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Serum Bone-Specific Alkaline Phosphatase as an indicator of the quantity of callus formation in mandibular fracture patients seen in a Nigerian Teaching Hospital

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Abstract

It is important to evaluate the level of bone-specific alkaline phosphatase as it relates to the quantity of callus formed in mandibular fracture healing. The objective of the present study was to assess Serum Bone-Specific Alkaline Phosphatase (BsALP) as an indicator of callus formation in patients with mandibular fracture and determine the relationship between BsALP and callus formation using two treatment methods. Fifty-five patients with isolated mandibular fractures were enrolled. BsALP was measured at presentation, 3rd and 6th week. The patients were recruited into two treatment groups: Closed Reduction with Mandibulomaxillary Fixation (MMF) and Open Reduction and Internal Fixation (ORIF). The Callus Index was measured at 3rd and 6th week after treatment using digital postero-anterior view of the jaws on DICOM viewer software. The mean value of BsALP was 26.2±9.5 ng/mL. BsALP concentration in patients with double site fractures was higher than those with a single fracture, $p=0.102$. Peak serum BsALP observed in the 3rd week post-intervention was (28.1±8.2 ng/mL). Statistically significant differences were observed between the BsALP concentration in the 3rd and 6th week, and between BsALP concentration at presentation and 6th week, $p<0.001$, respectively. There was no significant correlation between the Callus Index and mean serum BsALP at 6 weeks ($r=-0.08$, $p=0.580$). MMF treatment group had higher levels of serum BsALP compared with ORIF group in the 3rd week ($p=0.14$) and in the 6th week ($p=0.18$). BsALP is an indicator of the amount of callus formed in patients treated for mandibular fractures. Hence, it could be used as an adjunct to monitor the healing of mandibular fractures.

Introduction

Fracture healing is a normal physiological process, although surgical intervention and fracture immobilization may facilitate the healing process.[1] The hallmark of fracture healing is a marked reduction in the abnormal mobility at the fracture site. This is due to the formation of a minimal or substantial amount of callus (depending on the mode of treatment) that bridges the fractured gap. The amount of callus formed in fracture healing reflects the osteogenic activity of the osteoblast. Bone formation markers are majorly products of the bone-forming cell, osteoblast. Bone formation markers are known to regulate the metabolic activity of bone-forming cells.[2] The use of bone formation markers has been reported in the monitoring of fracture healing in other human long bones. However, more studies need to be conducted in assessing this bone-forming marker in facial bones healing like the mandible, which is considered a long bone in spite of being horseshoe-shaped.[3] Its similarities with other long bones include: the presence of an articular surface covered by cartilage, length greater than width, the presence of a medullary canal within the bone, and the presence of endosseous and extraosseous blood supp.[1] The mandible differs from other long bones in the body in that it is the only long bone in the body that houses the dentition. Hence, fractures involving the mandible are always compound fractures, and this may affect the healing of the fractures.

Bone alkaline phosphatase is an ectoenzyme that is anchored to the outer plasma membrane by a glycan linkage to phosphatidylinositol. It is released into circulation by the action of a glycan-inositol phosphate-specific hydrolase.[4] The biological half-life of circulating bone isoenzyme is between 1.12 and 2.15 days.[5] The bone isoenzyme has two binding sites, the metal-binding domain, and the crown domain. Bone-Specific Alkaline Phosphatase (BsALP) has been successfully used to monitor the healing of fractures of other long bones in the body by measuring the serum levels of this biomarker at different stages of healing.[2,6] A study by Ross *et al*.[7] showed a rise in the mean baseline of Serum Bone-Specific Alkaline Phosphate (BsALP) in women with osteoporotic fracture compared with women without fracture. It is worth noting that only one group of researchers, Ushtan *et al*.[8] has reported on the use of bone-specific alkaline phosphatase in the management of mandibular fractures.

