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## **Serum Insulin like Growth Factor (IGF-1) and Leptin Levels in Osteosarcoma**

Simmi Kharb, S. Chaudhary and Z.S. Kundu

Department of Biochemistry, Orthopedics, Pt B.D. Sharma PGIMS, Rohtak, India

*Corresponding Author: Simmi Kharb, Department of Biochemistry, Orthopedics, Pt B.D. Sharma PGIMS, H. No. 1447, Sector-1, Rohtak, Haryana, 24001, India*

### **ABSTRACT**

Osteosarcoma along with other tumors like osteoma, osteoid osteoma and osteoblastoma are grouped as bone forming tumors. Several studies have shown link between serum concentrations of IGF-I and increased risk of common cancers namely breast, prostate, colorectal and lung cancer. Conflicting data are available regarding leptin effects on bone with both positive and negative effects being reported. No reports are available regarding leptin levels in osteosarcoma and its correlation with IGF-I. Hence, the present study is planned to study serum IGF-I and leptin levels in patients of osteosarcoma. Serum IGF-1 and leptin were analyzed in thirty cases of osteosarcoma and these patients were compared with thirty age and sex matched controls with musculoskeletal pain. Serum calcium and phosphorous levels were decreased in patients with osteosarcoma as compared to controls. Serum alkaline phosphatase levels were significantly raised in patients with osteosarcoma as compared to patients of musculoskeletal pain ( $p < 0.001$ ). Serum IGF-1 and leptin levels were significantly decreased in osteosarcoma patients (group-II) as compared to the patients of musculoskeletal pain (group-I), ( $p < 0.001$ ,  $p < 0.05$ , respectively). Inverse correlation was observed between IGF-1 and calcium, IGF-1 and ALP, leptin and calcium leptin and ALP in group II as compared to group I. Lowered serum IGF-1 and leptin levels observed in osteosarcoma patients as compared to control in the present study and could be due to their possible utilization in tumor formation. The present study suggests that these parameters can serve as useful markers for diagnosis and follow up of disease.

**Key words:** IGF-1, leptin, osteosarcoma

### **INTRODUCTION**

Osteosarcoma, the most common primary malignant bone tumor in both children and adults, is characterized by the development of bone or osteoid substance by the tumor cells (Lamoureux *et al.*, 2007).

Bone is the second richest source of insulin like growth factors (IGF). In the skeleton, IGF-1 can act as a systemic hormone or as an autocrine/paracrine growth factor, IGF-1 modulates growth and differentiation of osteoblasts (Liotta *et al.*, 1991).

In biological fluids, IGFs are normally bound to IGF-BPs that are six in number, IGF-BP-1-6. IGF-BPs act as carriers of IGFs, serve as modulators of IGF availability and activity. Any perturbation in each level of IGF signaling proteins can lead to pathological conditions, mainly cancer formation and progression (Pavelic *et al.*, 2007). Several studies have shown link between serum concentrations of IGF-I and increased risk of common cancers namely breast, prostate, colorectal and lung cancer (Renehan *et al.*, 2004).

Increased levels of IGF-I have been associated with breast, prostate and colon cancer growth. Also, IGF-I stimulates growth of a variety of human malignant cells in tissue culture including breast cancer, prostate carcinoma and osteosarcomas cells (Schmid *et al.*, 2001). However, no reports are available in literature where IGF-I have been reported in osteosarcoma.

Leptin, a cytokine like hormone is a pleiotropic hormone with well-known angiogenic properties involved in regulation of a variety of physiological processes including protective effects exerted by fat mass on skeleton. There is new growing evidence that leptin may have significant peripheral effect on bone mass and may increase bone formation and decrease bone resorption (Gordeladze *et al.*, 2002) indicating its possible role in metastasis (Yang *et al.*, 2009).

There are reports of interrelationship between serum leptin and IGF-I and breast cancer risk (Lautenbach *et al.*, 2009). However, no reports are available regarding leptin levels in osteosarcoma and its correlation with IGF-I.

Hence, the present study is planned to study serum IGF-I and leptin levels in patients of osteosarcoma.

## MATERIALS AND METHODS

The present study was conducted in thirty histopathologically confirmed cases of osteosarcoma (localized without metastasis). These patients were compared with thirty controls which included age and sex matched thirty patients with musculoskeletal pain. Five milliliter of venous blood was collected aseptically from antecubital vein and serum separated by centrifugation and serum IGF-1 and leptin were analyzed by solid phase Enzyme-Linked Immunosorbent Assay (ELISA) based on the sandwich principle (Bondy *et al.*, 1994; Maffei *et al.*, 1995).

SPSS ver.18 was applied for various statistical analysis and student's t-test and regression analysis was carried out.

## RESULTS

In the present study, serum calcium levels were decreased in patients with osteosarcoma as compared to controls ( $p > 0.05$ ), (Table 1).

Serum phosphorus levels were lower in the group II as compared to group I ( $p > 0.05$ ), (Table 2).

