Correlation between plasma asymmetric dimethylarginine and different types of coronary heart disease

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Abstract: Objective To monitor the changes of plasma asymmetric dimethylarginine (ADMA), nitric oxide (NO), and von Willebrand factor (vWF) levels in patients with stable angina pectoris (SAP) or acute coronary syndrome (ACS) and to evaluate the correlation between ADMA and different types of coronary heart disease. **Methods** A total of 143 subjects were divided into a non-CHD group, a SAP group and an ACS group. Plasma levels of ADMA, NO and vWF were examined and their correlation with SAP or ACS was analyzed. **Results** Compared with the non-CHO or the SAP group, ADMA level was elevated in the ACS group ($P<0.05$). The ADMA level tended to increase in the SAP group compared with the non-CHD group, but had no significant difference ($P>0.05$). Compared with the non-CHD group, NO level was decreased in both the SAP and ACS group ($P<0.05$), and it decreased more in ACS group than that in the SAP group ($P<0.05$); vWF levels were increased in both the SAP and ACS group compared with the non-CHD group ($P<0.05$). There was no significant difference in the plasma levels of vWF in the SAP and the ACS group ($P>0.05$). **Conclusion** The change of plasma ADMA level is closely correlated with acute coronary syndrome. ADMA might be a clinical marker for acute coronary syndrome.

Key words: asymmetric dimethylarginine; nitric oxide; coronary heart disease; acute coronary syndrome

不对称二甲基精氨酸在冠心病临床分型中的应用

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[**摘要**] **目的:**测定冠心病患者中稳定型心绞痛和急性冠脉综合征的血浆不对称二甲基精氨酸(ADMA)、一氧化氮(NO)和血管性血友病因子(vWF)水平,探讨其与冠心病各临床类型之间的关系。**方法:**按纳入与排除标准入选了143例研究对象,其中包括非冠心病组64例,急性冠脉综合征组54例和稳定性心绞痛组25例。检测各组血浆ADMA,NO和vWF水平,并分析它们与急性冠脉综合征和稳定性心绞痛之间的相关性。**结果:**与非冠心病组和稳定型心绞痛组比较,急性冠脉综合征血浆ADMA水平显著升高($P<0.05$)。与非冠心病组相比,稳定型心绞痛组血浆ADMA水平有升高趋势但差异无统

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计学意义($P>0.05$);与非冠心病组相比,稳定型心绞痛和急性冠脉综合征组血浆 NO 水平均显著下降($P<0.05$),急性冠脉综合征组下降的程度明显大于稳定型心绞痛组($P<0.05$);与非冠心病组相比,稳定型心绞痛组和急性冠脉综合征组血浆 vWF 水平均显著升高($P<0.05$)。稳定型心绞痛组和急性冠脉综合征组之间血浆 vWF 水平差异无统计学意义($P>0.05$)。**结论**:血浆 ADMA 水平变化与冠心病中急性冠脉综合征的发生发展密切相关,血浆 ADMA 水平的变化可能是急性冠脉综合征的预测因子。

[**关键词**] 不对称二甲基精氨酸; 一氧化氮; 冠心病; 急性冠脉综合征
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Endothelium-derived nitric oxide (NO), which possesses various biological properties such as anti-oxidation, anti-inflammation and anti-apoptosis, plays an important role in maintaining the integrity of blood vessel endothelium and preventing the development of atherosclerosis. In the development of coronary heart disease (CHD), impaired function of blood vessel endothelium is closely related to the decline of NO level in plasma. The generation of NO is from L-arginine catalyzed by NO synthases (NOS). Recently, it was reported that L-arginine analogue asymmetric dimethylarginine (ADMA), a major endogenous inhibitor of NOS, plays a central role in regulation of NO synthesis, and the plasma level of ADMA significantly increased and accompanied by the decreased NO levels in CHD patients[1], supporting that ADMA was a novel independent risk factor for CHD.

CHD patients are classified as 2 types in clinic, stable angina pectoris (SAP) and acute coronary syndrome (ACS). Although the positive correlation between changes of plasma ADMA level and the occurrence of CHD has been previously reported[1], the correlation between the changes of plasma ADMA level and different types of CHDs remains largely unknown.

In this study, CHD patients were classified as ACS and SAP groups according to clinical symptoms. By measuring and analyzing the changes of plasma ADMA, NO and endothelial activating factor vWF level, we evaluated the correlation between ADMA and different types of CHDs, or the degree of atherosclerotic stenosis.

