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Correlation Between Serum Calcium, Phosphorus, And Alkaline Phosphatase Levels and the Stages of Fracture Healing

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ABSTRACT

Background: Fracture healing is a complex physiological process involving sequential inflammatory, reparative, and remodeling phases, during which biochemical changes reflect ongoing bone regeneration. Among various markers, serum calcium, phosphorus, and alkaline phosphatase (ALP) play crucial roles in bone mineralization and osteoblastic activity. Evaluating these parameters provides valuable insight into the metabolic dynamics of bone healing. **Aim:** To correlate serum calcium, phosphorus, and alkaline phosphatase levels with different stages of fracture healing. **Materials and Methods:** This prospective observational study was conducted in the Departments of Orthopaedics and Physiology at MGM Medical College & LSK Hospital, Kishanganj, Bihar, on 50 patients aged 18–60 years with fresh long bone fractures. Serum calcium, phosphorus, and ALP were measured at three stages—initial (0 week), reparative (3 weeks), and remodeling (6 weeks)—using standard colorimetric and kinetic enzymatic methods. Radiological assessment of fracture union was performed at each stage. Statistical analysis was done using SPSS v26.0, with ANOVA and Pearson's correlation tests applied; $p < 0.05$ was considered significant. **Results:** Mean serum calcium levels increased significantly from 8.18 ± 0.40 mg/dL at week 0 to 9.11 ± 0.38 mg/dL at 6 weeks ($p < 0.001$). Serum phosphorus showed minor, non-significant changes ($p = 0.09$). ALP levels peaked significantly at 3 weeks (182.4 ± 34.2 IU/L) and declined during remodeling ($p < 0.001$). A strong positive correlation ($r = 0.73$, $p < 0.001$) was found between ALP levels and radiological callus formation. **Conclusion:** Serum ALP is a reliable biochemical marker reflecting osteoblastic activity and fracture healing progression, while calcium shows supportive trends and phosphorus remains stable. Combined biochemical and radiological evaluation offers a simple, economical, and non-invasive approach for monitoring fracture repair in clinical practice.

***Keywords**: Fracture healing, calcium, phosphorus, alkaline phosphatase, biochemical markers, osteoblastic activity*

INTRODUCTION

Fracture healing is a remarkable biological process that restores the structural integrity and function of bone following injury. It represents one of the most complex regenerative phenomena in the human body, involving the coordinated participation of cells, growth factors, cytokines, and extracellular matrix components under strict temporal and spatial regulation.[1] The healing of a fracture progresses through three overlapping but distinct stages—inflammatory, reparative, and remodeling phases. During the inflammatory phase, hematoma formation and cytokine release set the groundwork for new tissue development. The reparative phase involves soft and hard callus formation, predominantly driven by osteoblastic activity and mineral deposition, while the remodeling phase restores the original lamellar bone architecture and mechanical strength.[2,3]

Biochemical processes occurring during these phases are closely reflected in serum concentrations of various markers of bone metabolism. Among these, serum calcium, phosphorus, and alkaline phosphatase (ALP) are among the most reliable and commonly studied indicators of osteoblastic and osteoclastic activity during bone repair.[4] Calcium, the major inorganic component of bone, plays a vital role in mineralization of the osteoid matrix and in maintaining skeletal rigidity. Immediately following a fracture, there is often a transient hypocalcemia, which can be attributed to increased utilization of calcium for callus formation and mineral deposition, as well as redistribution of calcium from serum into the bone matrix.[5,6]

Phosphorus, a key component of hydroxyapatite crystals ($Ca_{10}(PO_4)_6(OH)_2$), works synergistically with calcium to impart structural stability and strength to the bone. Its concentration in serum may fluctuate subtly during the course of fracture healing, as phosphate ions are consumed during ossification and callus maturation.[7,8] On the other hand, alkaline phosphatase (ALP), an enzyme secreted primarily by osteoblasts, serves as a crucial marker of bone formation. It hydrolyzes organic phosphate esters, releasing inorganic phosphate that participates in hydroxyapatite formation. Serum ALP levels typically rise during the reparative phase, peak during callus mineralization, and gradually decline as remodeling progresses and the bone regains its pre-fracture morphology.[9,10]

Conversely, calcium and phosphorus levels exhibit less pronounced but physiologically relevant fluctuations, serving as complementary markers that reflect mineral turnover during healing. Monitoring these parameters at sequential intervals can therefore provide valuable insight into the metabolic status of fracture repair, particularly in cases where radiographic union is delayed or inconclusive.

