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Correlation between serum free fatty acids levels and Gensini score in elderly patients with coronary heart disease

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Abstract

Objectives To investigate the relationship between serum free fatty acids (FFAs) levels and the severity of coronary artery lesions in elderly patients with coronary heart disease (CAD). **Methods** A total of 172 elderly patients who underwent coronary angiography were divided into CAD group (n = 128) and non-CAD group (n = 44) according to the results of coronary angiography. Serum FFAs and lipid levels were measured and the Gensini score were calculated. **Results** No matter the differences between age, gender and the usage of statins or not, there was no statistical significance in FFAs levels ($P > 0.05$). In terms of the Gensini score, it was higher in patients aged 70–79 years than in patients 60–69 years old [15.00 (5.00, 34.00) *vs*. 10.00 (2.00, 24.00), $P < 0.05$], higher in men than women [14.00 (4.00, 34.00) *vs*. 7.00 (2.50, 19.75), $P < 0.05$], and higher in patients on statins [13.50 (4.25, 33.50) *vs*. 6.50 (2.00, 18.00), $P < 0.05$]. The serum FFAs levels [449.50 (299.00, 624.75) mEq/L *vs*. 388.00 (258.50, 495.25) mEq/L, $P < 0.05$] and Gensini score [17.50 (8.00, 41.75) *vs*. 1.00 (0, 5.00), $P < 0.05$] were higher in the CAD group than in the non-CAD group. In the CAD group, there was no statistical significance in FFAs levels among patients with different numbers of diseased coronary vessels ($P > 0.05$). Furthermore, the FFAs levels were positively correlated with the Gensini score (r = 0.394, P = 0.005). Regression analysis showed that the FFAs levels were related to the Gensini score independently after adjusting for the other risk factors. **Conclusions** The serum FFAs levels were associated with the Gensini score in elderly patients with CAD. It might indicate FFAs as a biomarker predicting the severity of coronary artery lesions.

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Keywords: Coronary heart disease; Free fatty acids; Gensini score; The elderly

1 Introduction

Coronary artery disease (CAD) is the most common type of heart disease and the cause of heart attacks. This disease is caused by plaque building up along the inner walls of the arteries of theheart, which narrows the arteries and reduces blood flow to the heart. The Gensini score calculation is based on the evaluation of the number of stenotic coronary artery segments, the degree of their lumen stenosis and the localization of stenotic changes,[1] and it has become the standard score reflecting the severity of coronary lesions.

Additionally, oxidative stress, endothelial dysfunction and insulin resistance play important roles in the development of atherosclerosis and CAD. As the sources of myocardium energy, serum free fatty acids (FFAs) can enhance the reaction of oxidative stress, cause endothelial dysfunction[2] and correlates with increased insulin resistance.[3] Over a 5-year follow-up, the Quebec Cardiovascular Study confirmed that increased plasma FFAs in healthy men predicted the subsequent development of CAD.[4] But the relationship between FFAs levels and the degree of coronary lesions in elderly patients was not well established. Our study aims to investigate the correlation between the serum FFAs and Gensini score, and explore new predictors of the severity of coronary artery lesions.

2 Methods

2.1 Patients

A total of 172 elderly patients (111 men and 61 women) who were 60 to 89 years old with an average age of 70.9 ±

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7.0 years and were suspected to have CAD in Zhejiang Hospital were enrolled in our study from October 2012 to February 2013. Patients with malignant tumor, severe liver or renal dysfunction, acute infection, peripheral vessel disease, known allergy to contrast media, known CAD, undergoing coronary artery bypass surgery or other cardiac interventions were excluded from the study. All subjects gave their written informed consent to be in the study.

2.2 Study design

All patients underwent coronary angiography, and then were divided into the CAD group (coronary stenosis ≥ 50%, n = 128) and the non-CAD group (coronary stenosis < 50%, n = 44) according to coronary angiography. There were 43 patients with single-vessel disease, 40 patients with two-vessel disease and 45 patients with three-vessel disease in the CAD group. Clinical data were collected and blood samples were drawn.

