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Serum alkaline phosphatase: A prospective biomarker for assessment of progress of fracture healing in diaphyseal fractures of long bones in adult patients

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Abstract
Introduction: Serum biomarkers such as Alkaline Phosphatase can be used to assess bone-forming activity. Studies have shown that the measurement of bone formation markers such as Serum Alkaline phosphatase during the fracture healing process could enhance the accuracy of the bone healing stage assessment, allowing early detection in patients at risk of the development of delayed union or non-union.
Aims: To evaluate the changes of serum alkaline phosphatase levels during healing of diaphyseal fractures of long bones in adults treated operatively.
Materials and methods: A hospital based prospective study was conducted in the Department of Orthopaedics, Silchar Medical College and Hospital, Assam. 91 adult patients ranging from 18 yrs to 50 yrs with diaphyseal fractures of the tibia or femur who met the inclusion criteria were recruited in our study. Patients with systemic diseases and drugs intake which could affect the bone turnover were excluded from our study. The fractures were treated with closed reduction or open reduction and internal fixation with intramedullary nailing. The biomarker serum Alkaliine Phosphatase was measured at definite intervals until bony union was achieved clinico-radiologically.
Results: Out of the 91 patients, 36 patients (Group A=normal healing group) achieved bony union by the end of 6 months. 50 patients (Group B=delayed healing group) where bony union was not completed by the end of 6 months but completed by 9 months. 5 patients (Group C=non-union group) where bony union was not seen even by the end of 9 months. At the time of admission, mean serum ALP levels remained within normal limits in all the three groups. Mean serum ALP levels followed the same pattern in Group A and Group B reaching the maximum levels at 3rd week post trauma but the mean serum ALP at every selected interval was significantly higher in Group A than Group B. In Group A, the mean serum ALP returned to baseline level by the end of 6 months, whereas, in Group B patients, the mean serum ALP remained elevated even by the end of 6 months. In group C patients, the mean serum ALP level did not rise significantly throughout the period of study.
Conclusion: Thus, the determination of serum ALP levels during the fracture healing could be an additional tool to help predict fractures at risk of delayed union or non-union of diaphyseal fractures therby allowing the clinician to step in early and take appropriate intervention.

Keywords: Alkaline phosphatase, non-union of diaphyseal fractures, biomarkers of fracture healing

Introduction
Fracture healing is a continuous physiological process to achieve bony union [1]. The expected time required for a complete and adequate fracture healing process depends on several factors, such as the severity of injury of the adjacent soft tissue, the displacement of the fracture ends, the degree of comminution, the mode of stabilization of the fracture and patient related factors, such as age, and the presence of co-morbidities [2, 3]. In clinical practice, the fracture healing process is normally evaluated by physical and radiographic examinations.
However, there is a lack of consensus in the assessment of this, rendering an estimation of the incidence of fracture healing complications difficult [4]. It is estimated that 5-10% of all patients with long bone fractures will develop impaired fracture healing processes, especially delayed union and non-union. The development of a non-union process which is the most commonly diagnosed fracture healing complication, corresponds to a failure of the fracture

repair process, generally due to extensive injury to the adjacent soft tissues and inadequate vascularisation of the trauma site [5-7].

Fracture healing complications are associated with prolonged pain and functional impairement, making the early diagnosis of such complications mandatory [8]. A prompt diagnosis of such complications could prevent prolonged patient distress and disability. The process of fracture union is characterized by the production of a new organic matrix, known as osteoid and its subsequent mineralization which bridges the gap between two bony fragments, known as bridging callus. The process of this fracture healing should be serially quantifiable or measurable. Therefore, a valid measurement is desired to measure the bony union process and the values yielded should be on a continuous numerical scale [9-10].

Serological bone turnover markers (BTMs) have been studied in fracture healing researches with the objective of monitoring the fracture callus development and providing prognostic value for the early detection of fracture healing complications. BTMs are products of bone cell activity and are generally subdivided into three categories: bone resorption markers, bone formation markers and osteoclast regulatory proteins [11-13]. Bone formation markers namely the bone alkaline phosphatase (BALP), Osteocalcin (OC), N-terminal propeptide (PINP) and C-terminal propeptide (PICP) of type-1 procollagen are derived from the osteoblastic activity during the different stages of osteoblasts proliferation, differentiation and of osteoid synthesis. Markers of bone resorption includes Hydroxyproline, Hydroxylysine-glycosides, Carboxy terminal cross-linked Telopeptides of Type 1 Collagen, Pyridinoline, Deoxypyridinoline, Cathepsins, Tartrate Resistant Acid Phosphatase (TRAP) [14-15]. In recent years, biochemical markers of bone formation and resorption have been developed to quantify bone turnover and remodelling, with possible applications in clinical practice.