In resource-limited settings, biochemical monitoring offers a simple, economical, and minimally invasive tool to assess fracture healing progress alongside routine radiographic evaluations. Despite several studies worldwide, limited regional data exist from Eastern Bihar, where nutritional factors and bone metabolism may differ due to socioeconomic and dietary variations. Hence, the present study aims to analyze and correlate serum calcium, phosphorus, and alkaline phosphatase levels with the different stages of fracture healing among patients attending the Department of Orthopaedics at MGM Medical College & LSK Hospital, Kishanganj, Bihar. The findings are expected to enhance understanding of metabolic changes during bone repair and aid in early detection of delayed or impaired healing.

Aim: To correlate serum calcium, phosphorus, and alkaline phosphatase levels with different stages of fracture healing in patients attending the Department of Orthopaedics at MGM Medical College & LSK Hospital, Kishanganj, Bihar.

Materials and Methods

Study Design and Setting:

The present study was designed as a prospective observational study and was conducted jointly by the Departments of Orthopaedics and Physiology at MGM Medical College & LSK Hospital, Kishanganj, Bihar, over a period of 12 months (March 2024 to February2025).

Study Population: A total of **50 patients** presenting with fresh, closed fractures of long bones confirmed by radiographic examination were enrolled in the study. Both male and female patients aged between **18 and 60 years** were included. The study population represented cases admitted to the Orthopaedic wards and outpatient clinics of the institution.

Inclusion Criteria

1. Patients with recent fractures (<7 days old) of long bones such as femur, tibia, humerus, radius, or ulna.
2. Age group between 18–60 years of either sex.
3. Patients willing to participate and provide written informed consent.

Exclusion Criteria

1. Patients with open or compound fractures.
2. Pathological fractures due to malignancy, metabolic bone disease, or osteoporosis.
3. Individuals with chronic kidney disease, liver disorders, or parathyroid dysfunction.
4. Patients receiving calcium or vitamin D supplementation or drugs affecting bone metabolism.

Sample Collection and Biochemical Estimation

For biochemical evaluation, **3 mL of venous blood** was collected aseptically from each participant at three different intervals corresponding to the stages of fracture healing:

- **Stage I (Inflammatory phase)** – At the time of fracture (0–7 days)
- **Stage II (Reparative phase)** – At the 3rd week after injury
- **Stage III (Remodeling phase)** – At the 6th week after injury

Blood samples were allowed to clot and centrifuged at 3000 rpm for 10 minutes to separate serum. Serum calcium and phosphorus were estimated by colorimetric methods using Arsenazo III and Ammonium molybdate methods respectively. Serum alkaline phosphatase (ALP) was measured using a kinetic photometric method employing p-nitrophenyl phosphate (p-NPP) substrate on an automated chemistry analyzer (Erba XL-640, Germany). All reagents and procedures followed the manufacturer's standard protocol, and internal quality control sera were run daily to ensure analytical precision.

Radiological Evaluation of Fracture Healing

Radiographic examination of the fracture site was performed at each follow-up interval using standard anteroposterior and lateral views. The stages of healing were assessed according to the presence of callus formation and cortical continuity and categorized as:

- **Stage I:** Inflammatory phase (hematoma and early fibrocartilaginous callus)
- **Stage II:** Reparative phase (appearance of bridging callus, 3–6 weeks)
- **Stage III:** Remodeling phase (cortical continuity and consolidation >6 weeks)

Data Management and Statistical Analysis

All data were recorded in Microsoft Excel 2021 and analyzed using IBM SPSS Statistics version 26.0. Quantitative variables were expressed as mean ± standard deviation (SD), while qualitative variables were presented as percentages. Comparison of mean serum calcium, phosphorus, and ALP levels across the three stages of fracture healing was done using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for inter-group comparison. The Pearson correlation coefficient (r) was applied to determine the relationship between biochemical markers and radiological stages of healing. A p-value < 0.05 was considered statistically significant for all analyses.

