

# Cholinesterase in osteosarcoma

Simmi Kharb, Zile Singh Kundu<sup>1</sup>, Sandeep Kumar

Departments of Biochemistry and <sup>1</sup>Orthopaedics, Pt. B.D. Sharma, PGIMS, Rohtak, Haryana, India

## ABSTRACT

**Background:** Several biochemical markers have been proposed to have diagnostic and prognostic value in the management of osteosarcoma. Cholinesterase affects cell proliferation, differentiation and responses to various insults, including stress, and might have functional role in bone tissues. Status of serum cholinesterase levels in osteosarcoma is not clear. Hence, the present study was planned to analyze the status of cholinesterase in patients with osteosarcoma. **Methods:** Serum cholinesterase levels were analyzed in 30 cases of osteosarcoma and 30 patients with musculoskeletal pain. **Results:** Serum calcium and alkaline phosphatase levels were significantly raised with osteosarcoma (group I) as compared with controls (group II) ( $P < 0.01$ ). Serum phosphorus levels were lower in group I as compared with group II and the difference was not statistically significant ( $P > 0.05$ ). Serum cholinesterase levels were decreased in osteosarcoma patients (group I) as compared with the patients with musculoskeletal pain (group II,  $P < 0.05$ ). **Conclusion:** Findings of low levels of serum cholinesterase levels in the present study demonstrate that cholinesterase secreted by osteoblasts is consumed in bone formation and tumorigenesis.

**Keywords:** Alkaline phosphatase, calcium, cholinesterase, osteosarcoma

## INTRODUCTION

Osteosarcoma is defined as a malignant mesenchymal tumor in which the cancerous cells produce bone matrix. Osteosarcomas develop at sites of greatest bone growth, where bone cell mitotic activity is at its peak. Osteosarcoma occurs in all age groups. Various etiological factors for osteosarcoma include ionizing radiation, chemicals (fluoride, beryllium and vinyl chloride), viruses and family history of bone disorders and bone cancers. Several biochemical markers have been proposed to have diagnostic and prognostic value in the management of osteosarcoma, namely, vascular endothelial growth factor (VEGF), bone alkaline phosphatase (BALP), osteocalcin, survivin, ErbB<sub>2</sub>, Ki67 antigen and alpha V integrins.<sup>[1]</sup>

Cholinesterases (ChE) are defined as enzymes, which catalyze the hydrolysis of choline esters, the most important of which is acetylcholine (ACh).<sup>[2]</sup> Apart from its catalytic function, acetylcholinesterase (AChE) affects

cell proliferation, differentiation and responses to various insults, including stress, and might have functional role in bone tissues.<sup>[3,4]</sup> Serum levels of ChE are reduced in acute hepatitis, cirrhosis of liver, organophosphate poisoning and in some malignant tumors.<sup>[3]</sup> Osteoblast-derived AChE has been reported as novel mediator of cell-matrix interactions in bone.<sup>[5]</sup> Immunohistochemical localization in tissue sections provided evidence that AChE is a novel bone matrix protein, capable of mediating cell-matrix interactions, and as such may be a principal participant in organized bone formation and the regulation of remodeling.<sup>[6,7]</sup> To the best of our knowledge, there is no study where serum ChE levels are analyzed in osteosarcoma.

Hence, the present study was planned to analyze the status of ChE in patients with osteosarcoma.

## MATERIALS AND METHODS

The present study was conducted in the Department of Biochemistry in collaboration with Department of Orthopaedics, Pt. B.D. Sharma, PGIMS, Rohtak. Sixty patients attending the Orthopaedics Clinic were selected for the study. They were divided into two groups of 30 each. Histopathologically confirmed cases of osteosarcoma (localized without metastasis) of all the ages were included in group I. In all these cases, X-ray chest, computed tomography scans of thorax/whole body and bone scan were performed to rule out metastasis. These patients were

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**Address for correspondence:** Dr. Simmi Kharb, #1447, Sector-1, Rohtak – 124 001, Haryana, India. E-mail: [simmikh@rediffmail.com](mailto:simmikh@rediffmail.com)

compared with group II as controls, which included age and sex-matched 30 patients with musculoskeletal pain.

Five mL of venous blood was collected aseptically from the antecubital vein, and serum separated by centrifugation and analyzed the same day. Routine investigations, namely, hemoglobin, serum alkaline phosphatase, serum calcium, serum phosphorus, radiographic examination of bone tumor site and serum ChE<sup>[8]</sup> were analyzed.