2.3 Demographic data

Age, gender, body mass index (BMI), smoking status, concurrent basal diseases (including hypertension, diabetic mellitus and stroke), and the usage of statins were all recorded. BMI = body weight (kg)/body height^2 (cm^2).

2.4 Biochemical analysis

Five milliliter venous blood samples were obtained on the second day of hospitalization after fasting for 8 h or over night, then centrifuged at 3000 r/min for 10 min and placed at room temperature for 0.5 h, the separated serum were stored at −60℃. Fasting blood glucose (FBG) was measured by glucose oxidase method. Fasting serum FFAs, homocysteine (HCY), total cholesterol (T-CHO), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C) were measured quantitatively by the cyclophorase method using Olympus AU 5400 auto biochemistry analyzer.

2.5 Coronary angiography

Selective coronary angiography was performed for all study patients according to standard Judkins techniques. The CAD was defined as luminal diameter narrowing of 50% or more in the major coronary vessels in at least 2 orthogonality postures of projection, and if not, was diagnosed as non-CAD. The major coronary vessels included the left main (LM), left anterior descending artery (LAD), left circumflex artery (LCX) and right coronary artery (RCA). The angiograms were evaluated using quantitative coronary angiography by an experienced observer who was unaware of the results of FFAs measurement. The extent of coronary atherosclerosis was coded as 1, 2 or 3, according to the number of stenosed major coronary vessels.

2.6 Coronary Gensini score

The severity of coronary atherosclerosis was determined by Gensini scoring. The Gensini score is computed by assigning a severity score to each coronary stenosis according to the degree of luminal narrowing and its importance based on location. A reduction in the lumen diameter, and the angiographic appearance of concentric lesions and eccentric plaques were quantitatively evaluated. More specifically, reductions of 25%, 50%, 75%, 90%, 99% and complete occlusion were given Gensini score of 1, 2, 4, 8, 16 and 32, respectively. Each principal vascular segment was assigned a multiplier in accordance with the functional significance of the myocardial area supplied by that segment, that is, the LM was assigned the significant multiplier × 5; the proximal segment of the LAD was given × 2.5; the proximal segment of the LCX was weighted by a factor of × 2.5; the mid segment of the LAD was assigned a factor of × 1.5; the RCA, the distal segment of the LAD, the posterolateral artery, and the obtuse marginal artery were all given × 1; and all other areas were assigned a factor of × 0.5.[1]

2.7 Statistical analysis

The SPSS 18.0 (SPSS, Chicago, IL, USA) software package was employed for statistical processing. Measurement data were presented as mean ± SD or median (interquartile range); numeration data were presented as constituent ratio. All continuous variables were tested for normal distribution and homogeneity for variance. Statistically significant differences were tested by chi-square test (for qualitative items), Student's t-test for unpaired observations (for normally distributed quantitative items), two independent samples rank test, Spearman rank correlation, partial correlation and stepwise multiple regression analysis. P < 0.05 was considered statistically significant.

3 Results

3.1 Comparison of clinical data between the CAD and non-CAD group

Compared with the non-CAD group, the patients in the CAD group were older and with a higher prevalence of male subjects (P < 0.05). There was no statistical significance between the two groups in smoking status, concurrent basal diseases, BMI or FBG (P > 0.05). Compared with the non-CAD group, the levels of T-CHO, HDL-C and LDL-C in the CAD group were lower (P < 0.05), and the levels of HCY, FFAs and Gensini score in the CAD group were higher (P < 0.05). Moreover, the ratio of the usage of statins in the CAD group was higher than in the non-CAD group (P < 0.05). Details were shown in the Table 1.

Table 1. Comparison of clinical data between the CAD and non-CAD group.