RESULTS AND ANALYSIS

A total of **50 patients** with recent long bone fractures were enrolled in the study and followed up for 6 weeks. Among them, all participants completed the study period and were included in the final analysis. The biochemical parameters—serum calcium, phosphorus, and alkaline phosphatase—were assessed at three stages of fracture healing (0 week, 3 weeks, and 6 weeks) and correlated with radiological findings.

Table 1. Demographic and Clinical Profile of Study Participants (n = 50)

Parameter	Mean ± SD / n (%)
Age (years)	38.6 ± 12.4
Sex (Male : Female)	34 : 16
Mean BMI (kg/m²)	23.1 ± 2.6
Site of fracture	Lower limb – 30 (60%) Upper limb – 20 (40%)
Mode of injury	Road traffic accident – 34 (68%) Fall – 14 (28%) Others – 2 (4%)
Side involved	Right – 27 (54%) Left – 23 (46%)

Most of the patients were middle-aged males, with road-traffic accidents being the most common cause of fracture. Lower-limb fractures were slightly more frequent than upper-limb ones.

Table 2. Mean Serum Calcium Levels at Different Stages of Fracture Healing (n = 50)

Stage of Healing	Mean ± SD (mg/dL)	ANOVA p-value
Stage I (0 week)	8.18 ± 0.40	< 0.001*
Stage II (3 weeks)	8.77 ± 0.44	
Stage III (6 weeks)	9.11 ± 0.38	

Serum calcium levels showed a **significant rise** (p < 0.001) from the inflammatory to the remodeling phase, reflecting increased mineralization activity during callus formation and consolidation.

Table 3. Mean Serum Phosphorus Levels During Fracture Healing (n = 50)

Stage of Healing	Mean ± SD (mg/dL)	ANOVA p-value
Stage I (0 week)	3.46 ± 0.37	0.092 (NS)
Stage II (3 weeks)	3.63 ± 0.39	
Stage III (6 weeks)	3.58 ± 0.35	

Serum phosphorus levels remained within normal physiological range with mild, non-significant fluctuations, indicating a stable phosphate balance throughout the healing process.

Table 4. Mean Serum Alkaline Phosphatase (ALP) Levels at Different Stages (n = 50)

Stage of Healing	Mean ± SD (IU/L)	ANOVA p-value
Stage I (0 week)	129.6 ± 26.8	< 0.001*
Stage II (3 weeks)	182.4 ± 34.2	
Stage III (6 weeks)	160.8 ± 27.5	

ALP levels demonstrated a **significant increase** ($p < 0.001$) during the reparative phase, peaking at the 3rd week, followed by a decline in the remodeling phase. This rise corresponds to peak osteoblastic activity and active callus mineralization.

Table 5. Correlation Between Biochemical Parameters and Radiological Stages of Healing (n = 50)

Parameter	Correlation Coefficient (r)	p-value	Strength of Correlation
Calcium vs. Radiological Grade	+0.59	< 0.001*	Moderate correlation
Phosphorus vs. Radiological Grade	+0.18	0.168 (NS)	Weak correlation
ALP vs. Radiological Grade	+0.73	< 0.001*	Strong correlation

A **strong positive correlation** was found between serum ALP levels and radiological evidence of callus formation ($r = 0.73$, $p < 0.001$), confirming that ALP is a sensitive biochemical marker of bone healing.

Table 6. Comparison of Mean Biochemical Values Between Healed and Delayed-Union Cases (n = 50)

Parameter	Healed (n = 45) Mean ± SD	Delayed Union (n = 5) Mean ± SD	t-test p-value
Calcium (mg/dL)	9.09 ± 0.36	8.44 ± 0.31	< 0.001*
Phosphorus (mg/dL)	3.59 ± 0.38	3.33 ± 0.29	0.042*
ALP (IU/L)	161.4 ± 28.1	125.6 ± 22.7	< 0.001*

Patients achieving normal radiological union had significantly higher mean calcium and ALP levels compared with delayed-union cases, underlining their prognostic value in fracture healing assessment.

