Relationship between sclerostin and coronary tortuosity in postmenopausal females with non-obstructive coronary artery disease

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A R T I C L E   I N F O
Article history:
Received 28 May 2020
Received in revised form 18 August 2020
Accepted 9 September 2020
Available online 12 September 2020

Keywords:
Sclerostin
Coronary tortuosity
Tortuosity score
Postmenopausal females

A B S T R A C T

Background: Coronary tortuosity (CT) is commonly encountered in postmenopausal females and is usually present without obstructive lesions. Circulating sclerostin levels are elevated in postmenopausal females. In view of its possible vasculoprotective effect, we aimed to study the association between circulating sclerostin and CT.

Method: We prospectively enrolled 273 consecutive postmenopausal females with non-obstructive coronary artery disease diagnosed by coronary angiography. Presence and severity (by tortuosity score) of CT as well as serum sclerostin levels were assessed for each patient.

Results: Patients with CT (128, 47% of study group) were significantly older ($P<0.001$), with higher prevalence of hypertension ($P=0.001$) and had significantly higher levels of both sclerostin ($P<0.001$) and hs-CRP ($P=0.001$). Multivariate binary logistic regression revealed that the presence of CT (dependent variable) was associated with high sclerostin level (OR 8.9, 95% CI: 4.9–16.2, $P<0.001$). Using ROC curve analysis, Sclerostin at a cut-off value of >650 pg/ml was found to be associated with presence of CT (AUC 0.69, 95% CI: 0.61–0.75, $P<0.001$) with sensitivity and specificity of 75% and 72.4%, respectively. Using Pearson’s correlation analysis, significant positive correlation between sclerostin and severity of CT was found ($r=0.29$, $P=0.001$).

Conclusion: High circulating sclerostin is associated with the presence and severity of CT in postmenopausal females. This may add to the literature on the incompletely understood pathogenesis of CT.

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1. Introduction

Recent evidence shows that atherosclerosis is a dynamic process that is actively regulated through numerous pathways [1]. For example, bone-vascular axis has been recently identified with many endocrine and metabolic pathways being involved e.g. Wnt/β-catenin pathway [2–3]. Bone-vascular axis is particularly perturbed in postmenopausal females [4–5]. In blood vessels, this pathway promotes vascular smooth muscle cell proliferation and transformation into osteoblast-like cells [6].

Sclerostin is the main endogenous inhibitor of Wnt signaling and acts through competitive binding to co-receptor lipoprotein receptor-related proteins 5/6 [7]. The association between high circulating sclerostin and vascular pathologies is still controversial. Many studies reported negative correlation between circulating sclerostin (and/or Dkk-1) and vascular stiffness [8], atherosclerosis [9]; and calcification [10]. Recently, the use of sclerostin inhibitors was shown clinically to be associated with a probable increase in cardiovascular events [11]. Some other studies, however, reported positive correlation between sclerostin levels and vascular stiffness and/or calcification [12–14]. Elevated levels of circulating sclerostin were found in postmenopausal females [15,16] and were correlated to postmenopausal osteoporosis and increased fracture risk [17].

Coronary tortuosity (CT) is frequently encountered in postmenopausal females and may be attributed to aging, hypertension and left ventricular hypertrophy [18,19]. CT is associated with myocardial ischemia [20], spontaneous coronary artery dissection [21] and, probably, takotsubo syndrome [22]. The exact pathogenesis of CT is yet to be elucidated. Some studies report negative correlation between CT and “coronary” atherosclerosis and calcification [23–25]. Mechanically, reduced arterial wall stiffness may facilitate reaching the luminal pressure necessary for arterial twisting, the critical buckling pressure [18].

In view of its possible vasculoprotective effects, we aimed to study the association between sclerostin and CT in postmenopausal females. This may shed light on the pathogenesis of CT.
2. Methods

1. Patient population. We prospectively enrolled unselected 273 consecutive postmenopausal female patients who had undergone coronary angiography for suspected stable ischemic heart disease and were found to have normal (defined as no visible disease) or near-normal (defined as minimal luminal irregularities <30% assessed visually) coronary arteries. We excluded patients with left ventricular ejection fraction <40% or severe valvular disease, patients with estimated glomerular filtration rate (eGFR) < 30 ml/min/1.73 m², and patients who received hormonal replacement therapy, recent calcium and/or vitamin D supplements within the previous 6 months. All patients provided written, informed consent and the study was approved by the Local Ethics Committee.

