Original Article
Association between serum adropin and subclinical atherosclerosis in patients with hyperlipidemia

Jian-Fang Cao†, Mao-Song Wang‡, Cong-Yan Bian‡, Yi-Fei Tao‡, Tao You‡, Wei-Ting Xu‡, Xiao-Song Gu‡

†Department of Anesthesiology, The Second Affiliated Hospital of Soochow University, No.1055 Sanxiang Road, Suzhou 215000, China; ‡Department of Cardiology, The Second Affiliated Hospital of Soochow University, No.1055 Sanxiang Road, Suzhou 215000, China. *Equal contributors.

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Abstract: Background: Hyperlipidemia has a close relationship with subclinical atherosclerosis (SA). Serum adropin levels are reduced in hyperlipidemic patients. However, the relationship between serum adropin and SA remains unknown. The current study aimed to evaluate circulating adropin levels in hyperlipidemic patients, assessing the association between serum adropin and SA. Methods: A total of 170 hyperlipidemic patients were consecutively collected and divided into two groups, SA (n=102) and non-subclinical atherosclerosis (NSA) (n=68) groups. In addition, 70 subjects without hyperlipidemia were recruited as controls. SA was defined as having a carotid artery intima-media thickness (CIMT) of ≥0.9 mm and/or a plaque on the common carotid artery, without clinical manifestations. Demographic and basic clinical characteristics, including age, gender, smoking history, and blood pressure, of all participants were collected from the hospital database. Blood lipid panels were determined using the enzymatic colorimetric method. Results: Hyperlipidemic patients had lower serum adropin levels, compared to control subjects (P<0.001). Patients in the SA group had lower serum adropin levels, compared to the NSA group (P<0.001). Pearson’s correlation analysis revealed that CIMT had a positive correlation with triglycerides (TG) and low-density lipids (LDL). However, it showed a negative correlation with adropin. Binary logistic regression analysis revealed that BMI, TC, LDL-C, and adropin were independently associated with SA in hyperlipidemic patients. Serum adropin significantly decreased in SA patients with hyperlipidemia, negatively correlating with CIMT. Conclusion: For hyperlipidemic patients, circulating adropin levels are independent biomarkers of SA.

Keywords: Adropin, subclinical atherosclerosis, hyperlipidemia

Introduction

Hyperlipidemia is an independent modifiable risk factor of cardiovascular disease. It is a leading contributor to morbidity and mortality, worldwide [1, 2]. Hyperlipidemia is also the most important atherosclerotic risk factor, mainly occurring through the facilitation of progression of carotid stenosis during the pathogenesis of atherosclerosis induced by a high-fat diet (HFD) [3]. Subclinical atherosclerosis (SA), defined as having a carotid artery intima-media thickness (CIMT) of ≥0.9 mm and/or a plaque on the common carotid artery, without clinical manifestations, has been closely associated with hyperlipidemia [4]. Furthermore, SA is highly prevalent in middle-aged populations [5]. Hence, prevention of SA development may reduce the risk of recurrent major cardiovascular events in patients with clinically manifested vascular diseases [6]. Understandably, the identification of SA may facilitate early intervention and diminish cardiovascular risks linked to hyperlipidemia [7, 8].

Adropin, a secreted peptide hormone discovered in 2008 by Kumar et al., consists of 76 amino acids [9]. Recent studies have suggested that adropin plays a pivotal role in metabolic homeostasis, including fatty acid metabolism, insulin resistance prevention, and development of dyslipidemia [10]. Correlation levels between serum adropin and hyperlipidemia have also been explored. For instance, hyperlipidemic patients have low circulating levels of adropin [11]. Intraperitoneal administration of adropin reduces serum levels of triglycerides (TG), total cholesterol (TC), and low-density lipoprotein
cholesterol (LDL-C), while increasing levels of high-density lipoprotein cholesterol (HDL-C), in rat models of type-2 diabetes mellitus [12]. Moreover, adropin has been shown to participate in the development and progression of atherosclerosis [13]. A previous study demonstrated that serum adropin levels were negatively associated with severity of atherosclerosis [14]. However, there are few reports concerning the relationship between serum adropin and SA.

