2013 © Curr. Issues Pharm. Med. Sci. Vol. 26, No. 3, Pages 309–312

Current Issues in Pharmacy and Medical Sciences

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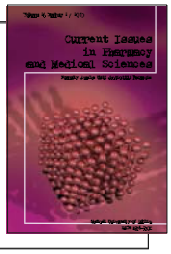

Could osteoprotegerin serve as a marker of metabolic syndrome?

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ABSTRACT

There has been a dramatic increase in the worldwide prevalence of obesity, which is associated with the development of several chronic diseases such as metabolic syndrome, type 2 diabetes and cardiovascular diseases. Osteoprotegerin is a glycoprotein mainly secreted by bone but produced also by heart muscle and blood vessels. It inhibits the recruitment, proliferation, and activation of osteoclasts. The role of osteoprotegerin in the pathogenesis of metabolic syndrome, type 2 diabetes and cardiovascular diseases is still discussed. The study was carried out on 62 patients with metabolic syndrome aged 35-83 (34F and 28M). Type 2 diabetes was diagnosed in 76% of subjects and 62% of them suffered from coronary artery disease as a macrovascular complication. Determinations of biochemical parameters and anthropometric measurements were performed in the studied group. The relationships between serum osteoprotegerin concentrations and components of metabolic syndrome and total cholesterol, LDL-cholesterol, HbA1C, BMI, levels of calcium and phosphate in the blood and 24-hour urinary calcium have been analysed. Diabetics had higher osteoprotegerin concentrations than patients without diabetes (5.570 pmol/l vs 4.690 pmol/l). Osteoprotegerin levels in patients with diabetes and coronary artery disease were significantly higher (6.640pmol/l) than in those without macrovascular complications (5.295 pmol/l) (Z=1.986; p=0.047). Furthermore, the associations between osteoprotegerin and calcium and phosphate levels in the blood and 24-hour urinary calcium have been shown. A lower calcium level in the blood was negative but a lower phosphate level was positive correlated with OPG serum concentration (respectively: 6.825 pmol/l vs 5.195 pmol/l, Z=2.656, p=0.008; 4.250pmol/l vs 5.640 pmol/l, Z=2.718, p=0.007). What's more, the inverse correlations between OPG concentrations and 24-hour urinary calcium and diastolic blood pressure have been observed. No associations between osteoprotegerin and waist circumference, BMI, cholesterol levels and HbA1C, were found. In summary, osteoprotegerin is not a useful marker of all components of metabolic syndrome. It is level depends on the presence of hypertension, type 2 diabetes and coronary artery disease. This glycoprotein may serve a a marker of calcium and phosphate homeostasis. We concluded that the relationship between osteoprotegerin concentrations and calcification of atherosclerotic plaques in patients with metabolic syndrome and type 2 diabetes should be analysed in further investigations.

Keywords: osteoprotegerin, metabolic syndrome, type 2 diabetes, calcium, phosphate, atherosclerosis

INTRODUCTION

There has been a dramatic increase in the worldwide prevalence of obesity [12]. It is estimated that about 34% of females and 30% of males are obese in Poland [9]. Apart from this, obesity is associated with the development of several chronic diseases such as metabolic syndrome (MetS), type 2 diabetes mellitus and cardiovascular diseases [12]. The consensus definition of MetS is coexistence 3 of 5 following criteria: central obesity, raised fasting plasma glucose or previously diagnosed type 2 diabetes, raised triglycerides or specific treatement of this lipid abnormality, reduced HDL-cholesterol or specific treatement of this disturbance and raised blood pressure or treatement of hypertension [14].

Osteoprotegerin (OPG), known also as osteoclastogenesis inhibitory factor (OCIF), is a glycoprotein which was first reported in rats by W.S. Simonet in 1997 as a protein involved in the regulation of bone density [4,6,7]. OPG has a molecular weight of 60 kDa as a monomer and

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DOI: 10.12923/j.2084-980X/26.3/a.15

120 kDa as a disulfide-linked dimer [15]. It belongs to the tumor necrosis factor receptor superfamily (TNFR) [7,10,12]. OPG hampers the binding of RANK to RANKL and thus inhibits the recruitment, proliferation and activation of osteoclasts [3,8,15]. This glycoprotein is mainly secreted by bone (osteoblasts) but is produced by variety of different tissues including heart muscle and vessels as well [3,7]. The role of OPG in the pathogenesis of metabolic syndrome, type 2 diabetes and cardiovascular diseases is still studied [12].

The aim of this study was to analyse relationships in humans between serum osteoprotegerin concentrations and the components of metabolic syndrome and total cholesterol, LDL-cholesterol, HbA1C, BMI, levels of: calcium and phosphate in the blood and 24-hour urinary calcium as well.

