Correlation between Biochemical Parameters and Radiographic Healing Score in Tibial Fractures

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Learning Point of the Article:

Serum biochemical markers, such as calcium, phosphorus, alkaline phosphatase, and Vitamin D can serve as valuable adjuncts for monitoring radiographic fracture healing progression in tibial fractures.

Abstract

Introduction: Fracture healing is a complex biological process influenced by systemic biochemical factors that regulate bone formation and remodeling. Evaluating the association between biochemical markers and radiographic evidence of union may enhance understanding of fracture repair and help predict healing outcomes.

Materials and Methods: This prospective observational study included 63 adult patients with tibial shaft fractures managed either surgically or conservatively. Serial assessments of serum calcium, phosphorus, alkaline phosphatase (ALP), Vitamin D, and parathyroid hormone (PTH) were performed at baseline, 6, 12, and 24 weeks. Radiographic healing was evaluated at corresponding intervals using the Radiographic Union Score for Tibial fractures (RUST). Correlation between biochemical parameters and RUST scores was analyzed using Pearson's correlation coefficient.

Results: Most participants were males (74.6%), with road traffic accidents as the predominant cause of injury. Progressive improvement was observed in calcium, phosphorus, ALP, and Vitamin D levels during follow-up, while PTH showed a gradual decline. The mean RUST score increased steadily from early to late follow-ups, indicating continuous radiographic healing. At 24 weeks, ALP demonstrated the strongest positive correlation with RUST (r = 0.61, P < 0.001), followed by Vitamin D (r = 0.46, P < 0.001), phosphorus (r = 0.42, P = 0.001), and calcium (r = 0.38, P = 0.002). PTH showed a weak negative correlation (r = –0.33, P = 0.008).

Conclusion: Serum biochemical parameters, particularly ALP and Vitamin D, reflect the biological progression of fracture healing and correlate significantly with radiographic union. Routine biochemical monitoring, in conjunction with radiographic evaluation, can serve as a valuable adjunct in assessing the healing potential of tibial fractures.

Keywords: Tibial fracture, alkaline phosphatase, radiographic union score, Vitamin D, bone healing.

Introduction

Tibial shaft fractures are among the most common long-bone injuries encountered in orthopedic practice and often carry a substantial risk of delayed union or non-union because of the limited soft-tissue envelope and the biomechanical load borne by the tibia. Reliable and reproducible methods to monitor healing are therefore essential for guiding management and identifying patients at risk of impaired recovery [1, 2].

Plain radiography remains the most widely available tool to assess structural repair, and the Radiographic Union Score for

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Author's Photo Gallery

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Table 1: Baseline demographic and clinical characteristics (*n*=63)

Parameter	Mean±standard deviation*h* (%)
Age (years)	38.6±10.7
Sex	Male: 47 (74.6)
	Female: 16 (25.4)
Type of fracture	Closed: 54 (85.7)
	Open (Grade I): 9 (14.3)
Mode of injury	Road traffic accident: 41 (65.1)
	Fall: 18 (28.6)
	Others: 4 (6.3)
Treatment modality	Intramedullary nailing: 46 (73.0)
	Conservative (casting): 17 (27.0)

Tibial fractures (RUST) – and its modified form RUST – provide standardized, validated metrics to quantify cortical bridging and loss of fracture line visibility on orthogonal radiographs. These scoring systems have demonstrated good inter- and intraobserver reliability and correlate with volumetric and biomechanical indices of callus mineralization in experimental and clinical studies [1, 2].

Concurrently, systemic biochemical markers of bone turnover and mineral metabolism offer objective information on the biological processes underlying fracture repair. Markers of bone formation (notably alkaline phosphatase [ALP] and its bone-specific isoform) and indices of mineral homeostasis (serum calcium, phosphate, 25-hydroxyvitamin D, and parathyroid hormone [PTH]) change during the different phases of healing and have been investigated as potential adjuncts to imaging for early detection of delayed union [3, 4].

