Journal of Diabetes and Its Complications 33 (2019) 592–597

Contents lists available at ScienceDirect

Journal of Diabetes and Its Complications

journal homepage: WWW.JDCJOURNAL.COM

# Serum sclerostin level and its relation to subclinical atherosclerosis in subjects with type 2 diabetes

Magui Abdel Moneim Shalash [a], Kamel Hemida Rohoma [a,*], Noha Said Kandil [b], Mohsen Ahmed Abdel Mohsen [c], Aya Abdul Fattah Taha [a]

[a] Department of Internal Medicine (Unit of Diabetes and Metabolism), Faculty of Medicine, Alexandria University, Alexandria, Egypt
[b] Department of Chemical Pathology, Medical Research Institute, Alexandria University, Alexandria, Egypt
[c] Department of Radiology, Faculty of Medicine, Alexandria University, Alexandria, Egypt

ARTICLE INFO

*Article history:*
Received 25 December 2018
Received in revised form 18 March 2019
Accepted 21 April 2019
Available online 27 April 2019

*Keywords:*
Sclerostin
CIMT
Subclinical atherosclerosis
T2DM

ABSTRACT

*Background:* Sclerostin, a Wnt-signalling inhibitor, is an established negative regulator of bone formation. However, data regarding its potential importance in vascular disease are less clear. Common carotid artery media thickness (CIMT) assessment and plaque identification using ultrasound imaging are well-recognized tools for identifying and monitoring atherosclerosis. The aim of the present study is to examine the relationship between serum sclerostin and subclinical atherosclerosis (as evidenced by CIMT).
*Methods:* This cross-sectional study included 50 subjects with T2DM and 20 subjects as a control group. Multivariable linear regression models were used to assess the association of sclerostin with subclinical atherosclerosis.
*Results:* Serum sclerostin levels in T2DM patients were significantly higher compared to the control group ($167.16 \pm 63.60$ versus $85.98 \pm 23.74$ pg/ml, $P < 0.0001$). A concentration of ≥162.5 pg/ml showed a sensitivity of 90% and a specificity of 86.67% to detect an increased risk of subclinical atherosclerosis. Univariate analysis revealed a significant positive correlation between serum sclerostin and CIMT ($r = 0.635$, $P < 0.001$). Sclerostin concentrations remained independently associated with CIMT ($\beta = 63.188$ [6.919–119.456], $P = 0.017$) after adjusting for age and gender.
*Conclusion:* Our data suggest a positive correlation between serum sclerostin level and subclinical atherosclerosis in subjects with type 2 diabetes mellitus.



## 1. Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder featuring hyperglycemia resulting from a combination of pathophysiological factors mainly resistance to insulin action and inadequate insulin secretion.[1] Patients with diabetes are at a higher risk of accelerated atherosclerosis which culminates in coronary artery disease, peripheral arterial disease as well as cerebrovascular disease seriously affecting morbidity and mortality.[2] Hyperglycemia, dyslipidemia and hypertension create a proinflammatory state that promotes an atherogenic milieu inducing endothelial injury, accelerating atheromatous plaque formation and decreasing its stability.[3] In the setting of atherosclerosis, vascular smooth muscle cells (VSMC) in arterial media have shown phenotypic switching acquiring macrophagal properties creating a VSMC that is pro-atherogenic.[4]

Canonical and non-canonical Wnt signalling has been demonstrated to impose adipogenic and myofibrotic phenotypic switches in pericytes and VSMCs.[5] The canonical Wnt or Wnt/b-catenin pathway is increasingly being linked to the regulation of VSMC proliferation, migration, and survival via modulation of the expression of matrix proteins.[6] Activation of the Wnt/b-catenin pathway depends on the subsequent association that occurs between Frizzled receptors and with the endogenous co-receptors low density lipoprotein (LDL) related proteins 5 and 6 (LRP 5/6).[7] The resulting complex causes disassembly of the B-catenin destruction complex causing rapid accumulation of the phosphorylated B- catenin and eventually translocation from the cytoplasm to the nucleus. B-catenin assists in the activation of T-cell factor/lymphoid enhancing binding factor (TCF/LEF) transcription of target genes involved with cell cycle activation and survival.[8]

Evidence in literature from other cell types presented different regulatory factors that activate Wnt in arterial and venous SMC including retinoic acid,[9] cytokines including IL-6,[10] low oxygen,[11] toll-like

Conflict of interest: None.

* Corresponding author at: Department of Internal Medicine (Unit of Diabetes and Metabolism), Faculty of Medicine, Alexandria University, 17 Champollion Street, El Messallah, Alexandria 2113, Egypt.
*E-mail address:* kamel.hemida@alexmed.edu.eg (K.H. Rohoma).

https://doi.org/10.1016/j.jdiacomp.2019.04.012
1056-8727/

receptor activators, lipopolysaccharide,[12] and LDL may be regulators of Wnt activity.[13]

The pervasiveness of aberrant Wnt signalling in cardiovascular disease (CVD) makes it a potential biomarker. Emerging experimental evidence suggests that some inhibitors of the canonical Wnt signalling pathway have been actively involved in bone formation and vascular calcification.[14] Extracellular antagonists of the Wnt signalling pathway including two main branches the first is the secreted Frizzled-related protein (sFRP) family while the second class comprises certain members of the Dickkopf (Dkk) family have shown involvement in vascular injury.[15] Nevertheless, Sclerostin produced by the osteocytes through the SOST gene have shown also major regulatory role of the canonical Wnt pathway.[16]

Sclerostin levels have been positively associated with abdominal fat, dyslipidemia particularly HDL and LDL cholesterol[17] as well as HbA1C[18] hence suggesting involvement of sclerostin in the pathogenesis of metabolic disease. Sclerostin had been highly expressed in calcified aortic tissue derived from three human aortic samples obtained from patients with atherosclerosis.[19] In a pro-calcifying environment VSMC undergo osteo/chondrogenic transdifferentiation. The resulting osteoblast-like cells express several osteogenic genes including SOST gene involved in the production of sclerostin.[20] Hence explaining higher sclerostin levels seen in diabetic patients with atherosclerosis due to the deleterious effect of hyperglycemia in vascular integrity.[21]

However, no previous study clarified the relationship of serum sclerostin with subclinical atherosclerosis in subjects with T2DM. Therefore, the usefulness of sclerostin as a serum marker of atherosclerotic risk and vascular lesions in patients with T2DM merits further studies. In this context our aim was to study the relationship between subclinical atherosclerosis and serum sclerostin level in T2DM.