MATERIAL AND METHODS

The study was carried out on 62 patients with metabolic syndrome aged 35-83, who were treated at the Endocrinology Department and in the Outpatient Clinic of the Independent Public Clinical Hospital No. 4 in Lublin. Among participants, there were 34 women (55%) and 28 men (45%). Type 2 diabetes was diagnosed in 76% of subjects and 62% of them suffered from coronary artery disease as a macrovascular complication. The duration of diabetes mellitus was estimated to be from newly diagnosed disease to about 30 years. In the analysed group determinations of biochemical parameters and anthropometric measurements were performed. Correlations between serum osteoprotegerin concentrations and components of MetS such as waist circumference (WC), fasting glucose, HDL-cholesterol and triglycerides levels, blood pressure and HbA1C, BMI, total cholesterol and LDL-cholesterol levels, have been studied. Moreover, an association between OPG concentration and levels of calcium and phosphate in the blood and 24-hour urinary calcium have been analysed as well. The material was the peripheral blood obtained from the ulnar vein (10 ml). Serum was separated from the collected blood samples by centrifugation in a standard way. Serum osteoprotegerin concentration was determined with the use of an enzyme immunoassay with sensivity of 0.4 pmol/l (MicroVue™ OPG EIA). For statistical analysis of the obtained results, Statistica 8.0 StatSoft was used. Distributions of the studied variables were tested using the W Shapiro-Wilk test, the Kolmogorov-Smirnov test, and the Lilliefors test. The Brown-Forsythe test was applied to check the equality of group variances. Lack of the normal distribution and/or the equality of variances were noted. For a comparison of the obtained results, the non-parametric tests U Mann-Whitney or Kruskal-Wallis were used. Correlations between variables were investigated by Spearman's test. A p value <0.05 was considered as statistically significant in all analyses.

RESULTS

The results of serum osteoprotegerin concentration (pmol/l) and selected parameters of the analysed group have been shown in Table1.

In diabetics serum OPG level was significantly higher (median= 5.570 pmol/l) than in patients without type 2 diabetes (median= 4.690 pmol/l) (Z=2.351; p=0.018). In addition, patients with coexistence of diabetes and coronary artery disease had higher serum OPG concentration (median= 6.640 pmol/l) than subjects without macrovascular complications (median= 5.295 pmol/l) (Z=1.986; p=0.047). To analyse associations between electrolytes and OPG, the studied subjects were divided into 2 groups based on the concentrations of calcium and phosphate in the serum (calcium groups: 9.0-9.5 mg/dl and 9.6-10.7 mg/dl; phosphate groups: 2.4-3.4 mg/dl and 3.5-5.3 mg/dl). A lower calcium level in the blood was negative but a lower phosphate level was positively correlated with OPG serum concentration (respectively: 6.825 pmol/l vs 5.195 pmol/l, Z=2.656, p=0.008; 4.250 pmol/l vs 5.640 pmol/l, Z=2.718, p=0.007). Furthermore, the inverse correlations between OPG concentration and calcium level in the blood, 24-hour urinary calcium and diastolic blood pressure have

Table 1. Serum levels of osteoprotegerin (pmol/l) and selected parameters in the studied group (n=62)

PARAMETERS	X ± SD	MEDIAN	MINIMUM	MAXIMUM
Osteoprotegerin (pmol/l)	6.127 ± 2.450	5.390	0.680	16.00
BMI (kg/m2)	33.821 ± 5.481	32.420	19.500	47.00
Waist circumference (cm)	F: 109.940 ± 12.340 M: 110.270 ± 9.210	F: 110.000 M: 109.500	F: 83.000 M: 100.000	F: 136.000 M: 134.000
HbA1C (%)	7.728 ± 2.153	6.755	5.400	14.000
Fasting plasma glucose (mg/dl)	132.855 ± 41.747	123.500	86.000	300.00
Systolic blood pressure (mmHg)	135.710 ± 17.209	130.000	110.000	180.000
Diastolic blood pressure (mmHg)	84.081 ± 11.409	80.5000	60.000	110.000
Triglycerides (mg/dl)	145.145 ± 90.252	119.500	47.000	532.000
HDL – cholesterol (mg/dl)	F: 56.410 ± 14.940 M: 45.290 ± 10.190	F: 52.500 M: 43.500	F: 36.000 M: 28.000	F: 97.000 M: 68.000
LDL – cholesterol (mg/dl)	118.677 ± 41.031	114.000	52.000	213.000
Total cholesterol (mg/dl)	198.823 ± 50.382	191.000	120.000	330.000
Calcium in the blood (mg/dl)	9.652 ± 0.394	9.600	9.00	10.70
Phosphate in the blood (mg/dl)	3.519 ± 0.541	3.500	2.400	5.300
24-hour urinary calcium (mg/day)	104.661 ± 78.643	79.00	6.00	336.00

