The relation between serum osteoprotegerin level and coronary artery calcification in type 2 diabetes mellitus patients

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Received 20 June 2017
Accepted 16 November 2017

Kasr Al Ainy Medical Journal
2017, 23:135–140

Background
Osteoprotegerin (OPG) is considered as a possible link between bone and vascular disease. It is important to establish noninvasive methods for monitoring vascular changes such as biochemical markers of increased risk for cardiovascular disease events such as OPG, as cardiovascular morbidity is high in diabetics.
Aim
The aim of this study was to determine the relationship between coronary artery calcification (CAC) and OPG levels in type 2 diabetic patients in comparison with healthy controls.
Patients and methods
Our study included 45 type 2 diabetic patients without evidence of previous cardiovascular disease (by history and ECG) and 45 healthy age-matched and sex-matched individuals as control. All were submitted to full history, clinical examination, and lab investigations. Serum OPG concentration was measured by an enzyme-linked immunosorbent assay and CAC imaging was performed using noncontrast multidetector computed tomography.
Results
Significant coronary artery calcification score (CACS) (more than 10 Agatston units) was seen in 23 (51.11%) patients.
OPG was significantly high in diabetic patients in comparison with controls, with a mean of 12.9±5.7 pmol/l in cases and 8.6±0.5 pmol/l in controls ($P<0.001$). The CACS was positively correlated with age and diabetes duration. The OPG was positively correlated with fasting blood sugar and diabetes duration. The CACS showed significant positive correlations with OPG.
Conclusion
Increased serum OPG in CAC that could be used for the early diagnosis of subclinical atherosclerosis allows for designing strategies to reduce the cardiovascular event rates in these patients. Further studies are important to establish the predictive value of increased OPG levels in diabetic vascular complications.

Keywords:
coronary artery calcification, multidetector computed tomography, osteoprotegerin, type 2 diabetes mellitus

Kasr Al Ainy Med J 23:135–140

1687-4625

Introduction

Poorly controlled type 2 diabetes is associated with vascular complications [1]. The risk of coronary heart disease (CHD) events is similar between individuals with diabetes mellitus (DM) and individuals with known CHD, which makes diabetes a coronary risk equivalent [2].

The major cause of death in Western countries is cardiovascular disease (CVD), especially CAD. There were more than 54 million deaths all over the world and 32% of them (17 million) were attributable to CVD in 2013 [2]. CVD, especially atherosclerosis, will become the leading cause of total disease over the world by 2020 [3].

Primary prevention of CAD requires identification of population at risk to ensure effective intervention. The presence of calcium in coronary arteries is indicative of atherosclerotic plaque [4]. Risk assessment using coronary artery calcification score (CACS) was shown to motivate individuals to change their lifestyle and allow for medications [5].

It is important to find noninvasive methods to follow vascular changes such as biochemical markers of

 DOI: 10.4103/kamj.kamj_24_17

increased risk for CVD events such as osteoprotegerin (OPG), as cardiovascular morbidity is increased in diabetics. OPG is a strong antiresorptive factor, member of the tumor necrosis factor receptor superfamily member 11B. It is a protein that in humans is encoded by the TNFRSF *11B* gene and is considered as a possible link between bone and vascular disease [6]. OPG is related to risk factors such as diabetes, systolic blood pressure, age, smoking, and prevalent CVD, as well as CVD-related mortality [7].

Aim

The aim of this study was to determine the relationship between coronary artery calcification (CAC) measured by the multidetector computed tomography (CT) and OPG levels in type 2 diabetic patients in comparison with healthy controls.

