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# Monitoring of bone fractured healing using biochemical markers among patients attending federal teaching hospital abakaliki and bone setter home in onueke ebonyi state, Nigeria

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ABSTRACT

**Background/Aim of study:** The monitoring of bone fractured healing using Alkaline phosphatase, calcium ion and inorganic phosphate was evaluated among patients with fractured bone in two different centers, Alex Ekwueme Federal University Teaching Hospital Abakaliki and Bone Setters Home, Onueke, Ezza in Ebonyi State between August 2017 and September 2018.

**Materials and Methods**: A total of 90 adults patients from 18 years to 78 years were examined using phenolphthalein monophosphate colorimetric end point method. Out of the 90 patients, 30 were healthy normal subjects, another 30 were patients in AE-FUTHA while the remaining 30 patients were in bone setter home.

**Results**: Patients without bone fracture had the least mean serum level of alkaline phosphatase, 28.5 ± 9.0$\mu$l followed by those admitted in bone setter home with a mean serum level of 38.2±17.9$\mu$l while patients admitted in AE-FUTHA had the highest mean serum level of 41.4±6.5$\mu$l (P<0.05). The mean serum level of calcium was significantly higher 10.9± 2.6mg/dl in healthy normal patients compared to mean serum level of 9.2 ± 3.3mg/dl and 7.4 ± 1.3mg/dl for patients admitted in AE-FUTHA and bone setter home respectively. The mean serum level of inorganic phosphate indicate that patients admitted in bone setter home had the highest mean of 4.1 ± 1.0mg/dl followed by patients admitted in AE-FUTHA 3.4 ± 0.2mg/dl while that of healthy normal individuals had the least mean serum level of 3.2 ± 0.5mg/dl.

**Conclusion**: Out of the three parameters examined, alkaline phosphatase test was more precise, reliable and patient doctor friendly; hence it can be used as a veritable tool to monitor the process of bone fracture healing effectively.



## 1. Introduction

The use of motor cycle popularly known as Okada, Tricycle popularly known as Keke and motor as a means of transport

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in the cities has increased in recent time in Nigeria and have since been implicated among the common causes of road accidents that could lead to bone fracture and this may be due to over speeding, high frequency of alcohol intake, smoking of tobacco, carrying greater than required number of passengers and getting angry easily.[1] Some other common causes of bone fracture include those who sleep and fall into gutter, as well as those who wanted to jump over the gutter on their walkway and mistakenly jump into the gutter and have bone fracture. The negative implication is the increase in the number of accidents among the operators and their passengers with many resulting in fracture and at times death. Some of these cases of fracture end up in local bone setter homes, while very few attend clinics for proper medical attention. In both cases, x-ray forms the only means of evaluating healing and monitoring of treatment; especially in hospitals. This is not patient friendly because of the danger of radioactive substances.[2] Despite being expensive, it cannot picture the minute collagen formation involved in fracture healing.[3] Normal fracture healing depends on the formation of granulation tissue between the fracture fragments, a sufficient callus synthesis, and the subsequent remodeling of the newly formed callus.[4] These complex mechanisms require a highly synchronized interplay between osteoblasts, osteoclasts, granulocytes and other cell types.[5–8] Plain radiography is not adapted to mirror this process adequately because radiological signs of fracture healing appear with delay and do not correlate with biochemical and histomorphologic data.[9] However, numerous efforts have been made to develop new and more conclusive tools for the monitoring of fracture healing. The methods include computed tomography (CT) and doppler sonography have been shown to provide additional information.[10] These methods are not suitable for use in daily routine, because of high cost and radiological effects. This has necessitated the development of bone markers that will be of help in the monitoring of fracture healing. Such markers should be patients' friendly, less expensive and adaptable for routine use.[11] Biochemical markers of fracture healing have been proposed to fulfill this need. This study therefore investigated the use of serum levels of Alkaline phosphatase, calcium ion and inorganic phosphate determination in serum of patients with bone fracture as biochemical markers in monitoring of bone fracture healing.