## 2. Methods

### 2.1. Study design

Fifty patients (23 men and 27 women) aged between 40 years and 60 years with a diagnosis of diabetes according to the American Diabetes Association criteria[22] were enrolled in the current study from among patients attending the outpatient clinics of the Diabetes and metabolism department of Alexandria Main University hospital. All patients had normal renal function (as assessed by serum creatinine levels). In addition, 20 apparently healthy subjects (7 men and 13 females) age-matched were included as a control group. All the controls included had normal glucose homeostasis as assessed by fasting glucose levels and measurement of glycated haemoglobin (HbA1c). The study design was approved by the ethics committee of Alexandria University. The study was conducted according to the criteria set by the declaration of Helsinki and each subject signed an informed consent before participating in the study.

### 2.2. Exclusion criteria

Patients with history of cerebrovascular disease (stroke, transient ischemic attack) or with peripheral vascular disease due to diabetes or any other cause. Patients with known history of coronary heart disease or severe cardiac decompensation. Presence of severe uncontrolled hypertension. Patients with end stage renal disease. Patients with Familial hypercholesterolemia, connective tissue diseases and vasculitis were also excluded.

### 2.3. Subjects and methods

All participants underwent the following.

#### 2.3.1. Clinical assessment

Full assessment of history was performed stressing on duration of diabetes, symptoms of vascular complications of diabetes, presence of chronic illnesses and detailed drug history. BMI was calculated using the Quetelet formula (weight in kg divided by the height in $m^2$).

The waist circumference was measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest, using a stretch-resistant tape. Hip circumference was measured around the widest portion of the buttocks, with the tape parallel to the floor. Each measurement was repeated twice; and the average calculated. Waist-hip ratio was calculated as waist measurement divided by hip measurement.

#### 2.3.2. Assessment of ankle brachial index using hand-held Doppler

Doppler examination of dorsalis pedis (DP) and posterior tibial (PT) arteries with calculation of ABI was done to both groups. Sphygmomanometer cuff was placed just above the ankle and a continuous-wave Doppler probe operating at 5 MHz (Nicolet Elite 200 R, VIASYS Healthcare Inc., Madison, WI, USA) was used to measure the systolic pressure of the PT and DP arteries of each leg. Then the ABI was calculated by dividing the ankle pressure (the higher pressure of the DP and the PT arteries) by the higher of the two brachial arterial systolic pressure measurements. Then the lower value of the two calculated ABI values for either limb was used for analysis.[23]

#### 2.3.3. Biochemical analysis

Blood samples were taken at the same time of the day for all subjects and in the fasting state. Blood samples were centrifuged within 20 min of collection at 4 °C. Ethylenediaminetetraacetic acid (EDTA) plasma was separated from cells immediately after centrifugation and stored at −20 °C until analysed. Total cholesterol, triglycerides, and HDL cholesterol were analysed using standard enzymatic methods. LDL cholesterol was calculated using the Fried Ewald formula. The percentage of glycated haemoglobin was measured by colorimetric method. A preparation of whole blood is mixed with a weakly binding cation- exchange resin. The non-glycated haemoglobin binds to the resin, leaving HbA1C to be removed by means of a resin separator in the supernatant.[24] Serum sclerostin was determined using a commercially available sandwich type enzyme labelled immunoassay (Assay kit Co. Ltd., Sunnyvale, USA) according to the manufacturer's instructions. Sclerostin measurements are reported in picogram per millilitre.

#### 2.3.4. Carotid studies

Measurements of carotid intima media thickness (CIMT) were performed on common carotid artery. All participants underwent carotid duplex scanning of both carotid arteries. CIMT was evaluated using a high-resolution 7–12 MHz linear transducer in B mode (Philips ClearVue 350). Far-wall measurements were obtained. CIMT of the far wall was defined as the distance between the leading edge of the lumen-intima interface and the leading edge of the media-adventitia interface. CIMT was measured on each side at three sites (thickest point, and at sites 1 cm upstream and downstream, free from plaques) on the longitudinal views. In cases with uniform CIMT, measurements were obtained 1 cm from the bifurcation to the end of the common carotid artery. CIMT was calculated as the average of measurements from three different sites on each side. C-IMT >0.9 mm was taken as a marker of asymptomatic organ damage.[25]

#### 2.3.5. ECG stress test

All participants enrolled for the study underwent exercise treadmill testing as an initial assessment to exclude coronary artery disease that can be evidenced by hemodynamic instability, arrhythmias or electrocardiographic signs of ischemia. A meta-analysis of 147 studies performed by the American College of Cardiology found exercise ECG to have a pooled sensitivity of 68% and specificity of 77% for detection of coronary artery disease (CAD).[26]

### 2.4. Statistical analysis

Data are presented as mean ± SD. The Student's *t*-test was used to compare parameters between the two groups. Kruskal Wallis test was used for abnormally distributed quantitative variables, to compare between more than two studied groups, and Post Hoc (Dunn's multiple comparisons test) for pairwise comparisons. For continuous variables, mean values between two groups were compared by unpaired Student *t*-test for normally distributed variables and Mann-Whitney *U* test for skewed variables. The $\chi 2$ test was used to compare categorical variables between groups. Pearson (normal distribution) and Spearman correlation analyses (non-normal distribution) were used to assess the correlations between serum sclerostin and other continuous parameters. All statistical analyses were carried out using the SPSS software (version 20.0; IBM Corporation, Armonk, NY, USA).

