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Original Research

Calcium homeostasis and acid phosphatase levels in giant cell tumour

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Key words: Giant cell tumour; calcium; acid phosphatase; tartrate resistant acid phosphatase.

Abstract

Objective: Giant-cell tumour of the bone is a relatively uncommon tumour. DMA cytometry, gene, and protein analyses of the tumor and contrast-enhanced dynamic MRI are important for predicting local recurrence but these markers are complicated. Moreover, local recurrence is missed by MRI when no appropriate range of interest is selected. Therefore present study was planned to find a marker which can be easily used to diagnose and to predict the local recurrence of giant-cell tumour of bone. Material: A total of 25 confirmed cases of giant cell tumour were enrolled for study. Total duration of study was 2 years. Acid phosphatase was determined by kinetic colorimetric method and calcium was measured using Cresolphthalein Complexone Method Results: The mean value of acid phosphatase in patients of giant cell tumour before surgery was 18.16±11.51 IU/L which decreased to 5.06±1.42I U/L after 3 months of surgery and the difference was highly significant. Tartrate resistant acid phosphatase levels were 8.63±3.71 U/L before surgery and after treatment it decreased to 3.20±1.50 U/L and the difference was statistically highly significant. A significant positive correlation was found of tumour volume with acid phosphatase and tartrate resistant acid phosphatase. Mean calcium levels were 16.43±3.09 mg/dl which decreased after treatment to 8.47±1.21 mg/dl with highly significant p value. Conclusion: A raised level of serum calcium indicates osteolytic activity. Serum tartrate resistant acid phosphatase is a more useful and more convenient specific marker for the diagnosis and prediction of the recurrence of giant cell tumour of bone than methods and markers presented in previous reports and may be useful in routine follow-up of patients treated for giant cell tumour.

INTRODUCTION

Giant cell tumour of the bone (GCT) is a relatively uncommon tumour. It is characterized by the presence of multinucleated giant cells (osteoclast-like cells). Giant cell tumours are normally benign with unpredictable behavior [1]. It is a heterogeneous tumour composed of three different cell populations. The giant cell tumour stromal cells constitute the neoplastic cells which are from an osteoblastic origin and are classified based on expression of osteoblast cell markers such as alkaline phosphatase and osteocalcin [2].

Acid phosphatase is an enzyme used to free attached phosphate groups from other molecules during digestion. It is basically a phosphomonoesterase. It is stored in lysosomes and functions when they fuse with endosomes which are acidified. Human acid phosphatase is normally found at low concentrations. However, pronounced changes in their synthesis occur in particular diseases where unusually high or low enzyme expression is seen as part of the pathophysiological process. This observation suggests that acid phosphatase could be diagnostically useful as serological and histological marker of diseases and could also be of use in the investigation of the pathophysiology of the associated diseases. During normal and pathological bone resorption, osteoclastic

ACP is synthesized in abundance by active osteoclasts of bone tissue [3].

The increase in osteoclast activity is accompanied by an increase in the synthesis and secretion of TRAP. This enzyme is resistant to the inhibitory influence of L(+) tartrate to which other acid phosphatase isoenzymes are sensitive. It is therefore commonly called as tartrate resistant acid phosphatase (TRAP) [4]. Osteoclasts are well known for containing a large amount of TRAP activity [5] and this phenomenon had been used for many years to identify osteoclasts in tissue samples using histochemical techniques. TRAP occurs in much higher concentrations in the serum of people with skeletal disease than in normal control subject. Furthermore, it increases with the rate of resorption taking place. There is a direct relation between excessive osteoclast facilitated bone resorption and increased amounts of TRAP in the circulation. Therefore, serum TRAP has been indicated as a disease associated marker for the clinical diagnosis of excessive bone resorption and for quantitatively monitoring the rate and progression of metabolic bone disorders [6].

To predict local recurrence, DMA cytometry, gene, and protein analyses of the tumor are useful in addition to contrast-enhanced dynamic MRI but an important problem is that the genetic and biochemical methodologies used to detect those markers are complicated. Moreover, local recurrence is missed by MRI when no appropriate range of interest (ROI) is selected [7]. Therefore present study was planned to find a marker which can be easily used to diagnose and to predict the local recurrence of GCT of bone.

MATERIALS AND METHODS

The present study was conducted in the department of Biochemistry in collaboration with department of Orthopaedics, Pt. B.D. Sharma University of Health Sciences, Rohtak. A total of 25 confirmed cases of Giant cell tumour were enrolled for study. Total duration of study was 2 years. A detailed case record containing information on age, sex, site of lesion, duration of pain and swelling, any history of trauma and history of similar kind of lesion in the past were documented. Abdominal, respiratory and neurological examination was also done.

