

Serum Alkaline Phosphatase Level As A Better Predictor for Metastatic Breast Cancer in Comparison to Acid Phosphatase And Calcium Activities

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Abstract: Early detection of breast cancer can provide patients a wider range of therapeutic options as well as a higher success rate of therapy that lowers mortality. Metastatic breast cancer is the principal cause of breast cancer death worldwide. Change in serum levels of some biochemical parameters could assist diagnosis, prediction and follow-up of metastatic breast cancer. Total 235 human subjects were taken in the study. Out of which 100 normal age matched healthy subjects were considered as controls and 135 breast cancer patients subjects. Alkaline phosphatase and acid phosphatase activities were determined by p-Nitrophenyl Phosphate method with stabilized substrate and a Naphthylphosphate kinetic method in serum respectively. Calcium was estimated by Ortho-Cresolphthalein complexone method, end point in plasma. The statistical differences between cases and control were determined by student independent sample t-test and one way analysis of variance (ANOVA). In order to determine correlation, statistical analysis was carried out by using spearman's rank correlation coefficient. Alkaline phosphatase was found statistically significant ($p < 0.05$) while acid phosphatase and calcium were non-significant in breast cancer patients. Alkaline phosphatase activity gradually increases as advancement of stages which showed that it can be used as a predictor for breast cancer metastasis.

Keywords: Alkaline phosphatase, Acid phosphatase, Breast cancer, Calcium.

I. Introduction

Breast cancer is the most common malignant disease in women. In these patients, it is not the primary tumor, but its metastasis at distant sites are the main cause of death.^[1] The process of breast cancer metastasis includes tumor cell seeding, tumor dormancy and metastatic growth.^[2] Bone is the first site of distant disease in 25–40% of patients with metastatic breast cancer, and up to 60–80% of patients with recurrent breast cancer eventually show evidence of skeletal involvement.^[3] More than 50% of patients with breast cancer develop overt bone metastasis and many of these subsequently develop liver metastasis.^[4] Survival from breast cancer may be improved by early detection of metastasis, as their treatment is usually reserved for symptomatic or progressive disease. Breast cancer like any other disease condition is associated with derangement in the body's physiological functions, alterations in the homeostasis and production of some biochemical metabolites. Some studies have shown increased serum levels of calcium and elevated activities of alkaline phosphatase (ALP) and acid phosphatase (ACP) in patients with malignancies including metastatic breast cancer.^[5-8] Assessment of the serum levels of these biochemical parameters could substitute for the classical tumor markers in areas where sophisticated facilities for these are not readily available.

The abnormal blood chemistry results also suggest the spread of breast cancer to the bone, kidney or liver. The assessment of these parameters is also cost effective, non-invasive and can be done routinely.

II. Methodology

The present study has been carried out in the Department of Biochemistry and Department of Radiotherapy, G.R Medical College & J.A. Group of Hospitals, Gwalior. Total 235 human subjects were taken in the study. Out of which 100 normal age matched healthy subjects were considered as controls and 135 breast cancer patients subjects as cases which were further divided into their respective stages according to TNM classification. Out of total 135 breast cancer patients subjects there were 40 patients of stage I, 30 patients of stage II, 30 patients of stage III and 35 patients of stage IV.

Inclusion criteria of the study:-

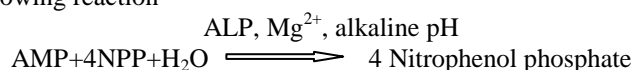
- Female patients (age >20 years) diagnosed with breast cancer.

- All patients with operable breast lumps and recurrent breast lump in a previously operated case of carcinoma breast.

Exclusion criteria of the study:-

- Pregnant women.
- Patients with benign breast diseases.
- Other diseases

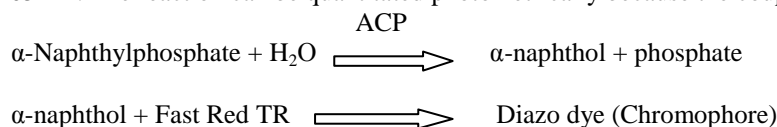
Before starting analysis, the written consent from all subjects was taken. The study has been approved by institutional ethical committee and was carried out by keeping all norms in mind. The clinical manifestations of disease, personal history of patients were recorded in study proforma. Blood sample was collected in plain vial and incubated at 37°C for 30 minutes. After incubation, clot was removed and remaining sample was taken in centrifuge test tube. Samples were centrifuged at 3000rpm for 10 to 20 minutes. Supernatant was collected in clean and dry serum test tube for alkaline phosphatase and acid phosphatase. Alkaline phosphatase was determined by p-Nitrophenyl Phosphate method with stabilized substrate. This method utilizes 4-nitrophenyl phosphate as the substrate. Under optimized conditions alkaline phosphatase present in the sample catalyzes the following reaction-