## 2. Materials and Methods

### *2.1. Study design*

This was a quasi experimented design such that three groups were compared but the participants were not randomly assigned to the group, because there were no interventions or treatment so as to put them into control groups and interventions. (It is quasi because it lacks control or randomization or both of them). A total of 60 osteoporotic patients with femoral neck or trochanter fracture whose serum concentrations of total ALP were examined at least four times at six periodic examination points (1,2,3,.4,6, and 8 weeks after surgery) and whose state of bone union were obtained within 24 weeks after surgery were selected for this study. The characteristic longitudinal changes of total ALP during the healing process were shown, and the possibility of total ALP as a predictive factor for the state of osteosynthesis of hip fracture.

### *2.2. Area of the Study*

The area of the study was the Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AE-FUTHA). The study was conducted in AE-FUTHA II because that is where the orthopaedic clinic is located and at Onueke in Ezza South Local Government Area where the bone setter home is located.

### *2.3. Population of the Study/ sample size*

The population for this study comprised an average of 30 patients who visited the clinic weekly. Since the research work covered a period of one month a total of 30 patients who were certified cases of orthopaedic (upper and lower limbs) were used for the study. An age and sex matched 30 certified cases of orthopaedic in bone setter homes at Onueke were used for the comparative study respectively. This gives a total of 90 sample size for the study.

### *2.4. Collection of samples*

About 5ml of venous blood sample was collected aseptically by venipuncture from each subject via the antecubital vein using a plastic syringe with minimum stasis into a plain container. The blood sample was allowed to clot and retracted; it was then centrifuged at 1000 rpm for 5 minutes and the serum separated and used for analysis of biochemical parameters under study (ALP, Calcium and inorganic phosphate) using the standard laboratory method. Samples that were not analyzed immediately were stored frozen at minus 20 degree centigrade until analyzed.

### *2.5. Ethical clearance*

Ethical clearance was obtained from the Ethical and Research Committee of Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Ebonyi State (FETHA/REC/VOL. 1/2016/451).Also informed consent were sought and obtained from the participants prior to the commencement of the study.

### *2.6. Laboratory methods*

Evaluation of alkaline phosphatase wasbased on the phenolphthalein monophosphate colorimetric end point method,[12] calcium ion determination was by

0–cresolphthalein complex one without deproteinization in an alkaline medium while evaluation of inorganic phosphate weredone according to the phosphomolybdate/ultra violet method.[13]

### 2.7. Statistical analysis

The data obtained was analyzed using SPSS statistics tool version 23.0 software to compare the changes in levels of the parameters studied using t-test. $P < 0.05$ was taken as significant.

## 3. Results

Fifteen male and fifteen female of healthy normal subjects, those admitted in orthopedic section of AE-FUTHA, and those admitted in bone setter home were analyzed for the serum level of ALP, $Ca^{2+}$ and inorganic phosphate. The mean serum level of ALP in healthy normal subject was 28.5±9.0$\mu$l, while those admitted in orthopedic section of AE-FUTHA was 41.4±6.5$\mu$l and those admitted in bone setter home was 38.2±17.9$\mu$l. The mean serum level of ALP in healthy normal subjects is significantly lower than that of the patients with bone fracture admitted in both locations (P<0.05). The mean serum level of ALP in healthy normal patients and patients with bone fracture is shown in Table 1. The mean serum level of $Ca^{2+}$ in healthy normal subject was 10.9±2.6mg/dl, while those admitted in orthopedic section of AE-FUTHA was 9.2±3.3mg/dl and those admitted in bone setter home was 7.4±1.3mg.dl. The mean serum level of $Ca^{2+}$ in healthy normal subjects was significantly higher than that of the patients with bone fracture admitted in both locations (P<0.05). Meanwhile, the mean serum level of $Ca^{2+}$ in patients admitted in orthopedic section of AE-FUTHA was significantly higher than that of the patients admitted in bone setter home. The mean serum level of $Ca^{2+}$ in healthy normal patients and patients with bone fracture is shown in Table 1. The mean serum level of inorganic phosphate in healthy normal subject was 3.2±0.5mg/dl, while those admitted in orthopedic section of AE-FUTHA was 3.4±0.2mg/dl and those admitted in bone setter home was 4.1±1.0mg.dl. The mean serum level of inorganic phosphate in healthy normal subject and that of the patients admitted in orthopedic section of AE-FUTHA was significantly lower than that of the patients admitted in bone setter home (P<0.05). The mean serum level of inorganic phosphate in healthy normal patients and patients with bone fracture is shown above.