Materials and Methods

This is a prospective cohort study conducted in Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria. The patients were recruited via the outpatient clinic of the Department of Oral and Maxillofacial Surgery and the Accident and Emergency Unit of the hospital from April 2018 to September 2019. The inclusion criteria are: patients with isolated mandibular fractures without other fractures in the body, patients who presented to the hospital within 72 hours of fracture, patients in whom fractures were secondary to road traffic crashes, assault, or sports injury, patients who consented to take part in the study. The following were excluded from the study: patients with an underlying systemic disease like liver disease, kidney disease, Paget's disease, pregnant women, condylar fractures, concomitant fractures in other parts of the body, and in whom hardware failed before 6 weeks.

Sample size calculation

The sample size was determined with the aid of the formulae below for sample size with a finite population correction factor.[9,10]

$$n = \frac{N(z^2)p(1-P)}{d^2(N-1)+(z^2)p(1-p)}$$

z = Confidence interval at 95% = 1.96
p = Prevalence = 0.571 (Ogunmuyiwa *et al*.)[11]
d = Degree of accuracy = 0.05
N = Study population (average number of mandibular fractures seen in the year 2014, 2015, and 2016 in OAUTHC Ile-Ife) = (37+41+38) /3 = 39
n = Sample size with a finite population

$$n = \frac{39(1.96^2)0.571(1-0.571)}{(.05^2)(39-1)+(1.96^2)0.571(1-0.571)}$$

$$n = \frac{36.7003}{1.0360}$$

$n = 35.42$
$n \approx 35$

10% attrition rate was added to make up 39 participants. However, 55 participants eventually constituted the study population.

The recruited patients had 5 mL of blood withdrawn from the antecubital fossa at three points in time: A1 (at presentation), A2 (three weeks after treatment), and A3 (six weeks after treatment). Recruited participants were treated by either closed reduction and mandibulomaxillary fixation, or open reduction and internal fixation. Blood was transported to the laboratory for BsALP assay using Xpressbio Human Bone Alkaline Phosphatase ELISA kit. Digital radiographic evaluation of the amount of callus formed was carried out at A2 and A3 using DICOM software. In order to standardize the radiographic process, CR 85-X AGFA digitizer was used, the same radiographer was responsible for the radiographs, DRYSTAR DT 2 B medical film was utilized, and patients were positioned facing the film with the head tipped forward so that the forehead and tip of the nose touched the film – the forehead-nose position. The radiographic baseline was horizontal and at the right angle to the film. The X-ray tube head was positioned with the central ray horizontal (0°) at the level of the mandibular rami; exposure time was the same for all research patients. The callus size was measured from the radiographic images, and the callus index was calculated with the formula.[12,13]

$$\text{Callus index} = \frac{\text{Maximum Callus diameter}}{\text{Diameter of basal bone cortex}}$$

The digital radiographs were viewed with the aid of a DICOM viewer computer application software. The diameter of basal bone cortex was measured by drawing a line from a point close to the tooth apex adjacent the fracture line down to the lower border of the mandible. The length of the line was automatically displayed on the digital image. This is depicted in Figures 1-3. The maximum callus diameter was measured adjacent to the point of reference of the diameter of the basal bone cortex to the opacity at the lower border of the mandible in the region of the fracture. This was done at points A2 (three weeks after treatment shown in Figure 2) and A3 (six weeks after treatment as shown in Figure 3). Participants were treated based on the traditional ways of treating mandibular fractures which included closed reduction with Mandibulomaxillary Fixation (MMF) or Open Reduction and Internal Fixation (ORIF).

Statistical method

The data collected was analyzed using IBM-SPSS version 25. The normality of quantitative data distribution was determined by Shapiro–Wilk test. Continuous variables were reported in means and standard deviation for normally distributed data, while the non-normally distributed data were presented as median and interquartile range. Tables and graphs were used to express the data. Inferential statistics were performed based on the parametric or non-parametric distribution of data. A repeated measure Analysis of Variance (ANOVA) was used to compare the mean values of bone-specific alkaline phosphatase at different presentation times. The changes in BsALPvalues at A1, A2, and A3 were compared using repeated measure ANOVA followed by a Bonferroni post hoc test. The relationship between BsALP and callus index at 3 weeks and 6 weeks (healing outcome) after treatment of mandibular fractures was determined using Spearman's correlation. The relationship between methods of treatment and serum levels of bone-specific alkaline phosphatase was assessed with the aid of an independent *t*-test. A $P<0.05$ was considered statistically significant.

