RED BLOOD CELL ARGINASE AND SERUM ALKALINE PHOSPHATASE LEVELS IN BONE TUMOUR PATIENTS

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Introduction

Arginase is a urea cycle enzyme catalyzing the cleavage of L Arginine to ornithine and urea. Alkaline phosphatase is an enzyme that transfers a phosphate moiety from a donor compound to an acceptor compound at an alkaline pH. Intact human red blood cell may be involved in the delivery of ornithine to peripheral tissues especially bone and cartilage lacking arginase ..Alkaline phosphatase is present in almost all tissues of the body, that transfers a phosphate moiety from a donor compound to an acceptor compound optimally at an alkaline pH. Its serum level is elevated mainly in hepatobiliary and bone diseases .The elevation in bone diseases denotes the degree of osteoblastic activity .

In the present industrialized world the incidence of bone tumors is on an increase .Even though the changes in arginase is reported in many malignancies, no material is available in the literature for the assay of red blood cell arginase in bone tumor patients. Literature about the levels of serum alkaline phosphatase in the bone tumors other than osteosarcoma is limited .These facts have urged me to embark upon the study of these enzymes in bone tumour patients as tumor markers.

Materials and methods:

This study is conducted during the period of January 1998 and February 1999.

Thirty patients with bone tumours and twenty normal persons, who served as control, form the material of this study. The patients were admitted in the department of orthopedics and oncology, kasthurba medical college Mangalore.

2.5 ml of blood sample is collected in heparinized bottle for RBC arganase and another 2.5 ml is collected in plain bottles for serum alkaline phosphatase. Samples were collected before the treatment and 1 month after the treatment.

The samples were subjected to measurement of red blood cell arginase and serum alkaline phosphatase.

Outcome:

This study showed the following observations

Age incidence

Patients were grouped in to six age groups

Age group in years	Number
10 -20	3
21 -30	5
31 – 40	3
41 – 50	8
51 – 60	4
61 – 70	7
Total	30

Sex distribution

Males predominated in this series with 60% as compared to 40 % of females

Sex	Number
Male	18
Female	12
Total	30

Types of tumour

Tumour	Number
Secondaries in bone	12
Giant cell tumour	6
Multiple Myeloma	4
Malignant fibrous histiocytoma	2
Multiple Osteochondromatosis	2
Osteosarcoma	1
Chondrosarcoma	1
Osteochondroma	1
Simple bone cyst	1
Total	30

Secondaries in bone

Primary site	Number
Prostate	3
Cervix	2
Lung	2
Breast	1
Thyroid	1
Leiomyoma uterus	1
Bladder	1
Unknown	1
Total	12

Site involved by bone tumour

Site	Number
Vertebra	7
Multiple sites	8
Upper Tibia	4
Lower Femur	3
Upper Femur	3
Lower Tibia	2
Pelvic bone	2
Skull	1
Total	30

Treatment given

Primary treatment	Number
Surgery	12
Radiotherapy	8
Chemotherapy	5
Conservative	5
Total	30

RBC Arginase levels

Giant cell tumours

	Mean	Standard deviation
Pretreatment level	1.203	± 0.448 u/ml
Post treatment level	1.98	± 1.423 u/ml
Control level	2.628	± 1.829 u/ml

Secondaries in bone

	Mean	Standard deviation
Pretreatment level	1.1071	± 6.729 u/ml
Post treatment level	1.4315	± 0.952 u/ml
Control level	2.744	± 1.045u/ml

Multiple Myeloma

	Mean	Standard deviation
Pretreatment level	1.98	± 1.652u/ml
Control level	3.74	± 1.856u/ml

Other malignant tumours

	Mean	Standard deviation
Pretreatment level	1.231	± 0.506u/ml
Post treatment level	1.307	± 0.185u/ml
Control level	2.73	± 0.461u/ml

Benign tumours

	Mean	Standard deviation
Pretreatment level	3.415	± 2.148u/ml
Post treatment level	3.13	± 0.297u/ml
Control level	3.18	± 0.906u/ml

Alkaline phosphatase

Giant cell tumour

	Mean	Standard deviation
Pretreatment level	16	± 11.49u/ml
Post treatment level	10.268	± 2.765u/ml
Control level	5.44	± 0.724u/ml

Secondaries in bone

	Mean	Standard deviation
Pretreatment level	13.287	± 3.168u/ml
Post treatment level	19.253	± 9.729u/ml
Control level	8.558	± 3.026u/ml

Multiple myeloma

	Mean	Standard deviation
Pretreatment level	13.175	± 1.556u/ml
Control level	7.9	± 2.62u/ml

Malignant fibrous histiocytoma

Pretreatment level	22.8u/ml
Post treatment level	expired
Control level	5.04u/ml

Osteosarcoma

Pretreatment level	16.07u/ml
Post treatment level	20.02u/ml
Control	8.7u/ml

Chondrosarcoma

Pretreatment level	8.4u/ml
Post treatment level	8.04u/ml
Control	9.8u/ml

Benign tumours

	Mean	Standard deviation
Pretreatment level	10.358	± 3.567u/ml
Post treatment level	11.72	± 1.810u/ml
Control	7.38	± 1.763

All giant cell tumors patients showed a decrease in the enzymes level and follow up sample showed an increase in the level in 83%. 91% of patients with secondaries in bone showed a decrease in the level of enzyme and follow up showed no significant elevation .75% of multiple myeloma patients showed a decrease .Other malignant tumor patients also showed a decrease in enzyme level. Benign tumor patients showed no difference from the controlled group.