	Non-CAD group (n = 44)	CAD group (n = 128)	*P*
Age, *n*(%)			
60–69 years	28 (63.6)	53 (41.4)	0.038
70–79 years	13 (29.5)	59 (46.1)	
80–89 years	3 (6.8)	16 (12.5)	
Male, *n* (%)	22 (50.0)	89 (69.5)	0.019
Smoking, *n* (%)	8 (21.2)	32 (27.4)	0.441
Concurrent basal diseases			
Hypertension, *n* (%)	30 (68.2)	98 (76.6)	0.272
Diabetes, *n* (%)	7 (15.9)	29 (23.8)	0.278
Stoke, *n* (%)	2 (4.5)	9 (7.0)	0.561
Using Statins, *n* (%)	24 (63.2)	96 (82.8)	0.011
BMI, kg/m^2	23.42 ± 0.34	24.22 ± 3.16	0.453
Biochemical parameters			
FBG, mmol/L	6.60 ± 1.09	5.86 ± 1.78	0.393
T-CHO (mmol/L)	4.57 ± 1.11	4.01 ± 1.04	0.003
TG (mmol/L)	1.26 ± 0.69	1.40 ± 0.74	0.273
HDL-C (mmol/L)	1.47 ± 0.47	1.31 ± 0.34	0.022
LDL-C (mmol/L)	2.52 ± 0.77	2.15 ± 0.78	0.008
HCY (μmol/L)	13.40 (10.53–16.25)	15.85 (11.40–20.80)	0.037
FFAs (mEq/L)	388.00 (258.50–495.25)	449.50 (299.00–624.75)	0.047
Gensini score	1.00 (0.00–5.00)	17.50 (8.00–41.75)	0.000

Data are mean ± SD unless indicated. HCY, FFAs levels and Gensini score are given as median (inter-quartile range). BMI: body mass index; CAD: coronary artery disease; FBG: fasting blood glucose; FFAs: fasting fatty acids; HCY: homocysteine; HDL-C: high density lipoprotein cholesterol; T-CHO: total cholesterol; TG: triglyceride; LDL-C: low density lipoprotein cholesterol.

Table 2. The influence of the difference of age, gender and the usage of statins on the serum FFAs levels and Gensini score.

	FFAs (mEq/L)	Gensini score
Age		
60–69 years, n = 81	400.00 (297.00–543.50)	10.00 (2.00–24.00)
70–79 years, n = 72	455.00 (249.00–642.00)	15.00 (5.00–34.00)*
80–89 years, n = 19	423.00 (241.00–601.00)	15.00 (5.00–41.00)
Gender		
Male, n = 111	404.00 (290.00–528.00)	14.00 (4.00–34.00)
Female, n = 61	436.00 (254.00–623.50)	7.00 (2.50–19.75)$^\#$
Using of statins		
Yes, n = 120	429.00 (259.25–595.25)	13.50 (4.25–33.50)†
No, n = 34	405.00 (320.75–591.00)	6.50 (2.00–18.00)

Data are given as median (interquartile range). $^*P < 0.05$ compared with 60–69 years group; $^\#P < 0.05$ compared with male; †$P < 0.05$ compared with not using of statins. FFAs: free fatty acids.

In order to investigate the influence of the difference between age, gender and the usage of the statins on the levels of FFAs and Gensini score, further analysis was performed. Results showed that there was no statistical significance between the FFAs levels attributed to age, gender, or statins use or not ($P > 0.05$). In terms of the Gensini score, it was higher in patients aged 70–79 years than in patients with 60–69 years old ($P < 0.05$), higher in men than women ($P < 0.05$), and higher in patients on statins than without ($P < 0.05$). Details were shown in the Table 2.