The mean serum level of ALP in healthy normal male subjects was significantly higher than that of the female subjects (P<0.05)(0.007). The mean serum level of $Ca^{2+}$ in healthy normal male subject was significantly higher than that of the female subjects (P<0.05)(0.012). The mean serum level of inorganic phosphate in healthy normal male subject was also significantly higher than that of the female subjects (P<0.05). See Table 2.

The mean serum level of ALP, $Ca^{2+}$, and inorganic phosphate (first date of analysis) in male patients admitted in the orthopedic section of AE-FUTHA was significantly higher than that of the female patients (P<0.05). However, the mean serum level of ALP (second date of analysis) in male patients was not significantly difference from that of the female patients (P>0.05). Yet, the mean serum level of $Ca^{2+}$and inorganic phosphate (second date of analysis) in male patients admitted in the orthopedic section of AE-FUTHA was significantly higher than that of the female patients (P<0.05). See Table 3.

The mean serum level of ALP and $Ca^{2+}$ (first date of analysis) in male patients admitted in the bone setter home was significantly higher than that of the female patients (P<0.05). However, the mean serum of inorganic phosphate (first date of analysis) in male patients admitted in the bone setter home was significantly lower than that of the female patients (P<0.05) = (0.000). The mean serum of ALP (second date of analysis) in male patients admitted in the bone setter home was significantly lower than that of the female patients (P<0.05) = (0.000); while the mean serum level of $Ca^{2+}$and inorganic phosphate (second date of analysis) in male patients was significantly higher than that of the female patients (P<0.05) = (0.006). See Table 4.

## 4. Discussion

In the estimation of Alkaline phosphatase, the mean serum level of ALP of 28.5i$\mu$/l (Table 1) healthy normal subjects was within the generally accepted normal range of (9-35)i$\mu$/l,[14] which was significantly lower than that of the patients admitted in AE-FUTHA (41.4i$\mu$/l) as well as those in bone setter home at Onueke (38.2i$\mu$/l). The study showed that there was an increase in mean serum level of ALP in patients admitted in AE-FUTHA and patients in the bone setter home. This is in line with the findings of Singh et al.[15] and Sousa et al.[16] In their respective research works, they found out that in normal healings groups, the mean values of serum ALP were higher than that of impaired groups. The increase in mean serum ALP which was more in significant in patients admitted in AE-FUTHA than those in the bone setter home, can be as a result of delay between injury and admission.

In the estimation of serum calcium ions, the mean serum of calcium ions in healthy normal subjects was 10.9mg/dl while those admitted in AE-FUTHA and in the bone setter home gave a serum level of 9.2mg/dl and 7.4mg/dl respectively (Table 2). The results obtained showed a higher serum level of calcium ions (10.9mg/dl) in normal subjects than in patients admitted in AE-FUTHA (9.2mg/dl) and in bone setter home. This indicates that there was a constant decrease in the serum level of calcium ions as the healing of the fracture progresses. This is in line with the findings

**Table 1:** Shows the levels of ALP, $Ca^{2+}$ and inorganic phosphate in healthy normal subjects and patients with bone fracture

| Parameter | Healthy Normal (n=30) | Patients admitted in AE-FUTHA (n=30) | Patients admitted in bone setter home (n=30) | P-value |
|---|---|---|---|---|
| ALP ($\mu$l) | 28.5±9.0 | 41.4±6.5 | 38.2±17.9$^b$ | 0.000* |
| $Ca^{2+}$ (mg/dl) | 10.9±2.6 | 9.2±3.3 | 7.4±1.3$^c$ | 0.000* |
| Inorganic phosphate (mg/dl) | 3.2±0.5 | 3.4±0.2 | 4.1±1.0$^b$ | 0.000* |

*Statistically significant at p<0.05.

**Table 2:** Gender comparison of serum level of ALP, $Ca^{2+}$ and inorganic phosphate in healthy normal subjects

| Parameter | Male (18-78yrs) (n=30) | Female(18-78yrs) (n=30) | P-value |
|---|---|---|---|
| ALP ($\mu$l) | 32.3±2.8 | 24.8±11.4 | 0.007* |
| $Ca^{2+}$ (mg/dl) | 11.9±3.4 | 9.8±0.8 | 0.012* |
| Inorganic phosphate (mg/dl) | 3.7±0.2 | 2.7±0.2 | 0.000* |

*Statistically significant at p<0.05.

