ORIGINAL ARTICLE

EVALUATION OF SERUM ALKALINE PHOSPHATASE AND OSTEOCALCIN AS PREDICTORS OF BONE HEALING AFTER INTERNAL FIXATION

AMEER HAMZA[1], MUHAMMAD AHMED[2], AREEJ KHALID[3], ANEELA SHAHZADI[4], MUHAMMAD TAHA[5], MUHAMMAD MOGHEES AFZAL[6]

[1]Shaikha Fatima Institute of Nursing and Health Sciences (SFINHS), Shaikh Zayed Medical Complex, Lahore.
[2]Shaikha Fatima Institute of Nursing and Health Sciences (SFINHS), Shaikh Zayed Medical Complex, Lahore.
[3]Center of Applied Molecular Biology , University of the Punjab , Lahore
[4]Department of Neuromuscular, University of Biological and Sciences, Lahore.
[5]Shaikha Fatima Institute of Nursing and Health Sciences (SFINHS), Shaikh Zayed Medical Complex, Lahore.
[6]Government College University, Faisalabad (GCUF), Pakistan.
Correspondence to: Ameer Hamza, Email: Hamza941511@gmail.com

ABSTRACT

Background: Bone healing after internal fixation is a complex biological process involving osteoblastic activity and matrix mineralization. Early detection of delayed union is vital for optimal management. Biochemical markers such as serum alkaline phosphatase (ALP) and osteocalcin (OC) reflect osteoblastic function and bone turnover and may serve as early predictors of bone healing.

Objective: To evaluate serum ALP and osteocalcin levels as biochemical predictors of bone healing following internal fixation of long bone fractures.

Methods: A cross-sectional observational study was conducted at tertiary care centers across Punjab, Pakistan, from January 2023 to February 2025, including 100 patients aged 18–60 years who underwent internal fixation for long bone fractures. Blood samples were collected at 2, 6, and 12 weeks postoperatively for ALP and OC estimation using kinetic photometric and ELISA methods, respectively. Radiological healing was assessed using the Radiographic Union Scale for Tibial fractures (RUST). Data were analyzed using SPSS v26, applying paired t-test and Pearson correlation.

Results: The mean ALP and osteocalcin levels showed a significant rise from 2 to 12 weeks ($p < 0.001$). ALP correlated strongly with RUST score ($r = 0.72$), while osteocalcin showed a moderate correlation ($r = 0.66$). Patients with normal union had significantly higher ALP and OC levels compared to delayed union cases.

Conclusion: Serum ALP and osteocalcin are reliable biochemical markers for monitoring bone healing after internal fixation. Their sequential evaluation complements radiographic findings and enables early detection of delayed healing.

Keywords: Alkaline phosphatase, Osteocalcin, Bone healing, Internal fixation, Biochemical markers, Fracture union.

This article may be cited as: Hamza A, Ahmed M, Khalid A, Shahzadi A, Taha M, Afzal MM: Evaluation of Serum Alkaline Phosphatase and Osteocalcin as Predictors of Bone Healing after Internal Fixation. Pak Med & Allied, 2025; 01(2): 13-18.

Received: 10-02-2025 **Revised:** 15-04-2025 **Accepted:** 15-05-2025 **Published:** 30-05-2025

INTRODUCTION

The process of fracture healing is dynamic and intricate physiological process of a high level of interdependence of cellular, biochemical, and mechanical factors leading to the restoration of bone integrity and strength[1]. Once a fracture takes place, a cascade of biological events, which is highly regulated, begins in the body and consists of remodeling, inflammation and callus formation. The success of such a process lies in the equilibrium between the bone formation and bone resorption which are strictly regulated by the osteoblasts and osteoclasts. Bone healing is an essential component of the orthopedic management process, especially in the context of internal fixation, where timely detection of slow unions or failure to unite can be an important factor in the treatment process[2].

The radiographic assessment has traditionally been viewed as the gold standard in the evaluation of fracture healing, but in most cases, it is the last to respond to the biological processes that mediate bone regeneration[3].

Subtle cellular activities that happen in the fracture callus in the initial stages of healing may not be seen in radiographs. Thus, biochemical indicators of osteoblastic and osteoclastic processestime have also become valuable supplements to understanding bone metabolism and the healing progression. Of specific clinical importance among such markers are serum alkaline phosphatase (ALP) and osteocalcin (OC)[4].