Patients and methods

This cross-sectional case–control study included 45 type 2 diabetic patients, 23 (51.11%) male and 22 (48.89%) female, with a mean age of 51.7 years (range: 40–60 years) and a mean duration of diabetes of 10.5 years (range: 2–19 years), without evidence of previous CVD (by history and resting ECG), who were recruited from Endocrinology and Diabetes Clinic, Faculty of Medicine, Cairo University hospitals. In addition, 45 healthy controls matched for age and sex were included. Patients with type 1 diabetes, peripheral vascular disease, renal disease, malignancy, acute, chronic inflammation, or prior evidence of coronary artery disease (CAD) such as myocardial infarction, angina pectoris, or an abnormal resting electrocardiogram were excluded. The protocol of the study was approved by regional ethics committee.

All individuals were submitted to full history, clinical examination including height, weight, BMI calculated by dividing the weight (kg) by the square of the height in meters, and laboratory investigations including fasting plasma glucose, 2-h postprandial plasma glucose, HA1c, total cholesterol, triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C).

Five ml of fasting (12–14 h) venous blood samples were taken from each individual in the study and divided into parts: the first part was 2 ml and added to a tube containing EDTA for the determination of HbA1c by cation exchange resin. The rest of the blood (3 ml) was left to clot and the serum was separated by centrifugation for 15 min at 3000*g* and fasting blood glucose was determined immediately on Dimension RxL Max analyzer (Siemens Healthcare GmbH, Erlangen, Germany). The rest of the serum was stored at −20°C for determination of the following: serum cholesterol, serum TG, LDL-C, HDL-C, and serum osteoprotegrin. Two hours after eating, 2 ml of blood was taken on fluoride for the determination of postprandial blood glucose.

The determination of fasting and postprandial blood glucose, total cholesterol, TG, and HDL was performed on Dimension RxL Max analyzer by colorimetric techniques. LDL cholesterol was calculated by Friedwald formula [8].

OPG was determined using sandwich enzyme immunoassay kit and the kit was supplied from BioVendor GmbH (Im Neuenheimer Feld 583, Heidlberg, Germany) [9].

CAC imaging was performed using noncontrast CT of the heart to detect and quantify CACs through the volume extended from below the carina to the apex of the heart, using the 64 multidetector row scanner Aquillion 64 (Toshiba Medical Systems, Tokyo, Japan). Acquisition parameters were ECG gated at 75% of RR interval, 400 ms gantry rotation, 64×0.5 mm collimation, 80 mA, and 120 kV. To minimize the total effective patient radiation dose, the scan was conducted with a relatively low tube current. CACSs were classified into four categories based on widely used cut-off values in the literature: 0–10 (minimal or insignificant), 11–100 (mild), 101–400 (moderate), and more than 400 (severe).

Statistical analysis

Statistical calculations were done using computer programs Microsoft Excel 2007 and statistical package of social science software program, version 21 (SPSS 21; SPSS Inc., Chicago, Illinois, USA).

Data were statistically described in terms of range, mean, SD, median, frequencies (number of cases), and percentages when appropriate. Comparison of quantitative variables between the study groups was done using Mann–Whitney *U*-test for independent samples. For comparing categorical data, χ^2-test was performed. Exact test was used instead when the expected frequency is less than 5. Agreement between osteoprotegrin and CACS was done using κ-statistics. Correlation between variables was done using Spearman's rank correlation equation for

non-normal variables. A *P* value less than 0.05 was considered statistically significant.

Results

Our patients had type 2 diabetes of 2–18 years duration. The demographic and laboratory data of our diabetic patients are shown in Table 1.

Comparison between cases and control as regards all parameters is summarized in Table 2.

CACS of all studied individuals ranged from a minimum of 0 mg/dl to a maximum of 1207 mg/dl with a mean of 199.8±325. Among controls, no calcification was detected ($P<0.001$), as shown in Tables 2 and 3, and Fig. 1.