2. Clinical evaluation and Anthropometric measurement. Diabetes mellitus (DM) was defined as HbA1c > 6.5 g/dl or use of oral hypoglycemic agents and/or insulin. Hypertension was defined as a systolic blood pressure > 140 and/or a diastolic blood pressure > 90 or prior use of an antihypertensive drug. Dyslipidemia was defined as fasting total cholesterol >200 mg/dl, low-density lipoprotein-cholesterol (LDL-C) > 130 mg/dl, high-density lipoprotein-cholesterol (HDL-C) < 40 mg/dl, triglycerides >150 mg/dl, and/or chronic use of lipid-lowering drugs. Current smoking was defined as cigarette smoking during the last month. Height and weight were measured for each patient. Body mass index (BMI) was calculated as the weight (kg) divided by height (m) squared (kg/m²). Dual X-ray absorptiometry was used to measure total body fat (TBF).

3. Sclerostin measurement and other laboratory measurements. A 5 ml sample of venous blood was collected from each patient after an overnight fasting. Sclerostin was measured using commercially available enzyme-linked immunosorbent assay kits (Biomedica Gruppe, Vienna, Austria). Intra- and inter-assay coefficients of variation were 5% and 3%, respectively [12]. Serum creatinine was measured. The Modification of Diet in Renal Disease Study formula was used to estimate glomerular filtration rate (eGFR) as: eGFR (ml /min/ 1.73 m²) = 175 x (serum creatinine) ^ -1.154 x (age) ^ -0.203 + 0.742 (with creatinine expressed in mg/dl and age in years). Total cholesterol, HDL-C, triglycerides, HbA1c and high sensitive C-reactive protein (hs-CRP) were measured.

4. Coronary angiography. Using 6 Fr Judkins diagnostic catheters, coronary angiography was performed for each patient. The coronary system was assessed by two independent operators. Evaluation for CT was performed on standardized angulations; at least 2 for each major vessel. Left anterior descending (LAD) artery was examined in antero-posterior and right anterior oblique with cranial angulation, left circumflex (LCX) artery in left and right anterior oblique with caudal angulation; and right coronary artery (RCA) in left and right anterior oblique view. All arteries were examined at end-diastole.

Angiographic definitions. CT was defined by the presence of ≥3 consecutive curvatures of ≥45° in an artery >2 mm or ≥90° in an artery <2 mm in diameter. Mild CT was defined as either ≥3 consecutive curvatures of 45° to 90° in an artery >2 mm in diameter, or ≥3 consecutive curvatures of 90° to 180° in an artery <2 mm in diameter. Moderate CT was defined by the presence of ≥3 consecutive curvatures of 90° to 180° in an artery ≥2 mm in diameter. Severe CT was defined as ≥2 consecutive curvatures of ≥180° in an artery ≥2 mm in diameter (Fig. 1). Tortuosity score was calculated as a sum of scores for each of the following: LAD, LCX, RCA, diagonal, obtuse marginal, right posterior descending, or right posterolateral; where 0 = no tortuosity, 1 = mild tortuosity, 2 = moderate tortuosity, and 3 = severe tortuosity [25]. The inter-observer agreement (calculated with weighted Kappa statistics) showed good agreement (k = 0.93, P = 0.01).

5. Statistical analysis. It was performed using SPSS for Windows version 25 (SPSS Inc., Chicago, Illinois, USA). Patients were classified into CT and non—CT groups. Continuous data were expressed as mean ± SD and categorical data as numbers (percentages). Independent t-test and Chi square test were used to assess differences between CT and non—CT groups regarding continuous and categorical data, respectively. Binary logistic regression was performed to detect association between the presence of CT (as a dependent variable) and variables that showed significant differences between groups (as independent variables). The results were expressed as odds ratio (OR) and 95% confidence interval (CI). Receiver operating characteristic (ROC) curve analysis was performed to estimate the best cut-off value of highest accuracy for each variable with significant difference between groups. Furthermore, Pearson’s correlation analysis was performed to test the relationship between tortuosity score (as a continuous variable) and clinical and laboratory variables.

3. Results

1. Comparison between patients with and without CT: Of the 273 postmenopausal females included in our study, 128 (47%) had CT. Mean age was 57.1 ± 7.9 years. 107 (39%) patients were hypertensive, 55 (20%) patients were diabetic, and 99 (36%) patients were dyslipidemic. Mean sclerostin level was 701.4 ± 425.4 pg/ml. Patients with CT were significantly older (P < 0.001) with significantly higher frequency of hypertension (P = 0.001). Regarding patients’ symptoms, patients with CT showed significantly higher prevalence of effort angina and dyspnea (P = 0.04 and 0.001; respectively). Levels of both sclerostin and hs-CRP were significantly higher in patients with CT (P < 0.001 and 0.001; respectively). Regarding echocardiographic data, patients with CT showed significantly higher left ventricular mass index as well as higher frequency of left ventricular diastolic dysfunction > grade 1 (P = 0.001 and 0.04; respectively) (Table 1).

