RESEARCH ARTICLE

The Relationship of Osteoprotegerin, Matrix Gla Protein, and HbA1C in Controlled and Uncontrolled Type 2 Diabetes Mellitus Patients

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Abstract

BACKGROUND: Many studies have reported that diabetes mellitus correlates with vascular calcification event that increases progressively in uncontrolled diabetes. Osteoprotegerin (OPG) is known to act as a promoter in vascular calcification, contrary to Matrix Gla Protein (MGP), which is an inhibitor in vascular calcification. The aim of this study was to observe the progress of vascular calcification in uncontrolled diabetes patients by assessing biochemical markers OPG as promoter and MGP as inhibitor in vascular calcification.

METHODS: This was an observational study with cross sectional design on adult male patients with type 2 diabetes mellitus, defined by DM Consensus Criteria Indonesia, 2006.

RESULTS: The results of this study showed that there was a positive significant correlation between OPG and HbA1c (r=0.261, p=0.030), in contrast with MGP that showed no significant correlation with HbA1c. OPG also correlated significantly with Fasting Plasma Glucose (r=0.261, p=0.014). In uncontrolled diabetes group there was positive significant correlation between OPG and HbA1c (r=0.397, p=0.014). There was no significant difference found in the levels of OPG in controlled and uncontrolled diabetes groups (p=0.567), but OPG/MGP index showed higher difference (p=0.259). The OPG/MGP index also had positive significant correlation with HbA1c (r=0.285, p=0.018) and Fasting Plasma Glucose (r=0.313, p=0.009).

CONCLUSIONS: This study suggested progress to vascular calcification in uncontrolled type 2 diabetes mellitus. The use of vascular calcification biomarkers are recommended to predict /detect vascular calcification event in type 2 diabetes mellitus patients.

KEYWORDS: Type 2 diabetes mellitus, vascular calcification, OPG, MGP, HbA1c.

Introduction

According to a WHO survey, Indonesia ranks fourth of all countries in the world in the number of patients with diabetes mellitus (DM) with prevalence of about 8.6% of the total population. Data from the Ministry of Health indicate that the number of inpatients and outpatients with diabetes in hospitals rank first among all endocrine diseases. Worldwide, the incidence of type 2 diabetes is increasing rapidly. In Indonesia, WHO estimates the increasing number of people with diabetes i.e. 8.4 million in 2000 to around 21.3 million in 2030 (1).

Two main subtypes of diabetes are type 1 and type 2 diabetes. Type 2 diabetes comprises about 80 to 90% of all cases (2). Diabetic patients may suffer a number

of debilitating complications such as retinopathy, nephropathy, neuropathy, and atherosclerosis resulting in cardiovascular, cerebrovascular, or peripheral vascular disease. These diabetic complications cause huge economic and psychosocial consequences (3). Diabetes mellitus, particularly type 2 diabetes mellitus, is associated with a markedly increased risk of cardiovascular events mainly due to premature and extensive atherosclerosis (2). Atherosclerotic disease is characterized by accumulation of lipid material in the arterial wall resulting from autoimmune and inflammatory mechanisms. More than 90% of the fatty plaques undergo calcification. Some studies have demonstrated a direct relationship between the degree of calcification of the atherosclerotic plaque and mortality due to cardiovascular events (4).

Vascular calcification, long thought as the result of passive degeneration, actually involves a complex, regulated process of biomineralization resembling osteogenesis. Evidence has indicated that proteins controlling bone mineralization are also involved in the regulation of vascular calcification. Vascular calcification is exacerbated in certain clinical entities, including diabetes mellitus, menopause, and osteoporosis (5). In patients with diabetes mellitus, arterial medial calcification is associated with an increased risk of cardiovascular complications (6). In a sub-sample of the population-based Framingham Offspring Study, subjects with insulin resistance or IFG/IGT and type 2 diabetes mellitus were found to have an increased burden of coronary artery subclinical atherosclerosis, as shown by using Electron-Beam Computed Tomography (7).

Recent data have suggested arterial calcification occurs as the result of an active, regulated process that involves inhibitors and promoters of vascular calcification. This idea is based partly on findings of high expression of bone-related macromolecules, such as alkaline phosphatase (ALP), bone sialoprotein (BSP), bone Gla protein (8) and osteoprotegerin (OPG) (9) as promoters of calcification in calcified areas of the arterial wall. Furthermore, direct evidence comes from gene knockout experiments of matrix-Gla protein (MGP) (the inhibitor of vascular calcification) and osteopontin-lacking mice, which develop extensive vascular calcification (8). OPG and MGP are promoter and inhibitor of vascular calcification involved in the development of diabetes mellitus complication, especially uncontrolled diabetes. This contradiction inspired us to study the differences in vascular calcification events in both controlled and uncontrolled type 2 diabetes groups. The aim of this study was to observe the progress of vascular calcification in uncontrolled diabetes mellitus patients by means of biochemical markers OPG as promoter and MGP as inhibitor for vascular calcification among Indonesian males with type 2 diabetes mellitus.

