Osteoprotegerin as a marker of atherosclerosis in type 1 and type 2 diabetic patients

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Background/aim: The aim of this study was to investigate the availability of osteoprotegerin (OPG) as a marker of atherosclerosis and compare serum OPG levels with ankle-brachial index (ABI) in diabetic patients.

Materials and methods: A total of 31 type 1 and 31 type 2 diabetic patients without macrovascular complications and 20 healthy volunteers were included. Serum OPG levels and ABI were measured.

Results: The duration of diabetes was significantly higher in type 1 diabetics than in type 2, although there was no significant difference between mean HbA1c levels. There was a weak and inverse correlation between OPG and atherosclerosis in type 1 diabetics only ($P = 0.046$, $r = -0.360$). There was a weak, positive correlation between ABI and HbA1c in all participant groups ($P = 0.047$, $r = 0.220$), and a weak-medium correlation in type 2 diabetics ($P = 0.021$, $r = 0.414$). After the adjustment of OPG levels to atherosclerosis risk factors, only the age factor was found to be effective on OPG.

Conclusion: The inverse correlation of serum OPG with atherosclerosis in type 1 diabetics suggests that atherosclerosis may be related to increased duration of diabetes. Since the study participants did not show macrovascular complications, future prospective studies on the development of diabetic complications and correlation with OPG might give further information about the availability of OPG as a marker of atherosclerosis.

Key words: Diabetes mellitus, atherosclerosis, osteoprotegerin, ankle-brachial index

1. Introduction

Diabetes mellitus (DM), the most common endocrine disorder globally, is increasingly prevalent. It causes serious medical and socioeconomic problems due to its microvascular and macrovascular complications. It is common knowledge that diabetes is an independent risk factor for atherosclerotic heart disease (1). Thus, early detection of atherosclerotic complications in diabetes is vital to decreasing morbidity and mortality.

Osteoprotegerin (OPG) is a glycoprotein and a member of the tumor necrosis factor receptor family initially found in the bone. Its main function is to inhibit both the differentiation and action of osteoclasts by binding and neutralizing the receptor activator for NF-kB ligand (RANKL), which is known to have osteoclast-inducing activity (2). Recent studies showed that increased OPG levels were an independent risk factor for atherosclerosis and cardiovascular diseases, and suggested that this cytokine could be used as a marker of coronary artery disease (3).

Ankle-brachial index (ABI) is the ratio of ankle systolic blood pressure to arm systolic blood pressure. ABI measurement is a noninvasive indicator of peripheral artery disease and atherosclerosis and is independent of symptom presence. Various studies have shown that low ABI (≤0.9) is related to increased mortality, coronary heart disease, congestive heart failure, stroke, and dementia (4–13).

In our study, we investigated the availability of OPG as a marker of atherosclerosis by comparing serum OPG levels with ABI in type 1 and type 2 diabetic patients with no macrovascular complications.

2. Materials and methods

This was a cross-sectional study. We recruited 31 type 1 DM and 31 type 2 DM patients without any macrovascular complications from our diabetes outpatient clinic, and 20 healthy consecutive patients, who did not have any chronical diseases and were not on any medications for

any reason, from our internal medicine outpatient clinic. Informed consent was obtained from all subjects and the investigation conformed to the principles outlined in the Declaration of Helsinki. The study protocol was approved by the ethics committee and was financed by the Scientific Research Fund of the hospital.

Sex-matched patients were involved regardless of their age. Diagnosis of cardiovascular disease (ischemic heart disease, myocardial infarction, previous coronary by-pass operation, and history of angina pectoris), cerebrovascular disease, carotid artery stenosis or peripheral artery disease, and a serum creatinine level of ≥1.4 were our exclusion criteria.

Each patient provided full personal data and medical history. All subjects underwent clinical examination, including measurement of weight and height. Blood samples were collected and fasting plasma glucose, hemoglobin A1c, LDL and HDL cholesterol, triglyceride, urea, creatinine, high sensitivity CRP (hsCRP), and fibrinogen levels were studied with Siemens Healthcare Diagnostics (USA). Urine was collected to detect microalbuminuria. For measurement of OPG levels, blood samples were collected in vacuum tubes with a cloth activator and kept for 30 min to coagulate. Then they were centrifuged to obtain serum samples, and were preserved at –80 °C until further study. The blood samples were studied at the Acıbadem Labmed (Altunizade, İstanbul, Turkey) laboratory with enzyme-linked immunosorbent assay (ELISA) (Catalog No: BI-20402, Biomedica, Germany) method. Coefficient of variation (CV) for intraassay (n = 6) for lowest level (mean: 4.59 pmol/L) was 10% and for highest level (mean: 10.76 pmol/L) it was 4%; for interassay (n = 10) for lowest level (mean: 5.53 pmol/L) it was 7% and for highest level (mean: 10.1 pmol/L) it was 8%.

