Iraqi Journal of Biotechnology, September 2018, Vol. 17, No. 2, 42-46

The relationship between levels of osteocalcin, calcium, vitamin K and vitamin D in sera of patients with bone fractures compared to apparently healthy control

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Received: June 7, 2018 / **Accepted**: September 10, 2018

Abstract: The production of osteocalcin is stimulated by 1**,** 25 dihydroxy vitamin D and depends on vitamin K. Vitamin K increases the carboxylation of osteocalcin, but it does not increase its overall rate of synthesis. Although its function is not completely understood, osteocalcin may exist as a deposition site for hydroxyapatite crystals. It may also affect energy metabolism via the production and action of insulin. The present study aimed at measuring serum levels of osteocalcin, calcium, vitamin k and vitamin D in sera of patients complaining from different types of fractures compared to levels in apparently healthy controls. Serum osteocalcin was within normal limits in patients and controls. Serum calcium generally lower than normal, vitamin D was lower than normal and vitamin K was within normal limits. The results indicated the importance of measurement of vitamin D and serum calcium in patients with fracture.

Keywords: Serum osteocalcin, Vitamin D, Vitamin K, bone fractures**.**

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Introduction:

Osteocalcin, also known as "bone gamma-carboxyglutamic acid (Gla) protein (BGP)," is the most abundant noncollagenous protein of bone matrix.(1).

In mature human bone, osteocalcin constituents 1-2% of the total protein. (2). It is a small protein of 49 amino acids including 3 residues of gamma-carboxyglutamic acid with a molecular weight of 5800 Da.(3). Because it is rapidly cleared by the kidney, the half life of circulating osteocalcin is short i.e. approximately 5 minutes (4).

Three glutamyl residues at position 17, 21, and 24 can be carboxylated in a posttranslational, vitamin K- dependent, enzymatic step producing γ-carboxyglutamyl (Gla) residues. Thus, levels of under carboxylated osteocalcin are influenced by vitamin K status, whereas total circulating concentrations of osteocalcin are influenced by bone cells activity independent of vitamin K.(5). Currently, seventeen members of the Gla protein family are known, including seven proteins involved in blood coagulation which are synthesized in the liver, as well as osteocalcin (OC) synthesized in bone, matrix Gla protein (MGP) synthesized in cartilage and vessel wall, Gas 6, Gla-rich proteins, periostin and periostin-like factor (6). Gla-rich protein (GRP) is the newest vitamin K dependent protein identified, and is being explored

as a potential new vitamin K target in cancer.(7,8). With the widespread occurrence of extra-hepatic Gla proteins there is interest in learning about the distribution of extra-hepatic K vitamins and the physiological importance of these proteins (9).

The aim of this study is to measure levels of osteocalcin, vitamin K and vitamin D in sera of patients with different types of fractures and to compare them to levels in apparently healthy individuals.

Materials and methods:

This is a case control study involving 50 patients with different fractures and 50 apparently healthy individuals. Patients together with their relatives were recruited at the Orthopaedic wards of Special Hospital/ Medical Campus during the period from 15 January to 15 September 2017.

Each participant donated 5ml of blood in a plane tube. Sera were obtained and submitted to the measurement of osteocalcin, calcium, vitamin K and vitamin D.

For measurement of all biochemical parameters, ELISA was implemented with a special kit for each. For the measurement of serum osteocalcin, the human osteocalcin ELISA assay kit was used with a reference normal value ranging from- 9-38 ng/mL to 8-32 ng/mL in males and 8 to 32 ng/mL in females. For serum calcium the calcium-MTB kit was used with a normal value of 8.6-10.3 mg/dL. For vitamin D, the human vitD ELISA assay kit was used with a normal value of 17 - 53 pg/ml For vitamin K, the human VitK ELISA assay kit was used with a normal value of 0.2 - 3.2ng/ml.

Results were tabulated and statistical analysis was applied to study the differences between the study groups. Student t test was used to differentiate between means of the biochemical parameters levels between study groups. Chi square was used to signify differences in the frequency distribution between study groups.

Results and Discussion:

The mean serum osteocalcin level in patients with fracture was 19.74 ±2.94ng/ml and that of the control was 15.03±2.85 with a significant difference, $p<0.05$. The mean serum calcium level in patients with fracture was 6.34±0.86 mg/dL and that for the control group was 9.88±0.57 mg/dL with a significant difference, $p<0.05$. The mean serum vitamin D level in patients with fracture was 13.86±2.69 ng/ml and that of the control group was 41.53±4.94 ng/ml with a significant difference, $p<0.05$. The mean serum vitamin K level was 131.86±9.73 pg/ml and that of the control group was121.41±6.41pg/ml with a non-significant difference, $p>0.05$. Data are presented in table (1).

Circulating osteocalcin level is an important marker indicative of osteoblastic activity. Patient with fracture included in the present study were all recently presented. The time lag was not enough for the healing process of fracture site to be established and to the followed by remodeling. This is why serum osteocalcin levels were also found within normal limits in patient with fracture.

The frequency distribution of study groups according to level of biochemical markers is shown table (2)

for the control group and table(3) for fracture group.

Serum osteocalcin was normal in 37 (92.5%) of the control group as well as 38 (95%) of patients with fractures. Higher than normal level was only detected in one (2.50%) person in the control group and one (2.50%) in the fracture group. While low levels were detected in 2 (5%) patients in the control group and one patient (2.5%) of the fracture group (Tables 2 and 3). Significant differences were shown between subgroups, $p<0.05$.