3.2 Comparison of the difference in FFAs levels and Gensini score among the different numbers of diseased coronary vessels in the CAD group

In CAD group, the Gensini score were successively higher in the three-vessel disease group (n = 45) than in the two-vessel disease group (n = 40) and the single-vessel disease group (n = 43) (43.00 (23.50, 68.00) *vs*. 17.00 (9.25, 38.00) *vs*. 8.00 (4.00, 15.00), $P < 0.001$, respectively). But there was no statistical significance among three-vessel disease group, two-vessel disease group and single-vessel disease group in serum FFAs levels (485.00 (305.00, 693.50) mEq/L *vs*. 402.50 (233.75, 666.75) mEq/L *vs*. 466.00 (321.00, 558.00) mEq/L, $H = 0.833$, $P = 0.660$).

3.3 Correlation analysis of the levels of FFAs and Gensini score

Spearman rank correlation analysis was used to detect the relationship between the Gensini score and serum FFAs levels and showed that there was a positive correlation between them ($r = 0.326$, $P < 0.001$) (Figure 1). After further controlling for age, gender, smoking status, BMI and con-

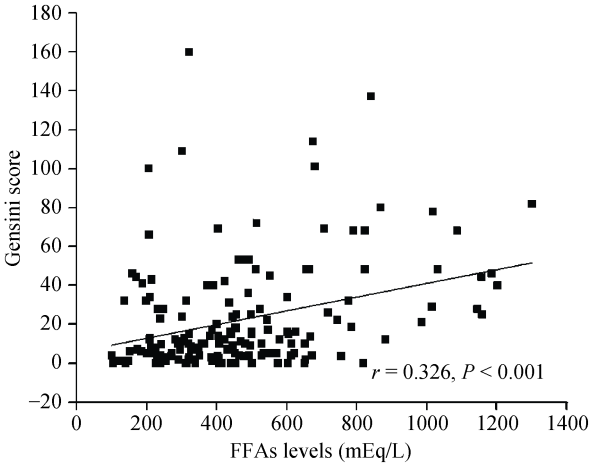

Figure 1. The correlation between the FFAs levels and the Gensini score. FFAs: free fatty acids.

current basal diseases, partial correlation analysis found only the Gensini score were positively correlated with the serum FFAs levels ($r = 0.394$, $P = 0.005$).

3.4 Multivariate analysis of the Gensini score of the elderly CAD patients

The stepwise multiple regression analysis found only the serum FFAs levels were correlated with the Gensini score ($\beta = 0.371$, $P = 0.029$), and age, gender, BMI, smoking status, concurrent basal diseases, statin use, FBG and HCY were all not correlated with the Gensini score ($P > 0.05$).

4 Discussion

CAD is one of the most common causes of death. The morbidity and mortality of the CAD increased year by year as living standards improved. As we all know, the occurrence and development of CAD are the results of many factors, and the mechanism has not been fully elucidated.

Many studies have demonstrated that atherosclerosis is an inflammatory disease, and the endothelial dysfunction, oxidative stress, and insulin resistance are the important pathogeneses of CAD.[5] Studies have shown that the serum FFAs were strongly correlated with carotid intimal media thickness (IMT) in diabetics.[6] FFAs could induce atherosclerosis through many pathways.

Increased FFAs would induce endothelial dysfunction and oxidative stress. Endothelium-dependent vasodilation would be impaired by increased FFAs.[7] Nitric oxide (NO) is the most vigorous endogenous vasodilator released from endothelium. The 2–3 folds elevation of FFAs could induce the generation and release of reduced NO and impair endogenous vasodilation.[8] FFAs could induce nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunit overexpression and reactive oxygen species (ROS) production and therefore resulted in the endothelial dysfunction.[2] High levels of FFAs induced ROS levels increased which reflex the oxidative stress states of the body.[9] At the same time, elevated FFAs were correlated with inflammation. Several *in vitro* studies found that adding IL-6 and TNF-α to isolated adipocytes resulted in increased lipolysis.[10,11] Furthermore, infusions of IL-6 or TNF-α in healthy subjects resulted in acutely increased circulating FFAs.[12,13] Reports also indicated insulin resistance is an independent predictor of atherosclerosis plaque progression in patients with coronary heart disease,[14] and the increased plasma FFAs levels were an important cause of obesity-associated insulin resistance.[15] In addition to these effects, elevation of some FFAs promotes uptake of oxidized LDL in macrophages, a critical step in development of atherosclerosis.[16]