Serum alkaline phosphatase levels were significantly raised in patients with osteosarcoma as compared to patients of musculoskeletal pain ( $p < 0.001$ ), (Table 1).

Table 1: Serum calcium and serum phosphorous levels in both the groups (Mean±SD)

Serum level	Group I (control)	Group II (study)
Calcium (mg dL <sup>-1</sup> )	9.63±1.10	8.94±1.25**
Phosphorous (mg dL <sup>-1</sup> )	3.41±0.45	3.24±0.71**
Alkaline phosphatase (IU L <sup>-1</sup> )	61.60±19.4	378.76±136.60*

\* $p < 0.001$  as compared to control, \*\* $p > 0.05$  as compared to control

Table 2: Serum IGF-1 and serum leptin levels in both the groups (Mean±SD)

Serum level (ng mL <sup>-1</sup> )	Group I (control)	Group II (study)
IGF-1	248.16±54.81	66.56±63.96*
Leptin	6.52±5.79	1.34±0.981*

\* $p < 0.001$  as compared to control

Table 3: Correlation between different parameters in group I and II

Parameters	Group I		Group II	
	r	P	r	P
IGF-1 vs. calcium	0.094	0.040	-0.026	0.890
IGF-1 vs. ALP	0.098	0.608	-0.127	0.505
Leptin vs. calcium	0.174	0.359	-0.089	0.642
Leptin vs. ALP	0.195	0.302	-0.055	0.774

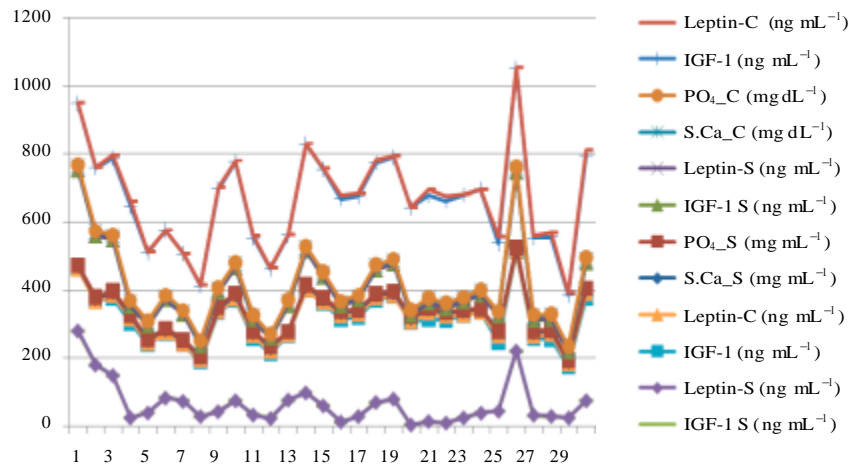


Fig. 1: Correlation between different parameters in both the group I and II

Serum IGF-1 and leptin levels were significantly decreased in osteosarcoma patients (group-II) as compared to the patients of musculoskeletal pain (group-I), ( $p < 0.001$  and  $p < 0.05$ , respectively; Table 2).

Inverse correlation was observed between IGF-1 and calcium, IGF-1 and ALP, leptin and calcium leptin and ALP in group II as compared to group I (Table 3, Fig. 1).

## DISCUSSION

IGF-1 has been shown to increase bone mass and evidence has shown that IGF-1 has direct effects on bone forming cells. IGF-1 exhibits direct influence on maintenance of normal immune function. The combined mitogenic and antiapoptotic effects have been found to have profound effect on tumor growth (Manral and Singh, 2011).

Insulin like growth factor 1 (IGF-1) has growth-promoting effects on myeloma cells *in vitro* as well as *in vivo*. Standal *et al.* (2002) found that healthy individuals and Myeloma (MM) patients at diagnosis have similar amounts of IGF-1 in the circulation and MM patients with low serum IGF-1 levels had a favorable prognosis. Serum levels of IGF-1 have been reported to be increased in patients with breast cancer compared to healthy individuals (Standal *et al.*, 2002), IGF-I and IGF-II are expressed by osteoblasts and exert similar biological actions but IGF-I is more potent than IGF-II.

In the present study, serum IGF-1 levels of osteosarcoma patients (group II) were decreased as compared to the patients of musculoskeletal pain (group I) were decreased and difference was

statistically significant ( $66.56 \pm 63.96$  vs.  $248.16 \pm 54.81$  ng mL<sup>-1</sup>, Table 2). The serum IGF-1 levels fell by four-fold in osteosarcoma patients. This could be due to possible utilization of IGF-1 by osteosarcoma cells during tumorigenesis. IGF-I serum levels of osteosarcoma patients have been reported to be significantly lowered than those of age matched controls by Giustina *et al.* (2008). Our findings are in agreement with this report.

In the present study, serum leptin levels of osteosarcoma patients (group II) were decreased as compared to the patients of musculoskeletal pain (group I) and the difference statistically significant ( $1.34 \pm 0.981$  vs.  $8.29 \pm 9.61$  pg mL<sup>-1</sup>,  $p < 0.001$ ; Table 2).