been observed. It has been presented in Table 2. Patients with higher diastolic blood pressure had lower concentration of OPG in the serum (Fig. 1). Subjects with lower calcium level in the blood, similarly to those with lower level of 24-hour urinary calcium, had higher OPG concentration in the serum (Fig. 2 and Fig. 3). Based on statistical analysis, in studied group, no associations between OPG and other components of MetS such as: WC, triglycerides, HDL-cholesterol and total cholesterol, LDL-cholesterol, HbA1C, BMI as well have been found.

Table 2. The correlations between osteoprotegerin and selected parameters in analysed patients

VARIABLES	R Spearman	t(N-2)	p
Diastolic blood pressure	-0.263	-2.112	0.039
Calcium in the blood	-0.390	-3.277	0.002
Urinary calcium	-0.337	-2.768	0.007

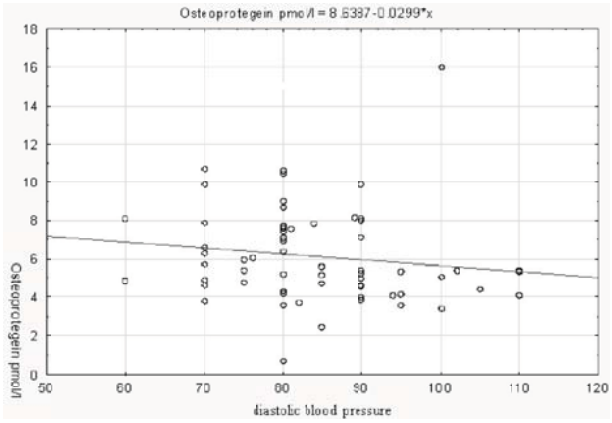

Fig. 1. The correlation between osteoprotegerin and diastolic blood pressure in patients with MetS

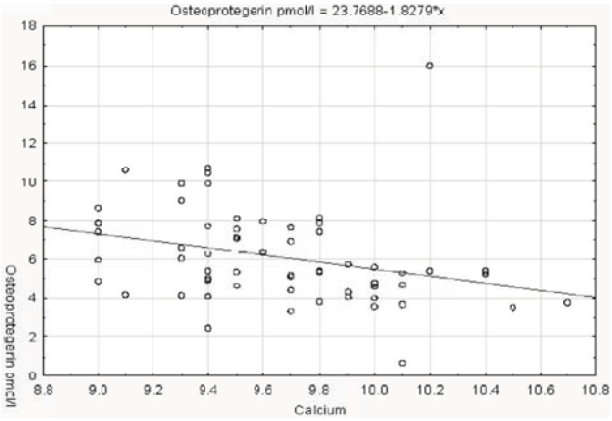

Fig. 2. The correlation between osteoprotegerin and serum calcium level in patients with MetS

DISCUSSION

Osteoprotegerin is a part of OPG/RANK/RANKL biochemical pathway which regulates osteoclasts differentiation and activation [3,7,15]. This glycoprotein plays a role as a soluble receptor for RANKL (*Receptor Activator of Nuclear Factor kappa B ligand)*, produced by osteoblasts and their precursors, and thus blocks binding

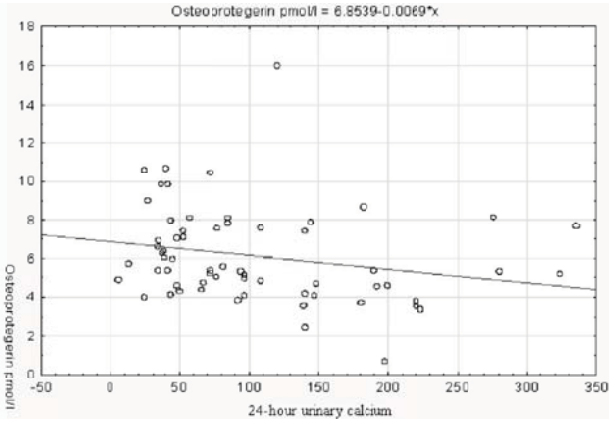

Fig. 3. The correlation between serum osteoprotegerin and 24-hour urinary calcium level in patients with MetS

RANKL to RANK (*Receptor Activator of Nuclear Factor kappa B)*, situated on osteoclasts surface, and leads to inhibition of osteoclasts formation, fusion, differentiation and activation [7,10,13]. In humans, the gene for osteoprotegerin is localized on chromosome 8q23,24 [15].