Since both SA or adropin have a close relationship with dyslipidemia and are involved in the development and progression of atherosclerosis, it was hypothesized that there could be a possible link between serum adropin and SA. The present study aimed to verify serum adropin levels in hyperlipidemic and normal subjects, evaluating correlation levels between serum adropin and SA by examining CIMT in SA and non-SA patients.

**Methods**

**Ethical approval**

All participants provided written informed consent prior to study initiation. The present study was approved by the Ethics Review Committee of the Second Affiliated Hospital of Soochow University and is in compliance with the principles of the Declaration of Helsinki.

**Study participants**

This cross-sectional study was conducted at the Second Affiliated Hospital of Soochow University, between January 2015 and August 2017. A total of 240 individuals, including 170 hyperlipidemic patients and 70 control subjects that did not have hyperlipidemia, were recruited. All participants were ≥18 years old. A pre-structured form was used to collect demographic data of these participants, including age, gender, body mass index (BMI, kg/m²), smoking history, blood pressure, medical history, and clinical examinations. Blood samples were collected before any medications were given. Subjects with one of the following were excluded from the present study: History of myocardial infarction; Angina or strokes; History of cancer or any other chronic disease; A glomerular filtration rate of <60 ml/minute/1.73 m²; Evidence of active infections.

**Laboratory measurements**

Blood samples were obtained from antecubital venipunctures of each participant after 12 hours of fasting. Plasma and serum were then collected by centrifugation at 3,000 × g for 15 minutes at 4°C. They were aliquoted and stored at -80°C for subsequent assays. Creatinine (Cr), fasting plasma glucose (FPG), baseline total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured from the obtained fasting blood samples. Measurements used the enzymatic colorimetric method via commercially available kits on an OLYMPUS AU2700 (Japan). Hyperlipidemia was defined according to the Third Report of the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III). High TC was defined as having a TC level of ≥5.18 mmol/L (200 mg/dL). Low HDL-C was defined as having an HDL-C level of <1.04 mmol/L (<40 mg/dL). High LDL-C was defined as having an LDL-C level of ≥3.37 (130 mg/dL). High TG was defined as having a TG level of ≥1.70 mmol/L (150 mg/dL). Hyperlipidemia was defined as having at least one of the following: High TC, high LDL-C, and high TG. Adropin was measured by enzyme-linked immuno-absorbent assays (Anti-body Online, USA), with a range of 0.156-10.000 ng/mL.

**CIMT assessment**

Detailed protocols for CIMT measurement were according to a previously published study [15]. Briefly, patients were placed in the supine position with their heads tilted back. Both common carotid arteries of each patient were evaluated with the subject’s head turned slightly to the contralateral side. The intima-media thickness (IMT) of the bilateral common carotid artery was measured using a GE Logiq7 linear array probe (7.5 MHz) in the outer wall of a predefined carotid segment of 1 cm in length and 1 cm below the carotid bifurcation for three cardiac cycles. All images were interpreted by a single technician. Plaque was defined as having a CIMT of ≥1.3 mm or a focal protrusion into the lumen with a thickness of at least 50% more than the adjacent intima-media complex. SA was diagnosed when a CIMT was ≥0.9 mm and/or a plaque was present on the common carotid artery, without clinical manifestations,
Relationship between serum adropin and SA in hyperlipidemic patients

Table 1. Comparison of demographic and baseline clinical characteristics of participants between control and hyperlipidemic subjects