ABI measurement was performed with bilateral upper and lower extremity color Doppler ultrasound. ABI was calculated by division of the highest blood pressure in the lower legs by the highest blood pressure in the arms. ABI ≤ 0.90 was accepted as atherosclerosis-peripheral artery disease presence, and values exceeding 0.90 were regarded as normal.

Statistical analysis was conducted with SPSS 12.0. P was significant at <0.05 and at a confidence interval of 95%. According to the nature of the variables, parametric or nonparametric statistical methods were used for secondary comparisons. For numeric parameters without normal distribution, the comparison of the two groups was performed with the Mann–Whitney U test, and of triple groups with the Kruskal–Wallis test. For numeric parameters with normal distribution, comparisons of three or more groups were performed with one-way ANOVA and Tukey HSD as posthoc test. Chi-square exact test was used for comparison of categorical variables. For the correlation of ordinal or nonsymmetrical distributed numeric variables, Spearman correlation was used. For the comparison of the mean numeric variables in two or more groups, covariance analysis was used.

3. Results

A total of 82 subjects were included in the study: 31 type 1 DM (37.8%) and 31 type 2 DM (37.8%) patients, and 20 healthy (24.4%) subjects as the control group. Distribution of age and sex, BMI, and HbA1c are shown in Table 1. There was a difference of age between groups. Diabetes duration was 13.6 years in type 1 DM group and 8.1 years in type 2 DM group, and was thus significantly longer for type 1 DM group. According to BMI values, obesity rate was higher in type 2 diabetic patients with a statistical significance of P = 0.002. According to HbA1c values, there was no significant difference between type 1 and 2 DM groups.

Patients were screened for atherosclerosis risk factors according to the National Cholesterol Education Program (NCEP) guidelines. Among risk factors for atherosclerosis, only hypertension was higher with a statistical significance of P = 0.004 in type 2 diabetic patients. There was no statistically significant difference for other risk factors between groups (Table 2). In addition, in type 2 DM group serum triglyceride levels were significantly higher when compared to type 1 DM and control groups (P = 0.001). When searched for additional risk factors for atherosclerosis, there was no statistically significant difference between groups for hsCRP and fibrinogen levels (P = 0.078 and 0.113). Again, for microalbumin/creatinine levels in spot urine samples, there was no difference between groups (P = 0.179) (mean values: 34 in type 1 DM group and 58.2 in type 2 DM group).

Table 1. Distribution of age, sex, BMI, and HbA1c (mean values).

	Male	Female	Age (years)	BMI (kg/m^2)	HbA1c
Total	37	45			
Type 1 DM	14	17	35.2	24.7	7.8
Type 2 DM	14	17	53.4	30.4	7.6
Control	9	11	43.7	28.7	5.4

Table 2. Atherosclerosis risk factors.

	Smoking (number of patients)	HT presence (number of patients)	LDL (mg/dL)	HDL (mg/dL)	TG (mg/dL)
Type 1 DM	17	3	96.6	55.6	124.8
Type 2 DM	13	10	118.9	49.2	204.8
Control	6	0	113.9	55.4	111.4

When OPG levels were evaluated, mean values were 5.1 pmol/L in type 1 DM, 6.1 pmol/L in type 2 DM, and 5.0 pmol/L in the healthy control group. However, there was no statistically significant difference between groups (P = 0.088).

ABI mean values were 1.08 in type 1 DM, 1.09 in type 2 DM group, and 1.06 in the healthy control group. There was no statistically significant difference between patient and control groups (P = 0.729).

In our study, according to ABI values, ≤0.9 was considered as atherosclerosis. Consequently, in type 1 DM group ABI ≤ 0.9 rate was 12.9%, in type 2 DM group it was 6.5%, and in the healthy control group 5%. There was no significant difference between groups regarding the presence of atherosclerosis (P = 0.535).

