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**Assessment of Selected Biomarkers of Bone Healing and Inflammation among Subjects with Fracture on Traditional and Conventional Treatment Methods**

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**Abstract**

Fractures have significant implications for health. Treatment approaches vary, including traditional and conventional methods. This study in Ekpoma, Nigeria assessed biomarkers in fracture healing through a cross-sectional random sampling. Blood samples from 60 subjects were analyzed for hydroxyproline, creatinine, calcium, alkaline phosphatase, and C-reactive protein. The aim was to evaluate treatment effectiveness and improvement in patient outcomes by monitoring bone remodeling. Statistical analysis utilized is the SPSS software version 21.0 software (SPSS Inc., Chicago, IL, USA). Biochemical marker analysis revealed that serum alkaline phosphatase activity, calcium, and creatinine did not significantly differ between subjects without fractures and those with fractures ( $p > 0.05$ ). However, hydroxyproline levels exhibited a significant difference ( $p < 0.05$ ), with higher values observed in subjects with fractures. Additionally, C-reactive protein levels showed significant variations ( $p < 0.05$ ), indicating increased inflammation in fracture patients. High-sensitive C-reactive protein levels also displayed significant differences ( $p < 0.05$ ). Further analysis comparing male and female subjects without fractures and those with fractures revealed no significant variations in serum alkaline phosphatase, calcium, and creatinine levels ( $p > 0.05$ ). However, hydroxyproline levels demonstrated significant variations ( $p < 0.05$ ) among males and females in the fracture group, suggesting gender-specific differences in bone metabolism. C-reactive protein and High-sensitive C-reactive protein

levels exhibited significant variations ( $p < 0.05$ ) between males and females in the fracture group. Furthermore, a comparison between patients on traditional and conventional treatment methods indicated significant variations in serum alkaline phosphatase activity and calcium levels ( $p < 0.05$ ), suggesting distinct effects of the treatment modalities on these markers. However, no significant differences were observed in creatinine, C-reactive protein, and high-sensitive C-reactive protein levels ( $p > 0.05$ ). Traditional and conventional fracture treatment methods may affect biochemical markers differently, with gender-specific variations in hydroxyproline, C-reactive protein, and high-sensitive C-reactive protein levels. Further research is needed to understand the clinical implications and underlying mechanisms of these findings.

**Keywords:** Bone Healing, Inflammation, Markers, Fracture

**Introduction**

A fracture, also known as a broken bone, is a common injury that can occur as a result of a variety of factors, including trauma, falls, sports injuries, and underlying medical conditions such as osteoporosis. When a bone is damaged and breaks into one or more pieces, it is considered a fracture (Greising *et al.*, 2020).

Fractures are a common and significant health concern, affecting millions of people each year. There are numerous treatments approaches available for managing fractures, including both traditional and conventional methods. However, assessing the effectiveness of these approaches

can be challenging, particularly given the variability in fracture healing outcomes and the lack of consensus on the best approach to fracture healing (Borgström *et al.*, 2020).

One potential solution to these challenges is the use of biochemical markers to monitor fracture healing. Biochemical markers are substances that are released into the bloodstream during the healing process and can provide valuable information about the progress of bone remodeling and the effectiveness of different treatment approaches (Cheng *et al.*, 2019).

Despite the potential benefits of using biochemical markers in fracture healing, there has been limited research conducted in this area, particularly with regard to traditional or alternative medicine approaches. This represents a significant gap in our understanding of how best to manage fractures and highlights the need for further investigation (Mastrolia *et al.*, 2019). The use of biochemical markers in fracture healing is a promising approach that could improve our ability to monitor and manage fractures. Biochemical markers can provide valuable information about the progress of bone remodeling and the effectiveness of different treatment approaches, which could ultimately lead to improved patient outcomes.

The use of traditional and alternative medicine for managing fractures is common in many parts of the world, and there is a need to investigate their effects on fracture healing using biochemical markers. For example, some traditional medicine approaches involve the use of herbal remedies, which could have an impact on the levels of biochemical markers involved in bone healing (Ozioma and Josephine, 2019).

