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Nagwa A. Lachine[1], Eman Y. Morsy[1], Abdelaziz Elnekiedy[2], Mohamed A. Sadaka[3], Gehan I. Khalil[4], Hesham G. Imam[1], Heba S. Kassab[1], Noha G. Amin[1]

[1]Department of Internal Medicine (Diabetes, Metabolism and Lipidology Unit), Faculty of Medicine, Alexandria University, Alexandria, Egypt
[2]Department of Radiodiagnosis, Faculty of Medicine, Alexandria University, Alexandria, Egypt
[3]Department of Cardiology and Angiology, Faculty of Medicine, Alexandria University, Alexandria, Egypt
[4]Department of Chemical Pathology, Medical Research Institute, Alexandria University, Alexandria, Egypt

Association of Bone Turnover Biomarkers and Subclinical Atherosclerosis in Subjects with Type 2 Diabetes: A Case-Control Study

ABSTRACT

Objective: The current study aims to assess the relationship between serum osteocalcin (OCN) and osteoprotegerin (OPG) levels and subclinical atherosclerosis.

Material and methods: This case-control study included 80 male subjects divided into 2 groups: 40 subjects with type 2 diabetes (T2D) without coronary artery disease and 40 control subjects without diabetes. To assess the association of OCN and OPG with subclinical atherosclerosis (defined as carotid intima-media thickness (cIMT) ≥ 0.9mm), multivariable linear regression models were applied.

Results: The mean age in the diabetes group was 54.1 ± ± 5.1 years while in the control group, it was 53.7 ± ± 6.6 years. The mean serum OCN level was significantly negatively correlated with hs-CRP, cIMT, HbA1c and FPG in the total sample (p = 0.001, < 0.001, < 0.001 and 0.006 respectively) while OPG level was significantly positively correlated with age and HbA1c (p = 0.047 and 0.009 respectively) in the total sample. Age and HbA1c were the only independent risk factors identifying subclinical atherosclerosis in multivariate analysis. A cut-off value of serum OCN level of ≤ 22 ng/mL was able to discriminate patients with subclinical atherosclerosis in the total sample (p = 0.003*) using receiver operator characteristic (ROC) curve analysis. Serum OCN level was significantly lower in the subclinical atherosclerosis group than in the control while OPG showed no significant difference between both groups.

Conclusions: OCN may be a better marker for subclinical atherosclerosis than OPG. This effect is attenuated in the presence of DM. (Clin Diabetol 2024; 13, 1: 43–51)

Keywords: atherosclerosis, osteocalcin, osteoprotegerin, carotid intima-media thickness, subclinical atherosclerosis

Introduction

Type 2 diabetes (T2D) is a multifactorial metabolic disease characterized by hyperglycemia that results predominantly from insulin resistance (IR) [1]. Patients with diabetes are at a higher risk of accelerated atherosclerosis, which is the major cause of cardiovascular morbidity and mortality [2].

Moreover, the accelerated atherosclerosis was attributed to several metabolic and vascular derangements. The presence of a pro-inflammatory state is highly responsible for driving the progression of accelerated atherosclerosis. Several vascular changes arise starting with endothelial dysfunction, in addition to platelet abnormalities and arterial smooth muscle changes along with the deposition of advanced glycated end-products (AGEs) resulting from long-standing hyperglycemia [3].

Address for correspondence:
Heba Sadek Kassab
Unit of Diabetes and Metabolism, Department of Internal Medicine, Faculty of Medicine, Alexandria University, 17 Champollion Street, El Messallah, Alexandria 21131, Egypt, Phone: +20 1005536874, e-mail: heba.kassab@alexmed.edu.eg
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Evidence is mounting for the relationship between atherosclerotic cardiovascular diseases (ASCVD) and bone metabolism [4, 5]. Among the early indicators of ASCVD is the detection of an increased carotid intima-media thickness (cIMT) [6]. It is suggested that atherogenic pathways modulate the arterial smooth cells into osteoblast-like cells. The transformed osteoblast-like cells produce several markers including osteoprotegerin (OPG), which in turn result in the advancement of vascular calcification [7]. OPG plays a role in bone mineralization by inhibiting RANKL and osteoclast recruitment [8]. In addition, bone remodeling is influenced by the adipose tissues through the leptin release and effects on osteoblasts. Thus, the bone system is suggested to be linked to energy metabolism, supporting the hypothesis of the endocrinal function of bones [9].