When the correlation of osteoprotegerin with ABI and atherosclerosis (ABI ≤ 0.9) was evaluated in patient and control groups, no significant correlation was found (P = 0.565). There was a weak-medium, inverse correlation between OPG and atherosclerosis only in type 1 diabetic patients (P = 0.046, r = –0.360). Additionally, there was a weak-medium correlation between OPG and ABI (P = 0.043, r = 0.366) (Table 3; Figure). When the correlation of ankle brachial index values with diabetes duration and HbA1c levels was evaluated, there was a very weak correlation between ABI and HbA1c in all subject groups included in the study (P = 0.047, r = 0.220). In the type 2 DM group, there was a weak-medium correlation between ABI and HbA1c (P = 0.021, r = 0.414). There was no
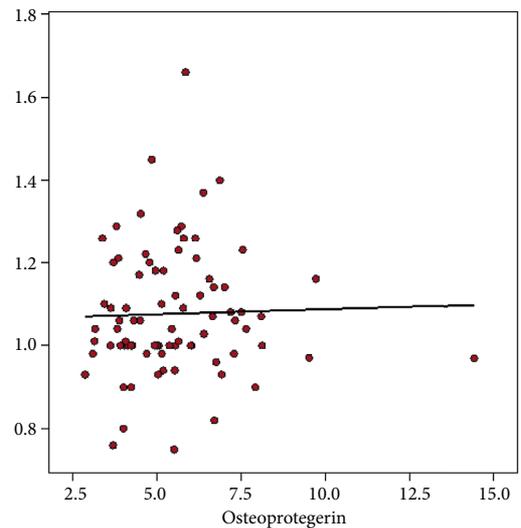

Figure. Relationship between osteoprotegerin and ankle brachial index in patient and control groups.

significant correlation between osteoprotegerin level and plasma fibrinogen, hsCRP, microalbuminuria, diabetes duration, and HbA1c.

In type 1 and type 2 DM patient groups, hypertension, smoking, male sex, high LDL, low HDL as atherosclerosis risk factors, and mean values of OPG cleared from the effect of obesity factors covariants were not different (P = 0.543). Instead, only the age factor affected OPG levels (P = 0.043) (Table 4).

Table 3. The relation of osteoprotegerin with ankle brachial index and atherosclerosis in patient and control groups.

	All groups		Type 1		Type 2		Control	
	Osteoprotegerin		Osteoprotegerin		Osteoprotegerin		Osteoprotegerin	
	R	P	R	P	R	P	R	P
Ankle-brachial index	0.077	0.494	0.366	0.043	–0.114	0.541	–0.102	0.668
Atherosclerosis*	–0.065	0.565	–0.36	0.046	0.132	0.479	0.259	0.271

Spearman correlation.
*Ankle-brachial index ≤ 0.9.

Table 4. Covariance analysis of atherosclerosis risk factors in diabetic patients.

	F	P
LDL (>100 mg/dL)	0.072	0.789
HDL (≤40 mg/dL)	0.009	0.925
Hypertension	0.405	0.527
BMI (≥30 kg/m²)	0.018	0.893
Smoking	0.690	0.410
Age (male ≥ 45, female ≥ 55)	4.321	0.043
Sex (male)	1.021	0.317
DM duration	0.942	0.336
Patient type	0.376	0.543

4. Discussion

Diabetes-related mortality is usually due to macrovascular complications (14). Asymptomatic coronary artery disease is common among diabetic patients, and this is a strong sign of possible coronary vascular events and even early death (15,16). Thus, it is important to make an early diagnosis of atherosclerosis development in high risk patients, so as to take the precautions needed to prevent related complications or mortality.

In our study, we found that atherosclerosis rates were low in all groups according to ABI levels, and there was no statistically significant difference between groups. However, when ABI values were compared to HbA1c, there was very weak correlation in all groups and weak-medium correlation in type 2 DM group. This could be related to the inclusion of only diabetic patients without any macrovascular complications. In a study carried out by Ishii et al. in diabetic and nondiabetic end stage renal disease patients, increased HbA1c levels were found to be related to increased peripheral artery disease (established by ABI) risk, and this study supports our assumption (17). Thus, these findings show that good glycemic control is the most important stage to prevent related complications.

OPG is a newly developing, strong, and independent marker of cardiovascular disease in high risk societies. Epidemiological studies show that serum OPG levels are related to age, diabetes, hypertension, vascular endothelium dysfunction (18), coronary artery disease (19), and cardiovascular mortality (20).