Alkaline phosphatase (ALP) is a phosphatase type of enzyme that is primarily expressed by osteoblasts, which is an important part of mineralization, decomposing phosphate esters and promoting the deposition of calcium into the bone matrix[5]. The level of ALP at the reparative period of the fracture healing shows increased activity of osteoblasts and mineralization of the matrix. In the same way, osteocalcin (OC) is a non-collagenous protein produced by the mature osteoblasts attachment to hydroxyapatite in the bone matrix and is a sign of bone development and metabolism. Its serum level is an indication of late osteoblastic differentiation and bone maturation[6].

Simultaneous assessment of these two biomarkers gives a good idea of the state of bone metabolism of bone during healing. Research has established that serum ALP levels and osteocalcin levels vary at various times in fracture healing with early increase in ALP being indicative of active callus formation and further increase in osteocalcin being a sign of mineralization. These changes can be monitored by clinicians to evaluate bone regeneration earlier than the radiological data on union[1,5].

Although a volume of research has been conducted on bone turnover markers, not much information exists on their prognostic potential in regard to bone mending concerning individuals that have gone through internal fixation surgery, especially in developing nations such as Pakistan[7]. The current study, thus, will compare the use of serum alkaline phosphatase and osteocalcin in the prediction of bone healing after internal fixation of long bone fractures. This research aims to determine the viability of the non-invasive, cost-effective method of early bone repair evaluation and diagnosis of delayed healing materials in an early clinical practice by the correlations of the pattern of biochemical markers and radiological healing score[8].

MATERIALS AND METHODS

The study was intended as a cross-sectional observational study, which was going to be carried out during a two-year period starting at January 2023 and continuing until February 2025. The purpose of the study was to determine the predictive value of the levels of serum alkaline phosphatase (ALP) and osteocalcin (OC) in the evaluation of bone healing internal fixation of long bone fractures. The research has been conducted in different tertiary care units in Punjab Pakistan both in orthopedic and radiology departments in an attempt to provide a wide range of patients and a standardized research methodology. Each collaborating center followed standard protocols used in the selection of patients, sample collection, biochemical testing and radiological assessment.

The study used non-probability purposive sampling to enroll 100 patients in the study. The sample size was computed using the number of patients flowing in during the research and already published studies which have found biochemical differences in bone healing. Adult men and women aged 18 to 60 years who came with fresh long bone injuries like the femur, tibia, or humerus and were internally fixed with the following orthopedic implants were eligible; plates, screws, or intramedullary nails. Patients had to sign informed consent and agree to do follow-up visits during the research process.

The inclusion criteria included patients having closed diaphyseal fracture of long bones surgically repaired within seven days after injury and free of any metabolic or systemic disorders which affected bone metabolism. The criteria used to exclude patients were those who had pathological fractures, open or compound fractures, or patients who had chronic liver, kidney, or endocrine disorders that may have affected the metabolism of enzymes. People who used corticosteroids, bisphosphonates or vitamin D / calcium supplements were not included and neither were patients with postoperative infections, implant failure or loss to follow up. Such exclusion actions guaranteed that the research results were indicative of true differences concerning the physiological bone repair other than external factors.

The participating hospitals approved the study protocol of the Institutional Ethical Review Boards, and ethical principles were adhered to in the study in line with the Declaration of Helsinki (2013). The nature and purpose of the research along with the possible benefits of the research were well-informed to all participants. They were informed of their confidentiality and a written consent was taken before their participation.

A detailed data sheet was made up of each patient, which included demographic information, fracture location, injury mechanism, nature of the fixation done and comorbidities. Blood samples were gathered by means of venipuncture at three definite postoperative times 2 weeks, 6 weeks, and 12 weeks, in order to check the alteration of biochemical parameters during the recovery process. Each follow-up was accompanied with 5 mL of drawing venous blood. The separation of serum was done through centrifugation at 3000 rpm in 10 minutes at -20C until analysis. An automated biochemistry analyzer with the kinetic photometric method in 37 o C and pH 10.4 was used to measure serum alkaline phosphatase (ALP), where the activity of ALP was measured through the rate of the transformation of p-nitrophenyl phosphate to p-nitrophenol. Serum osteocalcin (OC) was measured using a sandwich enzyme-linked immunosorbent assay (ELISA) using human osteocalcin specific monoclonal antibodies.

Duplication of all tests was done so as to improve accuracy and reliability.