Serum calcium levels were within normal limits in 13 (32.5%) of the control group. Nine (22.5%) had lower than normal levels and 18 (45%) had higher than normal readings. A significant difference between subgroups was noted as well, $p<0.05$. Table(2).

Serum calcium was within normal limits in only 2 (5%) patients with fracture. The majority of them, 35 (87.5%) had lower than normal levels. A significant difference between subgroups was noted, $p<0.05$(Table-3).

Levels of vitamin D and K were within normal limits in all subjects 40 (100%) of the control group (Table-2). Vitamin D levels were lower than normal in 37 (92.5%) of patients with fracture and only 3 patients (7.5%) had normal levels with a significant difference, $p<0.05$. Vitamin K was within normal limits in all 40 (100%) patients with fracture (Table-3).

Reviewing results in tables (2) and (3) reveals low mean serum calcium in patients with fracture than the controls with significant differences between subgroups groups.

Sato *et al*., (10) in their work had conduce that increased bone resorption and decrease bone formation and hypercalcemia are present by 1 week following hip fracture and some resorption increase persists for at least 3 months. Li *et al*., (11) found that the capability of reservation and restoration of serum calcium in patients with femoral neck fracture is better than that in patients with femoral inter trochanteric fracture they also suggested that a low s.calcium level may increase the patients susceptibility to intertrochanteric fracture. Fischer *et al.*, (12) have referred to the contribution of low serum Ca and vit. D to bone healing compromise. Gau*et al.*, (13) in their case control study suggested the lower serum calcium level was significantly associated with a higher risk for fractures of all types.

The majority of controls in the study group showed low levels of vit.D. The mean level was significantly lower than the mean of the control groups table. Veldurthy *et al.*, (14) stressed on the role of vitamin D as a principle factor that maintains calcium hemostasis, blaming inadequate vit.D levels in elderly patients to be responsible for disturbed calcium balance with age.

Ettehad*et al.*, (15) found that the overall reduction of vit.D level was significantly more evident in the first week of fracture versus the following weeks. This is in agreement with the present study. Iolascon *et al.*, (16) found sever vitamin D deficiency<30n mol / L is common in patients with fragility fractures.

Serum vitamin K levels were found to be normal in all study groups with mean level of fracture group higher than the mean of the control group.

In the study of Fusaro *etal.*, (17) a mention to the effect of vitamin K to osteocalcin was noted. The author also

refer to the effect of Vitamin K deficiency in fracture .Vitamin K was shown to have anti calcification, anti cancer, bone-forming and insulin sensitizing molecule. Ferreira *et al*., (18) included it under the term of bone remodeling markers.

In table (4) the results shows that the correlation of biomarker with in Cancer group between age shows a non-significant change (p>0.05), and Os.Calcin/age, ALP, Calcium, Vit D and Vit K/age shows a non-significant change (p>0.05), and ALP, Calcium ,Vit D and Vit K/ Os.Calcin shows a non-significant change Calcium, Vit D and Vit K/ ALP shows a non-significant change (p>0.05), and Vit D and Vit K/ Calcium shows a non-significant change (p>0.05). Vit K / Vit D shows a non-significant change (p>0.05).

Table (1): Mean serum levels of biochemical parameters between control group and fracture group by unpaired T-test.

Parameter	Control N=40 Mean±SE	Fracture N=40 Mean±SE	P value
S. Osteocalcin (ng/mL)	15.03+2.85	19.74+2.94	**0.039**
S. Calcium (mg/dL)	9.88+0.57	6.34+0.86	**< 0.001**
S. Vit. D (ng/mL)	41.53+4.94	13.86+2.69	**< 0.001**
S. Vit. K (pg/mL)	121.41+6.41	131.86+9.73	0.199

Table (2): Frequency distribution of subjects within the control according to serum levels of osteocalcin, calcium, vitamin D and vitamin K.

The Group	S. Osteocalcin	Calcium	Vit D	Vit K
Lower than normal	2 (5.00%)	9 (22.50%)	0 (0.00%)	0 (0.00%)
Within normal	37(92.50%)	13 (32.50%)	40 (100.00%)	40 (100.00%)
More than normal	1 (2.50%)	18 (45.00%)	0 (0.00%)	0 (0.00%)
P-value	0.0001 **	0.0001 **		
**** (P<0.01).**				

Table (3): Frequency distribution of subjects within the fracture group according to serum levels of osteocalcin, calcium, vitamin D and vitamin K.

The Group	S. Osteocalcin	Calcium	Vit D	Vit K
Lower than normal	1 (2.50%)	35 (87.50%)	37 (92.50%)	0 (0.00%)
Within normal	38 (95%)	2 (5.00%)	3 (7.50%)	40 (100.00%)
More than normal	1 (2.50%)	3 (7.50%)	0 (0.00%)	0 (0.00%)
P-value	0.0001 **	0.0001 **	0.0001 **	
**** (P<0.01).**				

Table (4): Correlation of biomarker within Fracture group

Parameters	Age	Os.Calcin	ALP	Calcium	Vit D	Vit K
Age	---					
Os.Calcin	-0.03 NS	---				
ALP	0.16 NS	0.11 NS	---			
Calcium	0.22 NS	-0.01 NS	0.16 NS	---		
Vit D	0.26 *	0.06 NS	0.07 NS	0.20 NS	---	
Vit K	0.20 NS	0.18 NS	-0.12 NS	0.11 NS	0.21 NS	--
NS: Non-Significant.						

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