ORIGINAL ARTICLE

Bone-Alkaline Phosphatase is a Potential Biomarker for Monitoring Femur Fracture Healing

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ABSTRACT

Background: Clinical management of fractures is often compounded by use of subjective and expensive methods such as radiological imaging using X-rays. In resource-constrained regions, Alkaline phosphatase testing could serve as an objective and inexpensive practical option for monitoring fracture healing and reduce healthcare costs. The expression pattern of Alkaline phosphatase reflects the biosynthetic activity of bone-forming cells.

Aim*:* To analyze the relationship between Alkaline Phosphatase serum levels and outcome of fracture healing in patients with femur fractures.

Methods: We examined the serum levels of Alkaline Phosphatase in 32 patients on skeletal traction in the first, third-, and sixth-week post-femur fracture using spectrophotometric and calorimetric quantitative methods. The outcome of femur fracture healing was assessed using radiological imaging at first- and eighth-week post-fracture.

Results: In the healing-group, serum Alkaline Phosphatase levels rose significantly ($p < 0.05$) from 76.5 IU/L to 540 IU/L at three weeks and then gradually decreased to 380 IU/L in the sixth week. In the non-healing group, there was no significant ($p > 0.05$) increase in Alkaline Phosphatase levels from the first (99 IU/L), third (180 IU/L), and sixth (276 IU/L) week. A significant increase in serum Alkaline Phosphatase levels in the third week corresponded to normal femur fracture healing. Conversely, an insignificant rise in serum Alkaline Phosphatase levels in the third week corresponded to non-healing of the femur fracture.

Conclusion: Our results show a direct relationship between serum Alkaline Phosphatase levels and femur fracture healing and render bone-Alkaline Phosphatase as a potential biomarker for monitoring fracture healing. These findings suggest that ALP testing could serve as an affordable and accessible biomarker for monitoring fracture healing in resource-constrained settings, where radiological imaging may not be readily available.

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INTRODUCTION

Clinical management of fractures has not been without challenges mainly due to dependence on subjective and costly methods that are employed in monitoring the process of fracture healing. In resource-constrained regions, these methods include physical assessment of patients and radiological imaging using X-rays. These methods are subjective as they depend on patients' opinions to determine whether fractures have healed or not and on the radiologists' expertise in interpreting the X-ray imaging results[1]. Furthermore, the accuracy of radiological imaging is time-dependent as progress in fracture healing is only visible through X-rays at about three weeks post-fracture. Costs associated with techniques such as X-rays, MRIs, and CT scans make them inaccessible to many patients, particularly in public healthcare settings where out-of-pocket expenses are common. Additionally, the availability of sophisticated equipment is limited, especially in rural areas, where healthcare facilities often lack the necessary tools, and those that do have such equipment may face frequent breakdowns due to poor maintenance. The reliance on highly trained professionals, such as radiologists and orthopedic specialists, further complicates fracture monitoring efforts, as there is a shortage of skilled personnel, particularly in rural regions. This shortage leads to prolonged waiting times for diagnostics, exacerbating delays in treatment. Inconsistent follow-up due to geographical barriers and limited transport options makes it difficult for patients to attend regular appointments, while patient compliance is further affected by socioeconomic factors. Radiation exposure from repeated X-rays also poses health concerns, especially for patients who require frequent monitoring. Collectively, these factors hinder effective fracture healing monitoring using current methods especially in resource-constrained regions. In rural settings, reliance on radiological imaging is hampered by limited access to radiology experts and functional equipment, increasing the need for alternative biomarkers like ALP. Therefore, to improve the quality of clinical management of fractures and prevent the dire consequences of delayed bone union, there is need to seek an objective and cost-effective method that complements radiological imaging in monitoring the process of fracture healing especially in resource- constrained regions such as Zambia.

When a fracture occurs, metabolic biomarkers are expressed at different stages of fracture healing and participate in several biochemical processes that restore the original anatomic structure and mechanical function of the bone. Previous studies have shown that some of these biomarkers can be used to assess the fracture healing process in addition to radiological imaging [2,3].

