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STUDY OF ADENOSINE DEAMINASE IN BREAST CANCER

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ABSTRACT

Introduction: There are numerous growing evidences for the increased oxidative metabolism in cancer cells which is reflected by the enzyme activities of the cancerous cells. Increased metabolic activity alter the normal DNA and RNA metabolising enzymes like Adenosine Deaminase (ADA) along other ubiquitous enzymes like Alkaline Phophatase, the levels of which can be used for diagnosis and prognosis. **Material And Methods:** In present study 50 cases of breast cancer were subjected for the estimation of serum activities of adenosine deaminase(ADA), heat stable alkaline phosphatase, and alkaline phosphatase. The 50 cases of breast were divided in four sub-groups of stage I to stage IV according to four stages of TNM classification. The results were compared with enzyme activities in normal age, sex matched healthy adults as controls. The enzyme activities was compared between operated and unoperated cases and also in cases with metastases and those without metastasis. **Stastisical Analysis**: The statistical analysis was done by using students "t" test for cases and controls. Annova was applied to compare the enzyme activities in different stages. **Result:** The study revealed a highly significant elevation of enzymes ADA, in breast cancer cases($p < 0.001$)as compared to controls with stage wise increase in enzyme activity. Presence of heat stable alkaline phosphatase was found in 98% of cancer patients. Activities of ALK though were higher in cancer patients the increase was not statistically significant($p=0.5$). The values of ADA were correlating well with the clinical stage of disease unlike alkaline phosphatase. **Conclusion:** The result of study indicates the increased activities of of ADA in breast cancer as well it's correlation with the clinical stage of disease. The re-expression of HsALK in breast cancer patients was found to be significant.

KEYWORDS: ADA, ALK, Breast cancer, HsALK.

INTRODUCTION

Breast cancer is first in the list of major gynecological killers.[1] The alteration in the enzymatic status in various malignancies has been under study since last 60 to 70 years. The fact that uncontrolled growth of cancerous cells requires more enzymes in DNA and RNA metabolism has been made use in various studies. The uncontrolled growth of cancerous cells might affect the enzymes in nucleotide metabolism. Amongst them Adenosine deaminase is most commonly studied enzyme and found altered.(Ajaykumar Khanna, Sujit Yadev et al.1996, Borzenco 1991).[2,3]

It has been shown that in human breast carcinoma the level of fatty acids is two to three times that in normal mammary gland (Szucowitz A.,J.Kwiatkowskiet al 1979)[4] which is consistent with the fact that activities of many mitochondrial and cytoplasmic enzymes in cancer tissues are 10 to 100 folds higher than in normal tissues The fact that there is increased oxidative processes in cancer is known since last few decades.[5] Enzymes are simple and easily available mediators to tell the altered metabolic processes in body. Based on this fact numerous studies have been carried to study the altered enzyme status in normal and cancer cells and it's use in the diagnostic and prognostic evaluation of breast cancer. Availability of simple enzymatic parameters for diagnosis of the disease and metastases if available may prove beneficial for breast cancer patients. With this in mind the study was carried out to evaluate the levels of established serum enzymes in breast cancer patients and to compare their values with age, sex matched healthy volunteers and also to correlate the levels of these enzymes with various stages of the disease.

MATERIAL AND METHODS

A case control study was carried out on 50 female patients (GROUP -A) belonging to adult age group, admitted to Mayo General Hospital, Government Medical College Nagpur, and Rashtra Sant Tukdoji cancer hospital Nagpur, with diagnosis of breast cancer. After approval of ethical Committee The study was carried out in Clinical Laboratory of Biochemistry Department, Indira Gandhi Govt. Medical College and Hospital, Nagpur. The diagnosis of breast cancer was confirmed by FNAC. Routine investigations like

Hemoglobin percentage, Peripheral smear (P.S.), ESR, blood urea, blood sugar were as performed in the hospital. The FNAC (Fine needle aspiration cytology) was performed to confirm the diagnosis. Biopsy was done in some cases as needed. Ultrasonography of abdomen was done for liver and other metastases. X-ray, bone scans and brain C.T were performed in a few cases where brain /skeletal metastases were suspected. Control group consisted of 50 age sex matched healthy volunteers. The selection of volunteers was done from the healthy hospital staff employees and their relatives, who were willing to participate in the study. Exclusion criteria's were autoimmune disorders, arthritis etc.

