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# Serum Level of Parathyroid Hormone, Alkaline Phosphatase, Calcium and Ionized Calcium After Forearm Fractures

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**Abstract:** Background: During the last decades, our understanding of fracture healing has evolved rapidly. Bone can heal without forming a fibrous scar and, as such, the process of fracture healing recapitulates bone development and may be considered a form of tissue regeneration. The cell and tissue proliferation and differentiation processes involved in fracture healing are regulated by growth factors, inflammatory cytokines, antioxidants, hormones, amino acids, and other nutrients. Objective: To identify changes in serum level of calcium, ionized calcium, alkaline phosphatase and parathyroid hormone according to mode of trauma (low and high energy) in forearm fractures. Patients and Methods: 50 Patients with forearm fracture were prospectively recruited from the emergency department within 48 hours of sustaining the fracture. Patients included in the study were asked to avoid calcium supplementation during fracture healing. Results: Mean PTH was elevated in both groups at Day 1 with low energy group was insignificantly higher than high energy group, whereas after 8 weeks mean PTH level decreased in both groups but in low energy group mean PTH level was significantly higher than high energy group. Mean ALP level increased in both groups at Day 1 whereas in low energy group it was insignificantly higher than that of high energy group, After 8 weeks mean ALP level insignificantly decreased to normal level in both groups. Mean serum calcium level was below normal level in both groups where it was insignificantly higher in high energy group than that of low energy group which increased after 8 weeks in both groups but remained insignificantly elevated in high energy than low energy group. Mean serum ionized calcium level was below normal level at Day 1 in both groups whereas it was insignificantly higher in high energy group than low energy group which after 8 weeks increased in both groups but remained insignificantly elevated in high energy group than low energy group. Conclusion: Serial monitoring of these markers reflect the actual status of bone resorption, and bone formation respectively over a short period. Thus, they can be used as an adjunct to clinical and radiological evidence of fracture healing.

**Keywords:** Alkaline Phosphatase, Parathyroid Hormone, Forearm Fracture, Biological Markers

## 1. Introduction

Fractures of both radius and ulna are one of the most common fractures in adults in upper extremity [1]. It increases in frequency secondary to rapid industrialization; road traffics and competitive sports [2].

Fracture healing is a proliferative and physiological process in which the body facilitates the repair of a bone fracture to restore the tissue to its original physical and mechanical properties [3].

During the last two decades, understanding fracture healing has evolved rapidly. The complex cell and tissue proliferation and differentiation processes involved in fracture healing are regulated by growth factors, inflammatory cytokines, antioxidants, hormones, amino acids, and other nutrients [4].

Parathyroid hormone (PTH) is an 84-amino acid polypeptide produced and secreted by the parathyroid glands [5]. Parathyroid hormone is a major regulator of calcium and phosphate homeostasis. The two major target organs of PTH action are bone and kidney [6].

Calcium is the most abundant mineral element in the body. Sufficient levels of calcium in the serum are necessary for the formation of the hydroxyapatite that gives bone its compressive strength [7]. About 50-60% of blood calcium is bound to plasma proteins, mostly albumin, or forms a complex with phosphate or citrate, while the rest is the ionized form, also termed as “free” calcium [8].

Only the ionized calcium fraction is physiologically active [9]. Once a fracture occurs, calcium is necessary for fracture repair to mineralize the fracture callus [10].

Bone alkaline phosphatase (BAP) is an isoform of alkaline phosphatase which is a glycoprotein that is found on the surface of osteoblasts [11]. Alkaline phosphatase is the biochemical markers which provide a dynamic view of the remodeling process of bone [12]. Clinical studies also show that the level of ALP in the serum correlates with the bone formation rate [13].

Delayed union and nonunion of the radius and ulna are major complication of forearm fractures, accounting for 2% to 10% of all forearm fractures [14].

The importance of classification of fractures according to mode of trauma into high and low energy trauma fractures come from there are some types of fractures that may progress to non union fractures such as low energy distal radius fractures and high energy diaphyseal forearm fractures [14].

The present study was conducted to measure serum level of PTH, alkaline phosphatse, calcium and ionized calcium as these factors that reflect actual status of fracture healing and may be used as an adjunct in patient monitoring to help in bone healing.