<table>
<thead>
<tr>
<th></th>
<th>Control (n=70)</th>
<th>Hyperlipidemia (n=170)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>68.53 ± 9.36</td>
<td>66.61 ± 10.50</td>
<td>0.185</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>49/21</td>
<td>86/84</td>
<td>0.07</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.58 ± 3.13</td>
<td>26.87 ± 3.89</td>
<td>0.014</td>
</tr>
<tr>
<td>Smoking history</td>
<td>19/51</td>
<td>76/94</td>
<td>0.013</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>125.68 ± 14.96</td>
<td>131.27 ± 9.97</td>
<td>0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>72.67 ± 10.96</td>
<td>80.05 ± 9.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FBG (mmol/l)</td>
<td>5.79 ± 1.55</td>
<td>6.17 ± 1.58</td>
<td>0.094</td>
</tr>
<tr>
<td>Cr (umol/l)</td>
<td>75.52 ± 17.90</td>
<td>71.04 ± 16.55</td>
<td>0.064</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>4.01 ± 0.68</td>
<td>5.83 ± 1.41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>1.04 ± 0.36</td>
<td>2.58 ± 1.91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>2.13 ± 0.55</td>
<td>3.55 ± 1.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CIMT (mm)</td>
<td>0.77 ± 0.11</td>
<td>0.86 ± 0.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SA n (%)</td>
<td>19 (27.1%)</td>
<td>102 (60%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adropin (ng/ml)</td>
<td>5.16 ± 2.52</td>
<td>3.63 ± 1.92</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

as defined by the European Societies of Cardiology and Hypertension [16].

Statistical analysis

Variables were tested for normality using the Kolmogorov-Smirnov Z statistic. Normally-distributed data are expressed as mean ± standard deviation (SD), while non-normally distributed data are expressed as medians (interquartile range). Between-group differences were analyzed using Student’s t-tests and Mann-Whitney U-tests, respectively. Spearman’s correlation analysis was used to appraise correlation levels between CIMT and various parameters. In addition, multivariate analysis and logistic regression analysis were used to evaluate independent variables for SA. P-values <0.05 indicate statistical significance, using two-tailed tests. All statistical analyses were performed using SPSS (version 22.0; SPSS Inc., Chicago, IL, USA).

Results

Comparison of demographic and baseline clinical characteristics of participants between control and hyperlipidemic subjects

Demographic and baseline clinical characteristics, including age, gender, blood pressure, lipids, fasting blood glucose (FBG), and other metabolic indexes of control are presented in

Table 1. Overall, there were no significant differences in terms of age, gender, FBG, and Cr. However, 102 (60%) of the 170 hyperlipidemic subjects and 19 (27.1%) of the 70 controls were diagnosed with SA. As expected, hyperlipidemic patients had significantly higher levels of TC (P<0.01), LDL-C (P<0.01), and TG (P<0.01), as well as greater CIMT (P<0.01). In addition, hyperlipidemic patients had higher BMI, SBP, and DBP, as well as lower levels of circulating adropin (P=0.001). Furthermore, a high percentage of hyperlipidemic subjects were smokers, compared to control subjects.

Comparison of demographic and baseline clinical characteristics of subjects between the SA and non-SA groups

Next, demographic and baseline clinical characteristics of subjects were compared between SA and non-SA groups. As shown in Table 2, there were no significant differences in terms of age, gender and blood pressure between SA and non-SA groups. However, subjects in the SA group had significantly lower adropin levels (P=0.001) and higher TC (P<0.001) and LDL-C (P<0.001) levels (Table 2), compared to the non-SA group.

Determination of correlation between CIMT and risk factors

Pearson’s correlation analysis was performed to examine correlation levels between CIMT and several risk factors, as shown in Table 3. CIMT was positively correlated with TC (r=0.16, P=0.04) and LDL-C (r=0.15, P=0.04), but negatively correlated with serum adropin (r=-0.17, P=0.03).

Determination of risk factors for development of subclinical atherosclerosis

In the multivariate model, age, gender, smoking history, SBP, DBP, BMI, TC, TG, LDL-C/HDL-C, FBG, and Cr were enrolled for analysis. Of these factors, BMI, TC, LDL-C, and adropin were shown to be significant indicators of SA when adjusted for age (Table 4).