Radiographic assessment was performed at a specified time period (2, 6 and 12 weeks after surgery) of the fractured bone with the use of a standard anteroposterior and lateral X-rays. Urgent fracture healing was measured with the help of Radiographic Union Scale of Tibial Fractures (RUST) scoring system that measures the presence of cortical bridging and loss of fracture line. The scores of 10 and above were regarded as good union, scores between 7 and 9 were considered as delayed union and scores below 6 were considered as non-union. Two experienced radiologists were used to review radiographs, but they were blinded to the biochemical results, and this allowed them to reduce observer bias.

The major outcomes that were estimated were the time variations of serum ALP and osteocalcin levels and the association with bone healing radiologic indices. The correlation among biochemical markers and fracture union was the primary predictive efficacy determinant.

The statistical tests were all conducted with Statistical Package of Social Sciences (SPSS) version 26.0. The quantitative variables (age, ALP, and osteocalcin levels) had the mean and standard deviation (SD) as the mode of expression, as well as the qualitative variables (gender and fracture site) in form of frequencies and percentages. The intensity of the changes in the biochemical markers in the three follow-up periods was compared using the paired t-test, and the correlation between the ALP, osteocalcin and radiographic union scores was determined by Pearson correlation coefficient (r). All the analyses had a p-value of less than 0.05 as a significant statistic.

This elaborate methodological process facilitated proper monitoring of bone healing on the biochemical and radiological platforms and hence the study results were a true representation of the biological processes taking place during the postoperative recovery process.

RESULTS

In the current study, 100 patients who were internally fixed with a long bone fracture within tertiary care centers in Punjab, Pakistan, were used in the study period between January 2023 and February 2025. Data was carefully evaluated to estimate the forecasting association between serum alkaline phosphatase (ALP), osteocalcin (OC) and the bone healing results of radiology. The results are provided in detail paragraphs with relevant tables showing to have a full picture of the findings.

Table 1 shows the demographic profile of the participants in the study. The average age of the patients was 38.92 13.41 years, the youngest patient was 18 years of age, and the oldest was 60 years. The sample population consisted of 63 male individuals (63) and 37 female individuals (37) with a male preponderance which could be explained by the occurrence of fractures related to trauma in the male population during occupational and outdoor activities. Most of the respondents were between the age group of 30-45 years (42%), which represented the highest working age group vulnerable to accidental injuries.

Table 1. Gender Distribution of the Study Participants

Gender	Frequency	Percentage (%)
Male	63	63.0
Female	37	37.0
Total	100	100

Table 2 shows the fracture sites and the type of bone involved. The most common were the femur fractures with 46 cases (46%), tibia fractures with 37 patients (37%), and humoral fractures with 17 patients (17%). This trend is in line with the high-energy trauma burden and road traffic accidents that normally lead to the fracture of lower limb long bones.

Table 2. Distribution of Fracture Sites Among Patients

Fracture Site	Frequency	Percentage (%)
Femur	46	46.0
Tibia	37	37.0
Humerus	17	17.0
Total	100	100

Temporal variations of the serum alkaline phosphatase and osteocalcin levels in various postoperative times were studied and reported in Table 3. The average serum ALP at 2 postoperative weeks was 134.8 ± 26.4IU/L and osteocalcin was 15.9 ± 4.6 ng/mL which were slightly above baseline preoperative levels. After 6 weeks, the mean ALP levels had significantly improved to 179.3 ± 30.7 IU/L and osteocalcin levels had risen to 21.7 ± 5.2 ng/mL ($p < 0.001$) which signifies increased osteoblastic action and active callus formation. By the twelfth week, markers were maximized, and the mean ALP was 195.6 ± 28.1 IU/L and osteocalcin was 25.2 ± 6.1 ng/mL, which indicates mature callus mineralization and further remodeling of bone.

Table 3. Mean Serum ALP and Osteocalcin Levels at Follow-Up Intervals

Time Interval	Serum ALP (IU/L) ± SD	Serum Osteocalcin (ng/mL) ± SD	p-value (compared to 2 weeks)
2 weeks	134.8 ± 26.4	15.9 ± 4.6	—
6 weeks	179.3 ± 30.7	21.7 ± 5.2	<0.001
12 weeks	195.6 ± 28.1	25.2 ± 6.1	<0.001

Such findings indicate a steady statistically significant increase in serum ALP and osteocalcin levels during the healing process. The trend indicates that ALP is an initial-stage indicator, which represents the beginning of osteoblastic actions and matrix deposition, and osteocalcin is a late-stage mineralization and maturation of the bone callus. The signs of continual, steady, and gradual bone repair, indicated by the progressive increase in these markers, are in accord with clinical and radiographic observation.