Results

Fifty-nine patients consented to be enrolled in the study, but only 55 (93.2%) completed the study. The four patients (6.8%) that dropped out were treated with open reduction and internal fixation, and their data were excluded from the statistical analysis. The patients' age ranged from 18 to 45 years, with a mean of 29.3±8.4 years. Twenty-two (40.0%) patients were within the age range of 20 to 29 years, while seven (12.7%) were aged less than 20 years. The mean age was 32.0±9.2 years for those treated with ORIF and 28.6±7.8 years for those treated with MMF. There was no significant difference (p=0.219) in mean age between the two treatment groups. The values of BsALP ranged between 6.7–47.5 ng/mL, with a mean value of 26.2±9.5 ng/mL. The value of bone-specific alkaline phosphatase varied with the time of presentation, with the highest value in patients who presented between 24–48 hours after sustaining mandibular fractures (the range was 10.8–47.5 ng/mL; the mean was 26.6±10.9 ng/mL). About two-thirds (36, 65.5%) presented between 49–72 hours of mandibular fractures with BsALP level of range 9.9–45.9 ng/mL, mean value 26.3±8.1 ng/ml. A one-way repeated measure ANOVA test to compare the mean values of bone-specific alkaline phosphatase at different times of presentation (≤24 hours, >24–≤48 hours, and >48–≤72 hours) after sustaining mandibular fractures showed no significant difference between the means of the three groups ($F(2,52) = 0.19$, $p=0.82$). Table 1 shows the mean, minimum, and maximum values of BsALP at different times of presentation.

The mean value of BsALP in 27 patients with single-site fractures was 24.1±8.3 ng/mL, with a range of 6.7 ng/mL to 44.4 ng/mL. Twenty-eight patients with double sites mandibular fractures had a mean BsALP value of 28.3±10.2 ng/mL and range of 9.9–47.5 ng/mL. Though the patients with double sites fractures had a higher mean value of BsALP compared to patients with single site fractures, the difference was not significant ($t(53) = -1.66$, $p=0.102$, 95% CI = -9.223–0.859). Table 2 shows the comparison of BsALP in patients that presented with a single site and double sites mandibular fractures.

A one-way repeated measure ANOVA conducted to determine if there was a statistically significant difference between the mean values of BsALP. showed a statistically significant difference in

Figure 1. Preoperative radiography.

Figure 2. Three weeks post operative radiography.

Figure 3. Six weeks post-operative radiography.

Table 1. Range of Serum Bone-Specific Alkaline Phosphatase (BsALP) at different presentation times.

Presentation time (hours)	Participants	Mean ± SD	Minimum	Maximum	P
≤24	3	22.9±19.4	6.7	44.4	0.822
>24 – ≤48	16	26.6±10.9	10.8	47.5	0.822
>48 – ≤72	36	26.3±8.1	9.9	45.9	0.822

One-way ANOVA.

the mean values of BsALP at presentation, 3 weeks and 6 weeks after treatment (F (2, 108) = 2.45, p<0.001).

A significant difference was observed between BsALP values at 3 weeks and 6 weeks post-treatment (t=-5.68, p<0.001), and between BsALP values at presentation and six weeks post-treatment (t=-6.94, p<0.001). The results are presented in Tables 3 and 4. A Spearman correlation test used to determine the relationship between the callus index at 3 weeks and BsALP at 3 weeks showed a positive correlation between callus index at 3 weeks and BsALP at 3 weeks. (Spearman rho = 0.340, p=0.011). This is presented in the scatterplot shown in Figure 4.

The callus index at six weeks was used as the treatment outcome of mandibular fractures' healing. A Spearman correlation test to determine the relationship between the callus index and BsALP at six weeks showed a very weak negative correlation between callus index and BsALP at six weeks. (Spearman rho = 0.08, p=0.580). This is presented in the scatterplot shown in Figure 5.