Methods

Our study protocol was approved for ethical clearance by the institutional review board of the Health Research Ethics Committee Faculty of Medicine, University of Hasanuddin, Makassar, Indonesia. All study participants provided written informed consent.

Sixty nine (69) type 2 diabetes mellitus (T2DM) male patients were enrolled in this cross sectional study. For gathering the baseline data, each participant completed a self administered questionnaires covering medical history, exercise, treatment for hypertension or diabetes, smoking habits, and alcohol intake. The questionnaires were checked by the researcher at the screening phase. Subjects who were consuming steroid or had been treated with anti-inflammatory drugs such as statins in the last 3 weeks; or had liver dysfunction, kidney dysfunction, fever and other acute inflammation were excluded from the study. Each subject was given explanation about the study and asked to sign informed consent prior to the commencement of the study.

All subjects were assessed after overnight fasting for a minimum of 10 hours. The parameters of anthropometric measurements (height, weight, body mass index (BMI), waist circumference, and blood pressure) and biochemical variables (fasting blood glucose, creatinine, SGOT, SGPT, HbA1c, OPG, MGP were measured in all subjects. Fasting serum samples were obtained and frozen at -20°C.

Assay of Biochemical Markers

Serum handling

Blood specimens were collected by venipuncture in serum tubes (10 ml; BD Vacutainer Systems) and in sodium citrate (10 ml; BD Vacutainer Systems) and stored for 20 minutes at room temperature before centrifugation. Serum and plasma were sub sampled in aliquots and frozen at –20° C until tested.

Serum MGP concentrations were quantified with the kit from Biomedica (Vienna, Austria). The kit is based on the competitive ELISA principle, with antibodies against non-phosphorylated MGP coated on the microtiter plate.

Serum OPG concentrations were quantified with the kit from IDS Lab Human OPG ELISA (Enzyme-Linked Immunosorbent Assay). The kit is based on the sandwich ELISA principle. This assay employs an antibody specific for human OPG coated on a 96-well plate.

All assays were performed according to the manufacturer's instructions.

Statistical Analysis

Statistical analysis was performed with the SPSS 11.5 statistical software package. Univariate analysis was performed to calculate mean, maximum and minimum value and SD. Spearman Significance levels were based on two-tailed tests, with the alpha level set at 0.05.

Results

Statistical tests in this study were performed on 69 subjects, which consisted of 31 subjects with controlled T2DM (HbA1c < 6.5%) and 38 subjects with poorly controlled T2DM (HbA1c > 6.5%). Creatinine and SGPT values that reflect respectively the index of renal function and SGPT liver function of the 69 study subjects were within normal limits. General description of the study subjects' baseline characteristics are shown in Table 1.

Table 2 shows significant correlation between OPG and HbA1c, significant correlation between OPG and Log FPG, and negative but not significant correlation between MGP and HbA1c. The trend was also shown in Figure 1 (A and B).

Table 1. Desciption of Subjects' Baseline Characteristics

Variables	Minimum	Maximum	Mean	SD
Age (years)	30	55	48.13	6.65
Clinical Variables				
BMI	14.9	33.5	23.71	3.18
Biochemical Variables				
ALT (U/l)	7.00	79.00	32.62	13.93
Creatinine (mg/dL)	0.60	1.70	1.01	0.17
HbA1c (%)	4.40	15.80	8.88	2.62
FPG (mg/dL)	80.00	359.00	179.95	68.44
OPG (pmol/l)	1.30	8.80	3.79	1.25
MGP (nmol/l)	5.00	14.30	10.64	1.79

Description: BMI = Body Mass Index; ALT = Alanine Aminotransferase; HbA1c = glycated hemoglobin = hemoglobin A1c; FPG = Fasting Plasma Glucose; OPG = Osteoprotegenin; MGP = Matrix Gla Protein.

Table 2. Correlation between variables

Variables		OPG	MGP
HbA1c	r	0.0680	-0.0221
	p	0.0300*	0.2230
Log FPG	r	0.0681	0.0402
	p	0.0300*	0.0980

Description: HbA1c = glycated hemoglobin, hemoglobin A1c; Log FPG = Logarithm of Fasting Plasma Glucose; * = significant at p< 0.05.