Bone-Alkaline phosphatases (ALP) are among the biomarkers that play a crucial role in bone regenerative processes. ALPs are membrane-bound metalloenzymes that are widely expressed in both plants and animals[4]. In humans, ALP contains two very similar subunits each of which contain a different binding site for magnesium ions that stimulate enzymatic activity. They also have a tightly bound zinc atom that adds to the structural integrity of the dipeptide and a less tightly bound zinc atom that is necessary for catalysis[5–7]. During fracture healing, ALP catalyses the hydrolysis of phosphate monoesters at an alkaline pH to release inorganic phosphates and calcium that are deposited onto the fracture callus, thereby promoting healing[8–10].

The expression pattern of ALP during fracture healing reflects the biosynthetic activity of bone-forming cells and some previous studies have shown changes in their serum levels in relation to fracture healing[11]. However, more evidence is needed to substantiate the usage of ALP as a prognostic marker of femur fracture healing especially in an African cohort. This study uniquely focuses on African populations, where healthcare resources are limited, contributing valuable data for regional fracture healing protocols. Therefore, this study sought to examine the relationship between ALP serum levels

and outcome of fracture healing in patients with fractures of the femur on skeletal traction at the University Teaching Hospital (UTH) and Levy Mwanawasa University Teaching Hospital (LMUTH) in Lusaka, Zambia.

METHODS

Ethical Approval

Permission to carry out this study was sought from the University of Zambia Biomedical Research Ethics Committee (UNZABREC; Reference Number: UNZA-208/2019) and the research was conducted in accordance with the Declaration of Helsinki. Consent to participate in the study was obtained from patients by signing a consent form after reading and/or understanding the verbal explanation with regards to the study as indicated on the information sheet.

Study Design

We conducted a prospective cohort study.

Study Site

The study was conducted in Lusaka, Zambia, at two sites: the UTH and LMUTH.

Sample Size Calculation

The Cochran formula was used to calculate the sample size[12]. This gave an estimated sample size of 384 participants. This formula is $n_0 = Z^2\ p\ q\ /\ e^2$ Where; n_0 = the sample size, Z^2 = the table value of chi-square for 1 degree of freedom at the desired confidence level of 0.05 ($1.96^2 = 3.84$), p = the estimated proportion of an attribute that is present in the population (assumed to be 0.50 since this would provide the maximum sample size), q = 1- p, e^2 = the desired level of precision (0.05).

The sample size (384 participants) was then adjusted using the Cochrane formula for calculating sample size of a finite population. An average of 25 patients with fractures of the femur had been attended to at UTH-Adult hospital and LMUTH two months prior to data collection. This number was then used as the population size that gave an adjusted sample size of 25 participants. The population correction formula that was used is: $n = n_0 / [1 + \{(n_0 - 1) / N\}]$, Where; n = the adjusted sample size, n_0 = the estimated sample size in the Cochran formula. N = the population size (25 participants)

The adjusted sample size of 25 participants was further adjusted to account for the number of participants who may leave (dropout) the study for any reason. Literature has shown that dropout rates in longitudinal studies can be as high as 42% [13] or as low as 9%[14]. With this in mind, this study estimated a dropout rate of about 20%. A new sample size was calculated with this factored. The final sample size was found to be 32 participants. The calculation of the adjusted sample size using the dropout rate is shown below.

If *n* is the sample size required as per formula and if d is the dropout rate then adjusted sample size N1 is obtained as $N1 = n/(1-d)$. Where N1 = Adjusted sample size (after factoring in the dropout rate), *n*= required sample size as per formula (adjusted Cochran formula above = 25 participants)

d = dropout rate (20%), Since $N1 = n/(1-d)$, Then $N1 = 25/(1-20\%)$, N1 = 31.25 ~ 32 participants.

Inclusion and Exclusion Criteria

To prevent selection bias and ensure that each patient had an equal chance of being included in the study, simple random sampling using the lottery method was used in the selection of participants. In this regard, every second patient that met the inclusion criteria was enrolled into the study. Both men and women aged 18 to 45 years with a body mass index (BMI) between 18.5 (kg/m^2) to 25 (kg/m^2) were included in the study. Furthermore, only patients who were on skeletal traction with non-complicated closed fractures were included in the study. This is because skeletal traction provides a more efficient fracture reduction method through adequate immobilization and stabilization. This reduces confounders that could result from poorly reduced

fractures. Patients with multiple fractures or pathological fractures of the femur were excluded from the study to reduce confounders that could affect the course of fracture healing. Furthermore, patients with osteoplastic diseases, liver disorders, gastro-intestinal tract inflammation, pregnancy and auto-immune disorders were excluded from the study as these could influence the levels of ALP.