The aim of the work was to identify changes in serum level of calcium, ionized calcium, alkaline phosphatase and parathyroid hormone according to mode of trauma (low Vs high energy) in forearm fractures and their role in fracture healing.

# 2. Patients and Methods

50 Patients with forearm fractures were prospectively recruited from the Accident and Emergency Department of Trauma Surgery within 48 hours of sustaining the fracture. This work has been approved by the appropriate ethical committees related to the hospital. Patients included in the study were asked to avoid calcium supplementation during fracture healing.

Patients were divided according to mode of trauma into 2 groups: High energy trauma group: 25 cases. Low energy trauma group: 25 cases.

### *2.1. Inclusion Criteria*

Adult age 18-50. Recent fracture. Isolated trauma. Radius or ulna or both.

### *2.2. Exclusion Criteria*

Multiple fractures. Old fractures (non united or mal united fractures). Pathological fractures.

### *2.3. Study Procedures*

#### *2.3.1. Parameters Studied*

Serum calcium level (ortho cresolphthalein complex method), serum ALP (IFCC kinetic method using DEA as buffer without PLP), serum parathyroid hormone level (ELISA-based technique) and ionized calcium by ion-selective electrodes [15].

Collection and processing of samples Five milliliters fasting venous blood were collected on the first day and 8$^{th}$ week. The collected blood were then centrifuged at 1500 RPM speed for 5 min for separation of serum.

i. Serum Total Calcium Measurement

*Principle:* Calcium in an alkaline medium combines with O-cresolphthalein complex one to form a purple-colored complex. Intensity of the color formed is directly proportional to the amount of calcium present in the sample. The color measured using 578 nm filters.

*Content of reagent:* Ortho cresolphthalein complex (color) reagent; DEA buffer; calcium standard, 10 mg/dL.

*Working reagent:* All reagents brought to room temperature. The working reagent was prepared by mixing equal parts of the color reagent and the buffer reagent. This is stable for 7 days at 2-8°C. Normal reference values: Serum/plasma: 8.5-11.0 mg/dL.

Serum ionized calcium measurement: Free ionized calcium was measured directly using ion-selective electrodes (direct potentiometry). These electrodes were not provided with a routine chemistry analyzer but were available with blood gas analyzers or point of care analyzers (e.g. iStat) [16].

Procedure: With this technique, an electrode containing an internal electrolyte solution was immersed in the patient sample, which is separated from the internal solution by a membrane that can detect the electromotive force (EMF) generated by the ions in both solutions. This EMF was determined by the difference in concentration of the test ion in the test solution and internal filling solution (test ion at fixed concentration). The EMF was predicted by the Nernst equation

Units of measurement: The concentration of free ionized calcium is measured in mg/dL (conventional units), mEq/L (conventional units), and mmol/L (SI units). The conversion formulas are identical to total calcium: *mg/dL x 0.2495=mmol/L. mEq/L x 0.5=mmol/L.*

ii. Serum Alkaline Phosphatase Measurement

*Principle:* Alkaline phosphatase at an alkaline medium hydrolyses p-nitrophenyl phosphate to form p-nitrophenol (PNP) and phosphate. The rate of formation of PNP is measured as an increase in absorbance that is proportional to the ALP activity in the sample.

*Procedure:* A clean dry test tube labeled as test (*T*) taken. Contents of the tube were mixed well. Initial absorbance after 1 min (Å) and absorbance after every 1-3 min measured at 405 nm wavelength. Mean absorbance change per minute ($\Delta A$/min) was calculated.

*Calculations:* Alkaline phosphatase activity in IU/L=$\Delta$A/min × 2754. In our auto-analyzer (XL 600), we have got direct value of serum ALP in IU/L as the system parameters for ALP were programmed in the instrument.

iii. Measurement of Parathyroid Hormone Level

*Principle:* The Access Intact PTH assay is a two-site immunoenzymatic ("sandwich") assay.

*Procedure:* A sample is added to a reaction vessel, along with a monoclonal anti-PTH antibody conjugated to alkaline phosphatase, TRIS buffered saline with proteins and paramagnetic particles coated with a goat polyclonal anti-PTH antibody. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of PTH in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

### 2.3.2. Radiological Evaluation

Plain X ray was done for assessment of fracture healing every 4 weeks.

