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Correlation of Osteocalcin Level with Blood Glucose Concentration and Insulin Level in Type II Diabetic Sudanese Patients

Ogail Yousif Dawod[1]*, Amar Babikir Elhussein[2], Mohamed Abd El-Rahim Ahmed[1], Nour Eldaim Elnoman Elbadawi[3] and Omer Abdel Aziz Musa[4]

[1] *Department of Physiology, Faculty of Medicine, University of Shandi, Shandi, Sudan*

[2] *Department of Biochemistry, Nile College for Medicine and Medical Science, Khartoum, Sudan*

[3] *Department of Biochemistry, College of Medicine, University of Shaqra, Shaqra, Saudi Arabia*

[4] *Department of Physiology, Faculty of Medicine, The National Ribat University, Sudan*

**Corresponding e-mail: ogail76@gmail.com*

ABSTRACT

Osteocalcin, a bone-derived protein, has recently been reported to affect energy metabolism, this study aimed to evaluate the effects of osteocalcin level on blood glucose concentration and insulin level in type II diabetic Sudanese patients. In this cross-sectional study, one hundred fifteen type II diabetic patients were enrolled in the study (34 males and 81 females) with ages ranging between 18 and 90 years. Total osteocalcin, undercarboxylated osteocalcin, and insulin were measured using ELISA technique. Fasting blood glucose and lipid profile were measured by a spectrophotometer. Mean serum osteocalcin concentration in diabetic patients was significantly lower than those in control subjects ($p \leq 0.001$). Undercarboxylated osteocalcin in diabetic patients was significantly lower than in control subjects ($p \leq 0.001$). Fasting insulin was significantly higher in diabetic subjects compared to control subjects ($p \leq 0.001$). Osteocalcin was negatively correlated with insulin and FBS ($r = -0.530$; $p \leq 0.001$), ($r = -0.373$; $p \leq 0.001$) respectively. Undercarboxylated was negatively correlated with insulin and FBS ($r = -0.634$; $p \leq 0.001$), ($r = -0.297$; $p \leq 0.001$) respectively. Total osteocalcin and undercarboxylated osteocalcin levels of diabetic patients were lower than those of the healthy control subjects. Fasting serum insulin was higher in patients compared to control subjects. Total osteocalcin and undercarboxylated osteocalcin were negatively correlated with Fasting serum insulin and FBS.

Keywords: Diabetes, Total osteocalcin, Undercarboxylated osteocalcin and Insulin

INTRODUCTION

Diabetes mellitus is a chronic disease which occurs either when the pancreas did not produce enough insulin or when the body tissues could not efficiently use the insulin [1]. The prevalence of diabetes world-wide in the ages between 20 to 70 years was estimated to be 8.3% in 2013 and 10.1% in 2035 [2]. Type II diabetes is a syndrome characterized by metabolic disorders and hyperglycaemia resulting from low levels of insulin with or without insulin resistance [3].

Organ damage is one of the long-term complications of diabetes mellitus; it is expected as a consequence of chronic hyperglycaemia. These complications result from atherosclerosis caused by the long-standing diabetes to the coronary arteries, cerebrovascular circulation, retinal and renal micro vessels and the vasa nervosa [4].

Animal studies and cell-based assays revealed that the skeleton acts as an endocrine tissue by secreting osteocalcin, which plays a role in the metabolism of glucose and lipids [5].

Osteocalcin (OC) is a type of non-collagenous protein which is produced and secreted by osteoblasts [6]. It is composed of undercarboxylated osteocalcin (ucOC) and carboxylated osteocalcin (cOC) [7] cOC is synthesized after the glutamic acid residues of OC on the 17th, 21th, and 24th sites are carboxylated by vitamin K-dependent carboxylase [8]. Osteocalcin with non-carboxylated glutamic acid residues is known as ucOC. OC play a crucial role in maintaining normal bone mineralization, inhibition of abnormal hydroxyapatite formation, and reduction of growth cartilage mineralization [7].

Previous studies had concluded that ucOC, but not cOC, is able to promote adiponectin and insulin secretion in mice [5,9]; other studies had shown independent associations between serum OC and metabolic traits in adults [10-13]. However, the carboxylated form of OC which is associated with metabolism in humans is considered a neglected area of research [14].