Discussion

The present study examined correlation levels between serum adropin and SA in hyperlipid-
Relationship between serum adropin and SA in hyperlipidemic patients

Table 2. Comparison of demographic and baseline clinical characteristics between SA and non-SA groups in hyperlipidemic subjects

<table>
<thead>
<tr>
<th></th>
<th>Non-SA (n=68)</th>
<th>SA (n=102)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66.98 ± 8.33</td>
<td>66.35 ± 11.76</td>
<td>0.7</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>30/38</td>
<td>56/46</td>
<td>0.21</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.25 ± 3.65</td>
<td>27.29 ± 4.0</td>
<td>0.08</td>
</tr>
<tr>
<td>Smoking history</td>
<td>19/49</td>
<td>37/65</td>
<td>0.31</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>130.17 ± 10.45</td>
<td>132.01 ± 9.62</td>
<td>0.24</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>79.91 ± 9.20</td>
<td>80.13 ± 9.46</td>
<td>0.88</td>
</tr>
<tr>
<td>FBG (mmol/l)</td>
<td>6.29 ± 1.65</td>
<td>6.08 ± 1.53</td>
<td>0.40</td>
</tr>
<tr>
<td>Cr (umol/l)</td>
<td>72.44 ± 17.56</td>
<td>70.11 ± 15.86</td>
<td>0.37</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>5.04 ± 0.99</td>
<td>6.35 ± 1.41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>2.92 ± 0.81</td>
<td>3.97 ± 1.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CIMT (mm)</td>
<td>0.75 ± 0.11</td>
<td>0.93 ± 0.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adropin (ng/ml)</td>
<td>4.29 ± 2.26</td>
<td>3.19 ± 1.52</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 3. Determination of correlation levels between CIMT and several risk factors

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adropin</td>
<td>-0.17</td>
<td>0.03</td>
</tr>
<tr>
<td>TC</td>
<td>0.16</td>
<td>0.04</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.15</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Table 4. Determination of risk factors for development of subclinical atherosclerosis

<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>95% CI</th>
<th>B</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>1.13</td>
<td>(1.01-1.26)</td>
<td>1.21</td>
<td>0.03</td>
</tr>
<tr>
<td>TC</td>
<td>2.08</td>
<td>(1.26-3.42)</td>
<td>0.73</td>
<td>0.004</td>
</tr>
<tr>
<td>LDL</td>
<td>1.95</td>
<td>(1.06-3.6)</td>
<td>0.67</td>
<td>0.033</td>
</tr>
<tr>
<td>Adropin</td>
<td>0.71</td>
<td>(0.57-0.87)</td>
<td>-0.34</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Hyperlipidemic patients had significantly lower serum adropin levels, compared to control subjects. Furthermore, SA patients had significantly lower serum adropin levels, compared to non-SA patients. In addition, circulating adropin levels were inversely associated with CIMT in a cohort of hyperlipidemic patients. Adropin was identified as an independent predictor of SA.

Detection of SA improves the risk prediction of cardiovascular diseases beyond traditional cardiovascular risk factors [17, 18]. At present, several approaches have been used to evaluate SA, including coronary calcification, measured by electron-beam computed tomography, and arterial stiffness, assessed by baPWV [19, 20]. Since the early 1990s, non-invasive assessment of CIMT with high-resolution B-mode ultrasonography testing has emerged as a powerful tool for evaluation of SA. At present, more advanced ultrasound systems with high-frequency transducers have allowed for easier and more accurate identification of the lumen-intima interface and intima-adventitia interface. This has allowed for reliable measurement of CIMT [21]. Therefore, using CIMT to predict the progression and regression of atherosclerosis has become widely accepted in clinical practice [22, 23]. In addition, CIMT has been shown as a marker of SA, independent of other cardiovascular risk factors [24, 25]. In line with the above findings, in the present investigation, CIMT was significantly higher in hyperlipidemic patients than in control subjects. Moreover, CIMT was positively linked to TC and LDL-C, but negatively associated with serum adropin. Overall, these findings suggest that CIMT may serve as a reliable predictor for hyperlipidemia, as well as development of atherosclerosis.