Osteocalcin (OCN) is another marker produced by osteoblastic cells and implicated in the development of bones [10]. OCN plays a vital role in glucose homeostasis and insulin sensitivity, explained by regulating the expression of an adipose-related gene [11, 12]. Several studies suggested the role of osteocalcin in the formation of atherosclerotic plaques, and the calcification of the coronary arteries [13, 14]. However, conflicting data exists regarding the direct role of OCN in endothelial and smooth muscle cell function [15].

Therefore, we aimed to shed light on the relationship between subclinical atherosclerosis and the serum levels of bone turnover biomarkers OCN and OPG and to investigate their values as early markers of ASCVD.

Materials and methods

Study design

This study is a case-control study.

Study participants

The present study included 80 male subjects. Subjects were recruited from those undergoing coronary angiography for a justified indication at the cardiology department, Alexandria University, and proved to be free of atherosclerotic coronary artery disease (ACAD).

The participating subjects were divided into two groups:

— group I: 40 subjects with T2D (diagnosis of diabetes according to the American Diabetes Association criteria) [1] aged 44–67 years;
— group II: 40 age-matched control subjects without diabetes aged 42–72 years.

Exclusion criteria

We excluded subjects with a history of an established ASCVD, including cerebrovascular strokes, a history of ACAD, and peripheral arterial diseases. Patients with a history of an endocrinal disease other than T2D, any bone disorder, or metabolic diseases, and subjects with a recent history of infection within the last 2 months were also excluded from the study. Based on previous studies, female subjects were excluded from the study to avoid any bias in OCN level related to female hormonal changes and sex differences in atherosclerosis [16, 17].

Ethical approval

The study was approved by the ethics committee of Alexandria University following the criteria set by the Declaration of Helsinki. The participating subjects signed an informed consent at the beginning of the study.

Materials and methods

The following was performed for all subjects:

Clinical assessment

A detailed history taking was performed focusing on the history of diabetes. Anthropometric measures were taken including BMI calculation (weight in kg divided by the height in m^2). The waist circumference (WC) and the hip circumference were measured, and the waist-to-hip ratio (W/H) was calculated as the ratio between the waist measurement and the hip measurement.

Assessment of ankle-brachial index (ABI)

Hand-held Doppler was applied to assess both the dorsalis pedis (DP) and posterior tibial (PT) arteries. The systolic pressure of the PT and DP arteries of each leg was measured using a Doppler probe of 5 MHz (Nicolet Elite 200 R, VIASYS Healthcare Inc., Madison, WI, USA). The lower value of the two calculated ABI ratios in both limbs was applied for statistical analysis [18].

Biochemical analysis

Blood samples were collected in the morning (8.00–10.00 a.m.) of the same day as the coronary angiography after an overnight fast of 10–12 hours. All subjects were advised not to smoke or exercise strenuously during the fasting period.

The collected venous samples were divided into 2 parts; one part in a plain vacutainer tube left to clot at 37°C: sera were separated by centrifugation and divided into 2 parts, one used for immediate assay of fasting plasma glucose (FPG), HbA1c, ALT, hs-CRP, insulin level, total cholesterol, triglycerides and HDL cholesterol. LDL cholesterol was calculated using the Friedewald formula. The other part was kept at –70°C for assay of OCN. Serum OCN was determined using sandwich-type

enzyme labeled immunoassay (Assay kit ab195214, USA) according to the manufacturer's instructions. OCN measurements are reported in ng/mL. The serum OPG level was measured using an ELISA assay kit according to the manufacturer's instructions. Homeostasis Model Assessment 2 (HOMA2) calculator was used to estimate insulin resistance (%S) (HOMA2-IR).

Measurement of the carotid intima-media thickness (cIMT)

All the study subjects were subjected to carotid duplex scanning of both carotid arteries. Using a high-resolution 7–12 MHz linear transducer in B mode (Philips ClearVue 350), the intima-media thickness (IMT) was measured on the common carotid artery. cIMT of the far wall was specified as the distance between the leading edge of the lumen-intima interface and the leading edge of the media-adventitia interface. We assessed three sites on each side and the average was calculated for the cIMT: thickest point and at sites 1 cm upstream and downstream, free from plaques on the longitudinal views. A measure of cIMT ≥ 0.9 mm was identified as a marker of subclinical atherosclerosis [19].