Several clinical and experimental reports have documented a temporal rise in total and bone-specific ALP in association with callus formation, supporting its use as a dynamic biochemical indicator of osteoblastic activity during the reparative phase. Similarly, disturbances in Vitamin D status and perturbations of calcium–phosphate balance have been linked to slower or suboptimal consolidation in some cohorts, although interventional trials of Vitamin D supplementation report mixed effects on final union rates. PTH has also been examined for its complex, context-dependent role in bone remodeling and repair [5, 6].

Given the complementary nature of radiographic scoring and circulating biochemical measurements, correlating serial biochemical profiles with validated radiographic healing scores could improve early identification of patients with impaired biological healing and refine prognostication. This study,

therefore, aimed to evaluate the temporal changes in selected biochemical parameters and to determine their correlation with RUST at 24 weeks in adults with tibial shaft fractures.

Materials and Methods

Study design and setting

This hospital-based prospective observational study was conducted at a tertiary care teaching hospital in India. Written consent was obtained from all participants before enrolment.

Sample size and selection criteria

A total of 63 patients with radiographically confirmed tibial shaft fractures were included in the study. The sample size was determined based on previous literature indicating a moderate correlation between biochemical parameters and fracture healing scores, assuming a correlation coefficient (r) of 0.35, with 80% power and 5% level of significance.

Inclusion criteria

• Adults aged 18–60 years with fresh, closed tibial fractures treated conservatively or surgically

• Willingness to participate and comply with follow-up visits.

Exclusion criteria

• Pathological fractures or fractures associated with metabolic bone disease

• Chronic renal or hepatic disorders affecting calcium or Vitamin D metabolism

• Patients on corticosteroids, bisphosphonates, or Vitamin D/calcium supplementation before injury

• Open or compound fractures (Gustilo-Anderson type II or higher).

Clinical management

Patients underwent either closed reduction or internal fixation using intramedullary nailing or conservative management with casting, as deemed appropriate by the treating surgeon. Standard post-operative and rehabilitation protocols were followed in all cases.

Assessment schedule

Clinical and biochemical evaluations were performed at the

Table 2: Temporal changes in biochemical parameters during fracture healing

Parameter	Baseline (Mean±standard deviation)	6 Weeks	12 Weeks	24 Weeks
Serum calcium (mg/dL)	8.72±0.62	8.96±0.58	9.14±0.55	9.28±0.51
Serum phosphorus (mg/dL)	3.34±0.46	3.48±0.42	3.61±0.39	3.73±0.36
Alkaline phosphatase (U/L)	117.3±25.4	144.5±30.6	159.7±33.2	135.2±26.8
Vitamin D (ng/mL)	21.8±6.9	23.5±6.4	26.3±6.0	28.6±5.7
Parathyroid hormone (pg/mL)	47.9±12.6	45.6±11.8	42.3±10.9	39.4±9.8

time of injury (baseline) and subsequently at 6 weeks, 12 weeks, and 24 weeks post-fracture. Radiographic assessment was done concurrently using standard anteroposterior and lateral views of the leg.

Biochemical parameters

Venous blood samples were collected after overnight fasting and analyzed for:

• Serum calcium (mg/dL) – by o-cresolphthalein complexone method

• Serum phosphorus (mg/dL) – by ammonium molybdate ultraviolet method

• Serum ALP (U/L) – by kinetic colorimetric method

• Serum Vitamin D (ng/mL) – by chemiluminescent immunoassay

• Serum PTH (pg/mL) – by electrochemiluminescence immunoassay.

All analyses were performed in the hospital's central biochemistry laboratory using standardized protocols and calibrated equipment.

Radiographic evaluation

Fracture healing was assessed radiographically using the RUSTs. Each cortex was scored from 1 to 3 (1 = fracture line visible with no callus, 2 = callus present but fracture line visible, 3 = bridging callus with no visible fracture line), with a total score ranging from 4 to 12. Radiographs were independently evaluated by two blinded orthopedic surgeons, and the mean score was used for analysis.