Total alkaline phosphatase activity in serum has been used commonly as a biochemical marker of osteoblast function, but lacks specificity because of the contribution of activity derived from the liver, in particular. Human alkaline phosphatases (ALP) are a group of enzymes of similar specificity coded for by at least four different gene loci that catalyse the hydrolysis of phosphate esters at an alkaline pH. The gene for tissue non-specific ALP encodes the isoenzymes expressed in liver, bone and kidney. In healthy individuals about half the activity of alkaline phosphatase in serum is derived from bone and the remainder from liver. The isoforms differ only in the degree of sialylation and glycosylation, reflected in differences in electrophoretic mobility, heat stability and precipitation by lectin. Methods to separate and quantify bone ALP in the presence of liver ALP, based on these properties, have not had sufficient specificity or sensitivity to be useful clinically.

Studies have shown that the measurement of bone formation markers such as Serum Alkaline phosphatase during the fracture healing process could enhance the accuracy of the bone healing stage assessment, allowing early detection in patients at risk of the development of delayed union or non-union. Since there are very few studies showing the role of serum alkaline phosphatase in assessment of fracture healing process, through our study we plan to evaluate the importance and relation of serum alkaline phosphatase with regard to fracture healing process.

Materials and methods

A hospital based prospective study was conducted in the Department of Orthopaedics, Silchar Medical College and Hospital, Assam from 01-06-2018 to 31-05-2019.

Data collection

91 patients with diaphyseal fractures of the femur or tibia attending the OPD and emergency of Department of Orthopaedics, Silchar Medical College and Hospital who met the inclusion criteria outlined below were recruited in the study. Inclusion criteria included traumatic fractures of diaphysis of femur or tibia less than 7 days old, age group between 18 years and 45 years, normal haemogram, blood glucose, kidney function and liver function, patients giving written consent. Exclusion criteria included Age less than 18 years(as we included only adults) and more than 45 years(osteoporosis may have occurred),pathological fractures, associated head trauma, compound fractures, Immunocompromised patients, uncontrolled Diabetes Mellitus, chronic liver disease, chronic renal failure, thyroid or parathyroid disorder, metabolic bone diseases and malignancy, inflammatory, septic or tubercular arthritis, Pregnant or lactating females, chronic inflammatory bowel disease, patients on medications such as Steroids, bisphosphonates etc. Patients refusing written consent, Patients with irregular follow up and those lost to follow up.

Consent

An informed written consent was obtained from each patient prior to participation in the study.

Ethical clearance

Ethical clearance for this study was taken from the ETHICAL Committee of Silchar Medical College and Hospital, Assam.

Biomarker examination

For biomarker examination, 2ml of peripheral blood was collected in Red coated vials under standard aseptic technique. Serum or heparinised plasma, free from hemolysis, is the recommended specimen. Serum should be separated from the cells within two hours after collection. Use of plasma with EDTA or oxalate is avoided. Quantitative determination of serum ALP (Alkaline Phosphatase) activity at ph 10.4, temp. 37*C will be done spectrophotometrically using a p-nitrophenyl phosphate as a substrate using Beckman Coulter AU analyzers.

Measurement of serum alkaline phosphatase done on the day of admission,14th day, 21st day, 1month, 2 month,3 month counted from day of trauma and last sample was collected when serum alkaline phosphatase level returned to its normal range. Reference range of serum Alkaline Phosphatase in normal adult is between 40-140 IU/L.

Statistical analysis

Statistical analysis was performed using Graph Pad Prism software (trial version) and Microsoft Excel. The datas in each category were presented as numbers (percent) and were compared among groups using Chi square test. The means and standard deviations were compared by unpaired t test and for more than two groups, ANOVA was used to find out the most significant groups among all the groups. The serum level of alkaline phosphatase was compared at different follow up of post injury from baseline. Probability value (p value) less than 0.05 was considered statistically significant.