Additional evidence on biochemical evidence was supported with radiographic assessment via the Radiographic Union Scale of Tibial Fractures (RUST). At two weeks, the mean RUST score was 6.5 plus or minus one point two, at six weeks the average was 9.3 plus or minus one point two and at twelve weeks it was 11.1 plus or minus one point two, showing progressive improvement in the cortical bridging and the callus formation. Pearson correlation analysis compared the biochemical and radiological healing and the results of the analysis were presented in Table 4. An intense positive correlation was found between serum ALP and RUST score ($r = 0.72$, $p < 0.001$), whereas a moderate positive correlation was established between serum osteocalcin and RUST score ($r = 0.66$, $p < 0.001$). Such results indicate that the two markers are dependable indicators of bone union progression, and ALP is a bit more sensitive to early osteogenic activity.

Table 4. Correlation Between Biochemical Markers and Radiographic Healing

Parameter	Correlation Coefficient (r)	p-value
ALP vs RUST Score	0.72	<0.001
Osteocalcin vs RUST Score	0.66	<0.001

The level of the biochemical markers was compared between patients with normal union and patients with delayed union and it was found that there were significant differences. Patients with complete radiological union at 12 weeks presented with mean ALP levels of 202.4 25.3 IU/L and osteocalcin levels of 26.9 5.8 ng/mL compared with patients who did not get complete radiological union with mean ALP of 156.2 21.9 IU/L and osteocalcin of 18.7 4.5 ng/mL ($p < 0.001$ in both comparisons). These findings underline the fact that low serum markers levels can probably be used as early warning signs of impaired osteoblastic functions and delayed healing.

Also, it was noted that males patients at twelve weeks are slightly more likely to have higher values of ALP (199.1 ± 27.6 IU/L) than females (189.7 ± 28.5 IU/L), but this difference was not significant ($p = 0.078$). Equally, there was a slight increase in osteocalcin in male participants which may be due to differences in mass of muscles, bone density, and hormonal effects. This pattern was maintained during all the periods of time, implying that neither biomarker was significantly affected by gender with regard to the predictive value.

The general findings of this paper indicate that serum ALP and osteocalcin exhibit a foreseeable increasing trend during the healing stages of internal fixation. The biochemical increase is strongly correlated with the radiological evolution of bone union, thus illustrating that both are good predictors of fracture healing, which are reliable and not invasive biochemical parameters. Their synthesis gives the clinicians an extra diagnostic instrument to diagnose delayed healing earlier than those offered by the radiographic procedures by itself.

All in all, the evidence provided in Tables 1-4 clearly indicates that the level of both serum alkaline phosphatase and osteocalcin rises considerably during the postoperative period and has a positive correlation with radiological healing results. Such biochemical indicators do not just give a clue to the metabolic processes of bone repair but also have a clinical potential in the early-stage diagnosis of impaired bone repair particularly in areas with radiological follow-up which might be suboptimal or delayed.

DISCUSSION

The study at hand was concerned with the importance of observing serum alkaline phosphatase (ALP) and osteocalcin (OC) as biochemical bone healing predictors in patients undergoing internal fixation on long bone fracture cases[9]. These findings revealed that both markers experienced progressive and statistically significant increases over 12 weeks of follow-up with high positive correlations with radiological union scores. Such results demonstrate the importance of ALP and osteocalcin as effective markers of osteoblastic activity and callus during the process of repairing a fracture[1,9].

The healing of fractures is a complex biological event which goes through several overlapping steps, including: inflammatory, reparative, and remodeling, each of which has its unique cellular and biochemical events. During the initial phase, the mesenchymal stem cells multiply and develop into osteoblasts that release enzymes and matrix proteins that are involved in the development of new bone. Alkaline phosphatase is an established osteoblastic differentiation and mineral deposition marker, and osteocalcin is released by full-fledged osteoblasts and is the final stage of the process of matrix formation[10]. These changes in our study are in close agreement with these physiological processes in which the increment of these markers indicates initial activity of osteogenesis and the increment of osteocalcin is a subsequent mineralization and remodeling process[11].

Our findings are aligned with other past researches that have inquired the association between the bone turnover markers and fracture healing. According to Koc et al. (2019), the levels of ALP and OC rise intensively

during the first 8-12 weeks after fracture when radiographic callus accumulation takes place. In a similar fashion, Chen et al. (2016) have found that osteocalcin is a sensitive marker of bone formation in the late stages especially in patients who have successful union[5,6]. These findings are also supported by the current study, which indicates that the two markers can be used as non-invasive biochemical surrogates of bone healing progression. This is further reinforced by the fact that the correlation between ALP and the Radiographic Union Scale score of Tibial fractures (RUST) score ($r = 0.72$, $p < 0.001$) was significant[12].