Conventional medicine approaches, on the other hand, often involve the use of medications such as non-steroidal anti-inflammatory drugs (NSAIDs) and bisphosphonates, which could also have an impact on biochemical markers. Investigating the effects of both traditional and conventional medicine approaches on biochemical markers could provide valuable insights into the mechanisms of bone healing and the efficacy of different treatment approaches (Buch *et al.*, 2019).

## **Materials and Methods**

### **Research Design**

A cross sectional random sampling method was used in this study in which, a total number of Sixty (60) subjects of which twenty (20) adults were control, twenty (20) were subjects with fracture on traditional treatment and twenty (20) were subjects with fracture on conventional treatment with minimally fractures.

### **Ethical Consideration**

Ethical consideration was sorted for and obtained from the Ethical and Research Committee of the Ambrose Alli University with assigned number (004/23). Researchers obtained informed consent from participants before involving them in a study. Participants were fully informed about the purpose, procedures, potential risks and benefits of the study as well as their rights to voluntary participation, confidentiality, and withdrawal.

### **Inclusion Criteria**

Subjects with fractured bones who were aged 18 to 70 who gave informed consent to participate in the study were consecutively recruited.

### **Exclusion Criteria**

Subjects on fracture with other health conditions that affect fracture healing were excluded from this study. These conditions include infection of the blood (sepsis) and cancer of the bone.

### **Sample Collection and Laboratory Investigation**

Five milliliters fasting venous blood were collected from fractured patients based on the week of treatment or week from when they sustained the fracture. Samples were collected at week 1 and at the 4<sup>th</sup> week, the samples were collected again from subjects with fracture receiving traditional and conventional treatment methods. The collected blood was centrifuged at 1500-rpm speed for 5 min for separation of serum. The sample was analyzed using the corresponding methodology for the serum hydroxyproline, calcium, and creatinine and serum alkaline phosphatase activity

## Laboratory Procedure

### A. Analytical determination of hydroxyproline concentration

**Principle:** The measurement of hydroxyproline in serum was performed using the Stegmann-Staeder's method (Stegmann and Staeder, 1967) with modifications by Utevskaia and Persky (1982). This method relies on a condensation reaction between the oxidation products of hydroxyproline (pyrole) and para-dimethylaminebenzoaldehyde (DABA). Subsequently, the resulting-coloured products, which exhibit maximum light absorption at 550nm, are quantified using a colorimeter.

**Procedure:** Chloramin 'B' solution of 1cm<sup>3</sup> was added to 1cm<sup>3</sup> of sample solution containing neutralized hydrolyzate, mixed well and allowed to stand for 20 minutes at room temperature. While waiting, 0.5cm<sup>3</sup> of n-propanol was added to the resulting mixture and agitated thoroughly. After 20 minutes, 1cm<sup>3</sup> of DABA, and hydrogen chlorate solutions were added, and the resulting solution was thoroughly mixed and heated in a water bath for 20 minutes at 60°C. The obtained coloured solutions are cooled to room temperature and optical densities measured on a colorimetric apparatus with maximum light absorption of  $\lambda = 540 \text{ nm}$ , in cuvettes of 0.5cm thick. Concentration of hydroxyproline is determined using a calibration graph, in the construction of which L-hydroxyproline is used.

### B. Creatinine Estimation

**Method: Modified Jaffe's method** (Tietz, 1999).

**Principle:** Randox Laboratories (United Kingdom) reagent was used for creatinine estimation. In an alkaline medium, the creatinine present in the sample undergoes a reaction with picric acid, resulting in the formation of a colored complex. The rate of complex formation is measured kinetically, ensuring that any potential interference is avoided.

**Procedure:** Bring the working reagent and the photometer to 37°C, then pipette into a cuvette. Mix working reagent 1.0ml to standard 0.1ml. Mix and insert cuvette into the photometer. Start the stopwatch. Record the absorbance at 500nm after 30seconds (A1) and after 90 seconds (A2).