Several studies showed that circulating osteocalcin level was associated with glucose/fat metabolism in humans [12,15]. Zhou, et al., showed that serum osteocalcin was inversely correlated with blood sugar and positively correlated with insulin secretion in the Chinese population [15]. Other researchers have also found a correlation between osteocalcin and glucose metabolism in humans [10,16], Previous clinical studies demonstrated that uncontrolled Diabetes could reduce serum OC level, while serum OC increased after blood glucose was well controlled [17] These data indicated that changes of glucose metabolism could influence OC levels [18].

The current study aimed to evaluate the effects of osteocalcin level on blood glucose concentration and insulin levels in Type II diabetic Sudanese patients.

METHODOLOGY

One hundred fifteen adults with type II diabetes mellitus patients were enrolled in this cross-sectional hospital based case-control study (34 males and 81 females) with ages ranging between 18 and 90 years who visited the diabetic centers in Khartoum (Sudan). Type II diabetes was confirmed according to WHO criteria and 65 healthy adults from Khartoum town were randomly selected as control group.

All patients were newly diagnosed with diabetes. They visited Khartoum diabetic centers during the period of March 2015 up to June 2015. Brief clinical history of present and past illness and medical therapy was recorded from all participants.

Patients using the following treatments or suffering from one of the following complications were excluded from the study:

(1) Insulin treatment. (2) Using agents such as Warfarin, Heparin,Vitamin D3, Glucocorticoids, Anticonvulsants drugs and drugs of osteoporosis. (3) Bone diseases such as multiple myeloma, osteomalacia, Paget's disease, and fracture up to one year. (4) Malignancy, cardiovascular disease, hypertension, and hyperthyroidism.

Venous blood was taken after overnight fasting and the level of fasting blood glucose was measured by a spectrophotometer; fasting insulin, lipid profile, and both total and undercarboxylated osteocalcin were measured by ELISA Method. Height and weight were measured and body mass indices were calculated.

Data analysis

Results of this study were statistically analysed using statistical package for social science (SPSS) program 64 bits for windows 8. Independent t-test, one-way analysis of variance (ANOVA) and Person correlation coefficients were used; significance levels were set at ($P<0.05$).

RESULTS

Total osteocalcin (TOC) level was significantly lower ($p \leq 0.001$) in diabetic subjects compared to controls, the levels were (2.4 ± 1.4 ng/ml) and (14.8 ± 2.1 ng/ml) respectively. The mean of ucOC level was significantly ($p \leq 0.001$) lower in diabetic subjects than in control subjects, the levels were (1.1 ± 0.7 ng/ml) and (2.6 ± 0.5 ng/ml) respectively. The mean insulin level was significantly ($p \leq 0.001$) higher in diabetic subjects than in control subjects, the levels were (19.7 ± 6.2 ml U/L) in diabetic subjects and (9.5 ± 2.4 ml U/L) in control subjects.

Table 1 shows highly significant ($p \leq 0.001$) difference in mean concentrations of FBS between diabetics and controls, the concentration was (195.4 ± 59.0 mg/dl) in diabetics and (85.4 ± 24.9 mg /dl) in controls.

Table 1 Comparison of mean concentrations of TOC, ucOC, insulin and FBS in diabetic and control subjects

Parameter	Diabetic patients Mean ± SD	Healthy control Mean ± SD	p-value
TOC ng/ml	2.4 ± 1.4	14.8 ± 2.1	0.00
ucOC ng/ml	1.1 ± 0.7	2.6 ± 0.5	0.00
Insulin ml U/L	19.7± 6.2	9.5 ± 2.4	0.00
FBS mg/dl	195.4 ± 59.0	85.4 ± 24.9	0.00

Mean of TOC showed highly significant negative correlation with insulin level in diabetic subjects ($r = -0.530$; $p \leq 0.001$), it also showed a highly significant negative correlation with FBS ($r = -0.373$; $p \leq 0.001$). Whereas ucOC levels was negatively highly significant correlated with insulin ($r = -0.634$; $p \leq 0.001$), it showed the same trend of correlation with FBS ($r = -0.297$; $p \leq 0.001$) (Table 2).