Radiological Imaging

Following standard protocol, radiological imaging of femur fractures was conducted using Quantum Digital System X-ray machine at week one and eight post-fracture. Participants whose radiographs showed bridging of the fracture by mineralized callus, bone or had an obliteration of the fracture line at eight weeks post fracture were allocated to the normal healing group while participants whose radiographs showed the fracture line or a radiolucent callus were allocated to the non-healing group.

Quantification of ALP Serum Levels

In the first, third-, and sixth-week post-fracture, 2 mls of venous blood was drawn from each participant in the morning after fasting and Sera was separated and stored at 2°C.

Following standard protocol and reagents (S1), total serum ALP was then quantified calorimetrically using the Beckman Coulter AU Analyser (Beckman Coulter, 2014) within two weeks after Sera collection.

Statistical Analysis

Data was analyzed using Stata version 23. Results were presented as means, median, confidence intervals and odds ratio. Univariate and multivariate logistic regression was conducted to ascertain relationship between ALP levels and the outcome of fracture healing. $P < 0.05$ was considered significant.

Potential Confounders

Confounding factors like age and sex were controlled through stratified analysis while comorbidities were ruled out through baseline clinical assessments.

RESULTS

Outcome of Fracture Healing

Skeletal traction provides a more efficient fracture reduction method that immobilizes and stabilizes fractures and hence minimises confounders that could result from poorly reduced fractures. To assess the outcome of fracture healing, radiological images of femur fractures of patients on skeletal traction were taken in the first- and eighth-week post-fracture, following standard protocol. Our results showed that in the eighth week, some patients had bridged fractures by mineralised callus, bone, or complete obliteration of fracture lines while others still showed fracture lines or radiolucent callus (Figure 1).

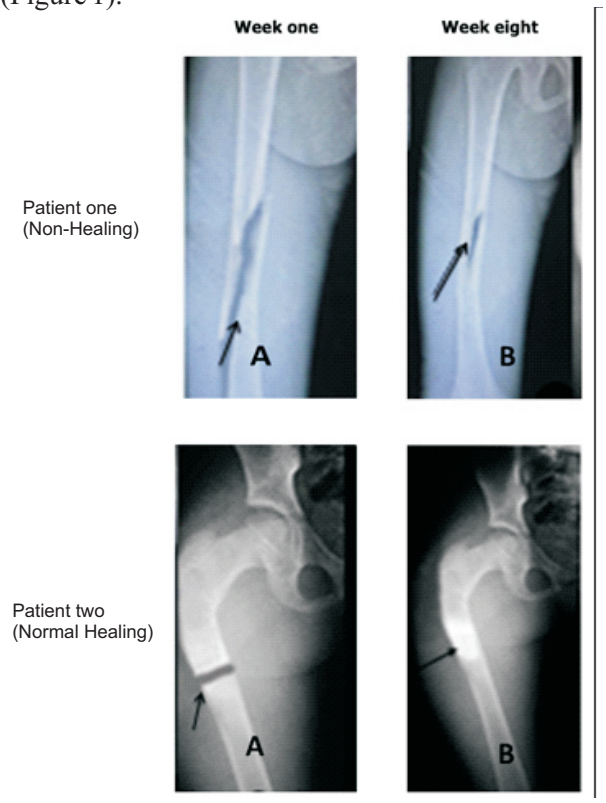

Figure 1: Radiographic images of the outcome of fracture healing

Patient one (1) A: X-ray showing a fracture of the femur at week one indicated by the arrow. B: X-ray showing a radiolucent femoral fracture that has not healed indicated by the arrow at eight weeks post fracture. Patient Two (2) A: X-ray showing a fracture of the femur at week one indicated by the arrow. B: X-ray showing a mineralized femoral shaft fracture that has healed indicated by the arrow at eight weeks post fracture.