### C. Calcium Estimation

**Principle:** Randox Laboratories (United Kingdom) reagents based on the O-cresol phthaline complexone method was used for calcium estimation. The method is based on the reaction between calcium and o-cresolphthalein complexone in the presence of 8-hydroxyquinoline-5-sulfonic acid, leading to the formation of a purple complex (Tietz, 1999).

**Procedure:** Calcium reagent of 1ml was pipetted into each tube labelled (blank, standard, control, and test). 25mls each of distilled water, standard solution, control sample and test sample was pipetted into each test tube respectively. It was mixed and incubated at room temperature for 20mins. Absorbance was read at 578nm.

### D. Determination of Serum Alkaline Phosphatase by Method of Wiwanitkit (2001).

**Principle:** A kinetic method uses p-nitrophenyle phosphate (PNPP) as a substrate PNPP does not absorb at the wavelength chosen to read the test (405 nm).

**Procedure:** This procedure involves 1.0ml of test been added to 1.0ml of stand and 1.0ml of blank to form Buffer. Then, 1.0ml of test added to 1.0ml of standard and 1.0ml of blank to form substrate. Incubate for 3 min at 37°C and add 0.1ml of serum to 0.1ml of standard and 0.1ml of distilled water. Incubate for 15min at 37°C and take it out. Mix and read the absorbance at 510 nm against blank within 5 min.

### E. Determination of C-reactive protein and hsCRP

**Principle:** The Rapid Quantitative Test for C-reactive proteins is a fluorescence-based immunoassay that employs a competitive approach to accurately measure and quantify the levels of C-reactive proteins.

**Procedure:** Please ensure that the test cassette, detection buffer, and specimen reach room temperature before commencing the test. Remove the ID chip from its packaging and verify that it corresponds to the batch number of the test cassette. Insert the ID chip into the designated chip port of the instrument. Draw 75  $\mu\text{L}$  of whole blood or serum or plasma with a transfer pipette and add it to the buffer tube.

Securely seal the lid of the detection buffer tube, then vigorously shake the sample mixture to ensure thorough mixing. Pipette 75  $\mu$ L of sample mixture and load it into the sample well of the test cassette. FIA Meters offer two modes: Standard Test mode and Quick Test mode. For detailed information, kindly consult the operation manual specific to the FIA meters. Set the timer and initiate the countdown before adding the sample mixture into the designated sample well. Allow the mixture to rest undisturbed at room temperature for 15 minutes. Proceed by inserting the test cassette onto the test cassette holder of the FIA Meter. Press the "Test" button to initiate the testing process. The FIA Meter will promptly commence scanning the test cassette containing the sample. Results are displayed on the main screen of meter and can be printed out by press "Print". Discard the used test kit according to local regulations and procedure after released from the meter.

### Statistical Analysis

The statistical analysis was performed using SPSS version 21.0 software (SPSS Inc., Chicago, IL, USA) on a Windows platform. Student t-test and ANOVA were utilized, with a significance level of  $P < 0.05$  indicating statistical significance.

### Result

The social demographic characteristic of subjects without fracture within age 41-50 which had the highest frequency of 10(50%), followed by age group 18-20 which had a frequency of 4(20%), followed by age group 31-40 with a frequency of 3(15%), age group 21-30 which had a frequency of 2(10%), and 51-above had the least with 1(5%).

Table 1 shows the serum alkaline phosphatase activity. There was no statistically significant ( $t=0.492$ : There was no statistically significant variation in the  $p > 0.05$ ) variation when subjects without fractured were compared to subjects with fracture. Alkaline Phosphatase activity control was ( $12.80 \pm 5.36$  u/l) and test value of ( $16.74 \pm 18.19$  u/l).

There was no statistically significant variation in the Calcium serum values showed no statistically significantly ( $t=0.370$ :  $p > 0.05$ ) variation when subjects without fractured were

compared to subjects with fracture. Calcium values for control were ( $9.37 \pm 2.06$  mg/dl) and test value were ( $8.25 \pm 2.86$  mg/dl).