An informed consent was taken from all the patients. Five ml of fasting venous blood sample was collected from antecubital vein under all aseptic conditions. Serum was separated by centrifugation and the sample was analyzed on the same day (and stored at -20 ºC if storage was required for more than 1 day).

Acid phosphatase was determined by kinetic colorimetric method. ACP at an acidic pH hydrolyses α-naphthyl phosphate to form α-naphthol and inorganic phosphate. The α-naphthol formed is coupled with Fast Red TR salt to form a diazo dye complex. The rate of formation of this complex is measured as an increase in absorbance which is proportional to the acid phosphatase activity in the sample. Tartrate inhibits prostatic ACP and the testing in its presence was done to find the non prostatic ACP i.e Tartrate resistant ACP [8]. Calcitonin was quantitatively assayed using Enzyme linked Immunosorbent (ELISA) kit [9,10]. Calcium was measured using Cresolphthalein Complexone Method [11]. Phosphorus was estimated by UV endpoint method [12]. Alkaline phosphatase was determined by kinetic (p-nitrophenyl phosphate) method [13]. Acid phosphatase, alkaline phosphatase, calcium and phosphorus were analysed on Konelab autoanalyser using Autopak kit. Three-dimensional tumour size was determined radiologically through X rays, CT scan and MRI scan taken at the time of diagnosis.

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 20 for windows. Values shown in the text, tables and figures are mean ±SD. Student t test were applied for comparison of means of study groups. p value < 0.05 were considered significant. Correlations between groups were analyzed using Pearson correlation coefficient (r) formula.

RESULTS

Mean age of patients was 33.6±9.12 years (range 17-60 years) with 36% males and 64% females. 40% of tumours were found to be around knee, 36% in lower limb and 24% in upper limb. It clearly shows that most common site of occurrence of tumour is around the knee. Biochemical parameters of patients are given in table.1 (Fig. 3). The mean values of acid phosphatase in patients of giant cell tumour before surgery were 18.16±11.51IU/L which decreased to 5.06±1.42IU/L after 3 months of surgery. The difference was highly significant ($p < 0.001$). Tartrate resistant acid phosphatase levels before surgery were 8.63±3.71 U/L after treatment decreased to 3.20±1.50 U/L and the difference was statistically significant ($p < 0.001$). The mean tumour volume was 63.35±47.00 cm^3 with a range of 2.7 to 170cm^3 and by using Pearson's correlation coefficient, a significant positive correlation was found of tumour volume with acid phosphatase ($r = 0.462$, $p < 0.05$) (Fig. 1) and with tartrate resistant acid phosphatase ($r = 0.467$, $p < 0.05$) (Fig. 2).

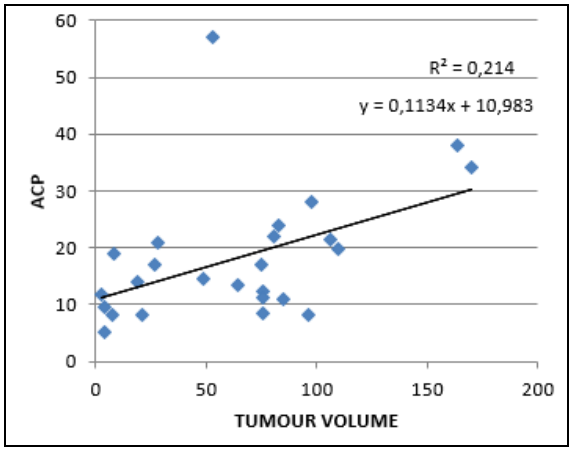

Fig. 1. Figure showing correlation between tumour volume and acid phosphatase levels (r = 0.462, p < 0.05).

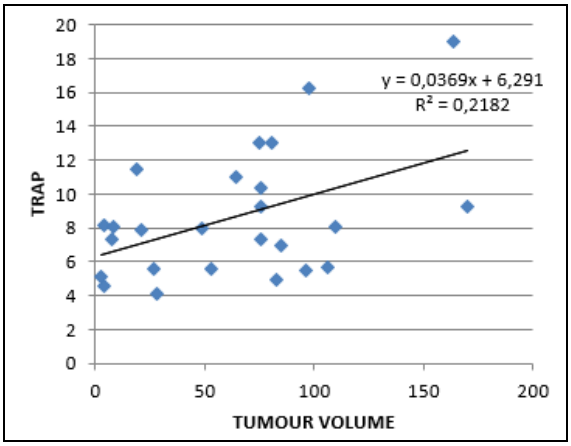

Fig. 2. Figure showing correlation between tumour volume and tartrate resistant acid phosphatase levels (r = 0.467, p < 0.05).

