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SERUM BONE ALKALINE PHOSPHATASE LEVEL AS A POSSIBLE BIOMARKER OF KNEE OSTEOARTHRITIS

Biochemistry

Subhra Sucharita Sahoo — Senior Resident, Department of Biochemistry, VMMC & SJH, New Delhi

Ankita Kabi — Assistant Professor, Department of Anaesthesia, AIIMS, Rishikesh

Jayeeta Bhadra — Senior Resident, Department of Biochemistry, VMMC & SJH, New Delhi

B. C. Kabi* — Director Professor, Department of Biochemistry, VMMC & SJH, New Delhi
*Corresponding Author

ABSTRACT

Serum levels of Bone Alkaline Phosphatase (BAP) , Calcium and Phosphorus was investigated in patients with knee osteoarthritis (K-OA), attending the OPD of Orthopedics Department of VMMC & Safdarjung Hospital, New Delhi. A total of 44 patients of knee OA(28 women, 16 men, mean age 76.0±7.8 yrs) with a Kellgren-Lawrence grade of atleast 3 or 4 for bilateral knee and age and sex matched equal number of healthy controls were enrolled in this study. The levels of Serum BAP (26.3±5.4µg/l) was significantly raised in patients compared with controls (10.03±6.2 µg/l). The levels of Serum Calcium and Serum Phosphorus (6.1±2.72 mg/dl and 2.7±1.35 mg/dl respectively) were significantly low in patients in comparison to the levels in healthy controls (9.9±1.04 mg/dl and 5.3±2.3 mg/dl respectively). A significant negative correlation between serum BAP and Serum Calcium (r=-0.35;P=0.0142); as well as with Serum Phosphorus (r=-0.29;p=0.0439) was observed. Further it was noticed that there was gradual rise of Serum BAP levels with increase in K/L grade from 6 to 8 (P-value=<0.001) suggesting accelerated bone synthesis (osteophytes) with the progression of K-OA.

KEYWORDS

INTRODUCTION

Osteoarthritis (OA) is a disease of joints characterized by pain, cartilage degeneration, and joint stiffness. This disease has an estimated incidence worldwide to be 18% in female and 9.6% in male. Incidence of OA in US population is 13.5% in male and 18.7% in female whereas in Indian population, incidence in age group >40yrs is 28.7% overall and 31.6% in Females.(1) Although details in the pathogenesis of OA remain elusive, it is clear that this is a complex multifactorial disease process ultimately leading to cartilage disturbed metabolism as well as changes in other tissues adjoining joints such as synovium, subchondral bone and tendons(2). Radiographic evidence of osteophyte formation in typical locations within the human knee along with joint space narrowing has historically been the hallmark of osteoarthritis diagnosis(3). But plain radiography provides direct information on bones but not on cartilage, and is characterized by limited sensitivity. Magnetic resonance imaging, which provides direct information on changes in the different joint tissues, is currently being optimized in OA (4). Moreover, many researchers are currently trying to evaluate OA using biomarkers. Biomarkers are molecules derived from the connective tissue matrices, which are released into the systemic circulation and urine during tissue turnover. Several biomarkers have been identified for bone, cartilage, and synovial metabolism in humans and it has been suggested that biomarkers are useful in identifying those subjects at risk of OA progression and also assessing the therapeutic responses in clinical trials, because the biomarkers are more sensitive than the radiographic change and reliable for measuring the progression (5).

Bone alkaline phosphatase (BAP) is the bone-specific isoenzyme of alkaline phosphatase. A glycoprotein that is found on the surface of osteoblasts, BAP reflects the biosynthetic activity of these bone-forming cells. BAP has been shown to be a sensitive and reliable indicator of bone metabolism(6). Normal bone is constantly undergoing remodelling in which bone degradation or resorption is balanced by bone formation. This process is necessary for maintaining bone health. But in almost all cases of OA there is loss of the articular cartilage, so in order to compensate for missing cartilage bones in the joint produce small bony growth called osteophyte along the margins of the bone surface in an attempt to stabilize the joint.

BAP plays a role in regulating extracellular inorganic Pyrophosphate (PPi) conc by hydrolysing PPi, which is a potent inhibitor of formation of Hydroxyapatite crystals; there by promoting bone formation.

This phenomenon is supported by a study done by Calvo MS et al (7) where BAP act an early osteoblastic marker that has been shown in the serum to correlate positively with bone formation. So can this function of BAP be used to evaluate the progression of OA in a patient ? Can it act as a Biomarker for the disease?