Table 1 Demographic and laboratory data of type 2 diabetic patients (*n*=45)

Items	Minimum	Maximum	Mean	SD
Age (years)	40	60	51.7	6.0
BMI (kg/m^2)	26	46	32.5	4.6
Diabetes duration (years)	2	19	10.5	8.5
FBS (mg/dl)	60	348	196.8	74.0
2 h PP (mg/dl)	155	473	261.5	85.6
HA1c%	6.5	14.5	9.5	2.5
Cholesterol (mg/dl)	160	235	201.7	20.1
Triglycerides (mg/dl)	75	486	194.3	103.4
LDL (mg/dl)	14	178	135.5	31.7
HDL (mg/dl)	26	89	38.3	10.0
CAC score	0	1207	199.8	325
OPG (pmol/l)	8	25.9	12.9	5.7

2 h PP, 2 h postprandial blood sugar; CAC score, coronary artery calcification score; FBS, fasting blood sugar; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; OPG, osteoprotegrin.

Table 2 Comparison of all parameters among cases and controls

Item	Case (mean ±SD)	Control (mean ±SD)	*P* value
Age (years)	51.7±6.0	50.0±6.0	0.166
BMI (kg/m^2)	32.5±4.6	33.5±5.0	0.484
FBS (mg/dl)	196.8±74.0	89.1±9.1	<**0.001**
2 h PP (mg/dl)	261.5±85.6	157.7±26.6	<**0.001**
HbA1c%	9.5±2.5	6.5±0.5	<**0.001**
Cholesterol (mg/dl)	201.7±20.1	193.0±67.9	0.082
Triglycerides (mg/dl)	194.3±103.4	156.1±54.8	0.242
HDL (mg/dl)	38.3±10.0	56.0±17.3	<**0.001**
LDL (mg/dl)	135.5±31.7	96.8±55.1	<**0.001**
CAC score	199.8±325.3	0±0	<**0.001**
OPG (pmol/l)	12.9±5.7	8.6±0.5	<**0.001**

2 h PP, 2 h postprandial blood sugar; CAC score, coronary artery calcification score; FBS, fasting blood sugar; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; OPG, osteoprotegrin. *Statistically significant.

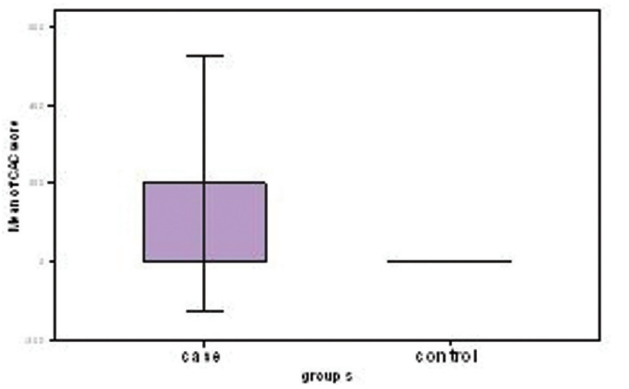
OPG was significantly higher in diabetic patients in comparison with controls. It ranged from a minimum of 8 pmol/l to a maximum of 25.9 pmol/l with a mean of 12.9±5.7 in diabetic patients, whereas in controls the mean was 8.6±0.5 pmol/l ($P<0.001$) (Fig. 2).

Significant CAC (more than 10 Agatston units) was seen in 23 (51.11%) patients: mild (11–100) in eight (17.7%) patients, moderate (101–400) in seven (15.5%) patients, and severe (>401) in eight (17.8%) patients.

The CACS was positively correlated with age ($P<0.033$) and duration of diabetes ($P<0.001$). OPG was positively correlated with fasting blood sugar and duration of diabetes ($P<0.001$).

