HIGH OSTEOPROTEGERIN SERUM LEVELS IN NEWLY-DIAGNOSED TYPE 2 DIABETIC MALES WITHOUT KNOWN CORONARY ARTERY DISEASE

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ABSTRACT

PURPOSES: Osteoprotegerin (OPG) is an inhibitor of osteoclastogenesis, but is produced from vasculature, too. There is recent evidence of increased circulating OPG levels in patients with diabetes as well as in patients with coronary artery disease (CAD). Up to date, there are no sufficient data about OPG concentrations in newly-diagnosed type 2 diabetes mellitus (nT2DM) patients. The aim of our study was to determine the serum OPG levels in males with nT2DM without known concomitant CAD and to investigate the association of OPG with intima-media thickness (IMT) of common carotid arteries and glucometabolic parameters.

MATERIAL AND METHODS: Serum OPG levels were measured in 31 nT2DM males and 15 age- and body mass index (BMI)-matched non-diabetic male subjects. IMT of common carotid arteries was measured by a 7,5-MHz B-mode ultrasonography. OPG was estimated by ELISA (BioMedica) in pmol/L.

RESULTS: OPG was significantly higher in nT2DM patients when compared to controls (4,59±0,28 versus 3,20±0,30 pmol/L; p=0,004). In the whole group of subjects, there was a positive correlation of OPG levels with glucose parameters: fasting plasma glucose (FPG) (r=0,38; p=0,01), 2-hour post-challenge glucose (r=0,45; p=0,003) and HbA1c (r=0,46; p=0,002). Moreover, OPG correlated significantly with carotid IMT (Pr 0,40; p=0,01).

CONCLUSION: Serum OPG is significantly elevated in nT2DM males without known CAD compared to non-diabetic controls. OPG levels show associations not only with some glucose indices but also with IMT, one of the earliest atherosclerotic markers. Probably, these glucose indices and this vascular parameter are involved in OPG regulation. We could suggest that OPG rises early in the evolution of diabetic disorders. However, further investigations are needed.

Key words: *osteoprotegerin, newly-diagnosed type 2 diabetes mellitus, post-challenge glycemia, coronary artery disease, intima-media thickness*

INTRODUCTION

Osteoprotegerin (OPG) is an inhibitor of osteoclastogenesis, but is produced from vasculature, too. OPG is a secreted member of the tumour necrosis factor (TNF) receptor superfamily and acts as a strong anti-resorptive factor through binding and neutralizing the receptor activator for NF-kB ligand (RANKL) (15). OPG is also present in the arterial wall and elevated plasma OPG is suggested to reflect the increased OPG content in atherosclerotic arterial

tissues (11). OPG is a promising biomarker of atherosclerosis (10). There is recent evidence of increased circulating OPG levels in patients with diabetes as well as in patients with coronary artery disease (CAD). OPG is associated with CAD severity and cardiovascular events independently of conventional risk factors (6,7). Moreover, the higher OPG level is an independent predictive factor in patients with intermediate coronary lesions (16). In type 2 diabetes mellitus (T2DM) patients, elevated serum OPG appears to be a powerful and independent predictor for cardiovascular morbidity and mortality during 17 years of follow-up (13). Notably, plasma OPG levels predict cardiovascular events in uncomplicated T2DM patients (2), too. Recent studies report that intima-media thickness (IMT) of common carotid artery, a well-known early atherosclerotic marker, is positively correlated to circulating OPG levels in diabetic patients (5,12). OPG is associated with the glycemic control, endothelial dysfunction and cardiovascular risk in T2DM (1,4). Up to date, there are no sufficient data about OPG concentrations in newly-diagnosed T2DM (nT2DM) patients.

The aim of our study was to determine the serum OPG levels in males with nT2DM without known concomitant CAD and to investigate the association of OPG with IMT and glucometabolic parameters.

MATERIAL AND METHODS

Serum OPG levels were measured in 31 nT2DM males and 15 age- and body mass index (BMI)-matched non-diabetic male subjects (controls). The patients with nT2DM were screen-detected by oral glucose tolerance test (OGTT) among risk individuals and had no history of CAD. IMT of common carotid arteries (CCAs) was measured by a 7,5-MHz B-mode ultrasonography (Fokuda) prior to the determination of OPG and glucometabolic parameters. Three manual measurements of each CCA were performed over 1-cm span ending 1 cm proximally to the transition between the CCA and bulb region. The distance between lumen-intima borderline and media-adventitia one on the far wall was measured. Mean IMT was calculated from the measurements on both sides. OPG was estimated by ELISA (BioMedica) in pmol/L according to the protocol of

manufacture. Data regarding risk factors, medical history and concomitant medications were collected. Systolic and diastolic blood pressure was measured three times on each arm. Anthropometric measurements included height, weight, waist circumference and BMI calculation. Venous plasma was centrifuged immediately after blood collection and glucose concentrations were determined by hexokinase method. Fasting plasma glucose (FPG) and 2-hour post-challenge glucose were measured. Glycated hemoglobin (HbA1c) was measured by immunoassay for the quantitative determination of percentage hemoglobin A1c in whole blood samples on the AxSYM System (Abbott, USA). Serum levels of triglycerides (TG), total cholesterol (TC), LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) were determined enzymatically. Fasting serum insulin was assessed by microparticle enzyme immunoassay (Abbott, USA). Insulin resistance was calculated using homeostasis model assessment of insulin resistance (HOMA-IR). Statistical analysis was done using GraphPad Prism version 5 and included calculation of correlations. Data are expressed as means±SD. Value of $p < 0,05$ was considered statistically significant. The study was approved by the Ethical Commission of the Medical University of Varna.