Table 2 Correlations of TOC and ucOC with plasma Insulin and Fasting Blood Sugar

Variables		Control group		Diabetic patients	
		TOCng/ml	ucOC ng/ml	TOCng/ml	ucOC ng/ml
Insulin ml U/L	r value	0.022	−0.307	−0.53	−0.634
	p-value	0.43	0.006	0.00	0.00
FBS mg/dl	r value	−0.042	−0.117	−0.373	−0.297
	p-value	0.369	0.177	0.00	0.001

Results presented in Table 3 shows a highly significant difference in mean TOC level ($p \leq 0.001$) in underweight group between diabetic subjects and healthy control subjects, the higher levels were in control subjects (13.8 ± 1.1 ng/ml) compared to (2.7 ± 0.7 ng/ml) diabetic subjects. The ucOC in underweight group showed significant difference ($p \leq 0.01$) between studied groups, the higher levels were shown in control subjects (2.3 ± 0.5 ng/ml) compared to diabetic patients (1.2 ± 0.5 ng/ml). In contrast mean insulin was highly significant ($p \leq 0.01$) higher in diabetic subjects (14.3 ± 0.5 ng/ml) than in control subjects (10.1 ± 2.0 ng/ml).

Table 3 Mean concentrations of TOC, ucOC and plasma Insulin according to BMI in diabetics and controls

Variables	Underweight		Normal weight		Overweight		Obese	
	Diabetic	Control	Diabetic	Control	Diabetic	Control	Diabetic	Control
TOC ng/ml	2.7 ± 0.7	13.8 ± 1.1	2.4 ± 1.4	15.1 ± 2.5	2.4 ± 1.4	14.8 ± 1.7	2.4 ± 1.5	14.6 ± 0.8
p-value	0.00		0.00		0.00		0.00	
ucOC ng/ml	1.2 ± 0.5	2.3 ± 0.5	1.1 ± 0.6	2.7 ± 0.5	1.1 ± 0.6	2.6 ± 0.4	1.1 ± 0.6	2.6 ± 0.56
p-value	0.013		0.00		0.00		0.001	
Insulin ml U/L	14.3 ± 0.5	10.1 ± 2.0	20.0 ± 0.4	9.7 ± 0.7	19.3 ± 5.6	9.0 ± 1.8	20.4 ± 6.8	10.0 ± 2.8
p-value	0.008		0.00		0.00		0.039	

TOC in normal weight group showed a highly significant difference ($p \leq 0.001$) the higher level was (15.1 ± 2.5 ng/ml) in control subjects compared to (2.4 ± 1.4 ng/ml) in diabetic subjects. UcOC in the same group also showed a highly significant difference ($p \leq 0.001$) in it mean level, the higher level was in control subjects (2.7 ± 0.5 ng/ml) followed by diabetic patients (1.1 ± 0.6 ng/ml). In this group insulin showed highly significant level ($p \leq 0.001$) in diabetic subjects (20.0 ± 0.4 ng/ml) compared to (9.7 ± 0.7 ng/ml) control subjects.

In overweight BMI group, TOC was significantly higher ($p \leq 0.001$) in control subjects (14.8 ± 1.7 ng/ml) compared to (2.4 ± 1.4 ng/ml) diabetic subjects. UcOC was also significantly higher ($p \leq 0.001$) in control subjects (1.1 ± 0.6 ng/ml) compared to diabetic subjects (2.6 ± 0.4 ng/ml). On the other hand, insulin level showed highly significant difference ($p \leq 0.001$) in both groups the higher level was seen in diabetic subjects (19.3 ± 5.6 ng/ml) compared to control subjects (9.0 ± 1.8 ng/ml).

Results in Table 3 revealed that in the Obese group, the TOC showed significantly higher level ($p \leq 0.001$) in control subjects (14.6 ± 0.8 ng/ml) compared to (2.4 ± 1.5 ng /ml) in diabetic patients. In the same group ucOC level was significantly increased ($p \leq 0.001$) in control subjects (2.6 ± 0.56 ng/ml) compared to diabetic subjects (1.1 ± 0.6 ng/ml). In the obese insulin showed a significant difference ($p<0.05$) in it mean level between diabetic subjects and control subjects, the higher level was in diabetic subjects (20.4 ± 6.8 ng/ml) compared to control subjects (10.0 ± 2.8 ng/ml).

