

EVALUATION OF SERUM OSTEOCALCIN IN ELDERLY PATIENTS WITH TYPE-2 DIABETES MELLITUS

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Abstract

Osteocalcin, the second most abundant protein in bone tissue after collagen, is secreted by osteoblasts and thought to participate in mineralization and calcium ion homeostasis.

In addition to its use as biomarker in osteoporosis, experimental studies on mice published in *Cell journal* revealed for osteocalcin new metabolic functions, as hormone, being involved in blood glucose regulation, in insulin secretion, and in adipose tissue shaping. The aim of this study, conducted on 85 elderly patients (21 males and 64 females, aged 63-78 years) was to examine the relationship between serum osteocalcin concentration and classical biomarkers of investigation for glucose and lipid metabolism, using data from a clinical evaluation in elderly patients with impaired glucose tolerance and type-2 diabetes mellitus.

Rezumat

Osteocalcina este, alături de collagen, o proteină de importanță majoră a țesutului osos, fiind implicată, în principal, în procesul de mineralizare osoasă și în homeostazia calciului. Pe lângă utilizarea sa ca biomarker în osteoporoză, studii experimentale realizate pe șoareci și publicate în revista *Cell* în anul 2007, au relevat pentru osteocalcină noi funcții metabolice, de tip hormonal, ea fiind implicată în reglarea glucozei sangvine, în secreția de insulină, și în modelarea țesutului adipos. În lucrarea de față, realizată pe 85 de pacienți vârstnici cu toleranță scăzută la glucoză sau diabet zaharat de tip 2 (21 bărbați și 64 femei, cu vârsta cuprinsă între 63 și 78 de ani), ne-am propus cercetarea interrelațiilor existente între nivelurile plasmatiche de osteocalcină și markeri clasici de investigare ai metabolismului glucidic și lipidic.

Keywords : type-2 diabetes mellitus (DM2); elderly; osteocalcin (OST)

Introduction

Osteocalcin (OST), the second most abundant protein in bone tissue after collagen, is secreted by osteoblasts and thought to participate in mineralization and calcium ion homeostasis, although its exact role is unknown. This small Ca²⁺-binding protein, 49-aminoacids residues long in

humans, employs 3 γ -carboxyglutamic acid (Gla) residues for tight adsorption to hydroxyapatite mineral surfaces in bone matrix [1, 2]. The carboxylation of glutamic acid residues in osteocalcin molecule is a vitamin K-dependent process.

It has been routinely observed that higher serum-osteocalcin levels are relatively well correlated with increases in bone mineral density during the treatment for osteoporosis with anabolic bone formation drugs [3, 4]. Osteocalcin, is a cell-specific molecule, synthesized as a pre-pro-molecule and secreted in the general circulation [5].

In August 2007, Gerard Karsenty and his colleagues from Columbia University Medical Center have identified a surprising important novel function of osteocalcin, as a hormone involved in energy metabolism [6]. In this published research, authors show that osteocalcin deficiency in knockout mice leads to decreased insulin and adiponectin secretion, insulin resistance, higher serum glucose levels and increased adiposity. This was the first experimental evidence suggesting that skeleton may regulate energy metabolism.

Few clinical observations showing that serum osteocalcin levels are significantly lower in type 2 diabetic patients, and become normal following improvement of glycemic control, are also consistent with this idea [7, 8, 9]. Recent studies on postmenopausal women with diabetes mellitus showed that serum osteocalcin was found to be an independent factor associated with glucose and glycated hemoglobin (HbA1c) [10].

A better understanding of this new role of osteocalcin in energy metabolism would be helpful for the prevention and treatment of diabetes and obesity in humans.

The aim of this study was to examine the relationship between serum osteocalcin concentration and biomarkers of glucose and lipid metabolism, using data from a clinical evaluation in elderly patients with impaired glucose tolerance and type-2 diabetes mellitus.

Materials and methods

Patients

The study group consisted in 85 elderly patients (21 males and 64 females, mean age 70 ± 8 years) hospitalized in "Ana Aslan" National Institute of Gerontology and Geriatrics, Bucharest - Romania. During the researches we carried out, subjects were differently grouped depending on their serum levels of glucose, as follows: 30 patients with type-2 diabetes mellitus (DM2), 30 patients with impaired fasting glucose (IFG), and a control group of 25 healthy subjects. Subjects with DM2 were defined by

fasting serum glucose >126 mg/dl or by having taken any hypoglycemic treatment. Subjects with IGF were defined by fasting glucose levels of 110 to 125 mg/dl. Studied patients were not taking any bone-active medication, hormone replacement therapy or insulin treatment, and gave their informed consent prior to joining this study.

