of cytogenetic and plasma protein analysis and hence are bound to convince a number of females to undergo screening who otherwise harbor a lot of anxiety and fear for the established methods of diagnostic screening like mammography. These techniques and tools have a huge potential to assist the established techniques in screening for the disease. Further long term and follow-up studies with large sample size are required to validate the suggested tumor markers further which have the potential to be used as promising markers.

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