As the major advantage of a Biochemical Biomarker is that it can not only be used for diagnosis, but can also be helpful in therapeutic management of the disease.

However, BAP has been examined in a few studies of human spinal and peripheral joint OA (8,9). Again these studies have very contrasting results like studies performed by Corrado A et al.(10) and Hulejová H. (11) showed higher serum BAP and urinary deoxypyridinoline levels in Advanced OA where as studies done by Nurdan P et al (12) and Kim HJ (13) suggested no increase in markers of bone turnover. Further more there is scanty information on Indian population in this regard.

Therefore, the purpose of this study was to measure serum BAP levels in patients with knee OA, and to correlate with the severity of the disease in Indian population.

MATERIAL AND METHODS

Patients with knee osteoarthritis. The study was approved by the Ethics Committee for VMMC & Safdarjung Medical College, GGSIP University, and informed consent was obtained from all patients and controls.

A total number of cases of 44 (28 women,16 men, mean age 76.0±7.8 yrs; range 54-88years, mean BMI 24.9±4.7) patients with a Kellgren-Lawrence (K/L) grade of 3 or 4 for bilateral knee were enrolled in this study (bilateral grade 3 K/L that sum up to a total grade 6, n=16; grade 3 and 4 K/L, sum up to a total grade 7, n=20; and bilateral grade 4 K/L sum up to a total grade 8, n=8). Patients with knee pain in the medial femoro-tibial compartment of the studied knee on the day examined, full filled the criteria of Knee OA of the medial femoro-tibial joint, as defined by the American College of Rheumatology (ACR) criteria (14). Standing, extended and antero-posterior view and lateral view radiographs were taken, and the staging of knee OA based on radiographic examination was assessed using K/L grading (15). All radiographs were taken by experienced technicians. Again a total of 44 controls with age and sex matched healthy individuals (mean age 70.76±*12.8;* range 58-84 years) having no evidence of OA either clinically or radiologically were taken in this study.

Measurement of biomarkers. Fasting Blood samples were obtained from all patients on the day that radiographs and all controls were taken. The serum samples were stored at -80 C until analysis. Serum Bone Alkaline Phosphatase(BAP); Serum Calcium and Serum Phosphorus were measured.

Assays including serum BAP level was performed with ELISA as follows: Serum BAP measured by Ostease BAP EIA Immunoenzymetric Assay Kit. Assays including Serum Calcium and Serum Phosphorus levels were performed by auto analyser in the lab.
Statistical analyses. Data are expressed as mean ± SD, unless otherwise noted, and analyzed for significant difference by a one-way analysis of variance (ANOVA) with the multiple comparisons test (Microsoft Excel Sheet software) or the Student's t-test (Graphpad Software, San Diego, CA). Correlation analysis was also performed with Graphpad Software. Differences were considered statistically significant at $P<0.05$.

RESULTS

Measurement of biomarkers. First, the levels of Serum BAP; Calcium and Phosphorus was examined in bilateral K-OA patients as well as in controls samples.

Table 1: Comparison of serum values among cases and controls

	Cases (n=44)	Controls (n=44)	P-value
Serum Bap	26.3±5.4ug/l	10.03±6.2ug/l	0.0001*
Serum Calcium	6.1±2.7 mg/dl	9.9±1.04 mg/dl	0.0001*
Serum Phosphorus	2.7±1.4 mg/dl	5.3±2.3 mg/dl	0.0001*

The levels of Serum BAP (a marker for bone synthesis) were significantly elevated in cases compared with healthy controls. However, the levels of Serum Calcium and Phosphorus were noticably low in cases as compared to controls as stated in Table 1.Secondly, the correlation between Serum BAP and Serum Calcium and Serum Phosphorus was analysed as in Table 2.

Table 2: Correlation between Serum BAP and bone mineralization parameters.

	r-VALUE	P-VALUE
SERUM CALCIUM	-0.359	0.0142*
SERUM PHOSPHORUS	-0.298	0.0439*

Of note, there was a significant negative correlation between Serum BAP and Serum Calcium(P=0.0142)as well as Serum Phosphorus,(P=0.0439)indicating accelerated bone mineralization in K-OA patients.

Further, the above levels were compared among three groups of severity of OA: K/L grade total 6 (n=16), K/L grade total 7 (n=20) and K/L grade total 8 (n=8).

Table 3: Correlation of Biomarkers with the severity of the disease by use of ANOVA.