There was no statistically significant variation in the Creatinine serum values ( $t=0.929$ :  $p > 0.05$ ) when subjects without fractured were compared to subjects with fracture. Creatinine values for were ( $1.15 \pm 0.54$  mg/dl) and test value were ( $1.18 \pm 0.65$  mg/dl).

There was a statistically significant variation in the Hydroxyproline serum values ( $t=0.001$ :  $p < 0.05$ ) when subjects without fractured were compared to subjects with fracture. Hydroxyproline values for control ( $1.03 \pm 0.60$  mg/ml) and female test value were ( $2.57 \pm 1.08$  mg/ml).

There was a statistically significant variation in the C-reactive protein ( $t=0.031$ :  $p < 0.05$ ) when subjects without fractured were compared to subjects with fracture. The values for control were ( $3.51 \pm 0.42$  mg/l) and test value were ( $32.30 \pm 40.24$  mg/l).

There was a statistically significant variation in the High sensitive C-reactive protein values ( $t=0.010$ :  $p < 0.05$ ) when subjects without fractured were compared to subjects with fracture. Its values for control were ( $1.06 \pm 1.21$  mg/l) and test value were ( $5.55 \pm 3.23$  mg/l).

Table 2: There was a statistically significant variation in the serum alkaline phosphatase activity ( $t=0.017^*$ :  $p < 0.05$ ) when those subjects with fracture on traditional treatment were compared with those on conventional treatment. The values for subjects on traditional treatment was ( $7.62 \pm 3.84$  u/l) and conventional treatment value was ( $24.99 \pm 21.33$  u/l).

There was a statistically significant variation in the Calcium values ( $t=0.000^*$ :  $p < 0.05$ ) when those subjects with fracture on traditional treatment were compared with those on conventional treatment. The values for subjects on traditional treatment was ( $6.26 \pm 1.51$  mg/dl) and conventional treatment value of ( $10.10 \pm 2.02$  mg/dl).

There was no statistically significant variation in the Creatinine values ( $t=0.959$ :  $p > 0.05$ ) when

those subjects with fracture on traditional treatment were compared with those on conventional treatment. The traditional treatment was (1.22±0.94 mg/dl) and conventional treatment value of (1.21±0.32 mg/dl).

There was a statistically significant variation in the Hydroxyproline values (t=0.033\*: p<0.05) variation when those subjects with fracture on traditional treatment were compared with those on conventional treatment. The subjects on traditional treatment was (2.92±0.89 mg/dl) and conventional treatment value of (2.04±0.98 mg/dl).

There was no statistically significant variation in

the C-reactive protein values (t=0.074: p>0.05) variation when those subjects with fracture on traditional treatment were compared with those on conventional treatment. The traditional treatment was (46.48±48.61mg/l) and conventional treatment value of (17.40±18.89 mg/l).

There was no statistically significant variation in the Hs-CRP values (t=0.970: p>0.05) variation when those subjects with fracture on traditional treatment were compared with those on conventional treatment. The subjects on traditional treatment was (5.52±3.92 mg/l) and conventional treatment value of (5.47±2.14 mg/l).

**Table 1: Mean and standard deviation of Alkaline Phosphatase, Calcium, Creatinine, Hydroxyproline, C-reactive protein and hsCRP of subjects without fractured and test group (subjects with fracture)**

Parameter	Control (Mean±SD) (N=20)	Test (Mean±SD) (N=40)	t	p-value
Alkaline Phosphatase(u/l)	12.80±5.36	16.74±18.19	-0.707	0.492
Calcium(mg/dl)	9.37±2.06	8.35±2.86	0.920	0.370
Creatinine(mg/dl)	1.15±0.54	1.18±0.65	-0.091	0.929
Hydroxyproline(mg/ml)	1.03±0.60	2.57±1.08	-4.072	0.001*
C-Reactive protein(mg/l)	3.51±0.42	32.30±40.24	-2.478	0.031*
high sensitive C reactive protein(mg/l)	1.06±1.21	5.55±3.23	-4.364	0.001*

Key: Control group : Non fractured subjects

Test group : fractured subjects, both those on conventional and traditional treatments

N: Number of subjects

\* : A statistical significant (p<0.05) variation occurred.