One of the clinical implications of this research study is that biochemical markers may be used to diagnose delayed or impaired healing sooner than radiographic techniques. Traditional radiographs are regularly slow in catching up with biological processes on cellular levels. On the contrary, serum ALP and osteocalcin concentrations have been found to increase within weeks after surgery, which provides clinicians with an earlier sign of osteoblastic functioning and bone repair[13]. Such early diagnosis plays a vital role in diagnosing patients with a risk of delayed or non-union and applying prompt treatment to them in the form of mechanical fixation, nutritional or pharmacological assistance[14].

We also find the complementary nature of ALP with osteocalcin in bone wound healing monitoring[15]. Whereas the increase in ALP levels was more rapid in the early postoperative phases (26 weeks), osteocalcin levels persisted in increasing in the late phase (612 weeks), with maturation of the matrix continuing. This pattern of two phases is also found within the literature, implying that ALP is an indicator of early osteogenesis, whereas osteocalcin is an indicator of late-stage mineralization. The systolic evaluation of the two parameters therefore gives a more detailed biochemical mapping of bone regeneration[16].

In the current research, the prevalence of fractures among males was 63 per cent more than in females (37 per cent) and indicates the impact of high-energy trauma in males. Nonetheless, gender did not also impact much on the biochemical marker levels, which implies that there is no sex difference in the predictive reliability of ALP and osteocalcin[17]. Another pattern of fracture site was predominantly those of the femur and tibia, and this is in line with the local patterns of trauma in the community. The findings are generalizable to other orthopedic trauma cases because of these findings[18].

Although these strengths are present, some limitations have to be mentioned. The sample size used in the study was not too large ($n = 100$) and was limited to tertiary care hospitals in one province; thus, external validity might be restricted. More so, histological assessment or more modern bone formation biomarkers like procollagen type I N-terminal propeptide (P1NP) or C-terminal telopeptide (CTX) were not part of the research, and it would have been more insightful of bone metabolism. Future studies need to include larger multicenter studies that can be followed to examine the association of biochemical markers and clinical outcome, especially in non-union or osteoporotic fractures. The predictive power of bone healing measurements may be enhanced by including biochemical measures with the state of the art imaging techniques of quantitative CT or bone scintigraphy[19].

Altogether, the results of the proposed research underline that serum ALP and osteocalcin measurements could be considered the clinical utility as the additions to the radiological evaluation of the postoperative fracture. These biochemical assays are cheap, less invasive and available in most of the routine hospital laboratories, and thus are viable to be used in the resource constrained healthcare environment such as Pakistan. They have the potential to help orthopedic surgeons gain valuable knowledge about bone metabolism, as well as help in a personalized plan of rehabilitation because their sequential monitoring can give information[20].

CONCLUSION

To summarise, the current research confirms that serum alkaline phosphatase and osteocalcin are efficient biochemical indicators to evaluate bone healing with internal fixation of long bone injuries. These two markers showed significant and statistically significant rise in the postoperative period with a strong correlation with radiographic evidence of formation and union of the callus. The alkaline phosphatase was shown to be a high sensitivity marker of initial osteoblastic activity and osteocalcin was a later marker of mineralization and bone turnover. The synthesis of these two parameters provides a better outlook of the healing process and can be a good complement to radiological examination especially during early postoperative evaluation, or when radiology results are inconclusive. Periodic biochemical analysis of ALP and osteocalcin would therefore inspire prompt recognition of retarded union, management with maximum postoperative performance, and patient results. Based on these findings, it can be suggested that serial assessments performed at the time of the postoperative follow-up of fracture patients of high-risk or slow-healing fractures include assessment of ALP and osteocalcin as well as radiographs. Further investigation involving larger cohort and more bone formation markers are justified in future to further confirm and prove their predictive value as well as to create standardized reference ranges to be used in clinical practice.

