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Evaluation of Serum Alkaline Phosphatase as a Biomarker of Healing Process Progression of Simple Diaphyseal Fractures in Adult Patients

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Abstract

In day to day practice, progression of bone healing is assessed mainly by clinico-radiological parameters. Furthermore, clinicians are unable to identify the delayed and nonunions early, and even advanced imaging techniques may not be helpful in these cases; hereby increasing the suffering period of the patients. Serum biomarkers, such as alkaline phosphatase (ALP) suggestive of bone formation, may be clinically useful in evaluating the progress of fracture healing. Changes in serum ALP levels were noted at definitive intervals in 95 adult patients (enrolled as per inclusion/exclusion criterion) with simple traumatic diaphyseal fractures of both bones of leg. Regular follow up of these patients was done till either bone union was completed or maximum up to the end of 09 month. The changes in serum ALP levels were recorded and correlated with the clinico-radiological progression of fracture healing in these patients.Depending on the clinico-radiological parameters of progression of fracture healing, all 95 patients were allocated into 02 groups by the end of active follow up; Group A (n = 69): clinico-radiological union achieved before or by the end of 06 months; and Group B (n = 26): clinico-radiological union not completed by the end of 06 months. The Group B was further subdivided into B_1 (n= 18): clinico-radiological healing not completed by 06 months but completed by the end of 09 months; and B_2 (n= 08): clinico-radiological healing not achieved even by the end of 09 months - Non-union. At the time of admission, mean serum ALP levels remained within normal limits in all included patients. Mean serum ALP levels followed the same pattern in group A and B_1, reaching a maximum level at post trauma 3rd week. But the mean levels of serum ALP at every selected interval was significantly higher in group A than group B_1. In Group A, serum ALP levels returned to normal values by complete union achieved (mean healing occurred at 19.2 weeks), where as in group B_1 values remained elevated even by the end of 06 months (mean time of union 28.5 weeks). In Group B_2, mean serum ALP level remained within normal limits throughout the followup. So, the determination of serum ALP levels during fracture healing could be an additional tool in predicting fractures at risk of delayed / nonunion of simple diaphyseal fractures, aiding the clinician to prefer the early appropriate intervention at appropriate period.

Keywords: Alkaline phosphatase, biomarker of fracture healing, nonunion of diaphyseal fractures.

Introduction

Fracture healing is a continuous physiological process to achieve union[1]. This process of fracture union is characterized by the production of a new organic matrix, known as osteoid and its subsequent mineralization, thus bridging the gap between two bony fragments (bridging callus). This fracture healing process should be serially quantifiable / measureable[2]. Till date, clinically validated method to measure healing progression is not available. So a valid measurement for bony union should be desired to measure the bony union process. Thus, the values yielded by measurement should be on a continuous numerical scale[3]. However till now, researchers have used an end point for completely healed fracture at a point of time without documenting the values signifying progress of union before that time[4]. Presently, fracture healing is commonly assessed clinically and radiologically. The probability of correct radiological evaluation of the stage of union in fractures of tibia has been shown to be only about fifty percent. So, the radiographic assessment is not an optimum method to assess the fracture healing, a fact borne out by a study on radiological evaluation of the stage of union in fractures of tibia[5]. None of the available measures of union will help in the early detection of problems in the bone healing process, resulting into problems in outcome i.e. bone union[6]. As the routine methods of diagnosis available may not be able to identify the complications like delayed / nonunion early, so patients will have to suffer for a longer period. Furthermore, it is sometimes hard to distinguish early, a delayed union from nonunion radiologically, and the use of more advanced imaging techniques, such as nuclear scintigraphy, is still limited to only few centers in our country[7]. So, the detection of specific serum biomarkers of bone formation, such as ALP can be clinically useful in evaluating the progress of healing process[8, 9].