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Categorical data were represented as numbers and percentages. **The chi-square test** was applied to investigate the association between the categorical variables. Alternatively, **Fisher's Exact or Monte Carlo correction test** was applied when more than 20% of the cells had an expected count of less than 5. For continuous data, they were tested for normality by **the Kolmogorov-Smirnov test**. Quantitative data were expressed as mean and standard deviation (SD). **Mann-Whitney test** was used to compare two groups for not normally distributed quantitative variables. **Student t-test** was used to compare two groups for normally distributed quantitative variables, and **Spearman coefficient** was used to assess the correlation between two distributed abnormally quantitative variables.

Logistic regression analysis was used to detect the strongest risk factors discriminating atherosclerosis (IMT ≥ 0.9 mm) in the total sample and diabetes group. The diagnostic performance of the markers was determined by the receiver operating characteristic curve (ROC), where an area greater than 50% defined acceptable performance, and an area of nearly 100% was considered the best performance for the test. The significance of the obtained results was judged at the 5% level.

Results

Baseline demographic and clinical characteristics of the study population

The mean age of the subjects in the T2D group was 54.1 ± 5.1 years while in the control group, it was 53.7 ± 6.6 years with no statistically significant difference. The mean diabetes duration in the T2D group was 11.1 ± 6.3 years. Subjects with T2D had significantly higher levels of OPG, hs-CRP and cIMT and significantly lower OCN and ABI than the control group. A comparison between the two studied groups is shown in Table 1.

Relationship of OCN & OPG serum levels with the studied parameters

The mean serum OCN level was significantly negatively correlated with hs-CRP, cIMT, HbA1c and FPG in the total sample ($p = 0.001$, < 0.001, < 0.001 and 0.006 respectively) and significantly negatively correlated with age and cIMT ($p = 0.005$ and 0.001, respectively) in the control group. However, there were no significant correlations between serum osteocalcin levels and the studied parameters in the diabetes group. On the other hand, the mean serum OPG level was significantly positively correlated with age and HbA1c ($p = 0.047$ and 0.009 respectively) in the total sample and significantly positively correlated with age ($p = 0.003$) in the diabetes group while in the control group, the mean serum OPG level was significantly positively correlated with total cholesterol, HDL-C and LDL-C ($p = 0.010$, 0.046 and 0.029 respectively).

On performing univariate logistic regression analysis to identify risk factors of subclinical atherosclerosis (cIMT ≥ 0.9 mm), age, smoking, OCN, HbA1c, FPG and UACR were the independent risk factors in the total sample. However, only age and HbA1c were the independent risk factors of subclinical atherosclerosis in multivariate analysis (Tab. 2). On the other hand, in the diabetes group, only HbA1c was the independent risk factor of subclinical atherosclerosis in univariate logistic regression analysis. (Data not depicted).

Sensitivity and specificity of OCN & OPG to identify subclinical atherosclerosis

Serum OCN level was able to discriminate patients with subclinical atherosclerosis in the total sample ($p = 0.003^*$) with a cut-off value of ≤ 22 ng/mL with good sensitivity, specificity, and AUC (64.71, 63.04 and 0.696, respectively) (Fig. 1). Regarding the control group, a cut-off value of serum OCN level ≤ 25 ng/mL was set discriminating patients with subclinical atherosclerosis ($p = 0.001^*$) with better sensitivity, specificity, and AUC (87.50, 68.75 and 0.873, respectively) while in diabetes group there was no significant discrimina-

Table 1. Comparison between the Two Studied Groups According to Different Parameters

