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## ENZYMES AND ANTIOXIDANT AS BIOMARKERS IN BREAST CANCER

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### ABSTRACT

**Objectives:** Current study was undertaken to determine the clinical utility of enzyme biomarkers like Alkaline phosphatase and Lactate Dehydrogenase as well as antioxidant like uric acid in diagnosis, prognosis and metastasis.

**Methods:** 50 clinically and histopathologically confirmed female breast cancer patients of the age group of 30-65 years served as cases and 50 normal healthy females in the same age group served as controls. The parameters were estimated by standard biochemical methods.

**Results:** Serum Alkaline Phosphatase and Lactate Dehydrogenase levels are significantly higher in cases than controls. Where as uric acid levels are non significantly increased in cases.

**Conclusions:** Significant increase of serum enzymes Alkaline phosphatase and Lactate Dehydrogenase along with simultaneous non significant increase of uric acid was observed in the study. These biomarkers can be utilized in remote areas where smaller labs operate at low cost and unexposed to sophisticated knowhow.

**Keywords:** Breast cancer, Alkaline phosphatase, Lactate Dehydrogenase, Uric Acid, Biomarker

### INTRODUCTION

Breast cancer is the most devastating cancer for women though for that matter any cancer is catastrophic. This breast cancer doesn't spare women of either developed or developing countries. It affects both countries and in developing countries like India higher than 40% of total breast cancers are prevalent. [1,2]

Oxidative stress causing cellular injury has been implicated in the initiation and progression of cancer.[3] Animal and human studies support the part of oxidative stress in the causation of breast cancer.[4,5,6] Antioxidants help in scavenging, disposal and inhibit the production of free radicals or oppose their action and increase with the degree of the disease. [7]

Current study is done to reveal the changes in oxidant antioxidant status in breast cancer patients

by estimating uric acid an antioxidant. Lactate Dehydrogenase mediates conversion of pyruvate to lactate which is the bottle neck step of anaerobic glycolysis. Increased rates of glycolysis is found in tumour cells producing more amount of lactate by upregulation.[8] Cancers have elevated rate of glycolysis causing high Lactate Dehydrogenase levels. High Lactate Dehydrogenase may mark presence of neoplasia.

Alkaline phosphatase is a serum hydrolase enzyme causing removal of phosphate group from nucleotides and proteins in an alkaline medium. Though present in all tissues, it is predominantly concentrated in liver, bile duct, kidney, bone and placenta. Osteoblasts produce high quantity of enzyme whenever there is bone repair activity like bone metastasis. Even a mild biliary obstruction is indicated by ALP which implicates liver

involvement. Half of all breast cancers go for distant metastases.[11]

Current study was undertaken to determine the clinical utility of enzyme biomarkers like Alkaline Phosphatase and Lactate Dehydrogenase as well as antioxidant like uric acid in diagnosis, prognosis and metastasis.

## MATERIALS AND METHODS

Our study consisted of 50 breast cancer women and 50 apparently healthy women who are age matched with them were selected. The cancer patients were from Manipal Super Specialty Hospital and City Cancer Centre, Vijayawada. Controls were randomly selected women attending the above hospital. The duration of study was from 2011-2012. Institutional ethical clearance was obtained and informed consent was taken from patients.

**Inclusion criteria:** freshly diagnosed female breast cancer Patients and controls in the age group 30-60 years.

**Exclusion criteria:** Female Patients or controls suffering from tuberculosis, rheumatic fever, hemolytic anemia, hypertension, diabetes mellitus, hepatitis, jaundice, pregnancy or breast feeding, bone diseases, pancreatic disease, congestive cardiac failure, myocardial infarction, ulcerative colitis, other malignancies and patients who had already received or were under treatment for malignancy were excluded from study.

Clinical investigations and questionnaires formed the basis of enquiry.

### Collection of blood

Under strictly aseptic conditions 5ml of fasting venous blood was drawn from median CUBITAL/BASILIC vein into BD red capped plain Vacutainers. Vacutainers were made to stand for 10 min at room temperature to allow clotting. Later they were centrifuged at 3000rpm for 10 minutes using Remi8RC centrifuge. Serum was separated and parameters assayed on same day.

The parameters were estimated by UV- Visible Spectrophotometer CHEM 7 [manufacturer TransAsia] using Tulip diagnostics (P) Ltd kits.

### Alkaline Phosphatase: pNPP method

ALP at alkaline pH hydrolyses p-nitrophenylphosphate to p-nitrophenol and phosphate. The rate of formation of p-nitrophenol is measured as an increase in absorbance which is proportional to the Alkaline phosphatase activity measured at 405nm

### Lactate Dehydrogenase: Modified IFCC method

Lactate Dehydrogenase catalyses the reduction of pyruvate with NADH to form NAD. The rate of oxidation of NADH to NAD is measured as decrease in absorbance which is proportional to activity of Lactate Dehydrogenase. The wavelength used is 340nm.

### Estimation of uric acid Uricase method

Uricase converts uric Acid to allantoin and hydrogen peroxide. The hydrogen peroxide formed further reacts with phenolic compounds and 4-aminoantipyrine by the catalytic action of peroxidase to form a red colored quinonimine dye complex. The intensity of color formed is directly proportional to the quantity of uric acid present.

### Statistical method

The results were tabulated and analyzed by SPSS software version 16.0 using Independent samples T Test.

## RESULTS

Significant stepping up of enzymes Alkaline Phosphatase ( $p=0.08$ ) and Lactate Dehydrogenase ( $p=0.02$ ) observed in cases than controls in association with non significant rise of uric acid in cases.