Alkaline phosphatase levels

83% of giant cell tumor patents showed elevated levels and declined with treatment.75% of patients with secondaries in bone showed increase in pre treatment and further increase in follow up. All patients with multiple myeloma showed marginal elevation. In other malignant tumors ALP level was high in initial and follow up sample. Benign tumors showed minimal increase in both samples.

Analysis:

The pre treatment level is compared with the post treatment level using wilcoxons signed rank sum test. The other comparison used mann.whitney –U- test.

RBC Arginase:

	Pre treatment vs. Control		Pretreatment vs. Post treatment	
Type of tumour	p value	Significance	p value	Significance
Giant cell tumour	0.05	Significant	0.05	not significant
Secondaries in bone	0.0004	highly significant	0.9453	not significant
Multiple myeloma	0.833	not significant		
Other malignant tumours	0.323	significant	1	not significant
Benign tumours	0.7728	not significant	0.5	not significant

Alkaline phosphatase

	Pre treatment vs. Control		Pre treatment vs. Post treatment	
Type of tumour	p value	Significance	p value	Significance
Giant cell tumour	0.1	not significant	0.3125	not significant
Secondaries in bone	0.0055	highly significant	0.0977	not significant
Multiple myeloma	0.0202	significant		
Other malignant tumours	0.288	not significant	0.05	not significant
Benign bone tumours	0.1489	not significant	0.5	not significant

Discussion:

Arginase is urea cycle enzyme which catalyzes the cleavage of L arginine to ornithine and urea. Ornithine is used for the production of proline and polyamines . Bone and cartilage is deficient of arginase . It is proposed that ornithine produced in RBC defuse to plasma and taken up by tissues devoid of arginase . Arginase is found to be elevated in many malignancies like colorectal carcinoma , carcinoma breast ,neurinoma , glioma ,gastric carcinoma and prostatic carcinoma. No material is available in the literature for the assay of RBC arginase in bone tumour patients.

Alkaline phosphatase is an enzyme which catalyzes the splitting of phosphoric acid from monophosphoric acids at an alkaline pH. The usefulness of this enzyme assay as a diagnostic and prognostic tool dates back to more than half a century. Its level is elevated mainly in hepatobiliary diseases and bone diseases .In bone disease it is released from osteoblast during the process of new bone formation. Its level helps in the prediction of prognosis of bone tumours especially osteosarcoma .literature about the levels in other bone tumours is not available.

No control study is available to compare the results of my study of RBC arginase levels in bone tumours .The reports of the works done in various other tumours showed elevated levels. A study in colorectal carcinoma by Shuh-Yan-Len (1991) found six times elevation of this enzyme in serum and two times elevation in tumour tissues. Straus et al (1991) analyzed the levels in carcinoma breast patients and found a preoperative elevation in 85% of patients and also found an abrupt decrease in one week.. The level comes to normal in 15 to 30 days. WacN.etal in 1992 showed elevated levels in gastric carcinoma and suggested the role of this enzyme in the spred of gastric carcinoma. In 1993 Vafa A.Z reported elevated level in prostatic fluid and prostate tissue with carcinoma .

In contrast to the to the studies done in tumours other than bone tumours , showing the elevation of the arginase my study showed a general decrease in the activity. The general decrease in tumours other than benign nature and the increase towards control level after initiating treatment is very interesting. The elevated levels in tumours other than bone tumours are explained by the fact that the tissues need poly amines for the tissue growth. The exact etiology of decrease found in my study is not known. I propose that this may be due to the inhibition of arginase activity in RBC by some inhibitors released from malignant bone tumour tissue.

The alkaline phosphatase activity is increased in bone tumours when the osteoblast is more actively laying down osteoid. LiceK.S and Leuing in 1996 found elevated level of ALP in 20 patients with osteosarcoma. Thorep.w (1978) followed up 30 patients with osteosarcoma and found normal preoperative level in 43% patients.

After surgery the level came to normal with in two weeks. Berruh et al (1996) reported increased levels in metastatic disease. Tobach (1969) found elevated level of ALP in patients with carcinoma lung and colon. Serum ALP level in most of the malignant bone tumours were high but not specific. Its level was influenced by the presence of pathological fracture.

SUMMARY AND CONCLUTION

Red blood cell arginase and serum alkaline phosphatase levels from bone tumour patients were analyzed to predict the prognosis and their usefulness as a tumour marker.

A general decrease in red blood cell arginase is found in almost all bone tumours. The decrease is proportional to the severity of the disease process. A persisted marked decrease denotes a bad prognosis. The increase in the level indicates a positive response to the treatment . Serum alkaline phosphatase is found to be raised in almost all bone tumours. A decrease in its level is noticed in response to the treatment.
Red blood cell arginase and serum alkaline phosphatase are not specific for any bone tumour. But their estimation helps in the follow up of patients to assess the response to the treatment and to detect the recurrence.

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