Mean ± SD	Group I (diabetes) (n = 40)	Group II (control) (n = 40)	p-value
Age [years]	54.1 ± 5.1	53.7 ± 6.6	0.763
Smoker [%]	29 (72.5%)	12 (30%)	< 0.001*
DM duration [years]	11.1 ± 6.3	—	
OPG [ng/mL]	506.4 ± 488.5	266.7 ± 272.7	0.029*
OCN [ng/mL]	20.3 ± 11.2	31 ± 13	< 0.001*
hs-CRP [mg/L]	12.3 ± 11.2	5.3 ± 6.1	< 0.001*
cIMT [mm]	0.95 ± 0.20	0.71 ± 0.18	< 0.001*
FPG [mg/dL]	140.7 ± 76.1	92.5 ± 18.9	0.005*
HbA1c [%] (mmol/mol)	8.6 (70) ± 2	5.7 (39) ± 0.5	< 0.001*
ABI	0.99 ± 0.08	1.05 ± 0.09	0.001*
Total cholesterol [mg/dL]	164.1 ± 44.8	167.8 ± 43.6	0.707
TG [mg/dL]	146.3 ± 52.4	131.4 ± 64.8	0.166
HDL-C [mg/dL]	36.5 ± 13.5	43.3 ± 16.4	0.048*
LDL-C [mg/dL]	98.3 ± 36.8	98.3 ± 32.3	0.994
BMI [kg/m^2]	28.4 ± 3.5	27.1 ± 3.6	0.104
WC [cm]	100.6 ± 4.6	97.5 ± 6.4	0.015*
Waist-to-hip ratio	0.97 ± 0.02	0.95 ± 0.03	0.005*
Albuminuria [%]	12 (30%)	0 (0%)	< 0.001*
HOMA2IR	1.67 ± 1.68	1.54 ± 1.21	0.946

*Statistically significant at $p \leq 0.05$
ABI — ankle-brachial index; BMI — body mass index; cIMT — carotid intima-media thickness; DM — diabetes mellitus; FPG — fasting plasma glucose; HbA1c — glycated hemoglobin; HDL-C — high-density lipoprotein cholesterol; HOMA2IR — Homeostasis Model Assessment 2 of insulin resistance; hs-CRP — high-sensitivity C-reactive protein; LDL-C — low-density lipoprotein cholesterol; OCN —osteocalcin; OPG — osteoprotegerin; SD — standard deviation; TG — triglycerides; WC — waist circumference

tion between patients with subclinical atherosclerosis and those without (p=0.580). On the contrary, OPG failed to discriminate against patients with subclinical atherosclerosis in the 3 studied groups.

The total sample (n = 80) was re-classified according to cIMT into 2 groups: a group with subclinical atherosclerosis (cIMT ≥ 0.9 mm) and a control group. Serum OCN level was significantly lower in the subclinical atherosclerosis group than control while OPG showed no significant difference between groups. A comparison between the 2 groups is shown in Table 3.

Discussion

Previous data supported the endocrine capacity of bone and its role in glucose and lipid metabolism. OCN and OPG are well-known bone turnover biomarkers. Their relation to vascular calcification in patients with vascular diseases such as myocardial infarction and diabetes was previously thoroughly studied. However, its relation to subclinical atherosclerosis in males without ACAD (proved by coronary angiography) has not been studied yet.

The present study showed a significantly lower level of OCN and significantly higher OPG level in the diabetes group than in the control group. This supports that diabetes is a state of low bone turnover. In agreement with the results of the present study, Hygum et al.'s [20] results showed significantly higher OPG and significantly lower OCN levels in patients with diabetes. Another study by Starup-Linde et al. [21] showed significantly lower OCN levels in patients with diabetes. They also observed higher levels of plasma OPG level in patients with increasing plasma glucose levels.

The current study showed a significant negative correlation of serum OCN level with hs-CRP, cIMT, HbA1c and FPG in the total sample and a significant negative correlation of OCN with age and IMT in the control group suggesting its important association with atherosclerosis, inflammation and diabetes. In agreement with the results of the present study, a study by Seidu et al. [22] stated an inverse association between OCN and cIMT and the risk of atherosclerotic outcome and CVD. Moreover, the Changfeng study [23] which included male participants with normal glucose tolerance, disclosed the presence of an independent association between serum OCN and carotid atherosclerosis in this cohort. Furthermore, in euglycemic males, carotid plaque prevalence decreased significantly with

Table 2. Univariate and Multivariate Logistic Regression Analysis to Detect the Strongest Risk Factors Discriminating Subclinical Atherosclerosis (cIMT ≥ 0.9 mm) in the Total Sample (n = 80)