Statistical analysis

Data were compiled in Microsoft Excel and analyzed using the IBM Statistical Package for the Social Sciences Statistics version 26.0. Continuous variables were expressed as mean ± standard deviation, while categorical data were represented as frequencies and percentages.

Correlation between biochemical parameters and RUST scores was assessed using Pearson's correlation. A $P < 0.05$ was considered statistically significant.

Results

A total of 63 patients with tibial fractures were included in the study. The demographic and clinical characteristics are summarized in Table 1. The majority of participants were middle-aged males, and road traffic accidents represented the predominant cause of injury. Most fractures were closed and managed surgically with intramedullary nailing.

Progressive improvement was observed in the biochemical profile over the follow-up period (Table 2). Calcium and phosphorus levels demonstrated a steady upward trend

Table 3: Mean RUST at follow-up

Follow-up interval	Mean RUST score±SD
6 Weeks	5.4±0.8
12 Weeks	8.2±1.1
24 Weeks	11.1±0.9

SD: Standard deviation, RUST: Radiographic Union Score for Tibial fractures

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Table 4: Correlation between biochemical parameters and RUST at 24 weeks

Parameter	Pearson's r	*P*-value	Interpretation
Serum calcium	0.38	0.002	Moderate positive correlation
Serum phosphorus	0.42	0.001	Moderate positive correlation
Alkaline phosphatase	0.61	<0.001	Strong positive correlation
Vitamin D	0.46	<0.001	Moderate positive correlation
Parathyroid hormone	−0.33	0.008	Weak negative correlation
RUST: Radiographic Union Score for Tibial fractures			

throughout the 24-week duration, while serum ALP exhibited a characteristic rise during the intermediate phase of healing (6–12 weeks) followed by a decline toward baseline values at 24 weeks, consistent with the phase of callus remodeling. Vitamin D concentrations also showed gradual improvement during follow-up, accompanied by a concurrent decline in PTH levels, reflecting normalization of bone metabolism as healing progressed.

Radiographic assessment using the RUSTs revealed a consistent increase across all time points, indicating progressive cortical bridging and callus consolidation (Table 3). The improvement in mean RUST scores over successive follow-ups confirmed satisfactory union in the majority of patients by 24 weeks.

Correlation analysis demonstrated statistically significant associations between biochemical parameters and radiographic healing scores at 24 weeks (Table 4). Serum ALP exhibited the strongest positive correlation with the RUST score, underscoring its utility as a dynamic biochemical marker of osteoblastic activity during fracture repair. Moderate positive correlations were also observed between calcium, phosphorus, and Vitamin D with RUST, suggesting their contributory role in bone mineralization and healing. In contrast, PTH levels showed a weak but significant negative correlation with radiographic union, indicating that persistently elevated PTH may reflect delayed mineralization or secondary hyperparathyroidism in slower healers.

Discussion

This study found that dynamic changes in routine biochemical indices of bone metabolism accompany radiographic progression of tibial fracture repair, and that serum ALP showed the strongest association with radiographic union at 24 weeks. These observations align with recent clinical case-series and cohort work that highlight a temporal relationship between

ALP behavior and radiographic indices of consolidation, suggesting ALP as a useful, easily obtainable adjunct to imaging when interpreting biological healing activity [7].

The validity of radiographic scoring systems, such as the RUSTs as surrogates of mechanical and structural healing has been repeatedly demonstrated in preclinical and translational work; RUST correlates with callus mineral density, bone volume fraction and biomechanical properties, supporting its use as a quantitative radiographic end-point in human studies and justifying its use here as the comparator for circulating markers [8]. Against that background, the concordant rise in ALP during the reparative phase observed in our cohort – together with its positive correlation with RUST – supports the interpretation that rising ALP largely reflects osteoblastic/callus activity rather than non-specific systemic processes [7,9].