Coronary angiography is the gold standard for the diagnosis of CAD. In our study, 172 elderly patients who underwent coronary angiography were investigated. We found the FFAs levels and Gensini score in CAD patients whose coronary stenosis ≥ 50% were higher than non-CAD patients, and there was no statistical significance in the FFAs levels among the diagnosed CAD patients with different numbers of diseased coronary vessels, which was similar to the study of Hao, *et al*.[17] Diabetic mellitus is one of the most important risk factors for CAD. There were 20.9% of patients with diabetes in our study. Elevated FFAs levels could lead to insulin resistance and therefore involved in the development of diabetes.[18] Diabetes might induce coronary artery endothelium dysfunction and then contribute to the development of CAD.[19] Studies had shown that diabetes was correlated with the Gensini score.[20,21] After analyzing the extent of coronary stenosis using quantitative computer analysis (QCA) and calculating the coronary artery score (CAS) in accordance with the Leaman CAS method, Liu, *et al*.[22] found FFAs levels were closely correlated with CAS and serum FFAs might be a good predictor for the occurrence and development of CAD. We also found the serum FFAs levels were positively correlated to the Gensini score in elderly CAD patients after controlling for many traditional factors, that is to say, the higher the serum FFAs levels, the higher Gensini score. But we are able to conclude the causation between them. Moreover, in our study, the regression analysis of Gensini score of elderly CAD patients found there was only serum FFAs levels correlated with the Gensini score, indicating the serum FFAs levels might be a precautionary indicator for coronary lesions and prompting the degree of coronary lesions indirectly.

As the first line drugs for the therapy of CAD and atherosclerosis, statins can reduce the levels of LDL-C, T-CHO and TG, and raise the levels of HDL-C, thus decrease the risks of cardiovascular and cerebrovascular events. In our study, 82.8% patients in the CAD group were using statins, which was higher than the non-CAD group (63.2%), and obviously, this might explain why the TG and LDL-C levels in the CAD group were lower than the non-CAD group. At the same time, many studies have shown that the HDL-C has vascular protective effects,[23–25] and low HDL-C was related to the increase in CAD and main cardiovascular events, although the statins have slight HDL-C raising effects, it remains to be determined the effect of the lower HDL-C levels in the CAD group in our study. Furthermore, the reports on whether statins could decrease the serum FFAs levels were not identical. The atorvastatin was proved to have the ability to decrease the FFAs levels.[26] But, Isley, *et al*.[27] found that high-dose simvastatin had no effect on

simvastatin had no effect on plasma FFAs concentrations. Sato, *et al*.[28] also found that pravastatin did not change the plasma FFAs levels. We did not find any difference in serum FFAs levels between use of statins or not.

Previous studies have demonstrated that the plasma HCY was significantly increased in CAD patients,[29,30] but the underlying mechanisms were still not very clear, and might be related to the proliferation and apoptosis of vascular smooth muscle cells, the injuries of vascular endothelium, the damage to clotting and the fibrinolysis system, and the effects on energy metabolism.[31–33] Although the HCY levels in CAD patients were higher than non-CAD patients in our study, no relationship was found between the HCY levels and the extent of coronary lesions using partial correlation and regression analysis. This was inconsistent with the results of previous studies.[34,35] and may be a conquence of the small sample size or the differences in study populations.

Above all, the serum FFAs levels were correlated with the coronary Gensini score in elderly CAD patients and might be an important indicator for the extent of coronary lesions in CAD patients. At the same time, how to reduce the serum FFAs levels might became an important direction in the treatment of CAD patients. In addition, our study was a cross-sectional study with small samples, further studies would augment the sample size and investigate the relationship between the serum FFAs levels and extent of coronary stenosis in elderly CAD patients by longitudinal studies.

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