2. Univariable and multivariable analyses: Presence of CT was found to be associated with presence of hypertension (OR 2.863; CI: 0.2–5.6, P = 0.001), older age (OR 2.289; CI: 1.3–4.1, P = 0.006), higher sclerostin levels (OR 8.909; CI: 4.9–16.2, P < 0.001) and higher hs-CRP (OR 3.269; CI: 1.8–5.9, P < 0.001) (Table 2).

ROC curve analysis to determine diagnostic accuracy of variables associated with CT: ROC curve analysis was performed for numerical variables showing significant differences between CT and non-CT groups. Sclerostin at a cutoff value ≥650 pg/ml was found to be associated with presence of CT (AUC 0.69, 95% CI: 0.61–0.75, P = 0.001) with sensitivity and specificity of 75% and 72.4%, respectively. Age at a cutoff value ≥57.5 years was found to be associated with presence of CT (AUC 0.65, 95% CI: 0.58–0.72, P < 0.001) with sensitivity and specificity of 64.8% and 52.4%, respectively. Hs-CRP at a cutoff value ≥22.5 mg/l was found to be associated with presence of CT (AUC 0.60, 95% CI: 0.53–0.64, P = 0.002) with sensitivity and specificity of 64.1% and 62.8%, respectively (Table 3 and Fig. 2).

4. Correlations between tortuosity score and other clinical and laboratory variables: Using Pearson’s correlation analysis, we found significant positive correlation between tortuosity score and HbA1c (r = 0.34, P < 0.001) as well as sclerostin levels (r = 0.289, P = 0.001) (Table 4).

4. Discussion

To the best of our knowledge, this is the first study addressing the relationship between sclerostin and CT in postmenopausal females with non-obstructive coronary artery disease. The chief finding in our study is that higher levels of circulating sclerostin are associated with the presence of CT and correlates positively with tortuosity score. We also found that patients with CT were older, with higher prevalence of hypertension; and had higher hs-CRP levels.

In postmenopausal females, both osteoporosis and atherosclerosis are major health problems leading to significant morbidity and mortality [26]. Several bone-derived mediators, messengers and signaling
pathways are involved in the complex and multifactorial link between bone homeostasis and vascular biology, the so termed bone-vascular axis [2–3]. Among key mediators involved in this axis is sclerostin which may have vasculoprotective role [8–11] and was found to be associated with lower vascular calcification [27].

Coronary arteries tend to be the most tortuous arterial system secondary to, probably, the repetitive cardiac motions [28]. In our study, older age was independently associated with the presence of CT. Previous studies have reported aging as a major predictor of CT [29,30]. We found that patients with CT showed significantly higher frequency of hypertension. This finding comes in agreement with previous studies [30,31]. Few studies [19,24], on the contrary, did not demonstrate association between CT and hypertension. The use of a different definition for CT by these studies may explain such discrepancy.

Despite lack of objective evidence of myocardial ischemia in the current study, we found that patients with CT had higher prevalence of effort angina and dyspnea. The association between CT and myocardial ischemia is still controversial. In previous studies, as compared to patients with negative provocative stress tests, those with positive tests and normal/near-normal coronary angiograms showed several fold increase in the prevalence of significant CT [19,20]. Regarding echocardiographic data, we found that CT was associated with increased left ventricular mass index and left ventricular diastolic dysfunction. Previous evidence [29,30,32] suggests an association between CT, chronic pressure overload and left ventricular diastolic dysfunction. This may support the hypothesis of a bidirectional interaction between CT and myocardial structure/dynamics.

In the current study, the unique finding was that high circulating sclerostin was associated with CT and correlated positively with tortuosity score. This is in line with previous work suggesting that sclerostin is protective against arterial stiffness, atherosclerosis and calcification [8–11,25]. Losing their mechanical stability, coronary arteries tend to buckle with elevation of intra-coronary pressure (hypertension) and reduction of wall stiffness and axial tension [33]. In postmenopausal females, age-related increase in sclerostin secretion by