These results led us to conclude that femur fractures heal at different rates and a number of factors could be responsible for the overall outcome of the healing process. Based on these results, we categorised patients whose fractures had mineralised callus by the eighth week as normal healing group while those with radiolucent callus as non-healing group.

Baseline Demographic Data of Study Participants

The baseline demographic data analysis showed that the continuous variables age and BMI had skewed data ($p < 0.05$). The median age in the normal healing group was 26 years while the non-healing group had a median age of 27 years. The Mann Whitney test indicated that this difference was not significant ($p = 0.06$). The majority (52.63%) of the males were in the non- healing group while the majority females (53.85%) were in the normal healing group. A Pearson Chi squared test showed that this difference in the two groups was not significant ($p = 0.53$). Lastly, 87.5% of the participants in the normal healing group were in informal employment while 12.5% were in formal employment. However, the Pearson's Chi-squared test showed that this difference was not significant ($p = 0.40$). Collectively, these demographic results showed that all participants in this study had equal chance of healing normally because of the non-significant differences in baseline data between the healing and non-healing group.

Baseline Clinical Data of Study Participants

The baseline clinical data analysis showed that the variables Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and age of fracture at time of admission were normally distributed ($p > 0.05$). In the first week, the normal healing group had a slightly higher mean ALT of 30.6 IU/L than the non-healing group which had a mean of 30.06 IU/L. The two-sample *t*-test showed that this difference was not significant ($p = 0.7506$). Furthermore, the mean AST for the normal healing group and the non-healing group was 23.02 IU/L and 23.96 IU/L respectively. This difference in mean AST was also not significant as indicated by the two-sample *t*-test ($p =0.53$) that was conducted. The results also showed that the median ALP in the normal healing group was 76.50 IU/L while that of the non-healing group was 99.00 IU/L. This difference was tested using the Mann Whitney test and was found to be not significant ($p = 0.31$). The median age of the fracture on admission in the normal healing group was 2 days (IQR = 2- 3.5 days) while that of the non- healing group was 2.5 days (IQR = 2-3 days). The Mann Whitney test showed that this difference was not significant ($p = 0.97$).

The clinical variables for all study participants were analysed in the third- and sixth-week post fracture. In the third week of observation the clinical parameters AST and ALT were normally distributed ($p > 0.05$) while ALP and BMI were not ($p < 0.05$). The mean ALT for the normal healing group was higher (31.06 IU/L) than in the non-healing group (30.44 IU/L). Similarly, the mean AST levels for the normal healing group was higher (26.63 IU/L) than in the non-healing group (24.25 IU/L). The median BMI readings in both groups in the third week were observed to be 23kg/m^2. The median ALP was also higher in the normal healing (540 IU/L) than in the non-healing group (180 IU/L); this difference was significant ($p <0.05$). There was no significant difference in the clinical variables AST, ALT, BMI for both the healing and non-healing groups ($p =$ 0.31, 0.82 and 0.60 respectively). These results signified the absence of any other underlying medical conditions that could have elevated these parameters, except for fractures. The clinical variables at 6 weeks showed that the variables ALT and AST were normally distributed for both groups ($p > 0.05$) while BMI and ALP were not normally distributed ($p < 0.05$). The mean ALT (30.06 IU/L), median ALP (380 IU/L) and BMI (21.50 kg/m^2) was higher in the normal healing group than in the non-healing group. The mean AST levels were higher in the non-healing group (23.31 IU/L) than in the normal healing group. It was also observed that the differences between the two groups in the clinical variables AST, ALT and BMIs was not significant ($p >0.05$). However, the difference in median ALP

levels between the two groups was significant ($p < 0.01$). These results showed that while there was no significant change in the physiological status of the participants, higher ALP levels were associated with normal femur fracture healing (Table 1).

Analysis of Changes in ALP levels

The changes in ALP levels were analyzed in the first, third- and sixth-week post fracture. The ALP levels in the normal healing group rose from a median of 76 IU/L in the first week to a median of 540 IU/L in the third week and then reduced to a median 380 IU/L in the sixth week. In comparison, the ALP levels in the non-healing group rose from 99 IU/L to 180IU/L in the third week and then to a median of 276 IU/L in the sixth week, as shown in Figure 2. A significant difference between the two groups in the median ALP levels was only seen at three weeks using the Mann Whitney test, as shown in Table 2. Outliers in ALP levels in the non-healing group may reflect variability in individual patient response or undiagnosed underlying conditions affecting bone metabolism.