Radiological examination

Standard radiographs in Antero-posterior and Lateral views of

the thigh and leg with adjacent joints above and below were taken. Closed reduction and internal fixation of the fractures were done with interlocking nail. Open reduction was done in those cases, where closed reduction was not possible. The radiographs were taken at 0 weeks (at the time of admission), on the day of surgery, then 3weeks, 2months, 3months, and then every month till bony union or till 9months whichever was earlier. The patients were followed up clinically for the signs of fracture union, pain and tenderness at the fracture site at 3weeks, 2 months and then every month till fracture union.

The fracture union was defined as the time when three of the four cortices showed bridging callus across the fracture site. An Antero-posterior and lateral radiographs were taken to assess the fracture healing. The presence or absence of callus and the number of cortices bridged by callus were observed in each radiograph and documented in case record sheet. Non union is defined as the cessation of all reparative processes of healing without bony union. A fracture that at minimum 9 months post occurrence and is not completely healed and has not shown radiographic progression for three months is labelled as non-union of diaphyseal fracture.

Observations and results

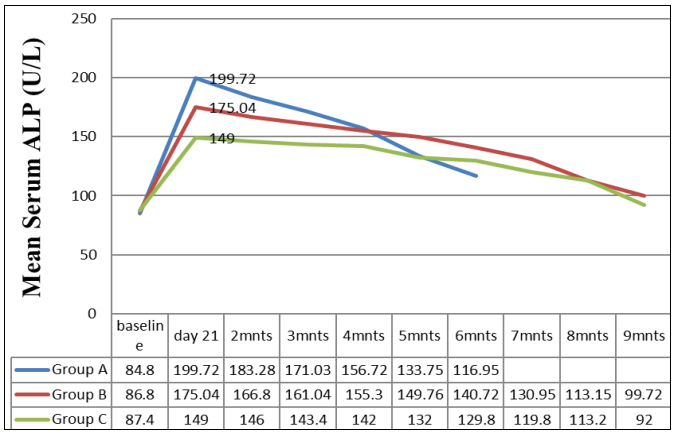
All the 91 patients were followed up for 9 months with no lost to follow up. The age ranges from 18 to 50 years in our study with a mean age of 31.5275 ± 10.6701 years. Maximum incidence of long bone fractures was found in 18 to 30 years. Of the 91 patients, 73(80.22%) patients were males and 18(19.78%) patients were females in our study. Of the 91 patients, 44(48.35%) patients had femoral shaft fractures, 43(47.25%) patients had fractures of both tibia and fibula of leg, and 4(4.40%) patients had isolated tibial shaft fractures. Out of the 44 femoral shaft fractures, 35 (79.58%) were due to road traffic accidents, 6 (13.67%) were due to fall from height, 2 (4.50%) were due to self fall and 1 (2.25%) due to physical assault. Out of the 43 fractures of both tibia and fibula of leg, 18 (41.86%) were due to RTA, 17 (39.53%) were due to self fall, 5 (11.63%) were due to fall from height and 3 (6.98%) were due to physical assault. 3 (75%) of the isolated tibial shaft fractures were due to self fall and 1 (25%) was due to RTA. Transverse fracture pattern was seen in 40 cases(43.97%), oblique pattern in 38 cases(41.76%) and spiral pattern in 13 cases(14.27%). Fractures in the upper 1/3rd of the diaphysis seen in 13 cases (14.29%), fractures in the middle 1/3rd seen in 60 cases (65.93%) and fractures in the lower 1/3rd seen in 18 cases (19.78%).

Group A consists of those patients where radiological union is completed by the end of 6 months. Group B consisted of delayed healing where radiological union is achieved between 7 to 9 months and Group C consists of those patients where radiological union is not achieved even by the end of 9 months

In Group A patients, the mean serum ALP on the day of admission was 84.8 IU/L. The mean reached a maximum at 3 weeks i.e 199.72 IU/L and was statistically significant (p value<0.001) when compared to the baseline. The value gradually decreased from the 2nd month onwards and at around 6 months, the mean serum ALP came down to the normal range. By the end of 6 months, there were 36 patients showing bridging callus in atleast three cortices on the AP and Lateral radiographs. These were classified as normal healing group. 26 of the patients had bridging callus in 3 cortices and the remaining 10 patients had bridging callus in all the 4 cortices.