At the pH of the reaction, 4-nitrophenol has an intense yellow color. The reagent also contains a metal ion buffer system to ensure that optimal concentrations of Zinc and Magnesium are maintained. The metal ion buffer can also chelate other potentially inhibitory ions which may be present. The reaction is monitored by measuring the rate of increase in absorbance at 405 or 415 nm which is proportional to the activity of ALP in the serum.

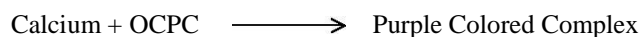
Acid phosphatase was determined by α Naphthylphosphate kinetic method in serum. In this method, α -Naphthylphosphate is hydrolyzed by serum acid phosphatase to α -naphthol and inorganic phosphate. The rate of hydrolysis is proportional to the enzyme activity present.

The α -naphthol produced is coupled with Fast Red TR to produce a colored complex which absorbs light at 405 nm. The reaction can be quantitated photometrically because the coupling reaction is instantaneous.



The blood samples collected in heparinized vial were centrifuged at 3000rpm for 15 minutes. Supernatant (plasma) was collected in clean and dry test tube for the estimation of calcium.

Calcium was estimated by ortho-cresolphthalein complexone method, end point in plasma. Calcium in an alkaline medium combines with o-Cresolphthalein Complexone to form a purple colored complex. Intensity of the color formed is directly proportional to the amount of calcium present in the sample.



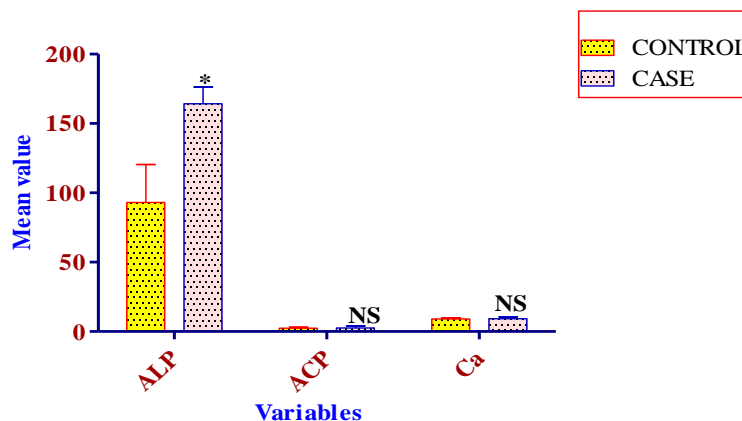
III. Statistical Analysis

The results were expressed as Mean \pm Standard Deviation. The statistical differences between cases and control were determined by student independent sample t-test and one way analysis of variance (ANOVA). Data analyses were performed with the Statistical Package for the Social Sciences, version 21.0 (SPSS, Chicago, Illinois, USA).

In order to determine correlation, statistical analysis was carried out by using spearman's rank correlation coefficient. The p value less than 0.05 were considered as significant.

IV. Results

Alkaline phosphatase (163.93 \pm 25.07) was found statistically significant (p<0.05) while acid phosphatase and calcium were non-significant in breast cancer patients as compared to control healthy subjects. (Graph 1)



Graph 1. Showing significant changes of biochemical parameters in healthy control subjects and breast cancer patients (case)

* Significant at 0.05(p<0.05), ^{NS} Non Significant

When all four stages were individually compared with control we found that alkaline phosphatase level was gradually increased with advancing stages. Following mean value of alkaline phosphatase level in different stages were observed in our study (Table 1)

Table 1: showing the mean value of Serum ALP level in different stages observed in the present study