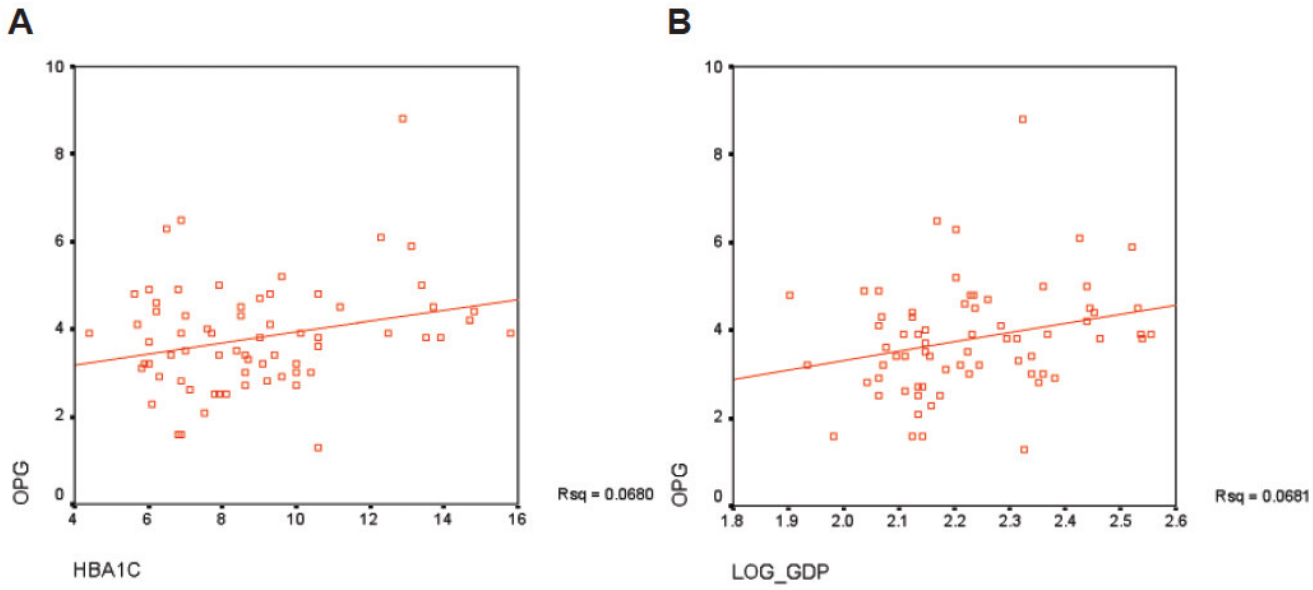

Figure 1. Plasma osteoprotegrin according to the plasma HbA1c and log FPG

Table 3 shows the relationship between HbA1c with each marker of vascular calcification in controlled type 2 DM group and uncontrolled type 2 DM group.

Independent t-test was applied to see the difference in markers of vascular calcification between controlled type 2 diabetes group and uncontrolled type 2 diabetes group, which showed the existence of differences but not significant. The concentration of OPG in uncontrolled type 2 diabetes group was higher than that in controlled type 2 diabetes group, but in the contrary the concentration of MGP was lower in uncontrolled type 2 diabetes. The ratio of OPG/MGP was higher in uncontrolled type 2 diabetes compared with controlled type 2 diabetes group. These results are shown in Table 4.

Table 3. Correlation between HbA1c with OPG and MGP in controlled type 2 DM group and uncontrolled type 2 DM group

Variables		OPG	MPG
Controlled Type 2 DM Group			
HbA1c	r	-0.0274	0.1155
	p	0.3740	0.0610
	n	31	31
Uncontrolled Type 2 DM Group			
HbA1c	r	0.1524	-0.0621
	p	0.0140*	0.1450
	n	38	38

Description: HbA1c = glycated hemoglobin, hemoglobin A1c; OPG = Osteoprotegerin; MGP = Matrix Gla Protein; * = significant at p< 0.05.

Table 4. T-test analysis of OPG and MGP in controlled type 2 DM group and uncontrolled type 2 DM group

Variables	Controlled Type 2 DM Group	Uncontrolled Type 2 DM Group	p
OPG (pmol/l)	3.59 ± 1.24	3.95 ± 1.25	0.567
MGP (nmol/l)	10.81 ± 1.53	10.50 ± 1.99	0.167
OPG/MGP	0.34 ± 0.13	0.40 ± 0.17	0.259

Description: OPG = Osteoprotegerin; MGP = Matrix Gla Protein.

Discussions

The results of this study showed a negative correlation between HbA1c with MGP levels although not statistically significant, and significant correlation between HbA1c with OPG levels (both in controlled and uncontrolled type 2 diabetes). It means that subjects with diabetes had a risk for vascular calcification. If opg-deficient mice, which have no measurable OPG in their blood, develop premature arterial calcification (mainly in the media of large vessels), that is preventable by restoration of the gene, then why are greater OPG levels in humans associated with diabetes and with an increased, rather than a decreased, risk for cardiovascular disease? One hypothesis is that increased serum OPG levels in humans are a "response" to rather than a "cause" of atherosclerosis or vascular calcification, perhaps as an attempt to regulate those processes. Another explanation is the greater OPG levels are a result of decreased clearance of OPG, perhaps because of increased binding of OPG ligand (10).