In one study, it was observed that serum bone ALP and creatinine corrected urinary collagen crosslinks were significantly higher among women who experienced an

osteoporotic fracture compared with those women who did not had fracture[10]. In a study, serum ALP activity correlated with the long bone fractures healing process in 83 dogs. It was also observed in the same study that serum phosphorus and calcium changes followed a proportional and inverse pattern to ALP changes respectively[11]. In another study[12], the changes in total alkaline phosphatase in femoral and trochantric fractures had been studied. It was observed that the changes were more in trochantric fractures than neck fractures and both fractures expressed similarly for serum ALP post surgically.

With this background, we made our research hypothesis, that as the serum alkaline phosphatase levels suggest the osteoblastic activity (responsible for both, bone matrix formation and its mineralization), then its serial serum estimations will correlate with progression of fracture healing process and thus may predict the fracture healing outcome early. We designed this prospective case series to analyse the correlation of serial serum ALP levels with progression of fracture healing process and to evaluate the relationship between serial serum ALP levels and fracture healing outcome.

Material and Methods

Total 95 adult patients in the age group of 18 years to 45 years with simple, fresh (< 7 days) traumatic diaphyseal fractures of both bones of leg managed conservatively were included in this study. Following was the exclusion criterion, age less than 18 yrs (as we included only adults) and more than 45 yrs (as at and after 45 years, osteoporosis may have occured), polytrauma, pathological fractures, compound or infected fractures immuno-compromised patients, patients with intact fibula, patients on prolonged drugs like anabolic steroids, thiazides, diuretics, hormonal therapy, NSAIDs, calcium, fluorides and immunosuppressive drugs, uncontrolled diabetes, patients with bile duct obstruction and chronic inflammatory bowel disease, clinically malnourished and those not willing for inclusion in study.

Study Protocol: After obtaining ethical clearance from departmental research review committee, all 95 patients were included in this study from 2006 to 2011. Informed consent was taken. After the recording of demographic characteristics, all 95 patients were managed conservatively (reduction setting and above knee POP was applied under regional / general anaesthesia by principal investigator AS / co-investigator RNS. All were discharged after 24 – 48 hours with a standard advice written on discharge card. We used following research tools in our study.

Biomarker Examination: : 2ml of peripheral blood collected into EDTA coated vials under standard aseptic technique. Quantitative determination of serum ALP activity (at pH-10.4, Temp-37°C) was done spectrophotometrically (405nm) using an p-Nitrophenylphosphate as a substrate and other reagent provided by LABKIT. According to LABKIT manual, reference range of normal adult was in between 98-279 U/L at 37°C. Follow ups were done at admission, 14^{th}, 21^{st}, 28^{th}, 45^{th}, 60^{th}, 90^{th} day counted from day of trauma and last sample at clinico-radiological union or at 6^{th} month.

Clinical Examination: Gentle clinical examination of the fracture site was done at 6^{th}, 10^{th} week, for the assessment of – skin condition, abnormal mobility, bony tenderness, transmitted movements. Further, follow up and management was decided by the investigator/ co-investigators as per progression of union.

Radiological Examination: Standard plain radiographs of the affected leg including knee and ankle joints (AP and Lateral views) were done and separately these were assessed by at least 02 investigators for evidence of progression of bony union, if any (as per RUST score)[13]. Radiological follow ups were done at 6^{th}, 10^{th} week (Further, follow up and management was decided by the investigator/ co-investigators as per progression of union).

Based on the above clinico-radiological evaluation, we allocated our patients into 2 groups – Group A: clinico-radiological union achieved by the end of 06 months, and Group B: clinico-radiological union not completed by the end of 06 months. Group B was further followed up, and subdivided into Group B_1 (clinico-radiological healing not completed by the end of 06 months but completed by the end of 09 months) and Group B_2 (clinico-radiological healing not completed even by the end of 09 months). Clinical bone union was defined as the stage in the healing process when the fracture site was painless (no tenderness), motionless (no abnormal mobility) with presence of transmitted movements. Radiographic bone was defined when bony callus was evident on at least 3 cortices in standard AP and Lateral views and with RUST score more than seven 13.