	OR (LL–UL 95% CI), p	OR (LL–UL 95% CI), p
Age [years]	1.137(1.040–1.242), p = 0.005*	1.159 (1.033–1.301), p = 0.012*
Smoker [%]	4.094 (1.583–10.587), p = 0.004*	2.625 (0.755–9.125), p = 0.129
DM duration [years]	1.103 (0.987–1.234), p = 0.084	
OPG [ng/mL]	1.00 (0.999–1.001), p = 0.816	
OCN [ng/mL]	0.942 (0.902–0.983), p = 0.006*	0.979 (0.925–1.035), p = 0.452
hs-CRP [mg/L]	1.023 (0.976–1.072), p = 0.345	
HbA1c [%](mmol/mol)	2.052 (1.455–2.894), p = < 0.001*	1.705 (1.077–2.698), p = 0.023*
ABI	0.051 (0.0–8.252), p = 0.252	
Total cholesterol[mg/dL]	1.001 (0.991–1.011), p = 0.831	
TG [mg/dL]	1.002 (0.995–1.010), p = 0.524	
HDL [mg/dL]	–0.029 (0.941–1.002), p = 0.067	
LDL [mg/dL]	1.006 (0.993–1.019), p = 0.376	
BMI [kg/m^2]	0.995 (0.878–1.127), p = 0.936	
WC [cm]	1.042 (0.964–1.128), p = 0.301	
Waist-to-hip ratio	2483189.856 (0.019–), p = 0.123	
FPG [mg/dL]	1.014 (1.003–1.024), p = 0.008*	1.010 (0.998–1.022), p = 0.101
UACR [mg/g]	9.167 (1.855–45.293), p = 0.007*	2.178 (0.266–17.852), p = 0.468
HOMA2IR	1.346 (0.943–1.922), p = 0.102	

*Statistically significant at p ≤ 0.05; #All variables with p < 0.05 was included in the multivariate
ABI — ankle-brachial index; BMI — body mass index; CI — confidence interval; cIMT — carotid intima-media thickness; DM — diabetes mellitus; FPG — fasting plasma glucose; HbA1c — glycated hemoglobin; HDL — high-density lipoproteins; HOMA2IR — Homeostasis Model Assessment 2 of insulin resistance; hs-CRP — high-sensitivity C-reactive protein; LDL — low-density lipoproteins; LL — lower limit; OCN — osteocalcin; OPG — osteoprotegerin; SD — standard deviation; TG — triglycerides; UACR — urinary albumin/creatinine ratio; UL — upper limit; WC — waist circumference

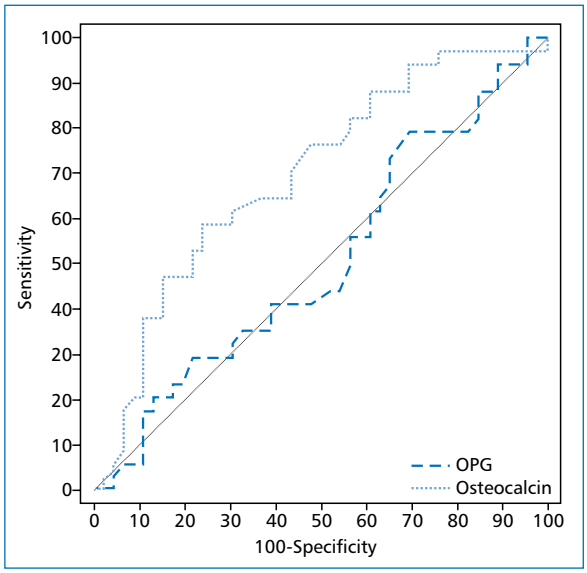

Figure 1. ROC Curve for Osteoprotegrin and Osteocalcin to Discriminate Patients with Subclinical Atherosclerosis (cIMT ≥ 0.9 mm) (n = 34) in the Total Sample
cIMT — carotid intima-media thickness; OPG — osteoprotegerin

increased OCN levels. Another study by Pennisi et al. [24], where half of their patients had DM, reported an association between lower serum levels of OCN and disorders in carotid arteries.

Moreover, Xu et al. [25] also found a significant negative correlation between OCN and cIMT in the total sample. Additionally, Zhang et al. [26] found a significant negative correlation between OCN, HbA1c and FPG. Bao et al. [17] similarly found that serum OCN levels had a significant negative correlation with HbA1c, FPG, BMI and HOMA-IR. These results go hand in hand with the results of the current study reflecting the important role played by OCN in the triad of diabetes, inflammation and atherosclerosis.