Elevated glucose levels (glucotoxicity) have been shown to be a direct sensitizer for osteogenesis. In vitro studies with vascular smooth muscle cells (VSMCs) and high glucose have been shown to induce cell proliferation and expression of osteopontin. Even though it is clinically known that diabetes is associated with an increase in medial vascular calcification, the pathogenesis is not completely understood. Glucotoxicity seems to play an important role by transforming VSMCs and possibly pericytes into osteoblast-like cells (11). This study showed a significant linear correlation between OPG concentration and Log FPG. The mechanisms behind the correlation between OPG and glycaemic status remained unknown, but this might relate to possible regulatory effects of OPG production from vascular cells and osteoblasts since OPG synthesis was regulated by insulin-like growth factor-I in osteoblasts, by insulin and TNF-α in vascular smooth muscle cells and by TNF-α in endothelial cells (12).

Cardiovascular disease is the most important cause of death in patients with T2DM and the risk starts very early during the stage of impaired glucose tolerance well before the clinical diagnosis of diabetes mellitus. While microvascular diseases affecting the eye, kidney, and nerves can occur in both type 1 and type 2 diabetes mellitus; patients with T2DM have a greater risk of developing macrovascular diseases, especially coronary artery disease. Macrovascular complications can occur in the brain, peripheral and coronary blood vessels (2). These clinical issues therefore have important implications as we address the issue of glycemic control. Controlling the blood glucose to normal levels result in preventing the development and progression of both microvascular and macrovascular disease (2). This study showed the differences between controlled and uncontrolled T2DM groups concerning regulators of calcification, although not statistically significant. Subjects in uncontrolled T2DM group were observed to have higher OPG and lower MGP concentration. This study also showed a significant correlation between OPG and HbA1c in uncontrolled type 2 diabetes group. The conclusion was there was a tendency for calcification in patients with uncontrolled T2DM.

Vascular calcification is a consequence of tightly regulated processes that culminate in organized extracellular matrix deposition by osteoblast-like cells. These cells may be derived from stem cells or differentiation of existing cells, such as smooth muscle cells (SMCs) or pericytes. Several factors induce this transition including bone morphogenetic proteins, oxidant stress, high phosphate levels, parathyroid hormone fragments, and vitamin D. Once the osteogenic phenotype is induced, cells gain a distinctive molecular fingerprint, marked by the transcription factor core binding factor 1. Alternatively, loss of inhibitors of mineralization such as matrix carboxyglutamic acid Gla protein, fetuin, and osteopontin, also contribute to vascular

calcification. The normal balance between promotion and inhibition of calcification becomes dysregulated in chronic kidney disease, diabetes mellitus, atherosclerosis, and as a consequence of aging (13). In this study, the ratio between promoter and inhibitor of calcification (OPG/MGP ratio) was greater in uncontrolled diabetic group although this difference was not statistically significant. Supporting this notion, recent reports have suggested a sequence of molecular events in vascular calcification beginning with the loss of expression by VSMCs, of constitutive inhibitory proteins, and ending with expression by VSMCs and macrophages of chondrocytic, osteoblastic, and osteoclastic-associated proteins that orchestrate the calcification process (14).

Conclusions

In conclusion, our study showed that HbA1c and high glucose concentrations were significantly associated with increased concentrations of OPG, and decrease in the concentration of MGP. Significant relationship between increasing concentrations of HbA1c with OPG levels in uncontrolled type 2 diabetes mellitus showed the progression of vascular calcification in diabetic subjects who were not yet controlled. There were differences in the concentration of the regulators of vascular calcification between controlled and uncontrolled type 2 diabetes mellitus, promoter of calcification (OPG) levels were higher in poorly controlled diabetes mellitus and calcification inhibitors (MGP) had lower levels in uncontrolled diabetes mellitus. The OPG/MGP ratio was higher in poorly controlled type 2 diabetes mellitus than in well controlled type 2 diabetes mellitus. The ratio of OPG/MGP had a better correlation with HbA1c and FPG than singly. In this study we didn't compare the serum OPG/MGP ratio with the calcium score degree, therefore, future studies are still required to examine whether the degree of OPG and MGP are associated with development of vascular calcification as assessed by EBCT (Electron Beam Computed Tomography).

Acknowledgements:

We thank the Prodia Foundation for Research and Training for the invaluable support given to this study.

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