Linear regression analyses were performed to examine relationships between CIMT and clinical variables. To determine the independent variables correlated with sclerostin (dependent variable), the parameters that correlate significantly in univariate analysis and others that are biologically linked to sclerostin were tested in multiple backward model linear regression analysis. The usefulness of serum sclerostin as a marker of high risk of subclinical atherosclerosis in T2DM was analysed using a receiver operating characteristic (ROC) curve. A *P* value <0.05 was considered significant.

## 3. Results

### 3.1. Baseline demographic and clinical characteristics of the study population

The mean serum sclerostin level in T2DM patients was higher compared with age- and gender-matched healthy controls (167.16 ± 63.60 versus 85.98 ± 23.74 pg/ml, $P < 0.0001$). CIMT was significantly increased in T2DM patients than in healthy subjects (0.94 ± 0.20 versus 0.65 ± 0.11, $P < 0.001$). Anthropometric measurements including BMI and Waist/Hip Ratio are significantly higher in the diabetic group than control (29.88 ± 3.56 versus 25.57 ± 1.84 kg/m$^2$ $P < 0.001$) and (0.96 ± 0.16 versus 0.80 ± 0.08, $P < 0.001$) respectively. Fasting Plasma glucose, HbA1C, serum urea, serum creatinine, total cholesterol and triglycerides were significantly higher in the diabetic group versus control. Meanwhile, HDL was significantly higher in the control group. There was no significant difference in ABI between both groups (Table 1).

**Table 1**
Baseline demographic and clinical characteristics of subjects.

| | Group I T2DM patients | Group II Non-diabetic controls | *P* value |
|---|---|---|---|
| n | 50 | 20 | |
| Men/women | 23/27 | 7/13 | 0.401 |
| Age (years) | 52.40 ± 5.12 | 50.3 ± 5.22 | 0.135 |
| BMI (kg/m$^2$)[a] | 29.88 ± 3.56 | 25.57 ± 1.84 | <0.001* |
| Waist circumference (cm) | 112.2 ± 11.29 | 92.50 ± 10.09 | <0.001* |
| Hip circumference (cm) | 118.6 ± 8.94 | 114.90 ± 3.81 | 0.017* |
| Waist/hip ratio | 0.96 ± 0.16 | 0.80 ± 0.08 | <0.001** |
| ABI[b] | 1.12 ± 0.09 | 1.12 ± 0.04 | 0.952 |
| CIMT[c] | 0.94 ± 0.20 | 0.65 ± 0.11 | <0.001* |
| FPG[d] | 149.1 ± 29.03 | 82.10 ± 8.52 | <0.001* |
| HbA1c[e] | 8.59 ± 1.72 | 5.62 ± 0.44 | <0.001* |
| Serum urea (mg/dl) | 28.52 ± 7.23 | 21.80 ± 7.24 | 0.001* |
| Serum creatinine (mg/dl) | 0.80 ± 0.14 | 0.69 ± 0.14 | 0.006* |
| Total cholesterol (mg/dl) | 199.2 ± 44.30 | 186.7 ± 30.79 | 0.253 |
| TG (mg/dl)[f] | 156.9 ± 79.17 | 113.5 ± 50.35 | 0.006** |
| HDL (mg/dl)[g] | 46.72 ± 10.21 | 60.0 ± 9.02 | 0.001* |
| LDL (mg/dl)[h] | 119.6 ± 34.10 | 111.3 ± 23.49 | 0.342 |
| Sclerostin (pg/ml) | 167.16 ± 63.60 | 85.98 ± 23.74 | <0.001** |

Data are means ± SD.
[a] BMI: body mass index.
[b] ABI: ankle brachial index.
[c] CIMT: carotid intimal media thickness.
[d] FPG: fasting plasma glucose.
[e] HbA1c: haemoglobin A1c (glycated haemoglobin).
[f] TG: triglycerides.
[g] HDL: high density lipoprotein.
[h] LDL: low density lipoprotein.
* Unpaired *t*-test: ˆ0.05 between groups.
** U: Mann Whitney test: *P* ˆ 0.05.

### 3.2. Risk factors for increased CIMT

CIMT was significantly positively correlated with age ($r = 0.642$, $P < 0.001$) diabetes duration ($r = 0.458$, $P = 0.001$), BMI ($r = 0.417$, $P = 0.003$), waist circumference ($r = 0.309$, $P = 0.029$), waist/hip ratio ($r = 0.305$, $P = 0.031$), FPG ($r = 0.400$, $P = 0.004$), HbA1C ($r = 0.596$, $P < 0.001$), total cholesterol ($r = 0.417$, $P = 0.005$) and LDL ($r = 0.290$, $P = 0.041$).

### 3.3. Relationship of sclerostin serum levels in T2DM patients with anthropometric and biochemical parameters

In T2DM patients, significant positive correlations were observed between serum sclerostin levels and age ($r = 0.559$, $P < 0.001$), diabetes duration ($r = 0.397$, $P = 0.004$), BMI ($r = 0.401$, $P = 0.004$), waist/hip ratio ($r = 0.433$, $P = 0.002$), CIMT ($r = 0.635$, $P < 0.001$), FPG ($r = 0.340$, $P = 0.016$), HbA1c ($r = 0.589$, $P < 0.001$) as well as total cholesterol ($r = 0.420$, $P = 0.002$). Meanwhile, sclerostin was significantly negatively correlated with HDL ($r = -0.311$, $P = 0.028$). On the other hand, we found no correlation between sclerostin and waist circumference, mean ABI, serum urea, serum creatinine, TG and LDL.