Table 1: Clinical Data of Study Participants at Three and Six Weeks

	Variable	Normal Healing Group (n=16)	Non-Healing Group (n=16)	P-Value
Three Weeks	ALT (IU/L) ?*	31.06 (9.15)	30.44 (6.56)	0.82
	AST (IU/L)[b]*	26.63 (7.14)	24.25 (5.76)	0.31
	ALP (IU/L)?**	540.00 (480.00-644.25)	180.00 (127.50-210.00)	0.01
	BMI (kg/m^2)?**	23.00 (20.00-23.60)	23.00 (20.00-24.00)	0.60
Six Weeks	ALT (IU/L) ?*	30.06 (6.91)	30.50 (9.58)	0.88
	AST (IU/L)?*	21.13 5.14)	23.31 (7.60)	0.35
	ALP (IU/L)?**	380.00 (367.00-475.00)	276.00 (172.50-240.25)	<0.073
	BMI (kg/m$^{2)}$?**	21.50 (19.25-23.75)	20.00 (19.00-23.00)	0.39
	* = Mean and standard deviation have been reported; **median and interquartile range have been reported; ?Mann Whitney test; ?two sample *t*-test with equal variances.			

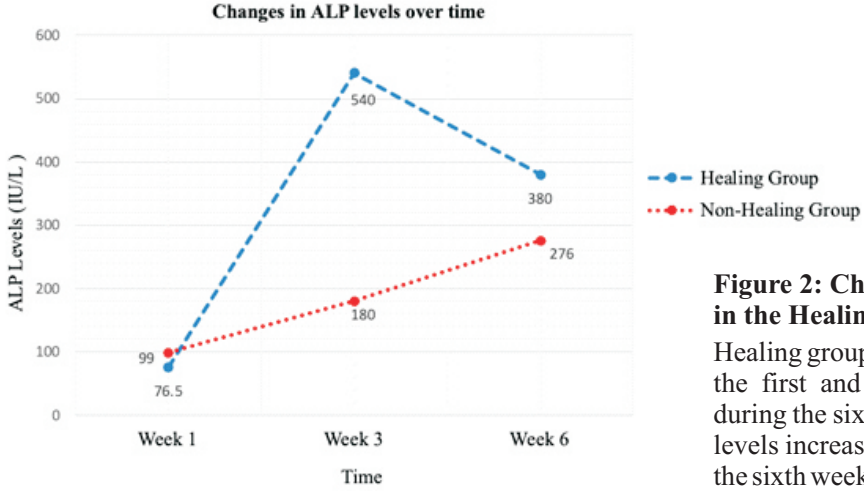

Figure 2: Changes in ALP levels over time in the Healing and Non-Healing Group

Healing group: ALP levels rose sharply between the first and third week, before plummeting during the sixth week. Non-healing group: ALP levels increased gradually between the first and the sixth week.

Table 2: Quantification of ALP Levels

Week	Healing Group	Non-Healing Group	*P*-value
Week 1*	76 IU/L (69-100.50)	99 IU/L (68-113)	0.064
Week 3*	540 IU/L (480.00-644.25)	180 IU/L (127.50- 210.00)	**<0.05**
Week 6*	380 IU/L (367.00-475.00)	276 IU/L (172.50-240.25)	0.073
*The median and IQR.			

Determination of the Relationship between ALP Levels and Outcome of Fracture Healing

Logistic regression analysis was used to determine the true predictors for outcome of fracture healing as well as to control for confounders. Both univariate analysis and multivariate logistic regression were conducted. The univariate analysis was conducted to determine the relationship between each variable and the outcome of fracture healing at one-, three- and six-weeks post fracture. The variables age, sex and ALP serum levels were significant at a p 0.05. The odds of not healing normally increased with age (OR=1.07, CI=0.997- 1.16) and being female (OR=1.33, CI=0.75-1.63), with p value of 0.04 and 0.01 respectively. In the normal healing group, the p value for ALP was less than 0.05, with an OR of 0.99 and a confidence interval of 0.99 – 1.00, indicating that the odds of healing normally increased in this group. The multivariate logistic regression model was performed and only the significant variables (age, sex, and ALP) from the univariate logistic regression were considered. The results (Table 3) indicated that only ALP serum levels were truly associated with the outcome of fracture healing with an odds ratio of 0.99 and p <0.05 (CI = 0.99-1.00), suggesting a huge difference between the normal and the non-healing group, with the odds of healing increased in the normal healing group