An independent t-test statistic to determine if a significant relationship exists between the mean values of BsALP at three and six weeks after treatment using the two different methods of treatment under study. The result is presented in Table 5. The mean BsALPvalue was lower among patients treated with Open Reduction and Internal Fixation (ORIF) compared to those treated with Mandibulomaxillary Fixation (MMF) at 3 weeks and 6 weeks after treatment. However, this difference was not significant at 3 weeks (t=1.49, p=0.14) and 6 weeks (t=1.35, p=0.18).

Table 2. Comparison of Serum Bone-Specific Alkaline Phosphatase (BsALP) in single site and double sites mandibular fractures in study participants.

Variable	Number of fracture site		P
	Single n=27	Double n=28	
	Mean ± SD	Mean ± SD	
BsALP	24.07±8.33	28.25±10.18	0.102

Independent *t-test.*

Table 3. Serum levels of Serum Bone-Specific Alkaline Phosphatase (BsALP) at presentation, 3 weeks and 6 weeks after treatment.

Variable	Mean ± SD	Minimum	Maximum
Presentation	26.2±9.5	6.7	47.5
3 weeks after treatment	28.1±8.2	12.0	50.6
6 weeks after treatment	17.5±8.3	2.7	36.2

Table 4. *Post hoc* test of significance between paired groups.

Time	*t*	P
3 weeks *versus* presentation	1.26	0.210
6 weeks *versus* presentation	-5.68	<0.001*
6 weeks *versus* 3 weeks	-6.94	<0.001*

*Statistically significant.

Table 5. Relationship between methods of treatment of mandibular fractures and Serum Bone-Specific Alkaline Phosphatase (BsALP) at 3 weeks and 6 weeks after treatment.

Timing	MMF Mean ± SD	ORIF Mean ± SD	*t*	P
BsALP at 3 weeks	29.1±8.4	25.5±7.1	1.49	0.140
BsALP at 6 weeks	18.5±8.1	15.1±8.6	1.35	0.180

Independent *t-test.*

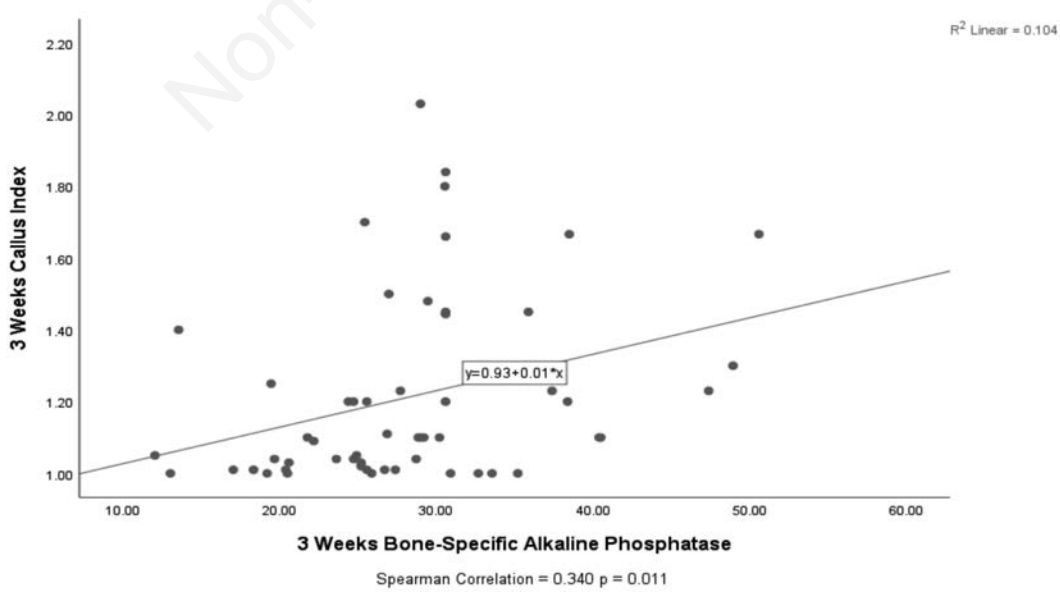

Figure 4. Scatter plot showing a positive relationship between 3 weeks' Serum Bone-Specific Alkaline Phosphatase (BsALP) and 3 weeks' callus index.