DISCUSSION

The current cross-sectional hospital based case-control study has revealed that serum TOC level was significantly low in diabetic patients compared to healthy control individuals. Several clinical studies have shown that serum TOC concentration was associated with glucose metabolism in human, [12] Rosato, et al., reported decreased levels of TOC in type II diabetic patients, [19] Zhou, et al., observed decreased TOC levels in diabetic patients compared to those

who has normal glucose tolerance test [15]. These data suggest that the changes in glucose metabolism could influence TOC levels [18]. An experimental animal study discovered that TOC could decrease blood glucose, stimulate insulin secretion, and increase insulin sensitivity [5]. Other previous clinical studies demonstrated that uncontrolled blood glucose could lead to lowering serum TOC level in diabetic patients, while serum TOC would increase after blood glucose is controlled [17]. Wang, et al., suggested that decreased serum TOC was related to hyperglycaemia and had some effect on insulin resistance [18].

Our results showed a negative correlation of TOC, ucOC with insulin and FBS, some studies carried on Caucasian and Asian populations showed that TOC level was independently correlated to FBS and fasting insulin [13,20-22]. In our study, the ucOC was low in diabetic subjects compared to control subjects and negatively correlated with insulin. This result is consistent with results of Sanchez-Enriquez, et al., who found lower levels of ucOC in diabetic patients than in control subjects [23]. Decreased TOC levels and the index ucOC/cOC are associated with increased FBS and insulin resistance and with risk for developing type II diabetes [21,22]. Both *in vivo* and *in vitro* previous studies demonstrated that it was probably the ucOC played a major role in decreasing blood sugars, enhancing insulin synthesis, and improving insulin resistance [5,9]. Takashi, et al., revealed that there is a stronger relationship between diabetes and skeletal metabolism through ucOC [24].

Diabetes is well known to affect bone integrity, because mature osteoblastic cells become weakened by abnormal glucose metabolism [25,26]. Thus, it is speculated that some humoral factors derived from bones, including ucOC, might stimulate β-cells for improving abnormal glucose metabolism. It is possible to consider that ucOC plays a crucial role in protecting bone degradation in disturbance of glucose metabolism by normalizing glucose metabolism, which is achieved by ucOC induced insulin secretion [24].

Certain studies have demonstrated that in hyperglycaemic, the osteoblast mass and function are decreased which suppresses osteocalcin synthesis and secretion [27,28]. Osteocalcin also has an effect on Blood sugar regulation. In line with results from experimental animal research, which showed that recombinant osteocalcin can enhance insulin secretion and β-cell proliferation [5,9]. Mice lacking osteocalcin gene develops a group of abnormalities such as reduced β-cell proliferation, low insulin secretion, insulin resistance and hyperglycaemia than wild-type mice [5]. Furthermore, administration of recombinant osteocalcin into wild-type mice increase pancreatic β-cell proliferation, insulin secretion and conserve them from weight gain and developing type II diabetes mellitus [9]. These results suggest that osteoblastic insulin signalling through osteocalcin can affect systemic glucose homeostasis [29].

Therefore, the association between TOC and glycaemic variability may be linked, in part, to the improvement in insulin secretion. Contrary, the improvement in insulin resistance may participate in the positive effect of osteocalcin on glycaemic control [30].

CONCLUSION

By estimating the circulating levels of TOC, ucOC, insulin and FBS in patients with type II diabetes, we found that the TOC and ucOC levels of patients were lower than those of the healthy control subjects. Fasting serum insulin was higher in patients compared to healthy control subjects. TOC and ucOC negatively correlate with Fasting serum insulin and FBS, therefore, TOC and ucOC may play an important role in regulating blood glucose and improving insulin secretion and insulin sensitivity. So, bones are not just a hard-calcified structure, but it may play an important role in controlling obesity, energy, and sugar metabolism.

DECLARATIONS

Ethical consideration

Written informed consent was obtained from diabetic patients and control subjects before entry into the study according to the guidelines of the Animal and Human Ethical Committee of Shendi University.

Conflict of interest

The authors and planners have disclosed no potential conflicts of interest, financial or otherwise.

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