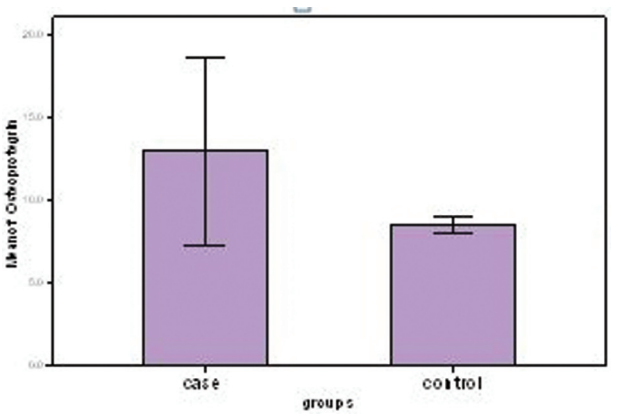
CACS and OPG showed significant positive correlations with each other after adjustment for age, sex, and other risk factors [odds ratio=0.043, 95% confidence interval (CI): 0.006–0.296; $P>0.001$] (Fig. 3). Cut-off value of OPG is 10 pmol/l with a sensitivity of 95.7% and specificity of 100%. Area under

Figure 1



Mean of coronary artery calcification score among cases and controls.

Figure 2



Mean of osteoprotegrin among cases and controls.

the receiver operating characteristic curve (AUROC) for serum OPG was 0.9, as shown in Table 4 and Fig. 4.

When OPG less than 10, CAC score showed one (4.3%) case with calcification and 22 (100%) cases without calcification. When OPG more than 10, CAC score showed 22 (95.7%) cases with calcification and no (0%) cases without calcification. This shows the strong correlation between OPG and CAC score, and a cut-off value of OPG 10 pmol/l is highly sensitive and specific in the detection of coronary calcification (Table 4 and Fig. 5).

Discussion

Calcium in coronary arteries is a sure indication of atherosclerotic plaque existence. The amount of calcium detected in the coronary arteries is converted to calcium score and the Agatston score is the used one. CACS correlates with the severity of the atherosclerosis and is determined by CT scanning. The main advantage of CACS is its high negative predictive value (95–99%) [4].

It is important to find noninvasive methods for following vascular changes such as biochemical markers of increased risk for CVD events as cardiovascular morbidity is increased in diabetic patients. OPG is a basic glycoprotein formed of 401 amino acids arranged into seven structural domains. It is found as either a 60 kDa monomer or a 120 kDa dimer linked by disulfide bonds. It has been hypothesized to be a link between bone and cardiovascular disease (CVD) [10,11].

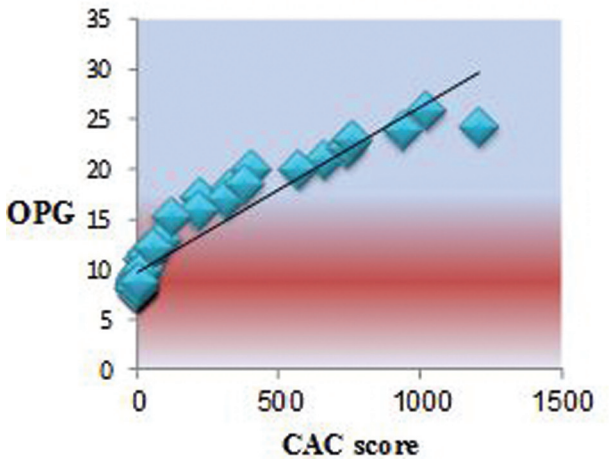
Increased circulating OPG level was found in individuals at higher risk of type 2 DM, and in patients with known CVD. Increased serum levels of OPG were found in patients with

Table 3 Frequency of coronary artery calcification detected in the study group by computed tomography scanning calculated coronary artery calcification score

Item	Cases [*n* (%)]	Control [*n* (%)]	*P* value
CAC score			
No calcification	22 (48.89)	45 (100.00)	<0.001*
Calcification	23 (51.11)	0 (0.00)	