### 3.4. Serum sclerostin levels and CIMT

Serum sclerostin level was significantly increased in relation to CIMT with a mean value of (211.7 ± 76.87 pg/ml) in (CIMT >0.9) compared to (137.4 ± 26.08 pg/ml) in (CIMT ≤0.9) ($P < 0.001$). Significant correlations were recorded between serum sclerostin levels and CIMT ($r = 0.635$, $P < 0.001$) in the diabetic group (Fig. 1).

### 3.5. Cut-off value of serum sclerostin that predicts subclinical atherosclerosis

In addition to analysing the association between serum sclerostin levels and the surrogate marker of subclinical atherosclerosis; the abnormal CIMT, a ROC curve analysis was performed to evaluate the usefulness of sclerostin as a marker for subclinical atherosclerosis. It showed an area under the curve of (0.093, $P < 0.001$) for abnormal CIMT thickness. A concentration of >162.5 pg/ml showed a sensitivity of 90% and a specificity of 86.67% to identify an increased risk for abnormal CIMT. The diagnostic performance of sclerostin revealed a sensitive rather than a specific marker (Fig. 2).

### 3.6. Linear regression analysis

Linear regression analysis was performed to determine the influence of independent factors identified in univariate correlation analysis, including age, duration of diabetes, BMI, waist/hip ratio, CIMT, FPG, HbA1c, total cholesterol, and HDL. The analysis demonstrated that only CIMT ($\beta = 63.188$ [6.919–119.456], $P = 0.017$) was positively independently associated with sclerostin (Table 2). Independent variables included in the multivariable model were the variables found to correlate significantly with CIMT (age, duration of diabetes, BMI, waist circumference, waist/hip ratio, sclerostin, FPG, HbA1c, total cholesterol, and HDL) and the variables found to have statistical significance in the univariate analysis were age ($\beta = 0.020$ [0.013–0.028], $P$ ˆ 0.001) and diabetes duration ($\beta = 0.026$ [0.010–0.042], $P$ ˆ 0.001) (Table 3).

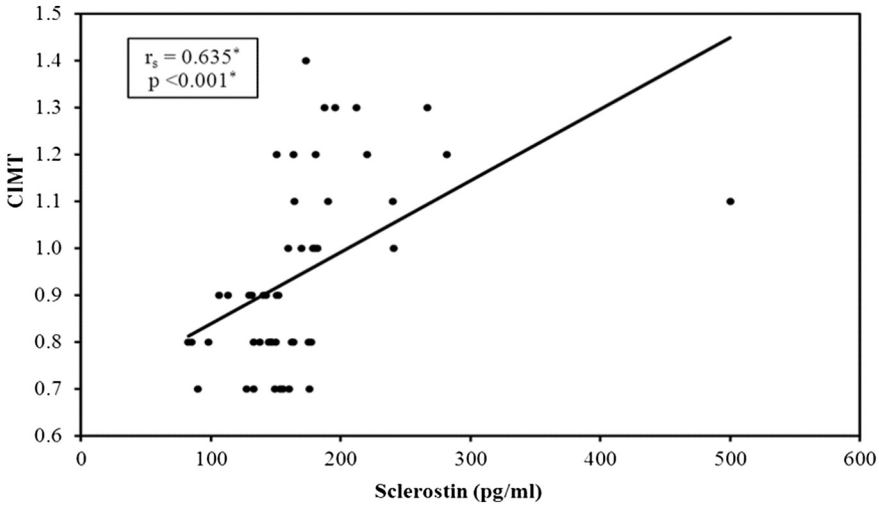


**Fig. 1.** Correlation between sclerostin (pg/ml) and CIMT in subjects with T2DM.

## 4. Discussion

Our study showed that higher sclerostin levels are independently associated with subclinical atherosclerosis in subjects with T2DM. Furthermore, high levels of serum sclerostin levels were associated with abnormal CIMT. Additionally, we found a significant independent positive correlation between age as well as diabetes duration with CIMT.

We demonstrated higher levels of sclerostin in T2DM with abnormal CIMT as compared to those with normal CIMT and healthy subjects. The results raise the possibility of the usefulness of sclerostin as a serum marker for subclinical atherosclerotic risk in T2DM. The role of sclerostin in the pathophysiological process in atherosclerosis had been raised in preclinical studies. The canonical Wnt signalling pathway activation is implicated in the pro-proliferation of arterial and venous vascular smooth muscle cells both in vitro and in vivo.[27] Furthermore, the Wnt pathway has been described to play an important role in the regulation of endothelial inflammation, vascular calcification and mesenchymal stem cell differentiation.[28] Sclerostin antagonizes the canonical Wnt signalling pathway and inhibits osteoblast activity and bone formation by sequestering LRP5 and LRP6.[29]

Previous reports on the Wnt signalling antagonist sFRP[30] illustrated that its upregulation after injury was involved in healing and homeostasis of vascular tissue. As a result, considering the fact that atherosclerosis is both an actively regulated and progressive process, we may speculate that high sclerostin levels might be indicative of a sort of defensive mechanism that may attenuate the upregulation of the canonical Wnt pathway leading to the restoration of quiescent Wnt signalling observed under healthy conditions and hindering the atherosclerotic progression.[31] This notion had been supported by previous studies that stated the possible role of sclerostin in counter regulatory mechanisms suppressing vascular calcification.[32]