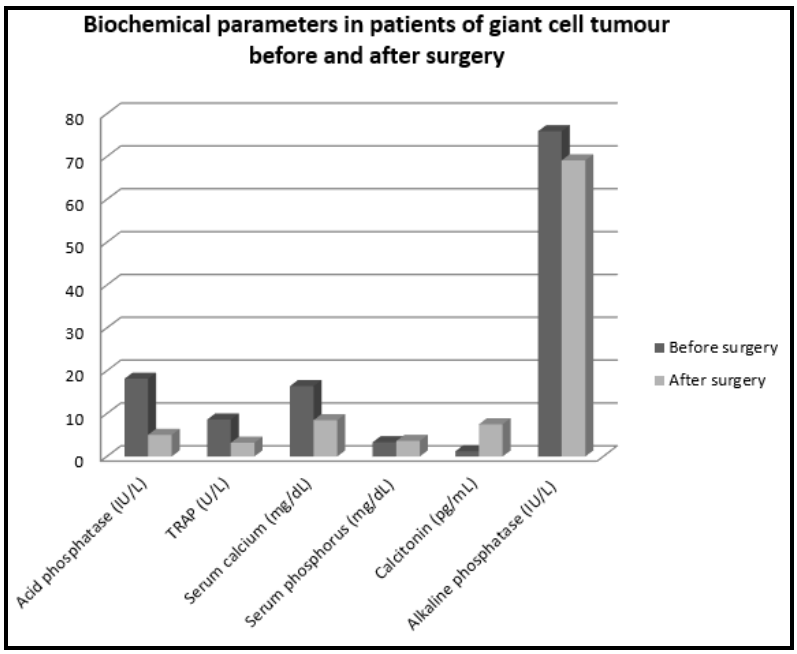

Fig. 3. Diagram showing biochemical parameters in patients of giant cell tumour before and after surgery.

Table 1. Biochemical parameters in patients of giant cell tumour:

Parameter	Before surgery	After surgery	p value	Significance
Acid phosphatase (IU/L)	18.16±11.51	5.06±1.42	<0.001	HS
TRAP (U/L)	8.63±3.71	3.20±1.50	<0.001	HS
Serum calcium (mg/dL)	16.43±3.09	8.47±1.21	<0.001	HS
Serum phosphorus (mg/dL)	3.34±0.83	3.64±0.83	<0.05	S
Calcitonin (pg/mL)	1.23±9.94	7.53±3.42	<0.001	HS
Alkaline phosphatase (IU/L)	75.92±25.91	69.12±19.59	<0.05	S

All values are in mean ± SD; HS: Highly Significant; S: Significant

Mean calcium levels were 16.43±3.09 mg/dl which decreased after treatment to 8.47±1.21 mg/dl. It clearly shows that after treatment mean values decreased significantly and found to be statistically highly significant ($p < 0.001$). Mean phosphate levels before treatment were 3.34±0.83 mg/dl and after treatment increased to 3.64±0.83 mg/dl and found to be statistically significant ($p < 0.05$). Alkaline phosphate level in the present study before treatment was 75.92±25.91 IU/L with a range of 13-135 IU/L and after treatment it decreased to 69.12±19.59 IU/L and found to be statistically significant ($p < 0.05$). Calcitonin levels before treatment were 1.23±9.94 pg/ml with a range of 0.3 - 4.02 pg/ml and after treatment it was raised to 7.5±3.42 pg/ml and found to be statistically highly significant ($p < 0.001$). No significant correlation was observed between tumour volume with calcium, phosphorus, alkaline phosphatase and calcitonin.

DISCUSSION

The elevation in acid phosphatase levels in giant cell tumour can be explained by increased secretion of acid phosphatase from lysosomes of multinucleated giant cells, which is a characteristic feature of GCT. A significant positive correlation was found between tumour volume and acid phosphatase levels. As the tumour size can influence acid phosphatase levels in serum. Therefore increased levels are seen with greater tumour volume and these levels decreased after surgery due to removal of tumour. TRAP has been found to be expressed in the multinucleated giant cells in GCT by immunohistochemistry in various studies. A recent study indicated high serum acid phosphatase in 5 of 9 cases of GCT and all of these 5 cases showed normal values postoperatively. They also found correlation between serum acid phosphatase and tumour size. The high preoperative acid phosphatase values in GCT patients became normalized after surgery, but reappeared in 3 of 5 patients with local recurrence. They included only adult GCT patients, as children and adolescents have higher serum acid phosphatase values because of higher bone turnover [14]. Similar findings were seen in other studies also [15,7]. This clearly indicates the role of acid phosphatase in GCT.