In contrast, Kushlinskii *et al.* (2000) demonstrated high leptin levels in osteosarcoma and neuroectodermal bone tumors (Kushlinskii *et al.*, 2000).

There are conflicting reports regarding action of leptin on bone formation and leptin has been reported to have antiosteogenic action. Leptin also stimulates the proliferation of cultured human osteoblasts and it has been shown to cause human bone marrow stromal cells to express alkaline phosphatase, collagen-I and osteocalcin and to mineralize matrix (Thomas *et al.*, 1999).

Finding of negative correlation between serum alkaline phosphatase and leptin levels in the present study probably occurred due to increased osteoblastic activity raising serum alkaline phosphatase and possible utilization of leptin by sarcoma cells as depicted by low serum leptin levels in osteosarcoma patients.

There is new growing evidence that leptin may have significant peripheral effects on bone mass. Gordeladze *et al.* (2002) have shown that leptin, as well as its receptor, are expressed by normal human osteoblasts and continuous leptin exposure of iliac crest osteoblasts, promoted collagen synthesis, cell differentiation and *in vitro* mineralization, as well as cell survival. *In vitro* data has supported the hypothesis that leptin may act locally to increase bone mass through the combination of an increase in bone formation and a decrease in bone resorption. The findings of the present study indicate possible utilization of leptin by tumor cells lends support to this hypothesis.

Burguera's data and the results of present study support the hypothesis that leptin may increase bone mass by stimulating osteoblast proliferation (Burguera *et al.*, 2006).

Findings of present study of inverse correlation between leptin and ALP in osteosarcoma patients and positive correlation between leptin and calcium lend support to the role of leptin in bone mineralization and tumorigenesis.

The growth hormone (GH)/insulin-like growth factor-I (IGF-I) system and leptin both play an important role in the regulation of body composition. Although the regulation of these two hormonal systems by insulin has been under intense investigation, the physiologic interactions between leptin and the GH/IGF-I system remain unknown. Jurimae and Jurimae (2006) observed a significant association between IGF-1 and leptin in healthy premenopausal women and it remained significant after controlling for age.

In the present study, serum IGF-1 and serum leptin levels were positively correlated in both the groups I and II ( $r = 0.137$ ,  $p = 0.472$  and  $r = 0.150$ ,  $p = 0.428$ , respectively Table 3). Findings of the present study are in accordance with reports of (Jurimae and Jurimae, 2006).

In the present study, serum leptin levels were correlated with serum calcium levels in group I and II and no significant correlation was observed in serum levels of both the groups ( $r = 0.174$ ,  $p = 0.359$  and  $r = -0.089$ ,  $p = 0.642$ , respectively Table 3). No reports are available in literature where leptin have been correlated with calcium in osteosarcoma.

The association of circulating leptin levels with ALP a specific marker of osteoblast activity suggests that leptin levels influence osteoblast activity. In contrast, Kim *et al.* (2006) observed a negative correlation between leptin and ALP in the obese children and no correlation in healthy children, concluding that leptin was a significant factor in bone formation but not in bone resorption in childhood obesity.

Serum alkaline phosphatase were inversely correlated with serum leptin levels in group II and positively correlated in group I, were statistically significant ( $r = 0.195$ ,  $p = 0.302$  and  $r = -0.055$ ,  $p = 0.774$ , respectively Table 3) in the present study.

Thus, leptin is emerging as central in two entirely different bone-controlling mechanisms. The first is the indirect one. It was discovered by Ducy *et al.* (2000). The second direct mechanism of leptin exerts its osteotrophic effects by promoting differentiation of bone marrow stromal cells into osteoblasts (Thomas *et al.*, 1999) and by inhibiting osteoclast generation (Holloway *et al.*, 2002). Circulating leptin penetrates the bone marrow, where it joins the autocrine/ paracrine leptin from early cells of the osteoblast lineage and from late-stage, matrix-mineralizing osteoblasts or from early osteocytes to stimulate the production of IGF-1. IGF-1 in turn stimulates the proliferation of osteoblast precursors making the osteoblastic lineage cells more resistant to apoptosis and further enhances bone formation through suppression of osteoclast generation.

In the present study an inverse correlation was observed between IGF-1 and calcium; IGF-1 and ALP; leptin and ALP in group II as compared to group I (Table 3). A positive correlation was observed between leptin and calcium in both the group (Table 3) and the values were higher in group II indicating possible link between these parameters namely, ALP, calcium, IGF-1 and leptin in bone formation, hence tumorigenesis.

As already discussed, there is presence of a feedback loop of leptin secretion and growth hormone correlating IGF-1 and leptin. The observed parallel increase in serum IGF-1 and leptin levels in osteosarcoma patients lends support to these studies.

Thus, the lowered serum IGF-1 and leptin levels observed in osteosarcoma patients in the present study suggests that these parameters can serve as useful markers for diagnosis, planning therapeutic modalities and follow up of disease.

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