Statistical analysis: Presentation of the data will be done in numerical, tabular and graphical forms as appropriate. Statistical analysis will be conducted by means of SPSS statistical software (version 16). For comparison of the groups, Student T-test will be used for quantitative variables, and Chi-square test for qualitative variables. Correlation and regression analyses will be used to describe the relationship between different variables in the study groups. Statistical significance will be determined at 95% level of confidence (i.e. differences will be considered significant for $P < 0.05$).

## 3. Results

The patients were further subdivided according to location of the fracture into 10 patients with proximal fracture, 13 patients with mid shaft fracture and 27 patients with distal fracture (Table 1).

***Table 1.*** *Showing classification of patients according to location of the fracture.*

| According to location | Groups | | | | | | Chi-Square | |
|---|---|---|---|---|---|---|---|---|
| | High energy | | Low energy | | Total | | | |
| | N | % | N | % | N | % | $X^2$ | P-value |
| Proximal | 5 | 20.00 | 5 | 20.00 | 10 | 20.00 | 1.026 | 0.599 |
| Mid shaft | 8 | 32.00 | 5 | 20.00 | 13 | 26.00 | | |
| Distal | 12 | 48.00 | 15 | 60.00 | 27 | 54.00 | | |
| Total | 25 | 100.00 | 25 | 100.00 | 50 | 100.00 | | |

It was found that mean PTH level in high energy group was 71.468 and SD 22.613 which decreased to 51.012 and SD 20.475 after 8 weeks while in low energy group mean PTH level was 77.056 and SD 20.106 at Day 1 which decreased to 71.404 and SD 16.520 after 8 weeks (Table 2).

***Table 2.*** *Shows PTH level at the first day and after 8 weeks in high energy group and low energy group.*

| PTH (pg/mL) | | Groups | | | | | | T-Test | |
|---|---|---|---|---|---|---|---|---|---|
| | | High energy | | | Low energy | | | t | P-value |
| After fracture | Range | 29.2 | - | 98 | 31.2 | - | 103 | -0.923 | 0.360 |
| | Mean ± SD | 71.468 | ± | 22.613 | 77.056 | ± | 20.106 | | |
| After 2 Months | Range | 25 | - | 92 | 24.6 | - | 107.6 | -3.876 | <0.001* |
| | Mean ± SD | 51.012 | ± | 20.475 | 71.404 | ± | 16.520 | | |
| Differences | Mean ± SD | 20.456 | ± | 26.937 | 5.652 | ± | 18.943 | | |
| Paired Test | P-value | 0.001* | | | 0.149 | | | | |

We found that mean PTH level in both groups at first day in proximal fracture was higher than mid shaft and distal fractures which remained elevated after 8 weeks in low energy group in proximal fracture, while in high energy group it was higher in distal fracture than mid shaft and proximal fractures after 8 weeks (tables 3, 4).

***Table 3.*** *Showing PTH level in high energy trauma group.*

| PTH (pg/mL) | | High energy | | | | | | | | | ANOVA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Proximal | | | Mid shaft | | | Distal | | | F | P-value |
| After fracture | Range | 74 | - | 81 | 43 | - | 96 | 29.2 | - | 98 | 0.311 | 0.736 |
| | Mean ± SD | 76.800 | ± | 2.775 | 73.600 | ± | 20.816 | 67.825 | ± | 28.397 | | |
| After 2 Months | Range | 25 | - | 75 | 27.6 | - | 92 | 35 | - | 90 | 0.314 | 0.734 |
| | Mean ± SD | 45.140 | ± | 25.079 | 50.300 | ± | 22.825 | 53.933 | ± | 18.141 | | |
| Differences | Mean ± SD | 31.660 | ± | 23.572 | 23.300 | ± | 25.941 | 13.892 | ± | 29.034 | | |
| Paired Test | P-value | 0.040* | | | 0.039* | | | 0.126 | | | | |