To predict local recurrence, DMA cytometry, gene, and protein analyses of the tumor are useful in addition to contrast-enhanced dynamic MRI but an important problem is that the genetic and biochemical methodologies used to detect those markers are complicated. Moreover, local recurrence is missed by MRI when no appropriate range of interest (ROI) is selected [7]. Therefore it is better to find a marker which can be easily used to diagnose and to predict the local recurrence of GCT of bone. Serum total acid phosphatase can be used for the diagnosis and the detection of local recurrence as we can see from our study also that it has positive significant correlation with tumour volume. However, it contains secretory acid phosphatase of various types including that from the osteoclasts. Therefore, more specific serum TRAP, a sensitive marker of bone resorption, which is secreted from osteoclasts, can be used as a tumour marker for diagnosing and monitoring response to the treatment of GCT.

Hypercalcemia is the most common life-threatening metabolic disorder associated with neoplastic diseases, occurring in an estimated 10% to 20% of all adults with cancer. It also occurs in children with cancer, but with much less frequency (approximately 0.5%–1%) [16-18]. It is believed that hypercalcemia results from the release of factors by malignant cells that ultimately cause calcium resorption from bone [19]. As giant cell tumour is an osteolytic condition serum calcium levels before surgery were more than normal value and came to normal values after surgery. Alteration in humoral regulation of calcium resulting from production of parathyroid hormone-related protein (PTH-rp) has also been implicated in tumour associated hypercalcemia [20, 21]. Plasma concentration of PTH-rp is rarely elevated in healthy individuals but elevated concentrations are detectable in about 80% of hypercalcemia patients with solid tumours. PTH-rp interacts with parathyroid hormone receptors on cell membranes, activating adenyl cyclase, which triggers an increase in cyclic AMP production and increases intracellular calcium. These actions are responsible for increasing bone demineralization and elevating serum calcium concentrations, decreasing reabsorption of phosphate in the proximal renal tubules, increasing calcium reabsorption in the distal tubules and increasing cholecalciferol production [22].

Under these circumstances, hypercalcemia and high calcium concentrations in urine (hypercalciuria) impair sodium and water reabsorption, causing polyuria (a calcium diuresis) with subsequent loss of circulating fluid volume (dehydration). As a consequence of dehydration, renal blood flow and the glomerular filtration rate decrease and proximal tubular calcium and sodium reabsorption increase, leading to further increases in serum calcium concentrations. Anorexia, nausea, and vomiting associated with loss of circulating fluid volume exacerbate dehydration [23]. Immobilization caused by weakness and lethargy may exacerbate calcium resorption from bone.

Bone-specific alkaline phosphatase isozyme, a member of the zinc metalloproteinase family, is produced from and located on the cell membrane of osteoblasts. Its activity involves dephosphorylation of an extracellular inorganic pyrophosphate which regulates the matrix

maturation and mineralization of bone. Increase in serum ALP levels is however non-specific as it is also frequently associated with a variety of other diseases. Its level is elevated mainly in hepatobiliary diseases and bone diseases. In bone diseases, it is released from osteoblast during the process of new bone formation. Its level helps in the prediction of prognosis of bone tumours. The alkaline phosphatase activity is increased in bone tumours when the osteoblast is more actively laying down osteoid. Nonetheless, BSAP (Bone specific alkaline phosphatase) can be monitored in patients who are known to be at risk of bone metastases [24,25].

Levels of calcitonin were in normal range after surgery as calcium levels got down to normal levels. Calcitonin acts both directly on osteoclasts resulting in inhibition of bone resorption and following attenuation of subchondral bone turnover and directly on chondrocytes, attenuating cartilage degradation and stimulating cartilage formation. The calcitonin-induced inhibition of osteoclast function is believed to be due to disruption of cytoskeletal organization (distraction of actin rings) and disappearance of the cellular polarity of osteoclasts. Calcitonin receptors are coupled to both cAMP-PKA and Ca^{2+}-PKC (protein kinase C)-mediated signaling pathways [26].

In conclusion, raised level of serum calcium, acid phosphatase and tartrate resistant acid phosphatase indicates osteolytic activity is taking place and low levels of serum calcitonin and alkaline phosphatase shows that osteoblastic activity has decreased. Serum TRAP is a more useful and more convenient specific marker for the diagnosis and prediction of the recurrence of GCT of bone than methods and markers presented in previous reports describing MRI and DNA analysis and may be useful in routine follow-up of patients treated for GCT.

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