Endothelial cell migration is essential in the context of angiogenesis. Endothelial cells originate from the differentiation of mesodermal cells forming primitive vascular structures called blood islands. Vascular endothelial growth factor (VEGF) influences the newly formed endothelial cells to migrate on a matrix allowing the fusion of the blood islands, their remodelling into tubular structures, and the formation of the first primitive vascular plexus.[33] Craig et al. demonstrated the role of sclerostin in vascular endothelial cell migration examined in Human umbilical vein endothelial cells (HUVECs). The combination of sclerostin together with cysteine rich protein 61 (potent stimulator of angiogenesis and mesenchymal stem cell expansion and differentiation) were shown to increases vascular endothelial cell migration but not by either protein alone; thus concluding that sclerostin potentiates Cyr-mediated vascular migration and cell growth.[34] Similarly, Oranger et al. displayed the role sclerostin in angiogenic activity in vivo elicited by binding to

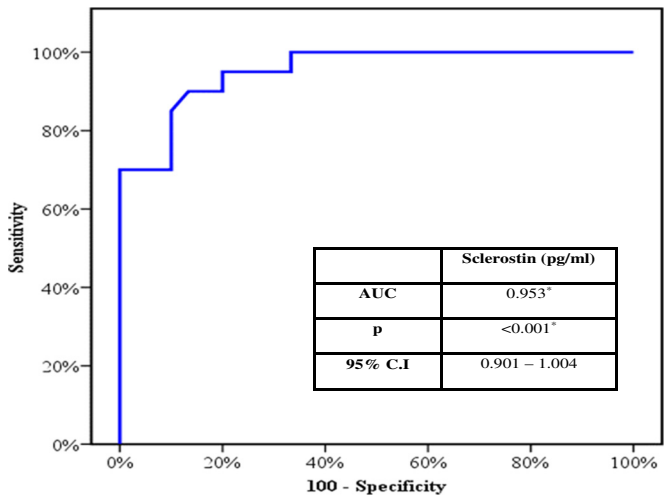


**Fig. 2.** ROC curve for sclerostin (pg/ml) to predict cases with CIMT >0.9 ($n = 50$). AUC = Area under the curve, CI = Confidence interval.

**Table 2**
Multivariate analysis linear regression for the parameters affecting sclerostin (pg/ml).

| | B | SE | Sig. | *t* | 95% CI | |
|---|---|---|---|---|---|---|
| | | | | | LL | UL |
| Age (years) | 1.366 | 1.747 | 0.439 | 0.782 | −2.165 | 4.897 |
| Diabetes duration (years) | −0.026 | 4.215 | 0.995 | 0.006 | −8.545 | 8.493 |
| BMI (kg/m2) | 1.095 | 2.995 | 0.717 | 0.366 | −4.958 | 7.148 |
| Waist/hip ratio | −5.470 | 56.511 | 0.923 | 0.097 | −119.683 | 108.744 |
| CIMT | 63.188 | 27.841 | 0.029* | 2.270* | 6.919 | 119.456 |
| FPG | −0.205 | 0.345 | 0.555 | 0.596 | −0.902 | 0.491 |
| HbA1c | 4.839 | 6.542 | 0.464 | 0.740 | −8.383 | 18.062 |
| Total cholesterol | −0.076 | 0.249 | 0.760 | 0.308 | −0.579 | 0.426 |
| HDL | −0.746 | 0.869 | 0.396 | 0.858 | −2.502 | 1.011 |

$R = 0.609$, $R^2 = 0.371$, $F = 2.626^*$, $P = 0.017^*$.
B: unstandardized coefficients.
OR: odds ratio.
CI: confidence interval.
LL: lower limit.
UL: upper limit.
* Statistically significant at $P \leq 0.05$.

**Table 3**
Multivariate analysis linear regression for the parameters affecting CIMT.

| | B | SE | Sig. | *t* | 95% CI | |
|---|---|---|---|---|---|---|
| | | | | | LL | UL |
| Age (years) | 0.020 | 0.004 | <0.001* | 5.450* | 0.013 | 0.028 |
| Diabetes duration (years) | 0.026 | 0.008 | 0.002* | 3.369* | 0.010 | 0.042 |
| BMI (kg/m2) | 0.015 | 0.008 | 0.081 | 1.792 | −0.002 | 0.032 |
| Waist circumference | −0.001 | 0.003 | 0.599 | −0.530 | −0.007 | 0.004 |
| Waist/hip ratio | 0.124 | 0.130 | 0.345 | 0.956 | −0.139 | 0.387 |
| Sclerostin | 0.000 | 0.000 | 0.851 | 0.189 | −0.001 | 0.001 |
| FPG | 0.000 | 0.001 | 0.525 | −0.642 | −0.002 | 0.001 |
| HbA1C | 0.022 | 0.013 | 0.115 | 1.613 | −0.006 | 0.049 |
| Total cholesterol | 0.001 | 0.001 | 0.169 | 1.401 | −0.001 | 0.003 |
| LDL | −0.001 | 0.001 | 0.297 | −1.058 | −0.003 | 0.001 |

$R = 0.859$, $R^2 = 0.737$, $F = 10.950^*$, $P < 0.001^*$.
B: unstandardized coefficients.
OR: odds ratio.
CI: confidence interval.
LL: lower limit.
UL: upper limit.
* Statistically significant at $P \leq 0.05$.