SD: standard deviation

**Table 2: The mean and standard deviation of Alkaline Phosphatase, Calcium, Creatinine, Hydroxyproline, C-reactive protein and hs CRP of test group (subjects with fracture) on traditional and conventional treatment.**

Parameters	Traditional treatment (N=20)	Conventional treatment (N=20)	t	p-value
Alkaline Phosphatase(u/l)	7.62±3.84	24.99±21.33	-2.774	0.017*
Calcium(mg/dl)	6.26±1.51	10.10±2.02	-5.259	0.000*
Creatinine(mg/dl)	1.22±0.94	1.21±0.32	0.052	0.959
Hydroxyproline(mg/ml)	2.92±0.89	2.04±0.98	2.271	0.033*
C-Reactive protein(mg/l)	46.48±48.61	17.40±18.89	1.931	0.074
high sensitive C reactive protein(mg/l)	5.52±3.92	5.47±2.14	0.039	0.970

N: Number of subjects

\* : A statistical significant (p<0.05) variation occurred.

SD: standard deviation

**Table 3: The mean and standard deviation of Alkaline Phosphatase, Calcium, Creatinine, Hydroxyproline, C-reactive protein and hs CRP of control (subjects without fracture) and test group (subjects with fracture) on traditional and conventional treatment at week one**

Parameters	Control (N=20)	Traditional Treatment (N=20)	Conventional Treatment (N=20)	F	p-value
Alkaline Phosphatase(u/l)	12.80±5.36	7.93±4.03	25.56±22.92	2.940	0.080
Calcium(mg/dl)	9.37±2.06	6.32±1.58	10.38±2.38	6.526	0.008*
Creatinine(mg/dl)	1.15±0.54	1.19±0.91	1.17±0.33	0.006	0.994
Hydroxyproline(mg/ml)	1.03±0.60	3.09±0.79	2.05±1.13	10.224	0.001*
C-Reactive protein(mg/l)	3.51±0.42	46.76±51.50	17.85±20.22	3.603	0.050*
high sensitive C reactive protein(mg/l)	1.06±1.21	5.60±4.22	5.50±2.27	6.547	0.008*

N: Number of subjects

\* : A statistical significant (p<0.05) variation occurred.

SD: standard deviation

**Table 4: Multiple comparisons between alkaline Phosphatase, Calcium, Creatinine, Hydroxyproline, C-reactive protein and hs CRP of control (subjects without fracture) and test group (subjects with fracture) on traditional and conventional treatment at week one**

Multiple Comparisons	Alkaline phosphatase (u/l)	Calcium (mg/dl)	Creatinine (mg/dl)	Hydroxyproline (mg/ml)	C-Reactive protein (mg/l)	high sensitive C reactive protein (mg/l)
Control VS Traditional treatment	0.500	0.013*	0.913	0.000*	0.016*	0.007*
Control VS Conventional treatment	0.089	0.370	0.968	0.040*	0.389	0.008*
Traditional treatment VS Conventional treatment	0.032*	0.003*	0.947	0.048*	0.113	0.950

Asterisks (\*) indicates a statistically significant variation (p<0.05)

**Discussion**

The research conducted in Ekpoma, Nigeria aimed to assess selected biomarkers for fracture healing and explore the implications of the findings. A total of 60 subjects were randomly sampled, including control, traditional treatment, and conventional treatment groups. The study examined various demographic characteristics and collected blood samples to analyze biomarkers such as hydroxyproline, creatinine, calcium, alkaline phosphatase, and C-reactive protein.