RESULTS

Table 1 shows the baseline characteristics of the study participants.

OPG is significantly higher in nT2DM patients when compared to controls (4,59±0,28 versus 3,20±0,30 pmol/L; $p=0,004$) (Fig. 1). In the whole group of subjects, there is a positive correlation of OPG levels with glucose parameters: FPG (r=0,38; p=0,01), 120-min. post-OGTT glucose (r=0,45; p=0,003) and HbA1c (r=0,46; p=0,002). Moreover, OPG correlates significantly with carotid IMT (Pr 0,40; p=0,01).

There are no correlations between OPG and fasting insulin, HOMA-IR, systolic and diastolic blood pressure, BMI, or waist circumference. When lipid profile parameters are concerned, OPG correlates positively with HDL-C (r=0,43; p=0,049) in nT2DM patients only.

Table 1. Characteristics of study participants

|	nT2DM (n=31)	Controls (n=15)	p
age (years)	53,2±7,8	53,8±7,1	0,8
BMI (kg/m^2)	33,0±3,9	31,0±3,0	0,1
waist circumference (cm)	111,5±10,9	107,0±9,0	0,2
FPG (mmol/L)	10,7±3,1	5,8±0,5	<0,0001
2-h plasma glucose (mmol/L)	14,96±5,43	5,68±1,45	<0,0001
HbA1c (%)	7,77±1,73	5,43±0,40	<0,0001
TC (mmol/L)	6,14±1,59	6,82±1,50	0,3
TG (mmol/L)	2,88±2,57	2,45±1,06	0,6
LDL-C (mmol/L)	3,06±1,01	4,60±1,22	0,002
HDL-C (mmol/L)	1,13±0,29	1,12±0,24	0,9
IMT (mm)	0,7±0,1	0,6±0,1	0,02
fasting serum insilin (µU/ml)	15,6±11,1	11,0±5,1	0,2

Data are expressed as mean±SD
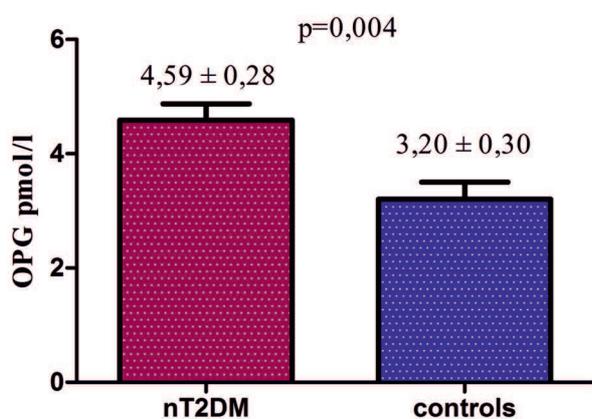
Fig. 1. Mean OPG serum levels in subjects with nT2DM and controls

DISCUSSION

We established higher serum OPG levels in nT-2DM patients without any history of CAD than in healthy controls. Although several studies indicate that serum OPG is significantly increased in T2DM (8,9,14), scanty data are available about serum OPG, especially in nT2DM patients without known CAD. We proved positive correlations between OPG and several parameters of glucose metabolism such as FPG, 2-h post-challenge glucose and A1c. That is why we could speculate that they may be involved in OPG regulation. An elevated serum OPG was demonstrated in recently diagnosed T2DM patients (within 2 years) as well as soon after diabetic induction in animal models (14). A positive correlation between OPG and glycemic levels was established, too (14).

On the other hand, we revealed a positive association of OPG with IMT, a well-known early atherosclerotic marker. In the diabetic population, OPG was associated with silent CAD (3) and predicted cardiovascular events in uncomplicated T2DM patients (2). Although the exact role of OPG in vasculature is unknown, the paradoxical OPG increase has been interpreted as a counter-regulatory protective response to atherosclerosis.

CONCLUSION

Serum OPG is significantly elevated in nT-2DM males without known CAD compared to non-diabetic controls. OPG levels show associations not only with some glucose indices but also with IMT, one of the earliest atherosclerotic markers. Probably, these glucose indices and this vascular parameter are involved in OPG regulation. We could suggest that

OPG rises early in the evolution of diabetic disorders. However, further investigations are needed.

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