LRP6, inducing pro angiogenic cytokines VEGF and placental growth factor PlGF production in human endothelial cells.[35]

Our study showed that age and diabetes duration were independent predictors of CIMT. Several studies have shown similar findings concerning association with age[36,37] and opposing findings with duration of diabetes.[36] We also found a positive correlation between CIMT and age, diabetes duration, BMI, waist circumference, waist/hip ratio, sclerostin, FPG, HbA1C, total cholesterol and LDL. This can be explained by the fact that cluster of lipid abnormalities associated with type 2 diabetes is defined by a high concentration of TG and small dense LDL and a low concentration of HDL cholesterol. Plasma LDL cholesterol levels are generally normal. Insulin resistance is believed to contribute to this atherogenic dyslipidemia by increasing the hepatic secretion of VLDL and other apolipoprotein (apo) B-containing lipoprotein particles, as a result of increased free fatty acid flux to the liver.[38] These anomalies are closely associated with an increased oxidative stress and an endothelial dysfunction, thereby reinforcing the proinflammatory nature of macrovascular atherosclerotic disease. Metabolic factors in diabetic patients including hyperglycaemia, dyslipidemia and insulin resistance lead to endothelial cell, vascular smooth muscle dysfunction exhibiting an increased risk of development of atherosclerotic CVD.[39] Sclerostin is independently associated with cardiovascular mortality according to a study by Novo-Rodriguez et.al; in which significantly high levels of sclerostin were found in patients with prevalent CVD. The survival analysis presented that an increase of 10 pmol/L in the serum sclerostin level resulted in a 31% increase in cardiovascular mortality proving sclerostin to be a strong predictor of cardiovascular mortality risk.[40]

We demonstrated significant positive correlation between sclerostin level and age, diabetes duration, BMI, waist/hip ratio, FPG, HbA1c and total cholesterol; however, sclerostin was significantly negatively correlated with HDL. The positive correlation between age and sclerostin irrespective of gender was supported by previous studies.[41] Granted that sclerostin is produced almost entirely by osteocytes,[42–44] this observation would be accordant with increased skeletal sclerostin production with aging in humans. On the other hand, this result is opposed by previous reports illustrating positive correlation of sclerostin with age that was observed in males but not females.

The association between sclerostin with HbA1c and diabetes duration had been demonstrated before[21] and can be explained by the fact that overproduction of reactive oxygen species (ROS) as a result of altered glucose metabolism and formation of advanced glycation endproducts (AGE) amplifies the process of atherosclerosis by activating nuclear factor κB (NFκB) and other proinflammatory pathways.[45] In combination with endothelial cell insulin resistance, these changes cause endothelial dysfunction manifesting itself by increased expression of adhesion molecules and other changes. This could partly explain the over expression of sclerostin in T2DM patients. Several recent reports[41] implicate Wnt signalling pathway in regulating adipogenesis, body fat distribution and, to a degree, susceptibility to obesity, being widely spread across tissues along with its natural inhibitor proteins sclerostin and DKK1.

The positive correlation between sclerostin and waist/hip ratio as well as BMI supports previous studies that demonstrated positive association of sclerostin with fat mass, body weight and ponderal index as well as negative association with HDL.[17] To determine the factors independently associated with sclerostin, we included all significant variables correlating with sclerostin (age, diabetes duration, BMI, waist/hip ratio, FPG, HbA1c total cholesterol and HDL) in a multiple linear regression analysis. We found that CIMT was the only factor independently associated with sclerostin levels. Given that CIMT is a strong predictor of vascular events.[46]

Limitations of the study included the cross-sectional design of the study so we are not able to establish the causative nature of the associations between sclerostin and CIMT. It remains to be confirmed, on a large scale, whether sclerostin level is independently associated with the occurrence of cardiovascular events (myocardial infraction, stroke …) and with the survival of patients with atherosclerotic disease.

## 5. Conclusion

In summary, our observation that T2DM patients with subclinical atherosclerosis presented higher levels of sclerostin supports the hypothesis that sclerostin action is not only on the regulation of bone formation but also in the pathophysiological process of atherosclerosis.

## Authors' contribution statement

Shalash MA and Rohoma KH conceived the presented idea and designed the study. They contributed to the collection, analysis, and interpretation of data; and wrote the manuscript. Kandil NS and Abdel Mohsen MA have participated to data collection and analysis; contributed to and revised the manuscript. Taha AA collected the data, contributed to its analysis, and interpretation; and participated in writing and revising the manuscript.

## Acknowledgment

This work was supported by Diabetes and Metabolism Unit, Internal Medicine Department, Faculty of Medicine, Alexandria University in collaboration with Chemical Pathology department, Medical Research Institute, Alexandria University as well the department of Radiology, Faculty of Medicine, Alexandria University, Egypt.

## References

1. American Diabetes A. Standards of medical care in diabetes-2018: summary of revisions. Diabetes Care 2018;41:S4-6. https://doi.org/10.2337/dc18-Sint01.
2. Kalofoutis C, Piperi C, Kalofoutis A, Harris F, Phoenix D, Singh J. Type II diabetes mellitus and cardiovascular risk factors: current therapeutic approaches. Exp Clin Cardiol 2007;12:17-28.
3. Srivastava SK, Ansari NH, Hair GA, Jaspan J, Rao MB, Das B. Hyperglycemia induced activation of human erythrocyte aldose reductase and alterations in kinetic properties. Biochim Biophys Acta 1986;870:302-11 https://doi.org/10.1016/0167-4838(86)90234-7.
4. Alexander MR, Owens GK. Epigenetic control of smooth muscle cell differentiation and phenotypic switching in vascular development and disease. Annu Rev Physiol 2012;74:13-40 https://doi.org/10.1146/annurev-physiol-012110-142315.
5. Yang L, Chu Y, Wang Y, Zhao X, Xu W, Zhang P, et al. siRNA-mediated silencing of Wnt5a regulates inflammatory responses in atherosclerosis through the MAPK/NF-κB pathways. Int J Mol Med 2014;34:1147-52 https://doi.org/10.3892/ijmm.2014.1860.
6. Mill C, George SJ. Wnt signalling in smooth muscle cells and its role in cardiovascular disorders. Cardiovasc Res 2012;95:233-40 https://doi.org/10.1093/cvr/cvs141.
7. Mikels AJ, Nusse R. Purified Wnt5a protein activates or inhibits b-catenin-TCF signaling depending on receptor context. PLoS Biol 2006;4:e115 https://doi.org/10.1371/journal.pbio.0040115.