SA, as an early stage of atherosclerosis, manifests early in life. For example, a study on the postmortem evaluation of hearts of young men, with a mean age of 22 years old, killed during the Vietnam conflict, revealed that approximately 45% had some evidence of coronary atherosclerosis. Moreover, 5% had evidence of severe coronary atherosclerosis [26]. Elderly people are usually associated with more advanced atherosclerosis [27]. Aging also plays an important role in CIMT increase. CIMT increases every 10 years in a healthy population of both genders in Slavonia (Eastern Croatia) [17]. Psoriatic patients with a mean age >45 years appeared to have thicker CIMT, compared to patients <45 years old [28]. Even in older patients, a 6-year follow-up study revealed aging as a significant factor for increases in CIMT [29]. These findings support the notion that age is an independent factor for SA [18]. However, in the present study, no differences in age between hyperlipidemic and control groups were detected. Results suggest that age is not a primary factor of SA associated with...
hyperlipidemia. Mechanisms underlying this discrepancy between the present findings and previous ones remain unclear. It is possible that the sample size and the method of subject selection played a role in this matter.

Smoking history, gender, and high blood pressure have been shown to be associated with SA [30-32]. In addition, gender, smoking history, and dyslipidemia have exhibited a closer link with femoral, as opposed to carotid disease burden [32]. In addition, males had significantly higher CIMT than females. BMI and LDL/HDL-C ratios were common factors of the best-fit regression models in both genders [33]. In the present study, Pearson’s correlation analysis was used to examine correlation levels between hyperlipidemia and cardiac disease risk factors. Smoking history, BMI, and blood pressure were significantly associated with hyperlipidemia, while age and gender were not associated. Furthermore, previous studies have shown that children with heterozygous familial hypercholesterolemia developed early signs of atherosclerosis, manifested by increased CIMT [34]. They also suggested that CIMT had a closer relationship with LDL-C concentrations, compared to other lipid parameters, in a Japanese population [35]. In addition, serum LDL-C/HDL-C ratios represent an independent index associated with increased CIMT. LDL-C combined with HDL-C has been shown to be a useful marker for predicting the presence of carotid plaque in a Chinese general population [36]. In agreement with the above findings, the current study found that BMI, TC, and LDL-C were closely correlated with CIMT, indicating that these three factors may be used to predict the risk of SA.

Due to the close correlation with serum adipon, the new adipokine and hyperlipidemia, the link between serum adipon and CIMT was examined. A negative correlation was found between serum adipon and CIMT, indicating that low serum adipon may be used as an independent predictor for SA, in accord with previous reports [37, 38]. However, the biological mechanisms underpinning this correlation remain to be elucidated. In a cross-sectional study, serum adipon levels were negatively correlated with carotid β-stiffness [39]. A previous study revealed that both arterial stiffness and serum adipon had negative relationships with endothelial function [40, 41], suggesting the participation of adipon in the development of carotid SA. Indeed, adipon has been revealed as an independent risk factor for endothelial dysfunction in individuals with type-2 diabetes mellitus [42]. Moreover, it has been well-documented that impaired endothelial function contributes to many cardiovascular diseases, including atherosclerosis [43, 44]. Given the roles of adipon in endothelial functional protection, adipon may play an important role in SA pathogenesis by protecting endothelial function.

There were several limitations to the current study, however. First, due to the small sample size, the predictive value of serum adipon needs to be further confirmed in studies with a large cohort. Second, due to the retrospective nature of the present study, a causal association between SA and serum adipon could not be established. However, present findings provide important information for development of new medicines for atherosclerosis, particularly SA. Third, studies that used other techniques, such as computed tomography (CT) and magnetic resonance imaging (MRI), to evaluate SA were not included in the present investigation. Hence, further investigations are necessary to determine whether present findings can be applied for all kinds of SA patients.

In conclusion, serum adipon is closely linked to CIMT and predicts SA in hyperlipidemic patients. Present findings support the notion that adipon plays a role in vascular complications of hyperlipidemia, providing a possible perspective for the use of adipon as a marker for detection of SA.

Acknowledgements

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Disclosure of conflict of interest

None.
Abbreviations

SA, subclinical atherosclerosis; NSA, non-subclinical atherosclerosis; CIMT, carotid artery intima-media thickness; Cr, Creatinine; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

Address correspondence to: Xiao-Song Gu, Department of Cardiology, The Second Affiliated Hospital of Soochow University, No.1055 Sanxiang Road, Suzhou 215000, China. Tel: +86158625-22568; E-mail: xiaosonggu@hotmail.com

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