## DISCUSSION

Reactive oxygen species (ROS) imbalance generation causes the promotion and progression of breast cancer. [12] Human body encounters these free radicals by enzymatic and non enzymatic antioxidant systems.[13] Purine metabolism end product is uric acid. It being an

antioxidant scavenges free radicals there by reducing oxidative stress. [14, 15, 16]

Uric acid enhances human life span by protecting against oxidative stress induced aging and cancer. Our study showed non-significant higher levels of uric acid in breast cancer patients. Greater amounts of serum uric acid observed in previous studies establish the protective action against oxidative stress.[17,18]. The elevated levels of serum uric acid may be a compensatory mechanism to regulate the increased oxidative stress.

Lactate Dehydrogenase exists in five isoenzymes in human tissue. It consists of four monomers composed of two major subunits M and H encoded by Ldh -A and Ldh -B. Ldh-A is mainly expressed in neoplastic cells was revealed by a immunohistochemical study. This property of Ldh-a can be exploited as a biomarker for malignancies.[19,20]

Ldh -A the important isoform is expressed in breast tissue. It essays a major role in glycolysis, growth properties as well tumour maintenance of breast cancer cells.[21]. Its activity is up regulated in cancer tissue and serum of breast cancer patients.[22] The up regulation gene for Ldh-A causes its elevated levels in serum. High levels of serum LDH enzyme activity is because of the rupture of cell membrane of a major portion of dividing malignant cells whose metabolism is characterized by anaerobic glycolysis.[23]

Our study revealed significant elevation in the serum Lactate Dehydrogenase in breast cancer patients than controls is in accordance with these studies.[24,25,26,27]

Alkaline Phosphatase is a very sensitive indicator of liver affection. High serum levels indicate bone or liver metastases. [28] Breast cancer death autopsies reveal 55-75% of them had hepatic metastases.[29] Patient survival relies mainly on hepatic metastases.[30] Hence early diagnosis of liver metastases leads to better prognosis by timely treatment.

CT, MRI, PET support in diagnosing liver metastases originating from breast cancer.[31,32] Symptoms of hepatic pathology like jaundice, hepatomegaly, ascitis are found at a later stage of involvement and they bring about worst prognosis. [33]

Our study had significant increased serum Alkaline Phosphatase in women affected with breast cancer is supported by these studies.[34,35,36].

## CONCLUSION

Our study demonstrated significant increase of serum enzymes Alkaline Phosphatase and Lactate Dehydrogenase along with simultaneous non significant increase of uric acid. Both these enzyme markers can be utilized as one of the biomarker in diagnosis of breast cancer in developing country like India and in remote areas where smaller labs operate at low cost and unexposed to sophisticated knowhow.

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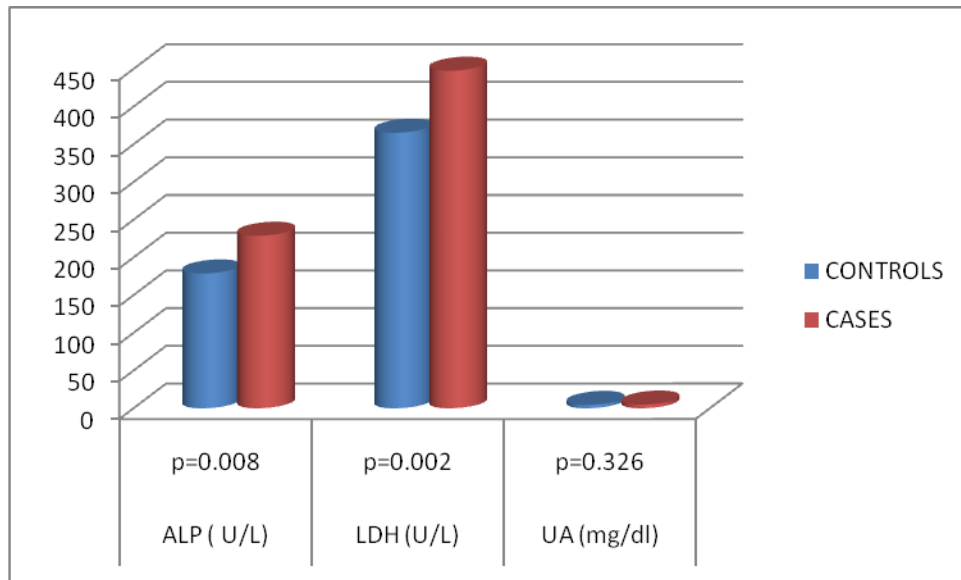
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**Table: Group statistics comparing results between breast cancer cases and controls cases**

| Parameter         | Groups   | N  | Mean  | Standard Deviation | Standard Error | P value |
|-------------------|----------|----|-------|--------------------|----------------|---------|
| ALP (U/L)         | Controls | 50 | 178.8 | 48.51              | 6.86           | 0.008*  |
|                   | Cases    | 50 | 228.8 | 120.77             | 17.07          |         |
| LDH(U/L)          | Controls | 50 | 365.4 | 66.13              | 9.35           | 0.002*  |
|                   | Cases    | 50 | 447.7 | 167.67             | 23.71          |         |
| Uric_Acid (mg/dl) | Controls | 50 | 4.5   | 1.11               | .16            | 0.326   |
|                   | Cases    | 50 | 4.9   | 2.75               | .39            |         |



P<0.05 significant

Position in text in results

**Figure: Graphical depiction of parameters outcome between breast cancer cases and controls**