On the other hand, Reyes-Garcia et al. [27] found no significant correlation between OCN, BMI, HbA1c or FPG. However, they reported that serum OCN is an independent predictor of coronary heart disease in logistic regression analysis. On the contrary, Millar et al. [28] found no significant difference in OCN levels between patients with atherosclerosis and the control group. This discordance between their findings and our results may reflect the role of OCN in subclinical but not established atherosclerosis. Another study by Luo et al. [29] found no association between OCN and cIMT in the metabolically healthy Chinese population. This difference between their study and ours may arise

Table 3. Comparison between Patients with Subclinical Atherosclerosis (cIMT ≥ 0.9 mm) and Patients without Atherosclerosis Regarding Different Parameters in the Total Sample (n = 80)

Mean ± SD	Carotid intima-media thickness		p-value
	< 0.9 mm (n = 46)	≥ 0.9 mm (n = 34)	
Age [years]	52.2 ± 4.9	56.1 ± 6.4	0.002*
Smoker [%]	29 (72.5%)	12 (30%)	< 0.001*
DM duration [years]	8.9 ± 6.1	12.7 ± 6.3	0.105
OPG [ng/mL]	377.4 ± 418.3	398.9 ± 407.2	0.915
OCN [ng/mL]	29.4 ± 13.6	20.6 ± 10.9	0.003*
hs-CRP [mg/L]	7.9 ± 9.1	10 ± 10.4	0.124
HbA1c [%](mmol/mol)	6.2 (44) ± 1.2	8.4 (68) ± 2.2	<0.001*
ABI	1.03 ± 0.09	1.01 ± 0.08	0.255
Total cholesterol [mg/dL]	165.1 ± 38.5	167.2 ± 51	0.833
TG [mg/dL]	135.2 ± 61.2	143.7 ± 56.5	0.411
HDL-C [mg/dL]	42.7 ± 16	36.2 ± 13.6	0.062
LDL-C [mg/dL]	95.4 ± 29.8	102.3 ± 40	0.380
BMI [kg/m^2]	27.7 ± 3.6	27.7 ± 3.6	0.937
WC [cm]	98.5 ± 6.1	99.8 ± 5.2	0.304
Waist-to-hip ratio	0.96 ± 0.03	0.97 ± 0.02	0.110
FPG [mg/dL]	99.3 ± 43.1	139.9 ± 71.8	0.001*
Albuminuria [%]	2 (4.3%)	10 (29.4%)	0.002*
HOMA2IR	1.36 ± 1.37	1.94 ± 1.53	0.020*

*Statistically significant at p ≤ 0.05; p-value for comparing between < 0.9 mm and ≥ 0.9 mm
ABI — ankle-brachial index; BMI — body mass index; DM — diabetes mellitus; FPG — fasting plasma glucose; HbA1c — glycated hemoglobin; HDL-C — high-density lipoprotein cholesterol; HOMA2IR — Homeostasis Model Assessment 2 of insulin resistance; hs-CRP — high-sensitivity C-reactive protein; LDL-C — low-density lipoprotein cholesterol; OCN — osteocalcin; OPG — osteoprotegerin; SD — standard deviation; TG — triglycerides; WC — waist circumference

from using different cohort (our cohort were only men) and the fact that in their study cardiovascular disease was excluded on the basis of medical history only while we excluded CAD by coronary angiography which is more specific. A study conducted by Kang [30] showed a significant negative correlation of OCN with serum glucose and HOMA-IR but not with coronary atherosclerosis reflecting its role in glucose metabolism and insulin resistance but not atherosclerosis.

The present study showed no significant correlations between serum OCN levels and other parameters in the diabetes group. Sheng et al.[31] in discordance with the results of the present study, found an association between low OCN levels and carotid atherosclerosis in people with T2DM. They found a significant negative correlation of OCN with FPG, age, fasting insulin, CRP and HOMA-IR. Kanazawa et al. [32] similarly found a negative correlation of OCN with cIMT, HbA1c and FPG in people with T2D. This difference may be due to the presence of other risk factors of atherosclerosis in the diabetes group and the present study cohort was free from CAD, which was confirmed by coronary angiography.

The present study showed a significant positive correlation of the mean serum OPG level with age and HbA1c in the total sample and a significant positive correlation with age in the diabetes group while in the control group, the mean serum OPG level was significantly positively correlated with total cholesterol, HDL-C and LDL-C. O'Sullivan et al. [33] reported similar results with higher levels of OPG, IL6 and hs-CRP in people with diabetes. However, in cases without vascular affection, OPG was the only significantly higher biomarker, suggesting a different pathophysiological process. They also found no significant correlation between OPG and all studied parameters in the diabetes group while in the control group, it correlated positively with age. Another study by Zwakenberg et al. [5] found no significant correlation between OCN, OPG and CVD in people with diabetes. The difference between their study and ours may result from using a different cohort including 82% females and the fact that their endpoint was established CVD.