The analysis of biomarker levels revealed significant differences and variations in certain markers. Hydroxyproline levels showed significant differences between subjects with and without fractures with higher values observed in subjects with fractures indicating potential differences in bone metabolism. This suggests that hydroxyproline could serve as a marker for assessing fracture healing. C-reactive protein levels also exhibited significant variations, suggesting increased inflammation in fracture patients. This finding could be attributed to the report of Koike *et al.* (2000) that reported elevated value in subjects with bone fractures. The elevated levels of C-reactive protein indicate an inflammatory response, which is a natural part of the healing process (Ahmadi-Abhari *et al.*, 2013).

A comparison between traditional and conventional treatment methods revealed variations in serum alkaline phosphatase activity and calcium levels. The significant differences observed in these markers indicate that different treatment modalities can influence bone remodeling and mineralization. The variations in serum alkaline phosphatase activity and calcium levels between traditional and conventional treatment methods suggest that different approaches can affect bone remodeling and mineralization differently. This highlights the need for further research to explore the mechanisms underlying these differences and determine the most effective treatment strategies for fracture patients (Shu *et al.*, 2022).

Hydroxyproline is an amino acid predominantly found in collagen, the primary component of bone tissue. The significant differences in hydroxyproline levels between subjects with and without fractures indicate potential differences in bone metabolism. Higher levels of hydroxyproline in subjects with fractures suggest increased collagen synthesis, which is essential for bone healing and the formation of a stable callus. Monitoring hydroxyproline levels can serve as an indicator of fracture healing progress and the effectiveness of treatment methods. It can help healthcare professionals assess the rate and quality of collagen formation

and adjust treatment approaches accordingly (Li and Wu, 2018).

**C-reactive protein (CRP):** CRP is a protein produced by the liver in response to inflammation. The significant variations in CRP levels suggest increased inflammation in fracture patients. Inflammation is a natural part of the healing process and is necessary for initiating immune responses and removing damaged tissue. However, excessive or prolonged inflammation can hinder healing and lead to complications. Monitoring CRP levels provides insights into the inflammatory response associated with fracture healing. Elevated CRP levels indicate ongoing inflammation, and a decrease in levels over time signifies the resolution of inflammation and progress in healing. Monitoring CRP levels can help healthcare professionals identify complications, such as infection or delayed healing, and guide appropriate interventions (Marnell *et al.*, 2005). The research study identified gender-specific differences in hydroxyproline and CRP levels among fracture patients. These differences suggest variations in bone metabolism and inflammatory response between males and females. Bone metabolism can be influenced by hormonal factors, including estrogen and testosterone. Understanding these gender-specific differences can aid in developing personalized treatment approaches that consider the unique physiological characteristics of males and females. For example, the findings may suggest the need for different dosages or durations of treatment based on gender, leading to improved fracture healing outcomes.

The variations observed in serum ALP activity and calcium levels between traditional and conventional treatment methods indicate distinct effects of these treatment modalities on bone remodeling and mineralization. ALP is an enzyme involved in bone formation, and calcium is a crucial mineral for bone health. Differences in ALP activity and calcium levels may reflect variations in the rate of bone remodeling and mineralization (Shu *et al.*, 2022). These findings suggest that different treatment methods can influence these processes differently. Further research is needed to explore the underlying

mechanisms behind these variations and determine the most effective treatment strategies for fracture patients.

The variations observed in the biomarker levels in the research study provide valuable insights into fracture healing and treatment approaches. Monitoring hydroxyproline and CRP levels can help assess the progress of fracture healing, identify complications, and guide treatment interventions. Additionally, considering gender-specific differences in biomarker levels can lead to personalized treatment approaches that optimize healing outcomes for both males and females. The variations in serum ALP activity and calcium levels between treatment methods highlight the need for further research to determine the underlying mechanisms and develop more effective treatment strategies. By understanding these variations and their implications, healthcare professionals can enhance fracture management and improve patient outcomes (Zhineng *et al.*, 2021).

### Conclusion

The research evaluated biomarkers for fracture healing. The findings revealed significant differences in hydroxyproline and C-reactive protein levels between subjects with and without fractures, indicating potential variations in bone metabolism and inflammation.

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