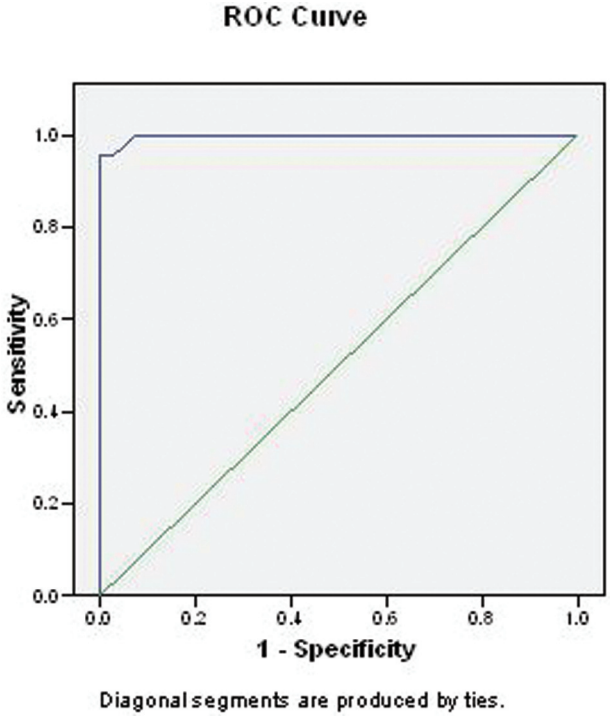
CAC score, coronary artery calcification score. *Statistically significant.

Figure 3



Correlation between osteoprotegrin and coronary artery calcification score.

Figure 4



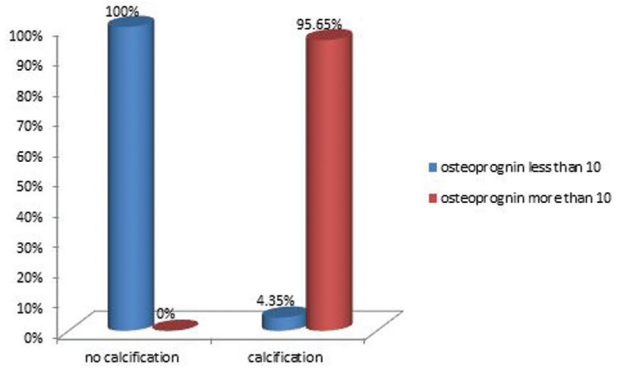
Receiver operating characteristic curve to explore the discriminating ability of plasma osteoprotegerin to differentiate among cases and controls.

Table 4 Correlation between cut-off value of osteoprotegrin and coronary artery calcification score

OPG	CAC [*n* (%)]			*P* value
	No calcification (*n*=22)	Calcification (*n*=23)	Total	
Less than 10	22 (100)	1 (4.34)	23 (51.11)	<0.001*
More than 10	0 (0)	22 (95.65)	22 (48.88)	

When OPG is less than 10, CAC shows no calcification in 22 (100%) patients. When OPG is more than 10, CAC shows calcification in 22 (95.5%) patients. CAC, coronary artery calcification; OPG, osteoprotegrin. *Statistically significant.

Figure 5



Relation between cut-off value of osteoprotegrin and coronary artery calcification score.

CVD and predicted CV outcomes including death [12].

Therefore, the current study aimed to increase our knowledge about the relation between CAC and OPG levels in type 2 diabetic patients.

In the current study, the comparison between OPG among cases and controls revealed a statistically significant difference with a mean of 12.9±5.7 pmol/l in cases and 8.6±0.5 pmol/l in controls ($P<0.001$). This is in agreement with the study of Jung *et al.* [13], who investigated the relationship between serum level of OPG, CACS, and arterial stiffness of 110 type 2 diabetic patients and reported that the mean OPG concentration was 10.2± 4.0 pmol/l.

Another study in which a total 151 patients with chest pain were evaluated (aged between 30 and 85 years) found that the mean value of serum OPG was 1.24 ±0.21 pmol/l, ranging from 0.3 to 30.8 pmol/l [14]; however, it differs from our study by measuring the predictive value of OPG for detecting CAC in symptomatic patients, whereas our study was about asymptomatic type 2 DM patients.