Changes in mineral indices (calcium and phosphate) and Vitamin D observed in our series echo prior clinical reports that document modest, time-dependent shifts in these analytes after long-bone fracture repair [9, 10]. Several prospective studies of femoral/tibial fractures have described early post-operative increases in calcium and phosphate and variable ALP kinetics during the interval of callus formation and remodeling; such trends are biologically plausible given the mineralization demands of new bone and are compatible with our finding of moderate positive correlations between mineral markers and radiographic union [9, 10].

The observed inverse relationship between PTH and radiographic union in our data is consistent with the complex role of PTH in bone biology. Pre-clinical work demonstrates that intermittent PTH administration can accelerate fracture repair and enhance callus formation in animal models, whereas chronically elevated endogenous PTH often reflects secondary disturbances in mineral metabolism (for example, Vitamin D

deficiency or renal bone disease) and may associate with impaired bone microarchitecture [11]. Thus, the negative correlation we report likely reflects that higher circulating PTH in some patients was a marker of relative mineral insufficiency or perturbed remodeling rather than a direct inhibitory effect on healing [11].

Several recent clinical investigations have attempted to use circulating biomarkers to predict impaired healing early; results have been mixed. Some reports and systematic appraisals show promising associations between specific bone-turnover markers and healing trajectories, yet heterogeneity in marker selection, assay methods, timing, and endpoints has limited translation into routine practice [12, 13]. Our results add to this body of evidence by demonstrating measurable, clinically accessible associations between widely available tests (total ALP, calcium, phosphate, Vitamin D, and PTH) and a validated radiographic score, but they also underscore the need for standardized assays and sampling protocols before biochemical monitoring can be recommended as a standalone predictor [13, 14].

Strengths of our study include prospective serial sampling, use of a validated radiographic outcome (RUST), and analysis of multiple, complementary biochemical indices. Limitations deserve mention: This was a single-center observational study with a modest sample size, and total (rather than bone-specific) ALP was measured; systemic illness, nutritional status, and unmeasured local factors (fracture gap, soft-tissue injury) can confound circulating markers. Furthermore, while RUST is validated and correlates with structural metrics, radiographs are imperfect surrogates for mechanical strength [8, 13].

In summary, our findings support a role for serial biochemical monitoring – particularly ALP, alongside mineral indices and Vitamin D – as an adjunct to radiographic assessment in tibial fracture follow-up. Future multicenter studies employing standardized BTM panels, bone-specific assays, and pre-specified thresholds are warranted to test whether early biochemical trajectories can prospectively identify patients at risk of delayed union and thereby guide timely interventions [13, 14].

Conclusion

The present study demonstrates a significant relationship between biochemical markers of bone metabolism and radiographic evidence of fracture union in tibial fractures. Among the parameters evaluated, serum ALP showed the strongest positive correlation with radiographic healing scores, indicating its potential as a reliable biochemical indicator of osteoblastic activity. Improvements in calcium, phosphorus, and Vitamin D levels were also associated with progressive fracture consolidation, whereas elevated PTH levels were inversely related to healing. These findings suggest that periodic monitoring of biochemical parameters, alongside radiographic evaluation, can provide valuable insights into the biological progress of bone repair and may assist clinicians in predicting the course of fracture healing.

Clinical Message

Monitoring biochemical markers, such as serum calcium, phosphorus, ALP, and Vitamin D can serve as valuable adjuncts in evaluating fracture healing progression. These parameters reflect underlying metabolic activity at the fracture site and correlate with radiographic healing outcomes. Incorporating biochemical assessment alongside imaging can enhance early detection of delayed union and guide timely therapeutic interventions.

Declaration of patient consent: The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given the consent for his/ her images and other clinical information to be reported in the journal. The patient understands that his/ her names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Conflict of interest: Nil   **Source of support:** None

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