The mean values of serum ALP, serially recorded throughout the follow up period, were compared with others subjects in the same group at different time intervals. Mean ALP values were also compared between these three groups. The data collected were entered in Microsoft Excel and were checked for any inconsistency. In each group, a hypothesis test of the same variable over time was carried out using analysis of variance on the repeated measures. Statistical analysis between groups was performed using a 95% confidence interval of mean. The 5% level was considered significant. All the analysis were done by using SPSS Software (15.0 version).

Results and Discussion

Total 95 patients were enrolled and studied, which were allocated into group A (N = 69), group B_1 (N = 18) and group B_2 (n = 8), depending on the progression, duration and type of bone healing. The average age of group A was 31.7 (range 28.4 – 39.8) years, group B_1 was 32.1 (range 26.2 - 38.9) years and was 32.8 (range 30.6 – 39.7) years for group B_2. The difference between the mean ages of these groups was not significant. The mean age of fracture at admission in these groups was 2.9 (range 1-5) days, 2.3 (range 1-6) days and 3.1 (range 1-5) days respectively (the difference between the age of fracture at

admission was insignificant). The bony healing occurred at 19.2 (range 15 – 22) weeks in group A and at 28.5 (range 27 – 34) weeks in group B_1. The mean serum ALP levels remained within normal limits (98-279 U/L) in all included patients at the time of admission. The variation in ALP serum levels followed the same pattern in group A and B_1, reaching a maximum level at 3rd week in both groups (maximum mean of group A 689 U/L and of group B_2 500 U/L) figure-1. Serum ALP levels remained elevated significantly till 06 weeks in group A and reached within normal range when clinico-radiological union occurred. In group B_1 mean serum ALP remained significantly elevated even till 06 months. Till bony union occurred in group A, at every interval, the mean values of serum ALP of group A were significantly higher than that of group B_1. The changes in mean serum ALP levels in group B_2 were never significant and remained within normal limits till 06 months.

Fracture healing is a continuous physiological process to achieve bone union. Conventionally treating doctors have relied on clinical and radiological assessment to remove plasters but these methods lack objectivity and hence are not a reliable indicator to be used to compare 'time to union' in different treatment methods. Hence, there is a need to develop an accurate, precise, reliable, reproducible, patient – doctor friendly and cost effective method to measure fracture healing objectively.

Skeletal turn-over can be assessed easily and non-invasively by the measurement of turn-over markers. They can be subdivided into; i. bone resorption markers ii. osteoclast regulatory proteins and iii. bone formation markers. On account of the rapidly accumulating knowledge of bone matrix physiology and biochemistry, interpretation and characterization of various stages of the fracture healing has been analyzed. Bone turnover markers level vary during the course of fracture repair with their rates of change being dependent on the size of the fracture and the time that it will take to heal. Thus, early knowledge of the individual progress of fracture could help to keep off delayed or nonunion by enabling modification of the host's biological response.

Normal fracture healing is generated by increased osteoblastic activity. Osteoblasts secrete large quantities of ALP, which is involved in the process of bone matrix formation and its mineralization[8]. ALP is believed to either increase the concentration of local inorganic phosphate or neutralize inorganic pyrophosphate, an inhibitor of hydroxyapatite crystal formation[14-15]. Although serum ALP levels correlate well with the process of fracture healing, the bone isoenzyme of ALP (BALP) is considered a more specific marker of bone formation[8]. The inclusion criteria set for this study eliminated the possibility of other ALP isoenzymes being responsible for the significant increases in ALP levels in these enrolled cases.