	F-VALUE	P-VALUE
SERUM BAP	211.5108	<0.001*
SERUM CALCIUM	0.904514	0.412
SERUM PHOSPHORUS	0.117976	0.889

There was a gradual significant rise of Serum BAP levels with increase in K/L grade 6 to 8 (p-value <0.001). However no significant change was noticed in Serum Calcium and Serum Phosphorus with the disease severity (Table 3, Fig.1 A,B,C,D).

These findings suggest that there is accelerated bone mineralization which is proportionate to the rise of BAP in K-OA patients.

DISCUSSION

It is a fact that BAP is a membrane anchored protein that is characteristic marker expressed in large amount by active Osteoblasts. Millan JL (16) stated that in mineralising tissues, BAP regulates extracellular inorganic Pyrophosphate (PPi) conc by hydrolysing PPi, which is a potent inhibitor of formation of hydroxyapatite crystals; there by indirectly promotes Osteoblastic activity. Hence Bone specific Alkaline Phosphatase (BAP) is a marker of Osteoblastic activity. Since the concentration of BAP in the serum from patients with bilateral K-OA significantly increased in comparison to healthy Controls, the rise in BAP levels must be from some osseous source within the knee joint. Similar finding was reported by Corrado A et al.(10) that suggested an increase in BAP production in Osteoarthritic cells.

Furthermore in this study there was a significant negative correlation between Serum BAP and Serum Calcium as well as Serum Phosphorus levels. The correlation of Serum BAP with Serum Phosphorus was also reported by Kemi et al (17) which suggested that high intake of Phosphorus adversely affect bone metabolism by decreasing the marker of bone formation, BAP activity, in healthy young females. Similar finding was also reported by M Haraikawa et al (18) which suggested significant negative correlation between Serum BAP with Serum phosphorus levels and Calcium intake.

Again when the increased serum BAP levels were compared within the group of K-OA patients with increasing severity. It was found that there was a significant positive correlation between the two i.e there was an increase in Serum BAP levels in K/L grade total 8 in comparison to K/L grade 6 and 7 suggesting that the likely osseous source of increased serum BAP in K/L grade 8 patients might be from Osteophyte formation.

In this study the serum BAP levels was also compared among equal number of Premenopausal females, Postmenopausal females and Males which showed no significant change although more female patients were present in the study suggesting that gender has no influence in the rise of serum BAP levels in K-OA patients.

Together these observations suggests the possible association of bone formation and Calcium regulation with the progression of Osteoarthritis providing a novel insight into the pathogenesis of OA, which can further be utilized for diagnosis of progression of the disease as well as may be helpful for therapeutic management.

However, in this study the number of cases taken is limited, to make the finding relevant, similar study's to be conducted over a larger population to strengthen the relevance of association. Furthermore, the cases taken in this study are advanced cases of K-OA having K/L grade of 6;7;8, so similar study to be conducted in lower K/L score patients to find its relevance as a biomarker in disease diagnosis.

The present study suggests a possibility that increase in Bone formation and Bone mineralization could be a factor for progression of K-OA and inclusion of Serum BAP, may be a useful marker for evaluating the severity of Osteoarthritic condition in case of K-OA mostly in advance state.

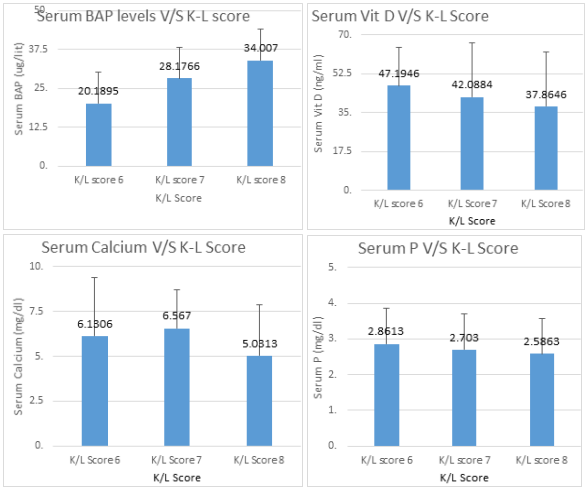


Fig1: Levels of Serum BAP; Serum Vitamin D; Serum Calcium and Serum Phosphorus in K/L grade total 6, 7 and 8 in patients with bilateral K-OA. Serum levels of (A) BAP, (B) Vitamin D were measured by ELISA and Serum levels of (C) Calcium, (D) Phosphorus were measured by Semi- Auto Analyser. Data represent the mean ± SD in each group (K/L grade total 6, n=16; grade total 7, n=20; grade total 8, n=8). Serum (A) BAP levels were only increase in K/L grade total 8 ($P<0.001$), compared with that in K/L grade total 6 and 7.

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