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Role of Vasodilator Stimulated Phosphoprotein (VASP) in Pathogenesis of Osteosarcoma and its Association with Alkaline Phosphatase Levels

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Abstract

Osteosarcoma is the most common bone tumour seen in the paediatric and adolescent age group. Most osteosarcomas are highly malignant tumours arising within the bone. Several markers for diagnosis and prognosis have been proposed in osteosarcoma namely, vascular endothelial growth factor (VEGF), bone alkaline phosphatase, osteocalcin. A new family of a protein known as Vasodilator-stimulated phosphoprotein, which is known to promote cell migration may also have a role in metastasis of osteosarcoma. So this study was planned to estimate the serum concentration of VASP in patients of osteosarcoma and to find the correlation of it with serum alkaline phosphatase and compare them with controls. Fifty patients attending the Orthopaedics clinics were selected for the study and were divided into two groups. Histopathologically confirmed cases of osteosarcoma (localized without metastasis) were included in Group I and age and sex matched twenty five patients with musculoskeletal pain in Group II as controls. Serum alkaline phosphatase levels and serum vasodilator-stimulated phosphoprotein (VASP) levels were estimated and the result was analysed using standard statistical methods. It has been found that serum VASP levels were significantly decreased and serum alkaline phosphatase levels were significantly raised in patients with osteosarcoma (Group I) as compared to the controls. Serum alkaline phosphatase levels showed a positive correlation with serum VASP levels in control, which got inverted in osteosarcoma cases. VASP, a member of ENA/VASP family, has been implicated in regulating key cellular functions (namely shape change, adhesion and migration) due to its ability to modify dynamic cytoskeleton. The negative correlation between VASP and ALP in osteosarcoma patients also supported the role of VASP in bone mineralization and tumorigenesis. So, VASP in osteosarcomas may lead to improved stratification of outcome and development of novel therapeutic modalities.

Keywords: Osteosarcoma; Vasodilator-stimulated phosphoprotein; Alkaline phosphatase; Malignant tumours; Cytoskeleton; Tumorigenesis

Introduction

Osteosarcoma is the most common bone tumour seen in the paediatric and adolescent age group. Osteosarcoma patient presents with painful swelling of bones [1]. Males are affected 1.5-2 times more than females with a predilection for metaphysis of the long bone of lower limb [1]. Most osteosarcomas are highly malignant tumours arising within the bone. According to their main histological component, they may be divided into various subtypes, such as osteoblastic, chondroblastic fibroblastic, telangiectatic, and small cell osteosarcoma [2,3]. In most patients, the etiology of osteosarcoma remains obscure. Various etiological factors for osteosarcoma are ionizing radiation, family history of bone disorders and cancer, chemicals (fluoride, beryllium, vinyl chloride) and viruses. Several markers for diagnosis and prognosis have been proposed in osteosarcoma namely, vascular endothelial growth factor (VEGF), bone alkaline phosphatase, osteocalcin, survivin, epidermal growth factor receptor Erb B_2 [3].

A family of proteins, known as the Ena/VASP family (enabled/Vasodilator-stimulated phosphoprotein), is known to promote cell migration. This protein becomes conjugated to the actin filaments within a cell and stimulates polymerization. Cancer cells, which are typically more motile than non-cancerous cells because they actively divide and spread rapidly throughout the body, may also have an increased amount of vasodilator-stimulated phosphoprotein (VASP) [4].

Various studies have reported that VASP can play an important role in the process of cell migration and tumour metastasis in cases of osteosarcoma [4,5]. However, till date, no study has examined serum VASP levels in osteosarcoma. So, the study was planned to estimate the serum concentration of VASP in patients of osteosarcoma and to find the correlation of it with serum alkaline phosphatase and compare them with controls.

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. Fifty patients attending the Orthopaedics clinics were selected for the study. They were divided into two groups. Histopathologically confirmed cases of osteosarcoma (localized without metastasis) of all the ages before starting any treatment were included in Group I. In all these cases X-ray chest, CT scans thorax and bone scan was performed to rule out metastasis. These patients were compared with Group II as controls which included age and sex matched twenty five patients with musculoskeletal pain.

Five ml of venous blood was collected aseptically from the antecubital vein and serum separated by centrifugation and analysed the same day.

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i) Serum alkaline phosphatase levels were estimated using the standard method on autoanalyzer.

ii) Serum vasodilator-stimulated phosphoprotein (VASP) levels were estimated by ELISA method.

Statistical Analysis

SPSS ver. 23 was used for statistical analyses. Results were expressed as Mean ± SE (SD) and unpaired 't' test and Pearson correlation test were applied.

Results

The present study was conducted in the Department of Biochemistry in collaboration with the Department of Orthopaedics, Pt. B.D. Sharma, PGIMS, Rohtak. Fifty patients attending the Orthopaedics clinics were selected for the study. They were divided into two groups. Group I consisting of twenty five cases of histopathologically confirmed osteosarcoma (localized without metastasis, before starting any treatment) of age 10-30 years. They were compared with age and sex matched twenty five patients with musculoskeletal pain (group II). Majority of osteosarcoma cases (n=19, 76%) were in the age group of 10-20 years, while six (24%) were in the age group of 21-30 years. Fourteen (56%) of the musculoskeletal pain cases were in the age group of 10-20 years, while the remaining 11 (44%) were in the age group of 21- 30 years (Tables 1-3).