In group B patients, the mean serum ALP on the day of admission was 86.8 IU/L. The value reached its maximum at around 3 weeks i.e 175.04 IU/L and was statistically significant (p< 0.001) when compared to the baseline. The value then starts to decrease and at around 6 months, the mean was 140.72 IU/L which was in the upper borderline of the normal range. The mean serum ALP came back to the normal range from the 7th month onwards and continued to be in the normal range till the end of 9 months. In group B patients, at the end of 6 months, bridging callus in three cortices was not seen in any of the patients. However, at the end of 7 months, 10 patients showed bridging callus in atleast three cortices. By the end of 8 months, 19 patients had bridging callus in three cortices and 5 patients had bridging callus in all the four cortices. At the end of 9 months, 33 patients had bridging callus in three cortices and bridging callus was seen in the remaining 17 patients in all the four cortices. Thus, these 50 patients which showed bridging calluses in atleast 3 cortices between 7 to 9 months were labelled as delayed healing group.

In group C patients, the mean serum ALP value at baseline was 87.4 IU/L. The value increased and reached its maximum to 149 IU/L on day 21 and was statistically significant (p value <0.0001). The value decreased gradually from the 2nd month and the increase in the mean ALP was lowered compared to that of Group B and Group C patients. The mean value at the end of 9 months was 92 IU/L which was not statistically significant (p value 0.0549). In Group C patients, bridging callus across one cortex was seen in 4 patients and 1 patient had bridging callus across two cortices by the end of 6 months and bridging callus in two cortices was seen in all patients by the end of 9 months. However, in all the 5 patients, there was no bridging callus in atleast three cortices at the end of 9 months. These 5 patients were thus classified as non union group.



	baseline	day 21	2mnts	3mnts	4mnts	5mnts	6mnts	7mnts	8mnts	9mnts
Group A	84.8	199.72	183.28	171.03	156.72	133.75	116.95			
Group B	86.8	175.04	166.8	161.04	155.3	149.76	140.72	130.95	113.15	99.72
Group C	87.4	149	146	143.4	142	132	129.8	119.8	113.2	92

Fig 1: Chart showing the variation of serum ALP among the three different groups

The maximum increase in mean serum ALP from the baseline in all the three groups was observed at 3 weeks (21 days) with the highest increase in group A (199.7 U/L) followed by group B (175.0 U/L) and group C (149U/L). The difference of mean Serum ALP among the three groups was statistically significant (p value <0.0001). The difference in mean between Group B and Group C was not statistically from 7th month onwards which was 0.0523(not significant), at 8th month it was 0.9865 (not significant) and at 9 month which was 0.1519 (not statistically significant)

Discussion

The outcome of our study is that estimation of serum alkaline

phosphatase co- relates with the clinico-radiological signs of fracture healing in diaphyseal fractures of long bones. Significant rise in the level of serum ALP demonstrates an increased osteoblastic activity. Osteoblasts secretes large quantities of ALP, which is involved in the process of bone matrix formation and its mineralization [16]. Although serum ALP levels co-relates well with the process of fracture healing, the bone-isoenzyme of ALP (BsALP) is considered a more specific marker of bone formation. However, the inclusion and exclusion criteria for this study rules out the possibility of other ALP isoenzymes being responsible for the significant increases in ALP levels.

Skeletal turnover can be easily assessed and non-invasively by the measurement of turn-over markers. Thus, early knowledge of a patient's progress of fracture can help to prevent delayed union or non-union by enabling modification of the host biological response. In our study, the serial serum ALP levels were co-related with the clinico-radiological progression of fracture healing in all the patients. We observed that the serum ALP at 3rd week was co-related with the future outcomes of these fractures and we may predict the future outcomes as early as the 3rd week. The findings of our study was similar to that of other studies [17-20].

Conclusion

The serial measurement of serum Alkaline Phosphatase levels during the fracture healing process in combination with clinico-radiological examination can be an additional, useful and cost effective tool in predicting whether fractures are at risk of developing complications like delayed union and non union. This in turn helps the clinician to intervene and take appropriate measures at an appropriate time.

The serum ALP level at the 3rd week could be co-related to the outcome of these fractures. The findings of our study was similar to that obtained by Singh Ajai *et al*. [18] and Prakash Anand *et al*. [17], in which they also co related the outcome of the fracture healing process by the estimation of mean serum ALP as early as the 3rd week.

The results of this study must be viewed within limitations of the methods used. The major limitation was the small number of patients studied. Also, the periodic estimation of serum ALP for assessing fracture healing till consolidation and remodeling could not be carried out due to the fixed time period. The bone isoenzyme of ALP (BsALP) is considered a more specific marker of bone formation, but the high cost involved and the unavailability at our set up was a limitation.

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