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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 two decades, our understanding of fracture healing has evolved rapidly. Bone is one of the few body tissues that 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 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. **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 and their role in fracture healing. **Patients and Methods:** 50 Patients with forearm fracture were prospectively recruited from the Accident and Emergency Department of Trauma Surgery, El Zaitoun specialized hospital 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 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 elevated in high energy group than low energy group. **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.

[Ahmed Mohamed Morsi, Tameem Mohamed Shafik, Ahmed Kamal Mohamed Hassan. **Serum Level of Parathyroid Hormone, Alkaline Phosphatase, Calcium and Ionized Calcium After Forearm Fractures.** *Nat Sci* 2019;17(9):190-195]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 23. doi:10.7537/marsnsj170919.23.

Keywords: Alkaline phosphatase, parathyroid hormone, distal radioulnar joint

1. Introduction

Fractures of both radius and ulna are one of the most common fractures in adults in upper extremity. [(1)] In this era or active life, rapid industrialization, increasing road traffic accidents, competitive sports, the incidence of fractures of forearm bones are increasing in frequency. [(2)]

Fractures of the forearm can occur at different levels: near the wrist at the farthest (distal) end of the bone, in the middle of the forearm or near the elbow at the top (proximal) end of the bone.

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

During the last two decades, our understanding of fracture healing has evolved rapidly. Bone is one of the few body tissues that 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 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 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 in fracture repair to mineralize the fracture callus. [10]

Bone alkaline phosphatase (BAP) is isoform of alkaline phosphatase which is a glycoprotein that is found on the surface of osteoblasts. [11] Alkaline phosphatase is one of 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. Non union is defined as absence of radiological and clinical signs of unions after an average period of six months. [14]

The management of non union fractures remain difficult due to the poor bone mass, the existence of previous implant material if present and joint stiffness that is associated with long-term immobilization.

Treatment options of non union fractures include surgical stabilization at the non union associated with the compression of the fracture site and stimulation of bone formation by bone grafting and bone-marrow injection. [15]

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], that is why we have chosen forearm fractures in our study because forearm fractures occur due to either high or low energy trauma.

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

Aim of the work

The aim of our study is 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 fracture were prospectively recruited from the Accident and Emergency Department of Trauma Surgery, El Zaitoun specialized hospital within 48 hours of sustaining the fracture. 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.

Inclusion Criteria:

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

Exclusion Criteria:

Multiple fractures. Old fractures [non united or mal united fractures]. Pathological fractures.

Study Procedures:

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. [16]

Collection and processing of samples

Five milliliters fasting venous blood was collected on first day and 8th week. The collected blood was then centrifuged at 1500 RPM speed for 5 min for separation of serum.

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 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 is measured directly using ion-selective electrodes (direct potentiometry). These electrodes are not provided with a routine chemistry analyzer but are 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 is 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 is 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 is 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.

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 are 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 (ΔA/min) calculated.

Calculations:

Alkaline phosphatase activity in IU/L= ΔA/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.

Measurement of Parathyroid hormone level

Principle:

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

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

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.

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 (1): 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				

Table [2] 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.

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 (2): 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*				

Table [3] 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.

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 (3): 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*				

Table (4): 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				

Table [4] 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.

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 serum calcium and ionized calcium level decreased significantly 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 agree with the Meller et al. [1984] and Hardy et al. [1993] that showed that serum PTH was significantly 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.

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.

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9/15/2019