5ml Fasting samples were collected from un-operated cases and age matched controls. The samples were centrifuged and serum was separated. The estimations of enzymes, Adenosine deaminase (ADA), heat stable Alkaline phosphatase (HsALK) and Alkaline phosphatase (ALK) were done within 24 hrs. All the cases having bone disorders, obstructive jaundice, tuerculosis, imuno-compromised diseases were excluded from the study. Samples were analysed for Estimation of ADA by colorimetric method of guisti.[6,7,8] Alkaline phosphatase was estimated by standard Kit provided by accucare diagnostic using phenyl phosphate as substrate.[9] and HsALK by temperature guarded inactivation analysis. Serum sample was kept in waterbath at 100˚C to inactivate all the iso-forms of alkaline phosphatase except heat stable isoform. Thereafter the estimation was done using phenyl phosphate as substrate. The results were tabulated for the statistical evaluation which was done on Microsoft windows 7 excel sheet by stepwise calculation of mean(m), standard deviation(SD), standard error of mean(SEM), and p- value was calculated by students t-test.[10] The level of significance was calculated between the enzyme activities of ADA, Alkaline phophatase amongst cases and controls. ANNOVA was applied to compare the activities of both the enzymes in different stages.

RESULTS

X-ray Chest was normal in all patients except one with lung metastases. USG was normal in 41 cases. In 9 cases with liver metastases. liver size had increased. Out of 50 cases, one died during the course of study.9 patients with distant metastases were undergoing chemotherapy plus radiotherapy. The age distribution shows maximum numbers of cases are in the age group of 45 to 54 year.

The activities of Alkaline phosphatase are slightly higher in cases than in controls but the increase is not stastistically significant (P = 0.05). The mean values of alkaline phosphatase in 9 patients with liver metastases were 109.3 IU/L, which was higher than controls. But again the increase is not significant. In the present study we could not get significant elevation of ALK activity in cases with liver metastases than those without liver metastases which may be due to small population (n=9) studied.

The mean values of ADA were found to be very significantly elevated in cases than in controls with P value less than 0.001. The activities of ADA, are correlating well with the clinical stage of the disease. ANNOVA The increase in ADA activity was significant in stage four as compared to stage two. No significant alterations were found in the activities of ADA in cases with metastases as compared with those without metastases.

In 50 controls studied we have found presence of heat stable alkaline phosphatase or placental isoenzyme in 4% of them whereas 96% of the cases were positive for heat stable alkaline phosphatase.

Study design of; Stagewise distribution of cases from stage 1 to stage IV.

Groups	No of cases	Clinical stage
Group I	3	Stage I
Group II	31	Stage II
Group III	7	Stage III
Group IV	9	Stage IV

Table No 1: Age distribution of cases.

Age group in year	No of cases	% of total cases
25 - 34	7	8%
35 – 44	15	32%
45 - 54	23	42%
55 and above	15	18%

Table no. 2: Mean enzyme activities in cases and controls.

Parameter	Mean values in cases	Mean values in controls
ALP	75.18 ± 29.17 #	67.3 ± 18.2
ADA	83.17 ± 60.40 **	7.4 ± 4
HsALP	Positive in ** 48cases	Positive in 2controls

*-significant

**-Highly significant
#-Not significant

Table No 3: Distribution according to stages

Parameter (IU/L)	Stage I	Stage II	Stage III	Stage IV
	n = 5	n = 29	n = 7	n = 8
ALK	71 ± 23	74 ± 47.5	72.4 ± 12.6	105 ± 29.15
HsAIP	Positive in 4 cases	Positive in 28 cases	Positive in all cases	Positive in all cases
ADA	46.7 ± 24	75.9± 62	103± 65.9	106.3 ± 48

Increase in activity of ADA is significant in stage IV as compared to stage II. Increase in activity of stage III not signigifant as compared to stage II.

Table No 4: Mean values in cases with and without metastases.

Parameter	Mean values in Liver metastases	Mean values in cases without liver metastases
ALP	100.23 ± 21.4	81.3± 25.2
A D A	92.1 ± 54.18'*	93.2± 61.15

'*' = P value significant.