Discussion

The current study reported an attrition rate of 6.8%. These four out of the fifty-nine patients opted for ORIF but did not keep their follow-up appointments at 3 weeks and 6 weeks. Radabaugh *et al*.[14] also found that the type of surgical repair of fractures has the greatest influence on patients' post-operative follow-up. The immediate restoration of jaw functions following ORIF could have accounted for the post-operative clinic default.

The age range of the patients was 18 to 45 years, with a mean age of 29.3±8.4 years. The patients in the ORIF group were older than those in the MMF group. This is because older patients are financially capable of procuring materials for the ORIF and payment for the expenses incurred in the operating room at will, while the younger patients depend on parents/guardians to offset the treatment bill, especially in a country like Nigeria which operates an out of pocket payment system.[15]

This study contributes to the body of knowledge on the use of BsALPin mandibular fracture healing.[8] The mean BsALP of patients at presentation was 26.2±9.5 ng/mL, ranging from 6.7–47.5 ng/mL. This is higher than Bhati *et al*.[16] who reported a lower mean value (16.56 µg/L = 16.56 ng/mL) in patients with closed diaphyseal fractures of long bones. The reason for the variation is unclear, but could be due to differences in the bone density of the mandible compared to the femur or in the ethnicity of the study participants.

The initial increase from the baseline value observed in this study is similar to the findings of Ushtan et al.[8], who reported a significantly higher activity of BsALP in patients with uncomplicated mandibular fractures when compared to the healthy population. Bhati *et al*.[16,] in another study on fracture healing of long diaphyseal bones, also reported significantly higher activity of BsALP. Although the mean values varied, the BsALP levels were raised during the healing of long bone fractures. A comparison was made between the patients who had single-site and double sites fractures. The difference in the mean levels of BsALP between the two groups was not statistically significant. This may suggest that the rise in the level of BsALP is independent of the number of fracture sites in mandibular fractures. We are not aware of any study with this finding.

The healing phase of bone is characterized by increased osteoblastic activities with resultant increased production of bone-specific alkaline phosphatase.[17] The present study showed an increase in the serum level of bone-specific alkaline phosphatase between presentation and 3 weeks after treatment, and a decline between 3 weeks and 6 weeks after treatment. The steady increase between presentation and 3 weeks could be as a result of increased osteoblastic activity during the formation of osteoids. The decline between three weeks and six weeks after treatment could be due to reduced osteoblastic activity after an abundant amount of osteoid had been laid down to allow for mineralization of the already laid down bone matrix.[18] These initial increase and later decline in the levels of BsALP in mandibular fractures is corroborated by the outcome of 24 weeks' follow-up studies on patients with tibial bone fractures.[13,16] Komnenou *et al*.[19] in their study on animals, and Ajai *et al*.[18], in a study on humans, found significantly higher levels of BsALP in cases of a normal union of long bones compared to the delayed-union group. However, the serum level of bone-specific alkaline phosphatase remained within the reference limit throughout the entire post-operative period for those with non-union. Laurer *et al*.[20] and Bowles *et al*.[21] independently showed an initial decrease in the serum level of bone-specific alkaline phosphatase within 24 hours of injury. This finding is in contrast with the present study. The reasons given for the initial decline in the serum level of bone-specific alkaline phosphatase include; the presence of type III collagen, which is non-osteoblastic collagen seen in the early post-fracture period,[22] acute phase of tissue response to trauma,[23] bone response to trauma and suppression of bone formation at the time of reorganization of the fracture site. Bowles *et al*.[21] concluded that the initial decline observed in the early post-injury period should be investigated further to ascertain whether these are specific responses to fracture or generalized responses of the body to tissue injury.