DISCUSSION

Clinical management of bone fractures requires quality and objective methods to avoid consequences of non-union such as permanent disabilities, joint stiffness, muscular atrophy, or reflex sympathetic dystrophy. In this study we show the relationship between ALP and the outcome of femur fracture healing in an exclusively African population on skeletal traction. Fracture healing was assessed using radiological imaging and was defined as bridging of the fracture by mineralized callus, bone or an obliteration of the fracture line at eight weeks post fracture. Normally, by as early as two weeks post fracture, mineralization of the bridging callus would have begun and so a radiolucent callus or a visible fracture line is not expected to be seen on x-ray at eight weeks post fracture[15].

Our data shows that participants in the normal healing group had mineralized callus, bone or an obliteration of the fracture line while those in the non-healing group had a visible fracture line or bridging callus that was translucent at eight weeks post fracture.

The baseline demographic and clinical data of the participants showed no significant differences in the parameters of the two groups, suggesting that all the

Table 3: Multivariate Logistic Regression of Study Variables

Variable	OR	*P*-value	(95% CI)
Age	1.05	0.32	0.95 - 1.17
Cause of fracture			
- RTAs	0.42	0.38	0.09 - 1.96
- Falls	1.36		0.00 -0.00
ALP*	0.99	**0.000**	0.99 – 1.00
*Indicates significant *P*-value at $P < 0.05$			

participants had equal chances of healing normally. Furthermore, the odds of not healing normally were significantly increased in the participants who were older. This is consistent with previous studies that have shown that increasing age decreases the healing potential of fractures [16–18]. The plausible reason for reduced fracture healing-potential in old age is the decrease in stem cell quantity, compounded by decrease in proliferation and differentiation potential of the bone[19–21]. This decline in osteoblast function and mesenchymal stem cell quantity decrease the ability to form a robust callus, resulting in decrease in bone cartilage content in a fracture callus and thus causing delay in fracture healing for the elderly[22].

We also found that the odds of not healing normally increased significantly with being female, consistent with previous studies involving animal models which have reported that females are more at risk for delayed and non-union of fractures than males[23–26]. The observed sex-based differences may be attributed to lower estrogen levels in females, which can impair bone regeneration by reducing osteoblast function and increasing osteoclast activity.

With reference to changes in ALP levels, it was observed that during the first week of the fracture occurring, the ALP levels remained within normal range of 40 IU/L - 140 IU/L in both groups. However, a significant increase in ALP levels at week three was only observed in the normal healing group. In the non-healing group, there was no significant increase in ALP levels at three and six weeks, consistent with previous studies which reported a significant increase in ALP levels in the normal healing group in comparison to the poor healing group[27–29]. The initial decrease in ALP has been attributed to the general stress response to trauma while the subsequent increase seems to depend on the magnitude of bone repair and the type of fracture healing[30].

It has been observed that the more severe and/or unstable fractures take long to reach their peak ALP levels as compared to less severe and/or stable fractures[30]. To establish the relationship between outcome of fracture healing and changes in ALP, conditional logistic regression was conducted. The univariate analysis of the study variables showed that only sex, age and ALP levels were significantly associated with outcome of fracture healing. The multivariate logistic regression on the other hand showed that the ALP level was the only factor that was truly associated with the outcome of fracture healing. In this study, a significant increase in ALP levels by the third week post fracture was associated with normal femur fracture healing while a non-significant increase was associated with delayed healing.

These results are similar to the ones obtained previously using animal models, where a significant increase in ALP correlated with normal healing[31]. However, other studies have reported that in the first two weeks post fracture, a minor increase or no change in the level of ALP indicated rapid bone healing while a major increase indicates inadequate fracture fixation and delayed bone healing.