***Table 4.*** *Showing PTH in low energy trauma group.*

| PTH (pg/mL) | | Low energy | | | | | | | | | ANOVA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Proximal | | | Mid shaft | | | Distal | | | F | P-value |
| After fracture | Range | 37 | - | 102 | 40 | - | 100 | 31.2 | - | 103 | 0.161 | 0.853 |
| | Mean ± SD | 80.960 | ± | 25.665 | 73.500 | ± | 23.346 | 76.940 | ± | 18.415 | | |
| After 2 Months | Range | 44 | - | 105.5 | 50 | - | 107.6 | 24.6 | - | 80 | 0.403 | 0.673 |
| | Mean ± SD | 76.040 | ± | 23.358 | 74.060 | ± | 21.171 | 68.973 | ± | 12.938 | | |
| Differences | Mean ± SD | 4.920 | ± | 23.091 | -0.560 | ± | 30.408 | 7.967 | ± | 13.374 | | |
| Paired Test | P-value | 0.659 | | | 0.969 | | | 0.037* | | | | |

By comparing two groups it was found that mean PTH was elevated in both groups at Day 1 with low energy group was higher than high energy group, whereas after 8 weeks mean PTH level decreased in both groups but in low energy group mean PTH level remain elevated above normal level. The decrease in mean PTH level in high energy group was significant with P value 0.001 while in low energy group it was insignificant decrease with P value 0.149.

Table 5 shows serum alkaline phosphatase at Day 1 and after 8 weeks in high energy and low energy groups. in the high energy group mean alkaline phosphatase at Day 1 was 106.200 and SD 23.523 which decreased after 8 weeks to 98.680 and SD 10.419, while in low energy group mean alkaline phosphatase was 107.760 and SD 18.485 at Day 1 which decreased to 99.440 and SD 16.691.

***Table 5.*** *Serum alkaline phosphatase after fracture and after 2 months in high and low energy trauma groups.*

| Alkaline phosphatase (IU/L) | | Groups | | | | | | T-Test | |
|---|---|---|---|---|---|---|---|---|---|
| | | High energy | | | Low energy | | | t | P-value |
| After fracture | Range | 52 | - | 134 | 65 | - | 135 | -0.261 | 0.795 |
| | Mean ± SD | 106.200 | ± | 23.523 | 107.760 | ± | 18.485 | | |
| After 2 Months | Range | 72 | - | 120 | 69 | - | 130 | -0.193 | 0.848 |
| | Mean ± SD | 98.680 | ± | 10.419 | 99.440 | ± | 16.691 | | |
| Differences | Mean ± SD | 7.520 | ± | 27.397 | 8.320 | ± | 18.386 | | |
| Paired Test | P-value | 0.183 | | | 0.033* | | | | |

We found that mean ALP in high energy group in proximal fracture higher than mid shaft and distal fractures at first day which remained elevated in proximal fracture after 8 weeks compared to other fractures, while in low energy group mean ALP was higher in mid shaft fracture than proximal and distal fractures at first day which also remained elevated after 8 weeks compared to other fractures (Tables 6, 7).

***Table 6.*** *Showing serum ALP in high energy trauma group.*

| Alkaline phosphatase (IU/L) | | High energy | | | | | | | | | ANOVA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Proximal | | | Mid shaft | | | Distal | | | F | P-value |
| After fracture | Range | 114 | - | 128 | 73 | - | 133 | 52 | - | 134 | 1.132 | 0.341 |
| | Mean ± SD | 119.400 | ± | 5.273 | 106.250 | ± | 19.862 | 100.667 | ± | 28.871 | | |
| After 2 Months | Range | 88 | - | 120 | 72 | - | 105 | 85 | - | 115 | 1.362 | 0.277 |
| | Mean ± SD | 101.400 | ± | 12.740 | 93.750 | ± | 12.021 | 100.833 | ± | 7.732 | | |
| Differences | Mean ± SD | 18.000 | ± | 14.595 | 12.500 | ± | 21.673 | -0.167 | ± | 33.599 | | |
| Paired Test | P-value | 0.051* | | | 0.147 | | | 0.987 | | | | |

***Table 7.*** *Showing serum ALP in low energy trauma group.*

| Alkaline phosphatase (IU/L) | | Low energy | | | | | | | | | ANOVA | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Proximal | | | Mid shaft | | | Distal | | | F | P-value |
| After fracture | Range | 82 | - | 122 | 67 | - | 125 | 65 | - | 135 | 0.097 | 0.908 |
| | Mean ± SD | 104.400 | ± | 14.775 | 108.200 | ± | 23.658 | 108.733 | ± | 18.930 | | |
| After 2 Months | Range | 77 | - | 129 | 69 | - | 130 | 85 | - | 125 | 0.034 | 0.967 |
| | Mean ± SD | 97.800 | ± | 21.765 | 100.600 | ± | 27.952 | 99.600 | ± | 10.842 | | |
| Differences | Mean ± SD | 6.600 | ± | 13.667 | 7.600 | ± | 23.533 | 9.133 | ± | 19.131 | | |
| Paired Test | P-value | 0.341 | | | 0.510 | | | 0.086 | | | | |

By comparing two groups, it was found that mean alkaline phosphatase level increased in both groups at Day 1 whereas in low energy group it was slightly higher than that of high energy group. After 8 weeks mean alkaline phosphatase decreased to normal level in both groups.