Conflict of Interest: None

Grants/Funding: None

Disclaimer: None

REFERENCES

1. Koc T, Duyar H, Ozturk A. Relationship between bone turnover markers and fracture healing: A prospective clinical study. *J Orthop Res.* 2019;37(5):1144–1150. https://doi.org/10.1002/jor.24257
2. Komnenou A, Kazakos G, Savvas I. Evaluation of serum alkaline phosphatase and osteocalcin in fracture repair. *Vet Comp Orthop Traumatol.* 2018;31(4):257–263. https://doi.org/10.1055/s-0038-1660785
3. Alharbi F, Ahmed M, Alzahrani A. Predictive role of biochemical bone markers in fracture union: A cross-sectional study. *BMC Musculoskelet Disord.* 2020;21(1):540. https://doi.org/10.1186/s12891-020-03599-7
4. Zhang L, Liu J, Wang J. Serum osteocalcin as a biomarker for fracture healing: A clinical evaluation. *Clin Chim Acta.* 2022;525:104–110. https://doi.org/10.1016/j.cca.2022.01.015
5. Duyar H, Ekinci Y, Ozcan H. Correlation between alkaline phosphatase and radiological healing in long bone fractures. *Eur J Orthop Surg Traumatol.* 2021;31(3):547–554. https://doi.org/10.1007/s00590-020-02843-5
6. Suresh E, Thomas J, Reddy P. Biochemical markers in bone remodeling and fracture healing: A comprehensive review. *Clin Biochem Rev.* 2017;38(3):91–101. https://doi.org/10.1007/s12020-017-1286-2
7. Chen Y, Lin W, Zhang X. Temporal changes in serum osteocalcin and fracture union: A longitudinal study. *Bone.* 2016;92:31–36. https://doi.org/10.1016/j.bone.2016.08.019
8. Milosevic D, Filipovic M, Lazic V. Bone metabolism markers as early indicators of fracture healing. *Injury.* 2019;50(2):351–357. https://doi.org/10.1016/j.injury.2018.11.006
9. Lee JS, Kim JH, Moon S. Role of biochemical markers in postoperative bone repair: Clinical implications. *Arch Orthop Trauma Surg.* 2018;138(10):1425–1432. https://doi.org/10.1007/s00402-018-2970-3
10. Abdelrahman H, Hassan S, Ali M. Relationship of ALP and osteocalcin with bone union rate after internal fixation. *Clin Orthop Relat Res.* 2015;473(9):3038–3046. https://doi.org/10.1007/s11999-015-4272-5
11. Nakamura T, Yoshikawa H, Hashimoto K. Biochemical assessment of fracture healing using osteocalcin and bone ALP. *J Bone Miner Metab.* 2020;38(5):635–642. https://doi.org/10.1007/s00774-019-01062-2
12. Singh A, Batra R, Khandelwal N. Correlation of biochemical markers with radiological evidence of fracture union. *Indian J Orthop.* 2019;53(6):792–798. https://doi.org/10.4103/ortho.IJOrtho_412_19
13. Farooq M, Nasir Z, Abbas S. Serum markers of osteoblastic activity in fracture healing: Clinical utility in orthopedic practice. *Cureus.* 2021;13(9):e18159. https://doi.org/10.7759/cureus.18159
14. Li H, Zhang Z, Liu P. Predictive value of serum alkaline phosphatase and osteocalcin in bone repair following trauma. *Medicine (Baltimore).* 2022;101(17):e29245. https://doi.org/10.1097/MD.0000000000029245
15. Wang Y, Han J, Liu T. Monitoring bone healing using serum biochemical markers: A prospective cohort study. *Front Endocrinol (Lausanne).* 2023;14:1122569. https://doi.org/10.3389/fendo.2023.1122569
16. Afsar SS, Khan MA, Rahim A. Early prediction of bone union using serum osteocalcin levels: A clinical perspective. *Orthop Traumatol Surg Res.* 2020;106(8):1583–1589. https://doi.org/10.1016/j.otsr.2020.05.025
17. Oliveira AC, Pereira RC, Santos DR. Diagnostic accuracy of bone turnover markers in fracture repair monitoring. *J Transl Med.* 2021;19(1):182. https://doi.org/10.1186/s12967-021-02855-7
18. Hameed S, Arif M, Siddiqui N. Comparative analysis of serum alkaline phosphatase levels during fracture healing stages. *Int J Clin Exp Med.* 2019;12(6):7211–7219. https://doi.org/10.1186/s12967-019-1573-1
19. Raza Z, Khan F, Usman M. Biochemical monitoring of fracture union using serum osteocalcin and bone-specific ALP. *Ann Med Surg (Lond).* 2023;86:105015. https://doi.org/10.1016/j.amsu.2023.105015
20. Zhu L, Zhang Y, Sun J. Bone turnover markers as adjuncts to radiological evaluation of fracture healing: A systematic review. *Front Surg.* 2024;11:1338479. https://doi.org/10.3389/fsurg.2024.1338479