Biochemical parameters

Our study was performed on blood samples which were collected after overnight fast. Blood was centrifugated at 1450xg at 4 °C for 10 min (using a Hettich centrifuge, Germany). Serum was stored at -20°C and evaluation of the samples occurred within 3 months. Serum glucose and lipid metabolism parameters, such as total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol were measured by enzymatic methods adapted on Olympus 400 Autoanalyzer (Japan), using commercially available kits. Insulin levels were assessed by chemiluminescence immunoassay, on Immulite 1000 (Diagnostic Products Corporation, USA) with specific kit (LKIN-2500, Siemens). Insulin resistance was estimated using the homeostasis model assessment (HOMA-IR), with the formula: fasting insulin (mU/l) x fasting glucose (mg/dl)/405 [11]. Serum osteocalcin was evaluated by enzymatic immunoassay, with monoclonal antibodies anti-hOST that recognize the intact molecule (DRG-3375), on Chemwell 2010 ELISA system (Awarness Technology INC, USA).

Statistical analysis

All results are expressed as mean $\pm$ standard deviation (SD). Clinical characteristics of patients were compared among the three studied groups of subjects using one-way analysis of variance (ANOVA). Pearson correlation coefficients were calculated to evaluate the relationship between osteocalcin and metabolic parameters. For all comparisons, p values < 0.05 were considered as statistically significant.

Results and discussion

A number of 85 subjects (21 males and 64 females) aged 63-78 years participated in this study. The metabolic characteristics in control subjects and in subjects with glucose metabolic disorders – type 2 diabetes mellitus (DM2) and impaired glucose tolerance (IGT), are shown in table I.

Table I
Metabolic parameters in control group and in elderly patients with impaired fasting glucose (IFG) and type 2 diabetes mellitus (DM2)

Serum biochemical parameters	Control group (n = 25)	Impaired fasting glucose group (n = 30)	Type 2 diabetes mellitus group (n = 20)
Glucose metabolism – related parameters			
- Fasting glucose (mg/dl)	89.70±6.33	122.13±4.40**	189.20±47.25***
- Fasting insulin (μIU/ml)	3.82±1.58	9.64±5.27***	12.22±6.14***
- HOMA-IR	1.04±0.63	2.93±1.65*	6.07±4.11**
Lipid profile parameters			
- Total cholesterol (mg/dl)	202.91±27.17	210.93±47.83	227.36±36.12
- Triglycerides (mg/dl)	112.75±24.49	158.75±60.40	128.68±26.19
- LDL-cholesterol (mg/dl)	117.5±25.39	153.5±35.99	147.37±37.35
- HDL-cholesterol (mg/dl)	54.04±10.36	52.59±13.35	49.37±15.85
Osteocalcin (ng/ml)	10.17±4.02	4.35±2.58***	3.06±2.86***

* Significantly different from control patients: * p<0.05; ** p<0.01; ***p<0.001

Serum osteocalcin levels measured in the groups of patients with DM2 and IGT were significantly lower than the levels found in healthy control subjects (p<0.001).

Significant correlation between serum osteocalcin and glucose metabolism- related parameters were pointed out for the studied patients. As shown in figures 1 and 2, simple linear regression analysis demonstrated that osteocalcin was inversely correlated with fasting serum glucose (r = - 0.587, p< 0.001) and HOMA-IR (r = - 0.511, p < 0.001).

The type 2 diabetes mellitus and impaired fasting glucose on elderly patients were characterized by a significant hyperinsulinemia, which represents a good marker of insulin resistance.

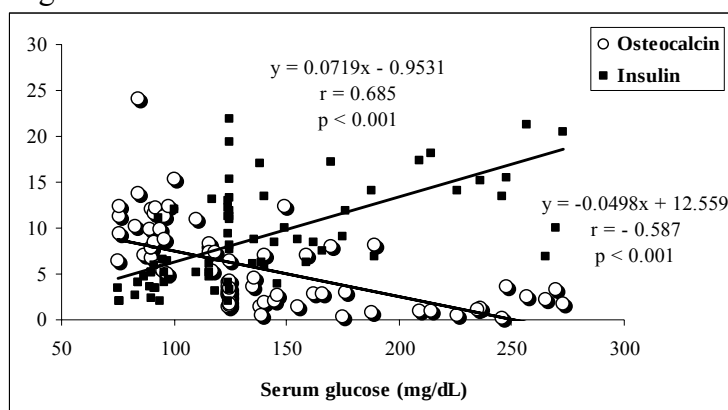
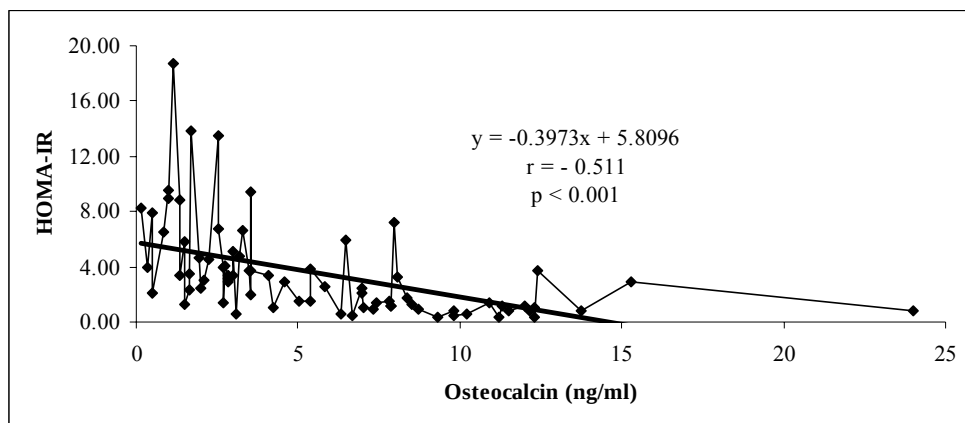