In our study, a weak-medium, inverse, statistically significant correlation was found between OPG and atherosclerosis in type 1 DM patient group. Furthermore, in type 1 DM group there was a weak-medium, significant correlation between OPG and ABI. It is noteworthy that the relation between OPG levels and atherosclerosis existed only in type 1 DM patients, according to our findings. This might be a consequence of the difference in diabetes duration of type 1 and 2 DM groups, and it suggests that diabetes duration might affect atherosclerosis development, even though diabetes is regulated. Although average age was lower in type 1 DM patients in our study, serum OPG–atherosclerosis relation was only found in that group. This suggests that the duration of diabetes is more important than the age of the patient for predicting atherosclerosis. The significant relation between diabetes duration and OPG levels, found in a study by Reinhard et al. and carried out in 283 type 2 DM patients, supports this assumption (21).

In our study, when OPG levels were compared to atherosclerosis risk factors such as hypertension, smoking, male sex, high LDL, and low HDL, and also to mean values of OPG cleared from the effect of obesity factors covariants, these were not found to be different. Only the age risk factor was found to affect OPG levels. In their study, Mogelvang et al. found no correlation between plasma hsCRP and OPG levels, which was consistent with our findings (22).

In a study by Altınova et al., it was found that OPG levels were high in poorly controlled type 2 DM patients and were related to patient age (23). In a study by Browner et al., results showed that OPG levels were not related to smoking, BMI, LDL-cholesterol, HDL cholesterol, or CRP levels, although high OPG levels were related to all cause and cardiovascular cause mortality (20). In their study, Güzel et al. found that serum OPG levels had a strong relation with silent myocardial ischemia in diabetics; age, sex, and smoking strengthened this relation (24). These results show the importance of other risk factors for atherosclerosis, as well as diabetes, which is considered to be a coronary artery disease equivalent.

In studies conducted by Allison et al. and Aboyans et

al., diabetes was associated with a high ABI; however, in our study we found no association between groups, which might be due to the lack of atherosclerosis signs in diabetic patients in our study (25,26). In a study by O'Sullivan et al., OPG was higher in peripheral artery disease regardless of the coexistence of type 2 DM. Moreover, we did not find an increase in OPG in type 2 DM patients (27). In contrast, Davenport et al. found an increase in OPG in diabetic patients. Nevertheless, those subjects had micro- and macrovascular complications, and the difference might be due to those complications (28).

There are several studies evaluating the relation of serum OPG levels with atherosclerosis (21,29). However, those studies were mainly carried out in type 2 DM patients, whereas our study was performed in both type 1 and type 2 DM patients. The inverse relation of OPG and atherosclerosis that we found in type 1 DM patients in our study shows that broader studies about the OPG–atherosclerosis relation should be carried out in that group.

In a review that evaluated studies examining OPG levels with indices of atherosclerosis, cardiovascular events, and glycemic control in diabetic patients, the existing data were found to be as yet sparse (30). In our study, we could only find a correlation between type 1 DM and OPG levels, but not for type 2 DM, which shows the lack of evidence for suggesting OPG as a certain biomarker of atherosclerosis for all kinds and stages of diabetes.

This study has several limitations. It is a cross-sectional study, and because the participants were without complications, they should be followed up prospectively for diabetic complications, related morbidity/mortality, and the effect of serum OPG levels. If coronary artery disease develops in the OPG high group, it might be more valuable to show that those patients need to be examined in more detail from the beginning. Reinherd et al. followed

up patients for 16.8 years and predicted OPG as a strong early marker of mortality (21). Moreover, another study showed that OPG was related to future risk of mortality caused by myocardial infarction, ischemic heart disease, stroke, and nonvascular reasons (31).

A study comparing diabetic patients with complications to patients without complications might help us to understand the role of OPG in relation to diabetic complications and atherosclerosis. In a study by Schoppet et al., coronary artery disease documented by coronary angiography and serum OPG levels was correlated with increasing coronary artery disease (19). In a study by Wang et al., OPG was found to be related to diabetic nephropathy presence and severity (32), and these studies support our assumption.

In conclusion, the results of our study show that regulation of diabetes is closely related to the development of atherosclerosis in diabetic patients. The inverse correlation of serum OPG with atherosclerosis in only type 1 diabetic patients suggests that atherosclerosis might be related to increasing duration of diabetes. In addition, it might be a promising agent to demonstrate atherosclerosis in patients with a longer duration of diabetes, especially in type 1 DM. However, it does not seem to reflect atherosclerosis in type 2 DM patients when their diabetes is regulated and they are without any complications. A future prospective study about the development of probable diabetic complications and its correlation with OPG can give more information about the availability of OPG as a promising agent and marker of atherosclerosis, but the present data do not confirm its availability so far. An early diagnosis with such a noninvasive tool would lead to early treatment and prevention of further damage in the cardiovascular system, thus helping to decrease morbidity and mortality caused by macrovascular complications.

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