The relationship between fracture healing and changes in ALP levels can be explained by the physiology of fracture healing in which bone formation markers such as ALP are inhibited from being produced in the earlier stages of fracture healing to allow for the body's initial response to inflammation and the removal of necrotic tissue by osteoclasts around the fracture margins[32,33]. This stage usually lasts up to seven days during which time osteoblasts that produce ALP are expressed[34]. Following its expression, ALP plays a critical role in bone mineralization by hydrolyzing phosphate esters thus increasing the concentration of phosphate ions necessary for calcium phosphate deposition in the fracture callus[35,36]. This process is essential for forming the mineralized matrix that supports bone regeneration. Additionally, ALP inactivates pyrophosphate, an inhibitor of bone mineralization, and binds to collagen fibrils, further aiding in the stabilization and deposition of calcium during fracture healing[37,38]. This phase of mineralization reaches peak at about two weeks post

fracture; the bone remodeling phase then follows when biomechanical stability of the formed hard callus is achieved by balancing hard callus resorption by osteoclasts and lamellar bone deposition by osteoblasts[39,40].

It is evident that ALP levels are significantly elevated only during normal bone healing and as such, monitoring of the changes in its levels can help determine the outcome of fracture healing at an early stage (as early as three weeks) in patients with fractures of the femur on skeletal traction. This can aid in reducing complications that are associated with delayed bone healing and/or non-healing of fractures.

Study Limitation

The primary limitation of this study was its small sample size which may limit the generalizability of the findings. Additionally, the inclusion of only patients with non-complicated closed femur fractures introduces potential selection bias, as more complex fracture cases were excluded. Furthermore, the study was restricted to patients on skeletal traction, which may not reflect all clinical presentations of femur fractures. Some medical records had incomplete entries such as patient diets and illnesses that they may have suffered from in childhood. Financial constraints prevented quick analysis of samples upon collection and as such they had to be stored at 2 ℃ until a full set of specimens needed to fully load the Beckman Coulter AU analyzer had been collected.

Recommendations

Future research should focus on conducting larger, multicenter studies to validate ALP as a biomarker for fracture healing. Additionally, exploring other biochemical markers such as osteocalcin, procollagen type 1 N-terminal propeptide (P1NP), and C-terminal telopeptide (CTX) could provide a more comprehensive understanding of the biochemical pathways involved in fracture healing. A longitudinal study examining both early and late-stage markers could further support the clinical integration of ALP testing.

CONCLUSION

A relationship between changes in ALP levels and fracture healing exists with an increase in ALP levels by the third week indicating normal healing while an insignificant increase in ALP levels indicating a delay in fracture healing. Collectively, our data renders bone-ALP a potential biomarker for monitoring femur fracture healing. These findings suggest that ALP testing offers a clinically viable and cost-effective method for monitoring fracture healing, especially in resource-limited settings where access to radiological imaging is restricted. By providing an affordable alternative, ALP testing could improve patient outcomes by allowing for earlier intervention in cases of delayed or impaired healing. We recommend incorporating ALP monitoring into routine follow-up care for patients with fractures especially in settings with limited access to radiological imaging. Further prospective studies are needed to establish definitive ALP thresholds for clinical use.

What is already known on this topic

i. In most African countries, and Zambia in particular, bone fractures are commonly managed by radiological imaging and physical assessment of patients and no speedy method is employed to monitor fracture healing to help prevent the consequences of delayed fracture healing.

ii. When a fracture occurs, metabolic biomarkers are expressed and participate in several biochemical processes that restore the original anatomic structure of the bone. Previous studies have shown that some of these biomarkers can be used to assess the fracture healing process. ALP is among the biomarkers that play a crucial role in bone regenerative processes.

What this study adds

I. The study shows the relationship between ALP levels and fracture healing in an exclusively African population.

ii. The study provides evidence that ALP can be used as a biomarker to complement radiological assessment of fracture healing especially in resource-limited places like Zambia and Africa at large.

Competing interests

The authors declare no competing interest.

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Authors' contributions

Gibson Sijumbila and James Munthali developed the research concept; Gibson Sijumbila, James Munthali, Lubinda Mukololo, and Musalwa Muyangwa-Semenova designed the experiments; Mercy Mukabila performed the experiments; Lubinda Mukololo, Musalwa Muyangwa-Semenova, and Mercy Mukabila analyzed data and wrote the paper.

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