DISCUSSION

The process of fracture healing is an intricate biological cascade involving cellular proliferation, differentiation, and matrix mineralization, orchestrated by osteoblasts and osteoclasts under the influence of systemic biochemical changes. The present study aimed to correlate serum calcium, phosphorus, and alkaline phosphatase (ALP) levels with different stages of fracture healing in 50 patients and to assess their utility as biochemical indicators of bone repair.

In our study, serum calcium levels were found to be significantly lower in the initial inflammatory phase and progressively increased during the reparative and remodeling phases. The mean calcium level rose from **8.18 ± 0.40 mg/dL at presentation** to **9.11 ± 0.38 mg/dL at six weeks**, which was statistically significant ($p < 0.001$). This rise reflects the physiological utilization of calcium during callus formation and subsequent mineralization of the osteoid matrix. Similar observations were reported by **Khan et al. (2016)**, who found a comparable upward trend in calcium levels during fracture healing and attributed it to enhanced osteoblastic activity and calcium incorporation into hydroxyapatite crystals.[5] **Shukla et al. (2016)** and **Kumar et al. (2020)** also noted that calcium levels, though initially low due to increased consumption in early bone repair, normalize with progression of healing, supporting our findings.[11,10]

Serum phosphorus levels in the current study showed mild fluctuations throughout the stages of healing, with no statistically significant difference ($p = 0.09$). The phosphorus level increased slightly from **3.46 ± 0.37 mg/dL** to **3.58 ± 0.35 mg/dL** by the sixth week. This observation is consistent with the findings of **Kaur et al. (2018)**, who reported that serum phosphate concentrations remain relatively stable during fracture repair, as phosphate mobilization and utilization for mineralization are tightly regulated by homeostatic mechanisms.[6] **Hurson et al. (2007)** also observed that changes in serum phosphorus were not as marked as those in calcium or ALP, suggesting that phosphorus may play a supportive rather than a primary role in the biochemical assessment of bone healing.[8]

In contrast, serum alkaline phosphatase (ALP) exhibited the most dynamic changes during fracture healing. In our study, ALP levels rose significantly from **129.6 ± 26.8 IU/L at the time of fracture** to a peak of **182.4 ± 34.2 IU/L at the third week**, followed by a decline to **160.8 ± 27.5 IU/L** at six weeks ($p < 0.001$). This pattern reflects the proliferative activity of osteoblasts during the reparative phase and gradual normalization as remodeling progresses. **Singh et al. (2019)** reported a similar peak in ALP activity during the 3–6 week interval post-fracture, associating it with increased bone-specific ALP synthesis by osteoblasts during callus mineralization.[9] Likewise, **Mahajan et al. (2019)** observed a significant elevation in ALP during early callus formation, followed by a decline as the fracture consolidated.[12]

Our findings align closely with those of **Gautam et al. (2021)**, who demonstrated a strong positive correlation ($r = 0.71$, $p < 0.001$) between ALP levels and radiological callus formation, similar to the correlation observed in our study ($r = 0.73$). They concluded that ALP is a reliable biochemical marker of osteoblastic activity and an indicator of union progression.[13] **Verma et al. (2020)** further confirmed that patients with delayed or impaired healing had consistently lower ALP levels compared to those with normal union, emphasizing the enzyme's diagnostic value.[14] In the present study, delayed-union cases also exhibited significantly reduced ALP levels (125.6 ± 22.7 IU/L) compared with normally healed fractures (161.4 ± 28.1 IU/L), reinforcing these findings.

Collectively, the results of this study indicate that **serum ALP** is the most sensitive and specific biochemical marker correlating with the rate and stage of fracture healing, while **serum calcium** shows supportive changes reflecting mineral deposition, and **phosphorus** remains relatively stable. These trends corroborate the mechanistic model proposed by **Seibel (2005)** and **Klein-Nulend et al. (2013)**, who described ALP as a direct enzymatic mediator of hydroxyapatite nucleation and mineralization, and calcium as an essential substrate for matrix calcification.[15,7]

From a clinical perspective, serial measurement of these biochemical markers offers a non-invasive, economical adjunct to radiological assessment. In resource-limited settings where advanced imaging is not always feasible, monitoring serum ALP and calcium trends can assist clinicians in predicting delayed union or non-union, allowing for timely intervention.
Thus, the present study supports the integration of biochemical monitoring into routine follow-up of fracture patients. The combination of radiographic and biochemical evaluation provides a more comprehensive understanding of bone repair and helps identify deviations from normal healing trajectories at an early stage.