In adult patients, under the ideal circumstances, the expected time of bone union of an uncomplicated diaphyseal tibial fracture is approximately 15.9-19.8 weeks [16], which is the case of group A (mean union at 19.2 weeks). Delayed union is considered as a fracture that requires more time for complete bone union than the usual but will unite ultimately[17], which is the case of group B_1 (mean union at 28.5 weeks). Nonunion is defined as the cessation of all reparative processes of healing without bony union[18]. A fracture that at minimum of 9 months post occurrence and is not completely healed, which is the case of group B_2 and has not shown radiographic progression for 3 months is labeled as nonunion of diaphyseal fracture tibia[19]. The incidence of nonunion of diaphyseal tibial fracture varies from 6 – 41% [16]. In the present study, the incidence of clinico-radiological nonunion at the end of 09 months was 8.5%. In our study, the serial serum ALP levels were correlated with the clinico-radiological progression of bony fracture healing in all patients. In all 95 patients, we were able to predict the fate of fracture healing process by the serial estimation of serum ALP levels. We observed that serum ALP level at third week was correlated with future outcome of these fractures. We may predict the future outcome of these fractures at as early as third week. Our finding related with ALP level variation during fracture healing were corresponding with that of other studies[12, 20].

In our study, changes in serum ALP level in group A and B_1 paralleled the process of fracture healing as documented by clinico-radiological (including RUST score) evidences. Though, in group A, mean ALP returned to normal reference limits by clinico-radiological fracture healing, in group B_2 these levels remained elevated till 06 months, suggestive of ongoing osteoblastic activity in these patients. At 06 months, clinico - radiologically it was not evident that which patients will fall into delayed union and which one will be in nonunion. Waiting 9 months to diagnose and treat a nonunion is patently unreasonable in modern world. This dilemma raised a question that whether a clinician should wait for radiological signs to settled down for an establish diagnosis of nonunion or one can predict and can intervene early by observing a biomarker, like ALP. We may predict the future outcome of these fractures at as early as third week by observing the serum ALP levels.

Conclusion

Simple diaphyseal, fresh traumatic fractures, the serial measurement of serum ALP levels during the fracture healing process in combination with clinico-radiological examination can be an additional, useful, reproducible, patient – clinician friendly and cost effective tool in predicting whether fractures are at risk of developing complications like delayed union / nonunion and in aiding the clinician to intervene properly at an appropriate time.

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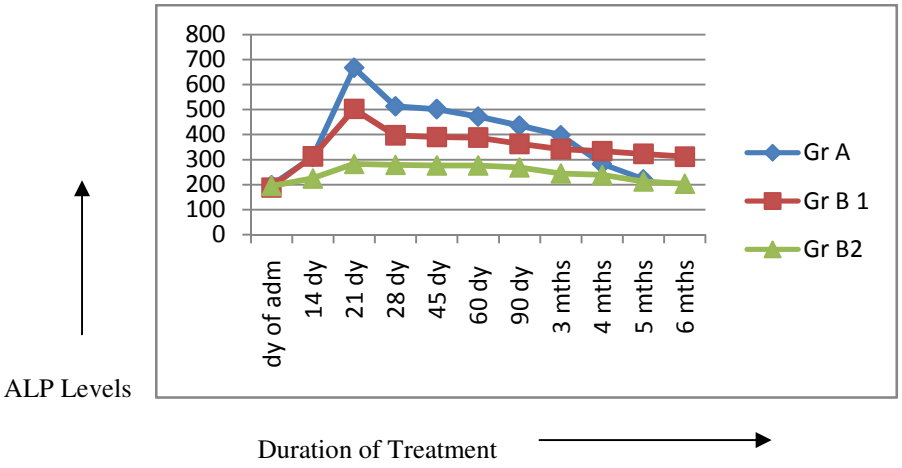

Figure-1

Serial ALP serum level changes showing variation in the groups. Maximum elevation was noted at 3rd week in group A and $B_1$with significant difference in the levels at each interval. Group B_2 showed no significant variation in serum ALP levels