Some authors reported no statistical difference of OPG values between subjects with or without MetS [12]. On the other hand, in a cohort of patients with peripheral artery disease, serum concentrations of OPG were elevated in those individuals with obesity and the MetS [10,12]. It is known that osteoprotegerin level is directly associated with bone mass and disturbances concerned with metabolic syndrome such as endothelial dysfunction and atherosclerosis [3,12]. In our study, we tried to answer if OPG would be a marker of MetS and its components.

Nabipour et al. [10] and Park et al. [11] reported a positive correlation between serum OPG level and fasting plasma glucose. What's more, higher serum OPG levels were independently associated with type 2 diabetes [10]. The relationships between OPG and HbA1C, fasting plasma glucose and diabetes have been also described by Kiechl et al. [6]. Nabipour et al. [10] also suggested that inflammation-driven hypergylcemia, rather than high glucose levels per se, is involved in the increase of OPG observed in diabetes. These findings are similar to our results because in our patients with type 2 diabetes OPG concentrations were significantly higher than in non-diabetic subjects. However, we did not find any connection between OPG and HbA1C.

It has been thought that OPG inhibits vascular calcification [3,7,10]. Animal studies have shown that osteoprotegerin knockout mice demonstrated osteoporosis and vascular calcifications of the same type as commonly seen in diabetes [4,7,8]. Paradoxically, clinical studies indicate that in humans elevated serum OPG level is connected not only with diabetes mellitus but also with coronary artery disease, future cardiovascular events and stroke [10,14]. Recent observational and cross-sectional studies confirmed a positive correlation between serum OPG and the severity and pro-

gression of coronary artery disease, atherosclerosis and vascular calcification in various patient cohorts [2,11,13]. Increase of OPG has been associated with higher mortality in diabetes, coronary artery disease, acute coronary syndrome and silent myocardial ischemia as well [5,8]. In our study, we observed that diabetics with coronary artery disease had higher serum OPG concentration than subjects without macrovascular complications which is consistent with mentioned reports. Owing to the fact that the protective role of OPG in atherosclerotic plaque formation is assumed, it has been thought that elevated level of osteoprotegerin in cardiovascular diseases is associated with impairment of compensatory mechanism [6,8,10]. What's more, an inflammation which usually coexists with cardiovascular diseases, may induce OPG secretion because pro-inflammatory mediators enhance OPG expression and production in vascular cells [5,7]. Some authors suggested that OPG is an independent risk factor for the progression of atherosclerosis and onset of cardiovascular disease and may predict cardiovascular events including a silent myocardial ischemia in asymptomatic diabetic patients [1,6,11].

It is well known that OPG regulates bone remodeling and thus may play a role in calcium and phosphate homeostasis [3,7]. In our analysed group of patients, the inverse correlations between OPG concentrations and calcium level in the blood and 24-hour urinary calcium have been observed. Moreover, subjects with a lower phosphate level in the serum had lower concentration of OPG. Further investigations should be conducted to clarify the association between levels of OPG, calcium, phosphate and calcification of atherosclerotic plaques in patients with MetS and type 2 diabetes.

The relationships between OPG concentrations and other components of metabolic syndrome such as WC, blood pressure, triglycerides and HDL-cholesterol level have been analysed in many studies [6,7,10]. Nabipour et al. [10] reported any association between OPG and these components in postmenopausal women. Similar findings have been shown in study conducted by Kiechl et al. [6]. In contrast to them, there are some clinical studies, which indicate positive correlations between OPG concentration and cholesterol level, blood pressure and WC [7]. Our findings are consistent with reports of Nabipour et al. and Kiechl et al. [6,10], except for blood pressure because we observed the inverse correlation between OPG concentration and diastolic blood pressure in patients with MetS. In accordance with other reaserch [6,10], any relationships between OPG, BMI and LDL-cholesterol level have been shown in our study.

CONCLUSIONS

To sum up, OPG is not a useful marker of all components of MetS. Many studies confirm that its level depends on presence of hypertension, type 2 diabetes and coronary artery disease. This glycoprotein may serve as a marker of calcium and phosphate homeostasis. We concluded that an association between OPG concentration and calcification of atherosclerotic plaques in patients with MetS and type 2 diabetes should be analysed in further investigations. The limitations of our study include a small group of patients and the lack of a control group, however our findings are consistent with some reports. Additional studies are required to clarify the relationships between OPG and components of MetS because there are only few reports conducted in such patients.

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