The current study showed a significantly lower OCN level in patients with subclinical atherosclerosis while there was no significant relation between serum OPG level and subclinical atherosclerosis. In agreement with the results of the present study, Deng et al. [34] showed a significantly lower level of OCN in patients with carotid atherosclerosis than in the control group

and a significant negative correlation between OCN and cIMT. Zhang et al. [26] found that serum OCN level was significantly lower in patients with coronary heart disease. Moreover, Xu et al. [25] reported similar results with significantly lower OCN levels in male patients with a moderate or high risk of ASCVD than those with low risk.

Maser et al. [35], in discordance with the results of the present study, suggested that OPG is better than OCN as a biomarker of arterial calcification in T2D. This difference may arise from the fact that most of their patients had established atherosclerosis, unlike our patients whose coronary angiography was free. These results may be complementary as OCN could be a better marker of subclinical atherosclerosis while OPG is better in advanced cases with established atherosclerosis. Davenport et al. [36] found significantly higher serum OPG levels in male patients with diabetes having CAD with multivessel disease. They concluded that serum OPG levels could help differentiate those high-risk patients.

Mogelvang et al. [37] studied serum OPG levels in patients with clinical and subclinical atherosclerosis and found that these patients had a significantly higher level of OPG. Their results showed a significant positive correlation between OPG and traditional risk factors of atherosclerosis including DM in both patients with and without clinical atherosclerosis. The difference between their results and ours may arise from the use of different cohorts and the fact that they (unlike our study) included both males and females and not all their patients had DM.

Our results showed that age, smoking, OCN, HbA1c, FPG and UACR were the independent risk factors of subclinical atherosclerosis (IMT $\geq$ 0.9mm) in the total sample. However, in multivariate analysis, only age and HbA1c were the independent risk factors of subclinical atherosclerosis. In the diabetes group, only HbA1c was the independent risk factor of subclinical atherosclerosis in univariate logistic regression analysis. Zhang et al. [26] showed a linear relation between OCN and CHD risk in regression analysis. Xu et al. [25] found that BMI and HbA1c were the predictors of low OCN levels after performing multivariate regression analysis. On the contrary, Sheng et al. [31] found that age, gender, OCN systolic blood pressure, LDL-C and HDL-C were independently associated with cIMT in patients with T2D in multivariate regression analysis.

To the best of our knowledge, this is the first study to set a cut-off value of OCN discriminating patients with subclinical atherosclerosis from those without atherosclerosis in subjects approved to have no ACAD by cardiac angiography done during subjects' recruitment.

However, the present study showed some limitations; including the small sample size, and that it included only the Egyptian population enrolled from a single center. Application of the study results to other races requires investigation. Study results could be generalized on males only because females were excluded due to the effect of gender on OCN level and different risk factors of atherosclerosis. Future follow-up research is required to confirm the independent association between OCN level and the development and progression of cardiovascular events and atherosclerotic diseases as the cross-sectional design of the study couldn't confirm the causal relationship between bone turnover biomarkers and subclinical atherosclerosis.

Conclusions

The present study showed a significantly higher level of OCN and a significantly lower level of OPG in subjects with T2D than the control group. This reflects the state of low bone turnover in T2D. The OCN level showed better correlations and better regression results than OPG in detecting patients with subclinical atherosclerosis. This effect is attenuated in the presence of T2D and may be due to the presence of other factors affecting atherosclerosis in T2D. A cut-off value of $\leq$ 22 ng/mL for OCN could predict subclinical atherosclerosis in the total sample with good sensitivity, specificity and AUC. We could suggest that OCN is a better marker for subclinical atherosclerosis than OPG while both may have a role in established atherosclerosis and CAD.

Article information

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics statement

The study design was approved by the ethics committee of Alexandria University.

Author contributions

Nagwa A. Lachine, Eman Y. Morsy and Noha G. Amin designed the study and defined the aim of work. They participated in the interpretation of data and writing the manuscript. Abdel Aziz Elnekiedy, Mohamed A. Sadaka, Gehan I. Khalil, Heba S. Kassab have participated to data collection and data analysis and revising the manuscript. Hesham G.Imam participated in data collection and analysis, in addition to writing the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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