**Table 3:** Gender comparison of serum level of ALP, $Ca^{2+}$ and inorganic phosphate in patients admitted in orthopedic section of AE-FUTHA

| Parameter | Male (20-68 yrs) (n=30) | Female(20-68 yrs) (n=30) | P-value |
|---|---|---|---|
| Pre-ALP ($\mu$l) | 44.4±7.5 | 38.4±3.3 | 0.002* |
| Pre-$Ca^{2+}$ (mg/dl) | 12.1±0.8 | 6.3±2.0 | 0.000* |
| Pre-Inorganic phosphate (mg/dl) | 3.5±0.1 | 3.3±0.2 | 0.002* |
| Post-ALP ($\mu$l) | 28.7±3.8 | 28.4±2.7 | 0.776 |
| Post-$Ca^{2+}$ (mg/dl) | 7.0±0.7 | 6.7±1.4 | 0.511 |
| Post-Inorganic phosphate (mg/dl) | 4.1±0.5 | 3.5±0.6 | 0.002* |

*Statistically significant at p<0.05.

**Table 4:** Gender comparison of serum level of ALP, $Ca^{2+}$ and inorganic phosphate in patients admitted in bone setter home

| Parameter | Male (18-78yrs) (n=30) | Female(18-78) (n=30) | P-value |
|---|---|---|---|
| Pre-ALP ($\mu$l) | 44.7±17.4 | 27.6±11.0 | 0.000* |
| Pre-$Ca^{2+}$ (mg/dl) | 7.8±0.8 | 7.0±1.5 | 0.037* |
| Pre-Inorganic phosphate (mg/dl) | 3.3±0.4 | 5.0±0.5 | 0.000* |
| Post-ALP ($\mu$l) | 29.6±6.0 | 52.6±21.0 | 0.000* |
| Post-$Ca^{2+}$ (mg/dl) | 7.1±0.7 | 5.7±0.3 | 0.000* |
| Post-Inorganic phosphate (mg/dl) | 3.0±0.3 | 2.7±0.4 | 0.006* |

*Statistically significant at p<0.05.

of Gulab et al. who observed a low level of calcium ions in patients with bone fracture in India.[17]

in the analysis of Inorganic phosphate, the mean serum level of inorganic phosphate in healthy normal subject was 3.2mg/dl while those admitted in orthopedic section of AE-FUTHA was 3.4mg/dl and those admitted in bone setter home was 4.1mg/dl. The analysis, the mean serum level of inorganic phosphate in healthy normal subject and that of the patients admitted in Orthopedic section of AE-FUTHA was significantly lower than that of the patients admitted in bone setter home. The findings in the present study are in agreement with earlier studies. Tisdal and Harris pointed out that the plasma phosphorus decreased in man after the cessation of growth, but that after fracture, the value increased for some weeks and approached the level of a growing individual.[18] Eddy and Heft, also found increased phosphorus levels after surgery other than bone surgery.[19] In the same vein, Speed, described blood phosphorus as constant findings in fracture patients.[20]

So out of the three parameters examined, alkaline phosphatase test was more precise, reliable and patient doctor friendly, hence it can be used as a veritable tool to monitor the process of bone fracture healing effectively.

## 5. Conclusion

Estimation of Alkaline phosphatase, calcium ions and inorganic phosphate appear to be a useful screening

procedure for assessment of the progress of fracture healing. In the present study, it was observed that, out of the three (3) parameters estimated, alkaline phosphatase activity was found to be considerably potential because it correlated well with the process of fracture healing. The results obtained from patients of the same age brackets and sexes admitted in Alex Ekwueme Federal University Teaching Hospital, and Bone Setter Homes Onueke showed a significant increase in serum ALP level more than other parameters analyzed specially in adult male and female patients, because calcium ion and Inorganic phosphate ion are reduced in female patients and in the youths as a result of loss of calcium and Inorganic ions in female patients during menstrual period and youths who indulge in smoking hard drugs and excessive alcohol intake respectively. Hence, the study suggests that it can be used as a veritable screening test in monitoring the progress of fracture healing in patients with fractured bone. In addition, alkaline phosphatase test was precise, reliable, reproducible, patient-doctor friendly and cost effective. Therefore, it can serve as accurate method to measure fracture healing objectively. Because there is increase in serum ALP level more than other parameters analyzed irrespective of age and sex.

## 6. Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

## 7. Source of Funding

None.

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