In our study, CACS revealed a statistically significant difference among cases and controls with a mean of 199.8 ±325.3 in cases and 0±0 in controls ($P<0.001$). In addition, CT coronary calculated CACS detected 22 (48.8%) diabetic patients with no calcification. CT coronary calculated CACS detected 23 (51.1%) diabetic patients with different calcification degrees. The CACS was mild (11–100) in eight (17.7%) patients, moderate (101–400) in seven (15.5%) patients, and severe (>401) in eight (17.8%) patients. Among controls, no calcification was detected ($P<0.001$), which is highly significant.

These findings are supported by Jung *et al.* [13], which reported that a total of 74 (67.3%) patients had minimal or insignificant CAC. The remaining 36 (32.7%) patients had different degrees of coronary calcification. The CAC was mild in 14 (12.7%) patients, moderate in 16 (14.5%), and severe in six (5.5%) [13].

Another study was done on 509 individuals and reported similar results: a total of 273 (53.5%) patients were found to have minimal or insignificant CAC. The remaining 236 (46.3%) patients had varying degrees of coronary calcification. The CAC was mild in 100 (19.6%) patients, moderate in 77 (15.1%), severe in 31 (6.1%), and extensive in 28 (5.5%) [15].

In the current study, correlations of tested variables with osteoprotegrin among cases show highly significant correlation with fetal bovine serum, duration of diabetes ($P<0.001$), but no correlation with age, BMI, 2 h postprandial, cholesterol, TGs, HDL, or LDL; however, there was no significant relation between CACS and OPG with sex or with treatment of individuals under study either oral or oral with insulin. Correlations of tested variables with CACS among cases show highly significant relation between CACS with age and duration of diabetes ($P<0.001$), but no correlation with BMI, FBS, 2 h postprandial, HbA1c, cholesterol, TGs, HDL, or LDL. These results agree with some studies but not others [13,15,16].

A very high statistically significant correlation between osteoprotegrin and CACS was detected in the current study ($P<0.001$), which is consistent with the results of studies that reported a highly significant correlation between osteoprotegrin and CACS [13–15,17].

The current study cut-off value of serum OPG was 10 pmol/l, which has a sensitivity of 95.7% and specificity of 100%. We found that the AUROC curve for serum OPG was 0.9. CACS and OPG showed significant positive correlations with each other after adjustment for age, sex, and other risk factors (odds ratio=0.043, 95% CI: 0.006–0.296; $P>0.001$). These results emphasize the usefulness of OPG to differentiate between cases (having coronary calcification) and controls.A recent study showed that the level of OPG is correlated significantly with the number of coronary arteries involved. A level of OPG had a weak positive correlation with CACS. ROC curve analysis showed that plasma OPG level had a prediction of CACS, with an AUROC curve of 0.62, which is close to our results. They concluded that plasma OPG is a valuable marker for coronary calcification [14].

Another study reported that the CACS and plasma OPG level were good predictors of short-term cardiovascular risk in comparison with the clinically derived UKPDS/Framingham risk scores, the authors used OPG level for prediction of short-term cardiovascular events at 19 pmol/l, with sensitivity 74%, specificity 91%. They reported that the OPG levels were significantly elevated in patients with increased CAC [15].

This supports what our study proved; when OPG less than 10 pmol/l, which was found in 23 patients, the CACS detected 22 (95.7%) cases without calcification and one (4.3%) case with calcification.

When OPG more than 10 pmol/l, the CACS detected 22 (100%) cases with calcification and no (0%) case without calcification. This shows that there is a highly significant relation between OPG cut-off value of 10 and CACS in the detection of CAC with 95% CI: 0.9–1.0 ($P<0.001$).

Conclusion

Findings suggest increased serum OPG in CAC patients and that it could be used for the early diagnosis of subclinical atherosclerosis, which would allow for designing strategies to reduce the cardiovascular event rate in these patients. Considering the cut-off value, it would help in the identification of patients at a high risk of vascular calcifications, and decision making for patients in need for aggressive management. Therefore, further studies are important to establish the predictive value of increased OPG levels in diabetes vascular complications.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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