The decrease in mean alkaline phosphatase level in high energy group was insignificant with P value 0.183 whereas in low energy group was significant decrease with P value 0.033.

Table 8 shows serum calcium level at Day 1 and after 8 weeks in both groups. It was found mean serum calcium level at Day 1 was 8.264 and SD 1.279 which increased after 8 weeks to 8.892 and SD 0.881 in high energy group while in low energy group mean serum calcium level at Day 1 was 8.024 and SD 1.121 which increased after 8 weeks to 8.524

and SD 0.649.

***Table 8.*** *Serum calcium after fracture and after 2 months in high and low energy trauma groups.*

| Serum calcium (mg/dl) | | Groups | | | | | | T-Test | |
|---|---|---|---|---|---|---|---|---|---|
| | | High energy | | | Low energy | | | t | P-value |
| After fracture | Range | 6 | - | 11.9 | 5.5 | - | 9.6 | 0.706 | 0.484 |
| | Mean ± SD | 8.264 | ± | 1.279 | 8.024 | ± | 1.121 | | |
| After 2 Months | Range | 7 | - | 10.7 | 8 | - | 10.6 | 1.681 | 0.099 |
| | Mean ± SD | 8.892 | ± | 0.881 | 8.524 | ± | 0.649 | | |
| Differences | Mean ± SD | -0.628 | ± | 1.582 | -0.500 | ± | 1.222 | | |
| Paired Test | P-value | 0.059 | | | 0.052* | | | | |

We found mean calcium level in high energy group was elevated in mid shaft fracture than proximal and distal fracture while in low energy group mean calcium level was higher in proximal fracture at first day, but after 8 weeks mean calcium level was significantly higher in proximal fractures compared to other fractures in high and low energy groups (Tables 9, 10).

***Table 9.*** *Showing serum calcium level in high energy trauma group.*

| Serum calcium (mg/dl) | | High energy | | | | | | | | | ANOVA | | TUKEY'S Test | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Proximal | | | Mid shaft | | | Distal | | | F | P-value | P&M | P&D | M&D |
| After fracture | Range | 6 | - | 8 | 7 | - | 11.9 | 7.2 | - | 9.8 | 1.554 | 0.234 | | | |
| | Mean ± SD | 7.460 | ± | 0.853 | 8.713 | ± | 1.684 | 8.300 | ± | 1.028 | | | | | |
| After 2 Months | Range | 8.7 | - | 10.7 | 7 | - | 9.1 | 7.5 | - | 9.8 | 8.993 | 0.001* | 0.002* | 0.003* | 0.842 |
| | Mean ± SD | 10.040 | ± | 0.847 | 8.500 | ± | 0.663 | 8.675 | ± | 0.625 | | | | | |
| Differences | Mean ± SD | -2.580 | ± | 0.576 | 0.213 | ± | 1.495 | -0.375 | ± | 1.265 | | | | | |
| Paired Test | P-value | 0.001* | | | 0.700 | | | 0.327 | | | | | | | |

***Table 10.*** *Showing serum calcium level in low energy trauma group.*

| Serum calcium (mg/dl) | | Low energy | | | | | | | | | ANOVA | | TUKEY'S Test | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Proximal | | | Mid shaft | | | Distal | | | F | P-value | P&M | P&D | M&D |
| After fracture | Range | 6 | - | 9.6 | 5.5 | - | 8.9 | 6.5 | - | 9.6 | 0.792 | 0.466 | | | |
| | Mean ± SD | 8.480 | ± | 1.534 | 7.580 | ± | 1.392 | 8.020 | ± | 0.886 | | | | | |
| After 2 Months | Range | 8.4 | - | 10.6 | 8 | - | 8.6 | 8 | - | 8.7 | 19.472 | <0.001* | <0.001* | <0.001* | 0.980 |
| | Mean ± SD | 9.540 | ± | 0.811 | 8.300 | ± | 0.255 | 8.260 | ± | 0.232 | | | | | |
| Differences | Mean ± SD | -1.060 | ± | 2.019 | -0.720 | ± | 1.308 | -0.240 | ± | 0.839 | | | | | |
| Paired Test | P-value | 0.306 | | | 0.286 | | | 0.287 | | | | | | | |