Discussion

Vasodilator-stimulated phosphoprotein (VASP), a member of ENA/VASP family, has been implicated in regulating key cellular functions (namely shape change, adhesion and migration) due to its ability to modify dynamic cytoskeleton. In addition to its role in normal cell growth, embryonic development and homeostasis, VASP plays an essential role in many diseases such as cancer metastasis, thrombosis, arteriosclerosis and nephritis [6]. In several types of malignancies, expression of VASP has been reported to be dysregulated and is also relevant to tumour metastasis [4]. In the present study, serum VASP levels of osteosarcoma patients (Group I) were decreased as compared to the patients of musculoskeletal pain (Group II) and the difference statistically significant (118.04 ± 28.46 vs. 216.70 ± 38.93 pg/mL, $p<0.05$, Table 1).

Parameters	Group I	Group II	p-value
Serum VASP (pg/mL)	118.04 ± 28.46 (142.32)	216.70 ± 38.93 (194.66)	0.046
Range (pg/mL)	7.31-601.81	8.71-1270.50	
Serum VASP levels were significantly decreased in osteosarcoma patients (group I) as compared to those with musculoskeletal pain (Group II, $p<0.05$, Table 1).			

Table 1: Serum VASP levels in both the groups [Mean ± S.E. (SD)].

Parameters	Group I	Group II	p-value
Serum Alkaline Phosphatase (U/L)	418.92 ± 47.54 (237.72)	76.20 ± 3.83 (19.17)	0.000
Range (U/L)	140-890	49-110	
Serum alkaline phosphatase levels were significantly raised in patients with osteosarcoma (group I) as compared to subjects of musculoskeletal pain (Group II, $p<0.001$, Table 2).			

Table 2: Serum alkaline phosphatase levels in both the groups [Mean ± S.E. (SD)].

Parameters	Group I	r value	Group II	r-value & p-value
Serum ALP (U/L)	418.92 ± 47.54 (237.72)	r=-0.155 p=0.583	76.20 ± 3.83 (19.17)	r=0.141 p=0.502
Serum VASP (pg/mL)	118.04 ± 28.46 (142.32)		216.70 ± 38.93 (194.66)	
Serum alkaline phosphatase levels showed a positive correlation with serum VASP levels in control, which got inverted in osteosarcoma cases, though the correlations in both the groups are statistically insignificant.				

Table 3: Correlation between serum alkaline phosphatase levels and serum vasp levels in both the groups [Mean ± S.E. (SD)].

There are conflicting reports regarding the action of VASP on bone formation and VASP has been reported to have angiogenic action [4,6,7]. Mounting evidence suggested that angiogenesis is regulated by cytoskeletal. alterations that lead to changes in cell morphology and in the rate of cell migration and proliferation. Oxidative stress and redox signalling have a role in cancer neovascularization, a process without which the tumour is not able to grow beyond few millimetres in size. ROS and NOS affect cell's response to hypoxia, a major for trigger angiogenesis switch in tumours and are important regulators and mediators of action of most potent proangiogenic factor-VEGF [8].

VASP has been reported to play an important role in the process of cell migration and tumour metastasis. However, no study has examined serum VASP levels in osteosarcoma till date [9,10]. A study reported that the effect of VASP on osteosarcoma cell migration, where two osteosarcoma strains (MG-63 and Saos-2 cells) having different metastatic potential were used and using RNAi, VASP gene expression was silenced. VASP silence MG-63 cells lost their migratory potential to 55%, by wound healing assay. Saos-2 cells had lower VASP expression than MG-63 cells and showed reduced cell migration, suggesting that VASP is at least partly responsible for motility of osteosarcoma cells. Also, VASP expression was determined in human osteosarcoma samples. In human, osteosarcoma specimens, VASP expression was determined using RT-PCR and specimen with metastasis had higher VASP expression as compared to those with the absence of metastasis suggesting that VASP may be involved in regulating osteosarcoma metastasis *in vivo*. VASP acts as a modulator of smooth cell proliferation by nitric oxide/cGMP. Both VASP deficiency and overexpression can regulate cell proliferation and can induce neoplastic transformation and promote tumour growth. VASP is a component of actin-based cytoskeleton and has been demonstrated to affect actin filament and may be involved in tumorigenesis. Normal cell growth may require maintenance of VASP expression within a narrow range and lowered serum VASP levels in osteosarcoma Group In the present study implicate VASP in tumorigenesis and/or cancer progression [4].

Abl kinase activity has been reported to be associated with VASP. Like VASP, abl is an important regulator of cytoskeletal dynamics and is activated and transiently localized to focal adhesions upon adhesion to extracellular matrix. VASP is a substrate of BCR-ABL oncoprotein and is tyrosine phosphorylated. Abl is required for actin cytoskeleton remodelling in response to growth factors and oncogene activation of abl induces transformations such as anchorage independent cell division and dramatic changes in cell morphology [9].

Osteoblasts are migratory cells that are recruited to resorbed bone surfaces and phosphorylation plays a role in osteoblast migration. It has been reported that dynamin GTPase activity is regulated by tyrosine kinase and its inhibition by dynasore (inhibitor) reduce c-fos and osterix expression, the markers of osteoblasts. Also, inhibition of dynamin GTPase activity promoted early osteoblast differentiation

leading to an increase in ALP activity and led to a decrease in osteoblast migration [10]. Finding of elevated serum alkaline phosphatase activity in osteosarcoma cases in the present study also lends support in these findings (Tables 2 and 3). The negative correlation between VASP and ALP in osteosarcoma patients also supported the role of VASP in bone mineralization and tumorigenesis.

Conclusion

The findings of the present study indicate that there is a possible utilization of VASP by tumour cells. Further investigations into the role of VASP in osteosarcomas may lead to improved stratification of outcome and development of novel therapeutic modalities A multi-targeted therapeutic approach involving modulation of redox species production, signalling and metabolism and/or modulation of cellular antioxidant response may be effective angiogenesis treatment in future.

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