8. Zhang T, Otevrel T, Gao Z, Gao Z, Ehrlich SM, Fields JZ, et al. Evidence that APC regulates survivin expression: a possible mechanism contributing to the stem cell origin of colon cancer. Cancer Res 2001;61:8664-7.
9. Schuuring E, van Deemter L, Roelink H, Nusse R. Transient expression of the proto-oncogene int-1 during differentiation of P19 embryonal carcinoma cells. Mol Cell Biol 1989;9:1357-61 https://doi.org/10.1128/MCB.9.3.1357.
10. Katoh M. Transcriptional mechanisms of WNT5A based on NF-kappa B, Hedgehog, TGF beta, and Notch signalling cascades. Int J Mol Med 2009;23:763-9 https://doi.org/10.3892/ijmm_00000190.
11. Ingraham CA, Park GC, Makarenkova HP, Crossin KL. Matrix metalloproteinase (MMP)-9 induced by wnt signalling increases the proliferation and migration of embryonic neural stem cells at low O2 levels. J Biol Chem 2011;286:17649-57 https://doi.org/10.1074/jbc.M111.229427.
12. Pereira C, Schaer DJ, Bachli EB, Kurrer MO, Schoedon G. Wnt5A/CaMKII signalling contributes to the inflammatory response of macrophages and is a target for the antiinflammatory action of activated protein C and interleukin-10. Arterioscler Thromb Vasc Biol 2008;28:504-10 https://doi.org/10.1161/ATVBAHA.107.157438.
13. Borrell-Pages M, Romero JC, Juan-Babot O, Badimon L. Wnt pathway activation, cell migration, and lipid uptake is regulated by low-density lipoprotein receptor-related protein 5 in human macrophages. Eur Heart J 2011;32:2841-50 https://doi.org/10.1093/eurheartj/ehr062.
14. Lu KC, Wu CC, Yen JF, Liu WC. Vascular calcification and renal bone disorders. ScientificWorld Journal 2014;2014 https://doi.org/10.1155/2014/637065.
15. Wu W, Glinka A, Delius H, Niehrs C. Mutual antagonism between dickkopf1 and dickkopf2 regulates Wnt/beta-catenin signalling. Curr Biol 2000;10:1611-4 https://doi.org/10.1016/S0960-9822(00)00868-X.
16. Van Bezooijen RL, ten Dijke P, Papapoulos SE, Löwik CW. SOST/sclerostin, an osteocyte-derived negative regulator of bone formation. Cytokine Growth Factor Rev 2005 Jun;16:319-27https://doi.org/10.1016/j.cytogfr.2005.02.005.
17. Urano T, Shiraki M, Ouchi Y, Inoue S. Association of circulating sclerostin levels with fat mass and metabolic disease—related markers in Japanese postmenopausal women. The Journal of Clinical Endocrinology & Metabolism 2012;97:1473-7 https://doi.org/10.1210/jc.2012-1218.
18. Sheth J, Shah A, Sheth F, Trivedi S, Nabar N, Shah N, et al. The association of dyslipidemia and obesity with glycated hemoglobin. Clin Diabetes Endocrinol 2015;1 https://doi.org/10.1186/s40842-015-0004-6.
19. Didangelos A, Yin X, Mandal K, Baumert M, Jahangiri M, Mayr M. Proteomics characterization of extracellular space components in the human aorta. Mol Cell Proteomics 2010;9:2048-62 https://doi.org/10.1074/mcp.M110.001693.
20. Viaene L, Behets GJ, Claes K, Meijers B, Blocki F, Brandenburg V, et al. Sclerostin: another bone-related protein related to all-cause mortality in haemodialysis? Nephrol Dial Transplant 2013;28:3024-30 https://doi.org/10.1093/ndt/gft039.
21. Morales-Santana S, Garcia-Fontana B, Garcia-Martin A, Rozas-Moreno P, Garcia-Salcedo JA, Reyes-Garcia R, et al. Atherosclerotic disease in type 2 diabetes is associated with an increase in sclerostin levels. Diabetes Care 2013;36:1667-74 https://doi.org/10.2337/dc12-1691.
22. American Diabetes A. 2. Classification and diagnosis of diabetes. Diabetes Care 2018;41:S13-27 https://doi.org/10.2337/dc18-S002.
23. Andoh N, Minami J, Ishimitsu T, Ohrui M, Matsuoka H. Relationship between markers of inflammation and brachial-ankle pulse wave velocity in Japanese men. Int Heart J 2006;47:409-20 https://doi.org/10.1536/ihj.47.409.
24. Khaw KT, Wareham N. Glycated hemoglobin as a marker of cardiovascular risk. Curr Opin Lipidol 2006;17:637-43 https://doi.org/10.1097/MOL.0b013e3280106b95.
25. Simova I, Katova T, Denchev S. Diagnostic accuracy of flow-mediated dilatation and intima-media thickness for the presence of significant coronary artery disease. J Am Soc Hypertens 2009;3:388-94 https://doi.org/10.1016/j.jash.2009.09.003.
26. Bourque JM, Beller GA. Citation: value of exercise ECG for risk stratification in suspected or known CAD in the era of advanced imaging technologies. JACC Cardiovasc Imaging 2015;8:1309-21 https://doi.org/10.1016/j.jcmg.2015.09.006.
27. Depner TA, Daugirdas JT. Equations for normalized protein catabolic rate based on two-point modeling of hemodialysis urea kinetics. J Am Soc Nephrol 1996;7:780-5.