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**Table 1**

Baseline characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No CT (n = 145)</th>
<th>CT (n = 128)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57.3 ± 7.9</td>
<td>61.3 ± 6.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>43 (29.7%)</td>
<td>64 (50.0%)</td>
<td>0.001</td>
</tr>
<tr>
<td>On beta-blockers therapy</td>
<td>21 (48.8%)</td>
<td>37 (57.8%)</td>
<td>0.42</td>
</tr>
<tr>
<td>On calcium channel blocker</td>
<td>23 (53.5%)</td>
<td>32 (50.0%)</td>
<td>0.65</td>
</tr>
<tr>
<td>On ACE/ARB therapy</td>
<td>28 (65.1%)</td>
<td>40 (62.5%)</td>
<td>0.38</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>25 (17.2%)</td>
<td>30 (23.4%)</td>
<td>0.2</td>
</tr>
<tr>
<td>Dyslipidemia, no (%)</td>
<td>58 (40.0%)</td>
<td>41 (32.0%)</td>
<td>0.17</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.7 ± 2.4</td>
<td>24.2 ± 2.9</td>
<td>0.20</td>
</tr>
<tr>
<td>TBF (%)</td>
<td>32.0 ± 3.6</td>
<td>31.8 ± 4.3</td>
<td>0.63</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>99.7 ± 17.4</td>
<td>103.6 ± 16.9</td>
<td>0.07</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.14 ± 0.31</td>
<td>1.17 ± 0.41</td>
<td>0.32</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>22.0 ± 10.4</td>
<td>23.2 ± 12.5</td>
<td>0.07</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>58.9 ± 20.4</td>
<td>59.4 ± 19.1</td>
<td>0.84</td>
</tr>
<tr>
<td>Hba1c (g/dl)</td>
<td>6.2 ± 1.3</td>
<td>6.3 ± 1.3</td>
<td>0.66</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>217.4 ± 69.4</td>
<td>220.7 ± 74.3</td>
<td>0.70</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>126.9 ± 44.8</td>
<td>118.1 ± 38.3</td>
<td>0.08</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>40.5 ± 5.5</td>
<td>41.6 ± 4.3</td>
<td>0.07</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>200.4 ± 63.3</td>
<td>189.9 ± 46.7</td>
<td>0.12</td>
</tr>
<tr>
<td>Hs-CRP (mg/l)</td>
<td>2.27 ± 0.8</td>
<td>2.67 ± 1.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Current aspirin use</td>
<td>121 (83.4%)</td>
<td>117 (91.4%)</td>
<td>0.70</td>
</tr>
<tr>
<td>Current statin use</td>
<td>76 (54.6%)</td>
<td>70 (54.7%)</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Data are represented as mean ± SD or numbers (%). ACEI/ARB: angiotensin converting enzyme inhibitor/angiotensin receptor blocker; BMI: body mass index; eGFR: estimated glomerular filtration rate; HDL-C: high density lipoprotein cholesterol; Hs-CRP: high sensitive C-reactive protein; LDL-C: low density lipoprotein cholesterol; TBF: total body fat. Bolded p values are significant.

**Table 2**

Binary logistic regression for prediction of presence of coronary tortuosity.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR 95% CI</td>
<td>P-value</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2.37 (1.4–3.9)</td>
<td>0.001</td>
</tr>
<tr>
<td>Age</td>
<td>2.03 (1.2–3.3)</td>
<td>0.004</td>
</tr>
<tr>
<td>Sclerostin</td>
<td>7.88 (4.6–13.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hs-CRP</td>
<td>3.004 (1.8–4.9)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Hs-CRP: high sensitive protein.
osteocytes was previously reported [15,16]. With the negative correlation between sclerostin and arterial stiffness in mind, this may provide a hypothesis to explain the common prevalence of CT in elderly females.

Compared with age-matched females, sclerostin levels are higher in males mostly due to larger bone mass [16]. However, these higher sclerostin levels are not associated with higher prevalence of CT in males. This may be explained by the higher prevalence of atherosclerosis [34] as well as larger diameter of coronary arteries [35] (and consequently, lower intra-coronary pressure) in males.

Finally, we found that higher levels of hs-CRP were associated with the presence of CT in our cohort of postmenopausal females. This may reflect heightened inflammatory state associated with CT and provide indirect link between CT and adverse cardiovascular events. So far, there is only one study showing higher levels of hs-CRP and higher incidence of lacunar infarcts in hypertensive patients with CT than hypertensive patients without CT [36]. This finding and ours stimulate active research on the possible link between CT, inflammation and adverse cardiovascular events.

5. Limitations

Our study has some limitations. The cross-sectional design prevents establishment of cause-effect relationship between sclerostin and CT. Although we believe that our results are consistent, the small sample size may reduce statistical power. The effect of body mineral content and drugs used by patients (e.g. statins and ACEI/ARBs) may probably affect circulating levels of sclerostin and/or modify its vascular actions.

6. Conclusions

The current study demonstrates that higher circulating sclerostin levels are associated with the presence and severity of CT in postmenopausal females. This may add to the literature on the incompletely understood mechanism of CT.

Funding

None.

Declaration of Competing Interest

The authors have nothing to declare.

Acknowledgement

None.

References