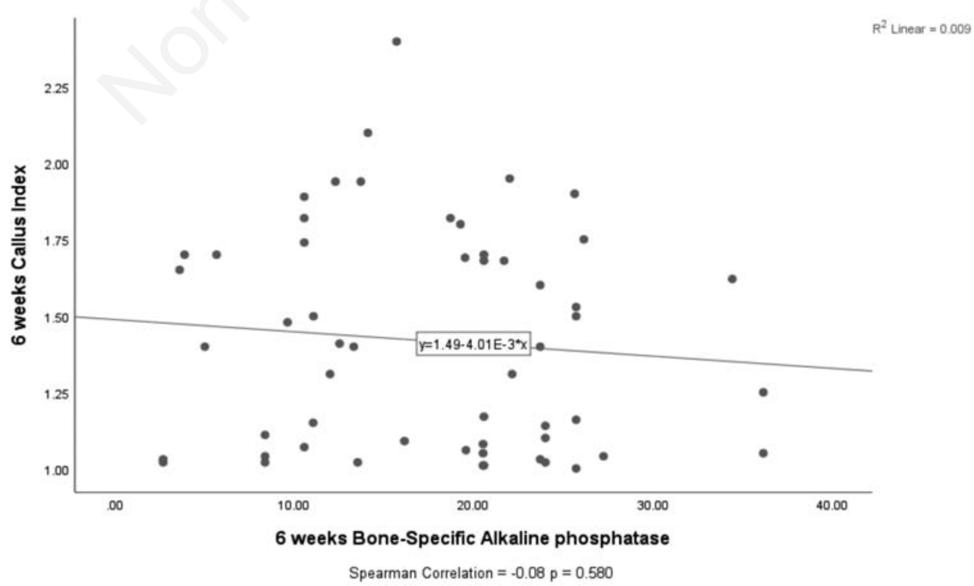

Figure 5. Scatter plot showing negative relationship between Serum Bone-Specific Alkaline Phosphatase (BsALP) and callus index at 6 weeks.

This study showed a significant difference between the levels of BsALP at presentation and 6 weeks after treatment and also between 3 weeks and 6 weeks BsALP like other studies.[16,18,21] Though the study showed a significant difference in the volume of BsALP at different healing times, the non-significant association between BsALP and callus index at six weeks implies that changes in BsALP do not translate to changes observed in the amount of callus formed on the radiograph during the healing of mandibular fractures. Similarly, Muljacic *et al*.[23] and Bhati *et al*.[16] found no relationship between the serum levels of bone-specific alkaline phosphatase and fracture healing outcomes in closed diaphyseal fractures. The absence of association between BsALP and callus formation, as also observed in a similar study[16] could be due to other possible factors that can affect the quantity of callus formed as revealed by the radiographic image such as the patients' age, method of treatment and types of bone involved in the fracture. The younger participants were observed to have more callus formed which could be adduced to the higher osteogenic potential that typifies these age groups.

The conservative method of treatment allows intervening callus formation between fractured ends. Patients treated with closed reduction by MMF had a higher mean level of BsALP compared to the ORIF group at 3 weeks and 6 weeks after treatment. Bhati *et al*.[16] and Muljacic *et al*.[23] also reported that patients treated by conservative methods had a higher serum level of BsALP after treatment compared to those who were treated with osteosynthesis. Serum levels of bone-specific alkaline phosphatase depend on the stability of the bone fragments during the healing phase of the fractured bone and the amount of granulation tissue formed between the fractured segments.[24] The difficulty in achieving a close approximation of the fracture ends in the MMF group results in more formation of granulation tissues, which is later replaced by callus as opposed to ORIF, where the fracture ends are brought together without intervening or minimal granulation tissue. Therefore, this results in minimal or no callus formation between the fractured ends. However, the mean serum levels of bone-specific alkaline phosphatase between the two treatment groups (MMF and ORIF) were not statistically significant. This could be due to the fact that the supposed rigid fixation of ORIF, which should have resulted in minimal or no callus formation between the fractured ends, was deficient in some areas and possibly permitted some callus formation.[24] The cambium layer of the periosteum around the fractured bone has a high osteogenic potential which can give rise to a limited quantity of callus formation compared to that formed in patients treated with MMF in whom both endosteal and periosteal osteogenic cells produce callus bone.[25]

Conclusions

The level of BsALP in the healing of mandibular fractures varies at 3 weeks and 6 weeks. It is directly proportional to the quantity of callus formed at the fracture site at three weeks and inversely proportional to six weeks. Hence, bone-specific alkaline phosphatase is an indicator of the amount of callus formed in patients treated for mandibular fractures.

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