Subjects	Mean ±SD of Serum ALP
Normal healthy controls	93.08±27.36
Stage I breast cancer patients	146.00±2.05
Stage II breast cancer patients	155.03±3.00
Stage III breast cancer patients	150.05± 5.25
Stage IV breast cancer patients	205.05±4.11

The one-way anova analysis between the biochemical parameters and all four stages in breast cancer patients showed that ALP was highly significant (p<0.001) in all stages as compared to other tumor markers. (Table 2)

Table 2: showing the one-way anova analysis between the biochemical parameters and all four stages in breast cancer patients

S.No.	Parameters	F	Sig
1	ALP	10.14	0.00**
2	ACP	1.53	0.21 ^{NS}
3	Ca	0.21	0.88 ^{NS}

** Highly Significant at 0.001 (p<0.001), * Significant at 0.05(p<0.05), ^{NS} Non Significant
ALP was also found positively correlated with the stages of breast cancer patients. (Table 3)

Table 3: showing correlation of all stages with biochemical parameters in breast cancer patients

With Stages			
S. N.	Markers	Pearson Correlation "r value"	P-value
1.	ALP	0.373	0.000**
2.	ACP	0.182	0.335 ^{NS}
3.	Ca	0.029	0.738 ^{NS}

** Highly Significant at 0.001 (p<0.001), * Significant at 0.05(p<0.05), ^{NS} Non Significant

V. Discussion

Alkaline phosphatase (ALP) is a serum enzyme whose total levels reflect the combined activity of several isoenzymes found in the liver, bone, kidney, and intestinal lining. In our study, alkaline phosphatase was found statistically significant (p<0.05) while acid phosphatase and calcium were non-significant in breast cancer patients as compared to control healthy subjects. A study conducted by the International Breast Cancer Study Group (IBCSG), ALP, aspartate transaminase (AST) and c-glutamyltransferase (GGT) were examined for their

sensitivity in detecting breast cancer recurrence, ALP alone was abnormal in a high proportion of breast cancer patients with bone and liver metastasis.^[9] Usoro N et al,^[10] reported the significant increase in serum calcium levels, ALP and ACP activities in breast cancer patients. Hypercalcemia and high ALP and ACP activities have also been reported in other malignancies.^[11-13] The hypercalcemia in breast cancer has been attributed by osteolytic bone metastasis and this accounts for 20-30% of the hypercalcemia cases in oncology patients. The skeletal invasion and destruction by tumor induced production of various cytokines and interleukins leads to increasing bone osteolysis^[14] and modification of the reabsorption, excretion and resorption of calcium and phosphate ion.^[15] ACP activity has been used to indicate that lysosomes participate in the execution of cell death in a variety of tissues and the regression of mammary carcinomas.^[5] Activities of lysosomal acid hydrolases have been demonstrated to be more marked in cancer cells than in homologous normal tissue.^[16,17] But in our study we did not find any significant elevation in serum calcium levels and ACP activities. This might be due to diagnostic insensitivity and non-specificity or supplementation and treatment regime of patients. Mishra S et al also found high level of ALP in breast cancer patients with metastasis as comparison to patients without metastasis.^[18] Many scientists reported elevated level of ALP in breast bone and liver metastasis.^[19-22] When all four stages were individually compared with control we found that alkaline phosphatase level was gradually increased with advancing stages.

This is in agreement with Singh AK et al.^[23] ALP levels were also found to be significantly positively correlated with advancing stages of breast cancer. Anova analysis between the biochemical parameters and all four stages in breast cancer patients also showed that ALP was highly significant ($p < 0.001$) in all stages as compared to other tumor markers.

Alkaline phosphatase [ALPs; orthophosphoric monoester phosphohydrolase (alkaline optimum) EC 3.1.3.1] are a group of phosphatidylinositol-anchored membrane proteins with wide substrate specificity.^[24,25] It comprises a group of enzymes that catalyze the hydrolysis of phosphate esters in an alkaline environment, generating an organic radical and inorganic phosphate.^[26,27] Elevation of serum ALP occurs because of the accelerated de novo synthesis of the enzyme and subsequent regurgitation into the serum

VI. Conclusion

We concluded from the study that the serum alkaline phosphatase level is a better predictor for metastatic breast cancer in comparison to acid phosphatase and calcium activities as its level gradually increases as the stages advances.

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