1 MATERIALS AND METHODS

1.1 Subjects

We choose 143 patients accepting selective coronary angiography, 79 patients with atherosclerosis as a CHD group and 64 patients without coronary atherosclerosis as a non-CHD group who were from the Department of Cardiology in the Third Xiangya Hospital due to chest pain, chest tightness, and palpitation from October 2006 to October 2007. The CHD group was further divided into a ACS group (54 patients) and a SAP group (25 patients) as according to clinical symptoms.

Inclusion criteria: The patients were inquired for history of diseases and accepted physical examinations, and meanwhile completed examinations including blood routine, liver and kidney function, electrocardiogram (ECG) and selective coronary angiography (without limits on anti-diabetes and anti-hyperlipidenmia therapy before being chosen).

Exclusion criteria: (1) severe infectious diseases or chronic inflammatory diseases last month, including severe upper respiratory tract, lung, and hepatobiliary duct infection; (2) active tuberculosis; (3) surgical operation or tissue trauma recently (within 3 months); (4) severe damaged liver or kidney functions; (5) malignant tumor; (6) connective tissue disease (CTD) and rheumatism; (7) inflammatory inhibitors such as non-steroidal anti-inflammatory analgesic and steroidal drugs was used; (8) with incomplete case history.

1.2 Major instruments and reagents

HITACHI 7170S automated biochemistry analyzer (Japanese Hitachi); ACS 180SE automatic electrochemical immuno-analyzer (USA Bayer); LC-10ADVP high performance liquid chromatograph (Japanese Shimadzu); Sequioa512 full digital color ultrasonic diagnostic apparatus and C-arm digital subtraction angiography (ANGELSTAR) (German Siemens); Waters chromatographic column nova-Pak C18 (Shanghai Shengxi Industry Co., Ltd.); Total cholesterol (TC) and triglycercide (TG) testing reagent (Sichuan Maker Biotechnology Co., Ltd.); High density lipoprotein (HDL) testing reagent (Chinese Wako Pure Chemical Industries Ltd.); Plasma vWF test kit (Shanghai Sun Biometric Technologies Co., Ltd.); Plasma NO test kit (Nanjing July Pharmaceutical Co., Ltd.).

1.3 Selective coronary angiography

Coronary angiography was conducted by radial artery approach with projection of multiple body positions. The angiography results were drawn by quantitative analysis on the blood vessel stenosis level of left main coronary artery (LM), left anterior descending coronary artery (LAD), left circumflex artery (LCX) and right coronary artery (RCA) by 2 experienced doctors with respective application of universal ocular estimate method of vessel diameter. In case of the blood vessel stenosis level of coronary artery was more than 50%, it was classified into the CHD group.

1.4 Blood collection and determination of biochemical parameters

1.4.1 Sample collection and determination of blood glucose and lipid

Before accepting coronary angiography, all patients kept hollow stomach for 8 hours, whose right femoral artery or radial artery was punctured by Seldinger method after conventional disinfection, draping, and local anesthesia with procaine or lidocaine, and put into artery sheath. Ten milliliter artery blood slowly drawed out along the artery sheath and then divided into 2 parts, which was placed into a 10 mL anticoagulant heparin solution pipe and a general centrifugal pipe, respectively. After centrifugated for 15 min at 3 000 r/min, supernatant plasma and serum respectively were placed into EP pipes in −80℃ refrigerator. In the meantime, the biochemical indicators including liver and kidney functions, blood glucose and lipid were examined in Clinical Laboratory Dept. of Third Xiangya Hospital.

1.4.2 Determination of plasma ADMA

The contents of ADMA in the plasma were measured by high-performance liquid chromatography (HPLC). Briefly, HPLC was carried out using a Shimadzu LC-6A liquid chromatograph with Shimadzu SCL-6A system controller and Shimadzu SIC-6A autosampler. O-Phthaldiadehyde adducts of methylated amino acids and internal standard ADMA produced by precolumn mixing were monitored using a model RF 530 fluorescence detector set at $\lambda ex = 338$ and $\lambda em = 425$ nm on a resolve C18 column. The proteins in the plasma were removed using 5-sulfosalicylic acid. Samples were eluted from the column using a linear gradient containing mobile phase A composed of 0.05 mmol (pH6.8) sodium acetate-methanol-tetrahydrofuran (volume ratio was 81∶18∶1) and mobile phase B composed of 0.05 mmol sodium acetate-methanol-tetrahydrofuran (volume ratio was 22∶77∶1) at a flow-rate of 1 mL/min.