Table No 5: Mean values in operated and unoperated cases

Parameter	Mean values in operated cases	Mean values in unoperated cases
ALP	70 ± 30.4	74
A D A	152.2 ± 8.07'*	75.9

'*' = P value significant

DISCUSSION

We could not get the significant elevation of ALK in cases as compared to controls. Our findings are similar to the study of Coombes et al (1981).wherein 94 patients of breast cancer with metastases the increase was insignificant.[11] Similarly in 387 cases studied by Kamby C. and Dirkson H.[12] the increase in the activity of ALK was not statistically significant. In a tangible study of Neo K. Kim and Walid G[13] although the increase was not significant in cases as compared to controls the increase was significant in 54 cases with distant metastases(liver or bone or brain metastases) than 40 cases without metastases. In the present study we could not get significant elevation of ALK activity in cases with liver metastases than those without liver metastases which may be due to small population (n=9) studied. Also the predominant form of alkaline phosphatase found in normal serum is of bone variety. The present study as was done in liver metastases, which may be other reason. In the study performed by Neculova M and Simikova activities of ALK were studied differently in cases with bone metastases and liver metastases. The increase was significant in cases with bone metastases unlike that of liver metastases.

Our findings for ADA are in accordance with the values of Walia M (1992)[14] et al who have also found the significantly (P <0.001) increased activity of ADA in 25 breast cancer patients. They have also found that values are decreased after surgery. Canabolt O. Durak I. et al (1996)[15], Borzenko B. G.(1999)[16] et al, Camici M, Tozzi M.G. et al (1990)[17] have also got similar results. We could not study the activities of ADA in cases after surgery. There is an increased catabolic process in cancer tissue. This metabolic alteration in cancer cells should lead to an increased synthesis of nucleotides in cancer cells. It might confirm selective growth advantages to neoplastic tissues leading to increased synthesis of ADA. They have also found decreased levels of serum adenosine deaminase after surgery. They have postulated that the increased anabolism in cancer cells may be an attempt of cancer cells to meet the increased metabolic need due to altered salvage pathway. Furthermore higher ADA activity might also play a part in detoxification process of high amounts of toxic adenosine and deoxyadenosine substrates produced from accelerated purine metabolism in tissues.

Nandini Seth et as 1976[18] have found significantly increased levels of ADA in mouse mammary cancerous tissues. They have postulated that Xanthine oxidase being very low in cancerous cells (Reid and Levin, 1957).[19] It is possible that an enzyme like Xanthine oxidase which is at terminal point in catabolism of pyrimidines is eliminated whereas these enzymes like ADA are ploughed for increased needs of tumors for nucleic acids.

The studies of Ajay Kumar Khanna et al 1996(3) are against our findings. They have not got the statistically significant alteration in the levels of ADA although the levels are high in cancer patients. It may be due to less study population ie 20 studied by them. The exact reason for this controversial result cannot be commented upon.

The presence of heat stable alkaline phosphatase or placental isoenzyme was found in 96% of the cases. Our studies are in accordance with the findings of Chang TC,

Wang JK, Hang MW et al (1994)[20] MC Dicken I.W., Stamp G.H., Mclaughlin P.J. et al (1983)[21], Kato M, Brijball D, Alder S.A et al (1992)[22], et al who have also found the presence of placental type or heat stable alkaline phosphates in 39 patients of breast cancer. The enzyme was not found in 35 healthy female individuals. (P< 0.001). The expression of placental alkaline phosphatase can be postulated to be oncofetal in development. Oncogenic alterations of the cancer tissue may be responsible for the re-expression of HsAlK which is generally found during fetal life.

Similar results were found by Murata T, Ihara S, Nakayama T et al (1999).[23] Statland BE.[24] Overall it can be said that As a result of increased oxidative metabolism in cancer tissue enzymes like ALK are increased in the cancer cells. Due to the encroachment of cancer cells over the surrounding parenchyma, enzymes leak out from the cells. This may be the possible reason for their increased activity in serum.

Increased synthesis of nucleotides, altered salvage pathway, increased, demands of nucleotides, more requirements for the catabolism of nucleotides may be responsible for increased synthesis of ADA and other nucleotide catabolising enzymes. Adenosine deaminase is found in high concentrations in T-lymphocytes. With the development of cancer, there is activation of cell mediated immunity which alters the enzymatic status of cancer cells. This may also be responsible for the increased activities of serum ADA. As an fetal enzyme, re-expression of heat stable alkaline phosphatase in cancer cells may be due to the altered genetic chemistry of the cancer cells during development of cancer.

CONCLUSION

The result of above study clearly illustrates the increased activities of serum ADA in breast cancer cases in a stage wise manner. Further studies are required to test the specificity and sensitivity of these parameters, there comparison with the other new markers of malignancies as a diagnostic and prognostic tool, and also there use to detect the recurrences after initial surgery.

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