By comparing two groups it was found that mean serum calcium level was below normal level in both groups where it was slightly higher in high energy group than that of low energy group which increased after 8 weeks in both groups but remain elevated in high energy than low energy group.

The increase of mean serum calcium level in high energy group was insignificant with P value 0.059 whereas it was significant in low energy group with P value 0.052.

Table 11 shows serum ionized calcium level at Day 1 and after 8 weeks in both groups. in high energy group mean ionized calcium level at Day 1 was 1.052 and SD 0.162 which increased to 1.207 and SD 0.204 after 8 weeks whereas in low energy group mean ionized calcium level was 1.009 and SD 0.183 which after 8 weeks increased to 1.057 and SD 0.098.

***Table 11.*** *Serum ionized calcium level after fracture and after 2 months in high and low energy trauma groups.*

| Ionized calcium (mmol/L) | | Groups | | | | | | T-Test | |
|---|---|---|---|---|---|---|---|---|---|
| | | High energy | | | Low energy | | | t | P-value |
| After fracture | Range | 0.7 | - | 1.48 | 0.7 | - | 1.35 | 0.891 | 0.377 |
| | Mean ± SD | 1.052 | ± | 0.162 | 1.009 | ± | 0.183 | | |
| After 2 Months | Range | 0.95 | - | 1.8 | 0.8 | - | 1.25 | 3.318 | 0.002* |
| | Mean ± SD | 1.207 | ± | 0.204 | 1.057 | ± | 0.098 | | |
| Differences | Mean ± SD | -0.155 | ± | 0.281 | -0.048 | ± | 0.232 | | |
| Paired Test | P-value | 0.011* | | | 0.306 | | | | |

We found that mean Ionized calcium level in high energy was higher in mid shaft fracture than proximal and distal fractures at first day which after 8 weeks was significantly higher in proximal fracture compared to other fractures, While in low energy group mean ionized calcium level was higher in proximal fracture at first day which after 8 weeks was higher in mid shaft fracture compared to other fractures (tables 12, 13).

***Table 12.*** *Showing serum ionized calcium level in high energy trauma group.*

| **Lonized calcium (mmol/L)** | | **High energy** | | | | | | | | | **ANOVA** | | **TUKEY'S Test** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Proximal** | | | **Mid shaft** | | | **Distal** | | | **F** | **P-value** | **P&M** | **P&D** | **M&D** |
| After fracture | Range | 0.7 | - | 1.2 | 0.9 | - | 1.45 | 0.85 | - | 1.48 | 0.405 | 0.672 | | | |
| | Mean ± SD | 0.994 | ± | 0.182 | 1.076 | ± | 0.180 | 1.061 | ± | 0.151 | | | | | |
| After 2 Months | Range | 1.15 | - | 1.8 | 0.95 | - | 1.4 | 1 | - | 1.21 | 9.728 | 0.001* | 0.003* | 0.001* | 0.964 |
| | Mean ± SD | 1.480 | ± | 0.280 | 1.150 | ± | 0.147 | 1.132 | ± | 0.076 | | | | | |
| Differences | Mean ± SD | -0.486 | ± | 0.349 | -0.074 | ± | 0.232 | -0.071 | ± | 0.177 | | | | | |
| Paired Test | P-value | 0.036* | | | 0.398 | | | 0.193 | | | | | | | |