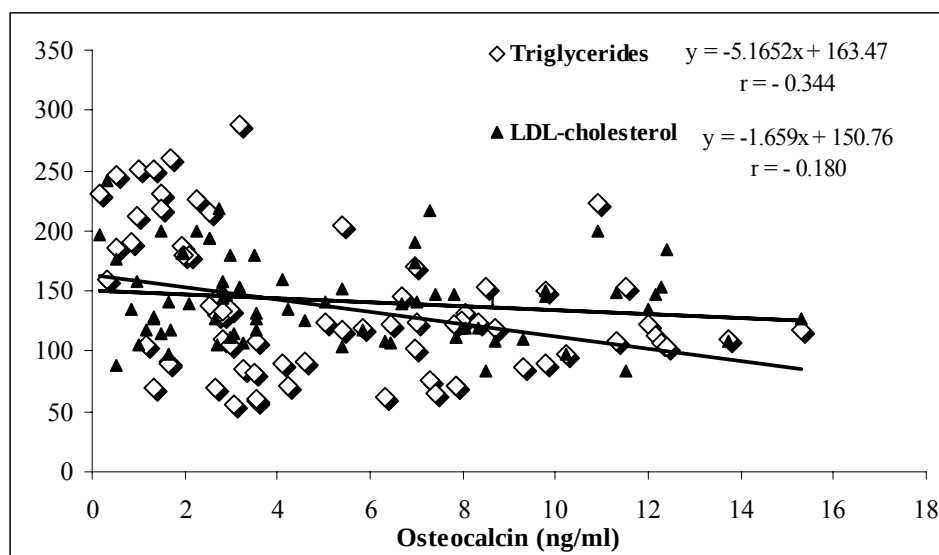
Figure 1

Relationship between fasting serum glucose, osteocalcin and insulin levels in a group of 85 elderly patients with normo- and hyperglycemia

**Figure 2**

Correlation between serum osteocalcin and insulin resistance estimated by the homeostasis model assessment of insulin resistance index (HOMA-IR), in a group of 85 elderly patients with normo- and hyperglycemia

However, no correlations between osteocalcin and lipid profile parameters, such as total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol, were found (figure 3).

**Figure 3**

Correlations between serum osteocalcin and lipid profile parameters – triglycerides and LDL-cholesterol in 85 elderly patients with normo- and hyperglycemia

These results suggest that osteocalcin may be involved in the glycemic homeostasis and are in agreement with previous studies [7, 8, 9].

Our data extrapolation is however limited by the fact that the patients' hypoglycemic therapy was not taken into consideration [12,13, 14,15]. Recent papers reported that osteocalcin levels are also negatively correlated with HbA1c in diabetes patients [10].

On the other hand, the endocrine and metabolic alteration in diabetes mellitus can trigger disorders of calcium homeostasis, skeletal metabolism and bone mass. However, recent findings suggest that although osteocalcin levels are lower and bone formation is decreased in type-2 diabetes, diabetic patients are not susceptible to bone resorption [9].

Osteocalcin has long been accepted as an osteoblast-specific product. The majority of osteocalcin secreted by the osteoblast is deposited in extracellular bone matrix; serum osteocalcin represents the fraction of total osteocalcin that has not been absorbed to hydroxyapatite [12]. It is postulated that newly synthesized osteocalcin is released into circulation as intact molecule.

Serum osteocalcin values are higher in children than in adults, rising during puberty and decreasing to adult levels. Most studies show a higher concentration of osteocalcin in adult males than in adult females, but there are no data available regarding the levels in osteocalcin during human ageing [12,16].

Lee et al. (2007) showed that diabetes mice without osteocalcin gene expression have a decreased beta-cell mass while mice engineered with an increase of active osteocalcin have increased beta-cell proliferation and increased insulin levels [6]. These studies provided a proof-of-principle that osteocalcin could be an important regulator of beta-cell mass and glucose homeostasis *in vivo*. The fact that the gene encoding osteocalcin is included in a region of the genome containing a determinant for type 1 diabetes (region Idd17) further suggests that this newly discovered factor might be important for understanding this disease.

These results raise new questions: why would a bone-specific hormone regulate energy metabolism, and what is the need for a hormone favoring β -cell proliferation and insulin secretion? In both cases answers can only be speculated. For the first question, given the large surface it covered, skeleton is an excellent site of hormone synthesis. For the second question, it is conceivable that the proliferation function of osteoblast-secreted hormones may have been required during evolution to maintain the size of the islets constant in periods of food deprivation [6].

Conclusions

The significance of this study is that serum osteocalcin levels are strongly correlated with pancreatic β -secretory function. This new relationship may help in the future to discover novel approaches for the treatment of type-2 diabetes in elderly patients. Further studies are necessary in order to find the causal effect of the osteocalcin on glucose and lipid metabolism in humans.

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