SPSS ver. 18 was used for various statistical analyses. Comparison of data between groups was done using Student's 't' test. Correlations between groups were analyzed using Pearson correlation coefficient (r) formula.

Serum calcium levels were significantly raised with osteosarcoma (group I) as compared with controls (group II) ( $P < 0.01$ ). Serum phosphorus levels were lower in group I as compared with group II and the difference was not statistically significant ( $P > 0.05$ ). Serum alkaline phosphatase levels were significantly raised in patients with osteosarcoma (group I) when compared with patients of musculoskeletal pain ( $P < 0.01$ ).

Serum ChE levels were decreased in osteosarcoma patients (group I) as compared with the patients with musculoskeletal pain (group II,  $P > 0.05$ ).

## DISCUSSION

Osteosarcoma, along with other tumors like osteoma, osteoid osteoma and osteoblastoma, is grouped as bone-forming tumors. The stromal mesenchymal cancer cells resemble osteoblasts and produce unmineralized bone or osteoid/bone matrix. The mean calcium levels in the present study in osteosarcoma were significantly higher ( $P < 0.05$ ) when compared with group II. Hypercalcemia leads to a decrease in PTH secretion by its action on the PT cell calcium receptor and no decrease in PTH mRNA levels. There is now convincing evidence that phosphate regulates the PT, independent of its effect on serum calcium and 1,25-dihydroxyvitamin D<sub>3</sub> [1,25(OH)<sub>2</sub>D<sub>3</sub>].<sup>[9]</sup> The levels of phosphorus were also comparable between both the groups with no significant differences ( $P > 0.05$ ). Farley *et al.*, demonstrated that skeletal alkaline phosphatase (ALP) in human osteoblast line cells is regulated by phosphates, phosphate esters and phosphate analogs, and release of ALP activity is inversely regulated by calcium.<sup>[10]</sup> In the present study, serum ALP was significantly raised in osteosarcomas when compared with controls ( $P < 0.01$ ). In osseous tissue, ALP is involved in calcification of bone matrix and with protein synthetic activity that is associated with bone matrix production. In the present study, a negative correlation was observed between ALP and serum calcium levels in

osteosarcoma ( $r = -0.216$ ,  $P > 0.05$ ) while the control group showed insignificant positive correlation in control group II ( $r = 0.143$ ,  $P > 0.05$ ). Studies have shown that serum alkaline phosphatase levels are elevated in 40–80% of patients with osteosarcoma, and increase of this enzyme is due to increase in osteoblastic activity and has a prognostic significance.

The non-neuronal cholinergic system has been identified in several tissues including keratocytes, cancer cells, immune cells, urinary bladder, airway epithelial cells, vascular endothelial cells and reproductive organs.<sup>[2]</sup> This non-neuronal cholinergic system is known to be involved in the regulation of function and that cholinergic dysfunction is related to pathophysiology of certain diseases.<sup>[3]</sup> Human AChE is expressed in osteoblasts and chondrocytes in a manner dependent both on their state of proliferation and differentiation.<sup>[11]</sup>

Nicotinic modulation of gene expression in osteoblast cells, MG-63, has been reported by Rothem *et al.*<sup>[12]</sup> Osteoblast-derived AChE has been reported as a novel mediator of cell-matrix interactions in bone. In the present study, serum ChE levels were decreased in osteosarcoma patients (group I) as compared with the patients of musculoskeletal pain (group II) [Table 1].

Bessmel'tsev *et al.*, demonstrated the therapeutic benefits of serum ChE levels to detect relapse and to verify response to chemotherapy in multiple myeloma patients. Initial low value of ChE detected in patients of multiple myeloma points showed that it has pathological role to play in tumors.<sup>[3]</sup> No study is available regarding ChE levels in osteosarcoma.