CONCLUSION

The present study highlights that biochemical markers such as serum calcium, phosphorus, and alkaline phosphatase (ALP) undergo distinct and predictable variations during different stages of fracture healing. Among these, serum ALP demonstrated the most consistent and statistically significant rise during the reparative phase, correlating strongly with radiological evidence of callus formation, thereby serving as a sensitive and reliable biochemical indicator of osteoblastic activity and bone regeneration. Serum calcium levels exhibited a gradual increase parallel to bone mineralization, reflecting its role in callus calcification, while serum phosphorus remained relatively stable, acting as a supportive parameter in the mineralization process.

Patients with delayed union exhibited lower calcium and ALP levels throughout follow-up, suggesting that serial estimation of these parameters may aid in early identification of delayed or impaired fracture healing. The combined biochemical assessment of ALP and calcium, along with routine radiological evaluation, thus provides a comprehensive, cost-effective, and non-invasive approach for monitoring bone healing progression, particularly valuable in resource-limited healthcare settings.

Further multicentric studies with larger sample sizes and inclusion of additional bone turnover markers such as bone-specific ALP, osteocalcin, and procollagen peptides are recommended to validate and strengthen the role of biochemical monitoring in clinical fracture management.

REFERENCES:

1. Einhorn TA, Gerstenfeld LC. Fracture healing: mechanisms and interventions. *Nat Rev Rheumatol.* 2015;11(1):45-54.
2. Marsell R, Einhorn TA. The biology of fracture healing. *Injury.* 2011;42(6):551-555.
3. Dimitriou R, Tsiridis E, Giannoudis PV. Current concepts of molecular aspects of bone healing. *Injury.* 2005;36(12):1392-1404.
4. Seibel MJ. Biochemical markers of bone turnover. *Clin Biochem Rev.* 2005;26(3):97–122.
5. Khan MR, Razaq S, Saleem M, Farooq M, Iqbal M. Serum calcium and phosphorus in fracture healing. *Indian J Orthop.* 2016;50(2):211–215.
6. Kaur S, Arora S, Singh J, Gill G. Serum phosphorus and calcium changes during bone healing. *J Clin Diagn Res.* 2018;12(6):BC06–BC09.
7. Klein-Nulend J, Bacabac RG, Bakker AD. Mechanobiology of bone tissue. *Clin Rev Bone Miner Metab.* 2013;11(2):123–132.
8. Hurson C, Butler JS, Keating DT, Nicholson P, Stack J, O'Byrne JM. Analysis of biochemical markers in fracture healing. *Injury.* 2007;38(3):357–363.
9. Singh A, Singh R, Sharma A, Gupta A. Role of biochemical parameters in fracture healing. *Indian J Clin Biochem.* 2019;34(1):59–64.
10. Kumar R, Patel V, Sharma A, Verma S. Evaluation of biochemical markers in fracture healing. *Int J Res Orthop.* 2020;6(2):219–223.
11. Shukla P, Kumar V, Gupta A, Singh S. Study of calcium and phosphorus during fracture healing. *J Orthop Allied Sci.* 2016;4(1):27–30.
12. Mahajan A, Kumar S, Sharma S, Gupta P. Serum alkaline phosphatase as a predictor of fracture union. *J Orthop Traumatol Rehabil.* 2019;11(2):87–91.
13. Gautam S, Singh P, Mehra A, Yadav A. Correlation between serum alkaline phosphatase and fracture healing. *Indian J Orthop Res.* 2021;5(4):219–223.
14. Verma P, Kaur R, Singh M, Kumar S. Correlation of biochemical markers with fracture healing. *J Clin Orthop Trauma.* 2020;11(5):853–858.
15. Seibel MJ. Biochemical markers of bone turnover. *Clin Biochem Rev.* 2005;26(3):97–122.