***Table 13.*** *Showing serum ionized calcium in low energy trauma group.*

| **Lonized calcium (mmol/L)** | | **Low energy** | | | | | | | | | **ANOVA** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Proximal** | | | **Mid shaft** | | | **Distal** | | | **F** | **P-value** |
| After fracture | Range | 0.8 | - | 1.35 | 0.7 | - | 1.23 | 0.7 | - | 1.3 | 1.346 | 0.281 |
| | Mean ± SD | 1.126 | ± | 0.213 | 0.996 | ± | 0.227 | 0.974 | ± | 0.153 | | |
| After 2 Months | Range | 0.9 | - | 1.2 | 1 | - | 1.18 | 0.8 | - | 1.25 | 0.007 | 0.993 |
| | Mean ± SD | 1.056 | ± | 0.110 | 1.062 | ± | 0.077 | 1.056 | ± | 0.107 | | |
| Differences | Mean ± SD | 0.070 | ± | 0.300 | -0.066 | ± | 0.266 | -0.082 | ± | 0.199 | | |
| Paired Test | P-value | 0.629 | | | 0.609 | | | 0.133 | | | | |

By comparing two groups it was found that serum ionized calcium level was below normal level at Day 1 in both groups whereas it was slightly higher in high energy group than low energy group which after 8 weeks increased in both groups but remain elevated in high energy group than low energy group.

The increase in mean ionized calcium in high energy group was significant with P value 0.011 while in low energy group it was insignificant increase with P value 0.306.

## 4. Discussion

Biochemical bone markers reflect bone metabolism and provide information regarding bone turnover.

With a bone fracture, bone turnover is increased to facilitate fracture repair and healing [17].

Although a lot has been achieved, our study is the first attempt to measure biochemical markers according to mode of trauma in adults not according to bone union and non union.

Das et al. (2015) and Mukhopadhyay et al. (2011) results found that Serum ALP was elevated more than 2 months and then decreased to normal reference interval until bone remodeling was complete [10, 18] and Paskalev et al. (2005) showed that serum ALP decreased with insignificant variation whether fracture healed or not [19] But on the other hand in the present study serum ALP showed the same pattern by increasing until 60 days and returned to normal levels in healed individuals and this agree with Ajai [2013], Varma et al. (2003) and Muljacic et al. (2010) who showed that Serum ALP reached maximum level at third week which remained elevated significantly and then reached within normal range when union occurred by clinical radiological findings [13, 20, 21].

In the current study we found that serum calcium decreased immediately after fracture in both groups and increased insignificantly to normal level after 2 months in high energy trauma group while it remained below normal reference range in low energy group and this agree with Das et al. (2015) who found that serum calcium level after 2 weeks was significantly higher than day 1 then reduced to normal level within 1 month in normal healed group, while Paskalev et al. (2005), Meller et al. (1984) and Hardy et al. (1993) found that showed serum calcium and ionized calcium level decreased signifcantly after one week then returned to normal level in less than 2 months [10, 19, 22, 23], however Varma et al.(2003) showed significantly increased serum calcium in normal individuals after 6 weeks and remained elevated up to 6 months (21).

The current study found that serum PTH was elevated immediately after fracture and significantly decreased to normal level in high energy group while it remained elevated above normal reference range in low energy trauma group and this agrees with the Meller et al. (1984) and Hardy et al. (1993) that showed that serum PTH was significant elevated following fracture and returned to normal level after 6 weeks [22, 23].

Gruys et al. (2005) and Theman Collins et al. (2009) explained local or systemic disturbances in its homeostasis caused by trauma. The vascular system and inflammatory cells are in active form. Cytokines caused decrease in serum calcium and ionized calcium level with elevated alkaline phosphatase level [24, 25].

The reduction of serum calcium and ionized calcium following fracture inactivate calcium sensing receptor in parathyroid cells causing increase PTH secretion which act on PTHR which stimulates calcium reabsorption from the kidney and bone resorption. These changes were consistent with the current study which found decreased serum calcium and ionized calcium with elevated serum ALP and PTH level immediately after fracture in both high energy and low energy trauma groups.

## 5. Conclusion

Serial monitoring of these physiological markers reflect the actual status of bone resorption, and bone formation respectively over a short period. Thus, they can be used as an adjunct to clinical and radiological evidence of fracture healing. By monitoring the changes of the biochemical parameters of alkaline phosphatase it is easily possible to detect the dynamics of the healing of the bone fracture early. Most probably there was disturbance in physiological markers of low energy trauma group before occurrence of fracture which was predisposing factor for incidence of fracture. Low energy trauma group should be better managed and followed up by giving calcium supplementation immediately after incidence of fracture as our study found that serum calcium and ionized calcium level were below reference range after 8 weeks. Follow-up studies or future work on this topic is recommended.

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