Since AChE expression occurs at sites of new bone formation and is regulated by osteogenic stimuli,<sup>[13]</sup> findings of low levels of serum ChE levels in the present study demonstrate that AChE secreted by osteoblasts is consumed in bone formation and tumorigenesis. AChE inhibitors have been reported to decrease osteoblastic adhesions in cultures of MC3T3-EI cells and HOBs.<sup>[14]</sup>

The study suggests that these parameters can serve as useful markers for diagnosis and follow-up of disease, and cholinergic inhibitors, along with newer antifolate derivative and drugs targeting FR $\alpha$  proteins, may be of

**Table 1: Serum calcium, phosphorus and cholinesterase concentrations in both the groups (mean  $\pm$  S.D.)**

Parameter	Group I	Group II
Serum calcium (mg/dL)	10.01 $\pm$ 0.58**	9.46 $\pm$ 0.70
Serum phosphorous (mg/dL)	4.41 $\pm$ 0.55+	4.58 $\pm$ 0.51
Serum alkaline phosphatase (U/L)	331.73 $\pm$ 173.69**	120.23 $\pm$ 31.78
Serum cholinesterase ( $\mu$ kat/L)	112.60 $\pm$ 40.13+	127.41 $\pm$ 28.87

\*\*  $P < 0.01$  as compared to group II

value in treatment of osteosarcoma in future.

## REFERENCES

1. Bajpai J, Sharma M, Sreenivas V, Kumar R, Gamnagatti S, Khan SA, *et al.* VEGF expression as a prognostic marker in osteosarcoma. *Pediatr Blood Cancer* 2009;53:1035-9.
2. Kawashima K, Fujii T. Basic and clinical aspects of non-neuronal acetylcholine: Overview of non-neuronal cholinergic systems and their biological significance. *J Pharmacol Sci* 2008;106:167-73.
3. Bessmel'tsev SS, Rybakova LP, Gritskevich NL, Golota GZ, Blinov MN, Abdulkadyrov KM. Diagnostic and prognostic significance of blood-serum ceruloplasmin, acetylcholinesterase and total proteolytic activity in patients with multiple myeloma. *Vopr Onkol* 1999;45:398-404.
4. Grisaru D, Lev-Lehman E, Shapira M, Chaikin E, Lessing JB, Eldor A, *et al.* Human osteogenesis involves differentiation-dependent increases in the morphogenetically-active 3'alternative splicing variant of acetylcholinesterase. *Mol Cell Biol* 1999;19:788-95.
5. Genever PG, Birch MA, Brown E, Skerry TM. Osteoblast-derived acetylcholinesterase: A novel mediator of cell-matrix interactions in bone? *Bone* 1999;24:297-303.
6. Layer PG, Willbold E. Novel functions of cholinesterases in development. *Physiol Dis Prog Histochem Cytochem* 1995;29:1-94.
7. Inkson CA, Brabbs AC, Grewal TS, Skerry TM, Genever PG. Characterization of acetylcholinesterase expression and secretion during osteoblast differentiation. *Bone* 2004;35:819-27.
8. Proposal of standard methods for the determination of enzyme catalytic concentrations in serum and plasma at 37 degrees C.II. Cholinestrace (acylcholine acylhydrolase, EC 3.1.1.8). Working Group of enzymes, German Society for clinical chemistry. *Eur J Clin Biochem* 1992;303:163-70.
9. Pasquini GM, Davey RA, Ho PW, Michelangeli VP, Grill V, Kaczmarczyk SJ, *et al.* Local secretion of parathyroid hormone-related protein by an osteoblastic osteosarcoma (UMR 106-01) cell line results in growth inhibition. *Bone* 2002;31:598-605.
10. Farley JR, Hall SL, Tanner MA, Wergedal JE. Specific activity of skeletal alkaline phosphatase in human osteoblast-line cells regulated by phosphate, phosphate esters, and phosphate analogs and release of alkaline phosphatase activity inversely regulated by calcium. *J Bone Miner Res* 1994;9:497-508.
11. Operskalski EA, Martin SP, Henderson BE, Vischer BR. A case control study of osteosarcoma in young persons. *Am J Epidemiol* 1987;126:118-26.
12. Rothem DE, Rothem L, Dahan A, Eliakim R, Soudry M. Nicotinic modulation of gene expression in osteoblast cells, MG-63. *Bone* 2011;48:903-9.
13. Genever PG, Birch MA, Brown E, Skerry TM. Osteoblast-derived acetylcholinesterase: A novel mediator of cell-matrix interactions in bone? *Bone* 1999;24:297-303.
14. Nosse ME, Hartmann S, Trinkaus K, Alt V, Stigler B, Heiss C, *et al.* Expression of non-neuronal cholinergic system in osteoblast-like cells and its involvement in osteogenesis. *Cell Tissue Res* 2009;338:45-7.

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