28. Askevold ET, Gullestad L, Aakhus S, Ranheim T, Tonnessen T, Solberg OG, et al. Secreted Wnt modulators in symptomatic aortic stenosis. J Am Heart Assoc 2012;1, e002261 https://doi.org/10.1161/JAHA.112.002261.
29. Li X, Liu P, Liu W, Maye P, Zhang J, Zhang Y, et al. Dkk2 has a role in terminal osteoblast differentiation and mineralized matrix formation. Nat Genet 2005;37:945-52 https://doi.org/10.1038/ng1614.
30. Barandon L, Couffinhal T, Ezan J, Dufourcq P, Costet P, Alzieu P, et al. Reduction of infarct size and prevention of cardiac rupture in transgenic mice overexpressing FrzA. Circulation 2003;108:2282-9 https://doi.org/10.1161/01.CIR.0000093186.22847.4C.
31. Gaudio A, Privitera F, Pulvirenti I, Canzonieri E, Rapisarda R, Fiore CE. The relationship between inhibitors of the Wnt signalling pathway (sclerostin and Dickkopf-1) and carotid intima-media thickness in postmenopausal women with type 2 diabetes mellitus. Diab Vasc Dis Res 2014;11:48-52 https://doi.org/10.1177/1479164113510923.
32. Claes KJ, Viaene L, Heye S, Meijers B, d'Haese P, Evenepoel P. Sclerostin: another vascular calcification inhibitor? The Journal of Clinical Endocrinology & Metabolism 2013;98:3221-8 https://doi.org/10.1210/jc.2013-1521.
33. Rousseau S, Houle F, Landry J, Huot J. p38 MAP kinase activation by vascular endothelial growth factor mediates actin reorganization and cell migration in human endothelial cells. Oncogene 1997;15:2169-77 https://doi.org/10.1038/sj.onc.1201380.
34. Craig TA, Bhattacharya R, Mukhopadhyay D, Kumar R. Sclerostin binds and regulates the activity of cysteine-rich protein 61. Biochem Biophys Res Commun 2009;392:36-40 https://doi.org/10.1016/j.bbrc.2009.12.143.
35. Oranger A, Brunetti G, Colaianni G, Tamma R, Carbone C, Lippo L, et al. Sclerostin stimulates angiogenesis in human endothelial cells. Bone 2017;101:26-36 https://doi.org/10.1016/j.bone.2017.03.001.
36. Baba MM, Talle MA, Ibinaiye PO, Abdul H, Buba F. Carotid intima-media thickness in patients with diabetes mellitus attending tertiary care hospital in Nigeria. Angiol 2018;6 https://doi.org/10.4172/2329-9495.1000210.
37. Taniwaki H, Kawagishi T, Emoto M, Shoji T, Kanda H, Maekawa K, et al. Correlation between the intima-media thickness of the carotid artery and aortic pulse-wave velocity in patients with type 2 diabetes. Vessel wall properties in type 2 diabetes. Diabetes Care Nov 1999;22:1851-7 https://doi.org/10.2337/diacare.22.11.1851.
38. Laws A, Hoen HM, Selby JV, Saad MF, Haffner SM, Howard BV. Differences in insulin suppression of free fatty acid levels by gender and glucose tolerance status: relation to plasma triglyceride and apolipoprotein B concentrations: Insulin Resistance Atherosclerosis Study (IRAS) Investigators. Arterioscler Thromb Vasc Biol 1997;17:64-71 https://doi.org/10.1161/01.ATV.17.1.64.
39. Suzuki LA, Poot M, Gerrity RG, Bornfeldt KE. Diabetes accelerates smooth muscle accumulation in lesions of atherosclerosis: lack of direct growth-promoting effects of high glucose levels. Diabetes 2001;5:851-60 https://doi.org/10.2337/diabetes.50.4.851.
40. Novo-Rodríguez C, García-Fontana B, Luna-Del Castillo JD, et al. Circulating levels of sclerostin are associated with cardiovascular mortality. PLoS One 2018;13, e0199504 https://doi.org/10.1371/journal.pone.0199504.
41. Mödder UI, Hoey KA, Amin S, McCready LK, Achenbach SJ, Riggs BL, et al. Relation of age, gender, and bone mass to circulating sclerostin levels in women and men. J Bone Miner Res 2011;26:373-9 https://doi.org/10.1002/jbmr.217.
42. Van Bezooijen RL, Roelen BAJ, Visser A, van der Wee-Pals L, de Wilt E, Karperien M, et al. Sclerostin is an osteocyte-expressed negative regulator of bone formation, but not a classical BMP antagonist. J Exp Med 2004;199:805-14 https://doi.org/10.1084/jem.20031454.
43. Poole KE, van Bezooijen RL, Loveridge N, Hamersma H, Papapoulos SE, Löwik CW, et al. Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. FASEB J 2005;19:1842-4 https://doi.org/10.1096/fj.05-4221fje.
44. Barons R, Rawadi G. Targeting the Wnt/b-catenin pathway to regulate bone formation in the adult skeleton. Endocrinology 2007;148:2635-43 https://doi.org/10.1210/en.2007-0270.
45. Rask-Madsen C, King GL. Vascular complications of diabetes: mechanisms of injury and protective factors. Cell Metab 2013;17:20-33 https://doi.org/10.1016/j.cmet.2012.11.012.
46. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. Circulation 2007;115:459-67 https://doi.org/10.1161/CIRCULATIONAHA.106.628875.