1.4.3 Determination of plasma vWF factor

Enzyme linked immunosorbent assay (ELISA) was applied to determine the content of plasma vWF. The detailed operations were carried out in accordance with directions of the test kit, and values were read at 492 nm by microplate reader. A standard curve was plotted at plasma content of vWF criteria, and the vWF content of sample was tracked down from the standard curve.

1.4.4 Determination of NO in plasma

The level of NO in plasma, reflected indirectly by the content of nitrite and nitrate, was measured. Briefly, nitrate was converted to nitrite with nitrite

reductase, and the total nitrite was measured with the Griess reagent. The absorbance was determined at 540 nm with a spectrophotometer.

1.5 Statistical analysis

The measurement data were presented as mean ± standard deviation ($\bar{x} \pm s$). All statistical analysis was carried out by SPSS software (version 11.0 for windows, SPSS Inc, Chicago, Illinois, USA). The enumeration data were assessed with χ^2 test. The independent-samples t test and one-way ANOVA were used to evaluate the statistical differences among different groups. $P < 0.05$ was considered as statistical difference.

2 RESULTS

2.1 Basic characteristics of subjects

TC and TG in plasma had no obvious difference among the SAP, ACS and non-CHD group; Ages of the SAP and ACS groups were older than that of non-CHD group, while HDL in the non-CHD group was significantly higher than that in the SAP and the ACS groups; Male/female ratio in the ACS group ranked the highest, that in the SAP group went second, and that in non-CHD group was the lowest (Tab. 1).

Tab. 1 Comparison between SAP, ACS, and non-CHD group

Groups	n	Age/years	Gender (male/female)	TG/(mmol/L)	TC/(mmol/L)	LDL/(mmol/L)	HDL/(mmol/L)
SAP group	25	62.6 ± 11.0[a]	17/8 *▲	1.58 ± 0.95	4.18 ± 0.94	2.49 ± 0.71 *	0.94 ± 0.27 *
ACS group	54	67.8 ± 9.8 *	41/13 *	1.43 ± 0.78	4.52 ± 1.33	2.70 ± 1.10 *	1.12 ± 0.28 *
Non-CHD group	64	59.8 ± 10.4	34/30	1.60 ± 0.75	4.42 ± 0.90	1.12 ± 0.30	2.56 ± 0.80

Compared with the non-CHD group, * $P<0.05$; compared with the ACS group, ▲ $P<0.05$.

2.2 Plasma ADMA level was increased in the ACS group

The plasma of ADMA level of the ACS group significantly increased compared with the non-CHD and the SAP groups ($P < 0.05$). However, the plasma ADMA level of SAP group tended to increase but the trend did not reach significance, compared with the non-CHD group ($P > 0.05$, Tab. 2).

Tab. 2 Comparison in plasma levels of ADMA, NO, VWF among SAP, ACS, and non-CHD group

Groups	Plasma ADMA concentration/(μmol/L)	Plasma NO concentration/(μmol/L)	Plasma vWF/%
SAP group	0.42 ± 0.13	6.62 ± 2.19 *	154.53 ± 37.34 *
ACS group	0.51 ± 0.18▲*	5.93 ± 1.99 *▲	157.01 ± 46.40 *
Non-CHD group	0.36 ± 0.12	7.38 ± 1.89	126.66 ± 48.04

Compared with non-CHD group, * $P<0.05$; compared with SAP group, ▲ $P<0.05$.

2.3 Decline of plasma NO levels in the SAP and ACS groups

Compared with the non-CHD group, the plasma NO level in the SAP and ACS groups both significantly decreased and the reduced degree of plasma NO level in the ACS group was more than that in the SAP group ($P < 0.05$, Tab. 2).

2.4 Increased plasma levels of vWF in SAP and ACS groups

Compared with the non-CHD group, the plasma

vWF level in the SAP and ACS groups both significantly decreased ($P < 0.05$). There was no significant difference in plasma levels of vWF in the SAP and ACS groups ($P > 0.05$, Tab. 2).

3 DISCUSSION

As a new marker of cardiovascular disease, the function of ADMA in facilitating atherosclerosis has been recognized[2]. ADMA is generated from hydrolysis of the proteins in which the arginine residues are methylated by arginine methyltransferase. In vivo, part of ADMA is cleared by renal excretion whereas most of ADMA clearance is dependent on the enzyme dimethylarginine dimethylaminohydrolase (DDAH).

ADMA can competitively inhibit the activity of NOS and decrease the synthesis of NO[3]. In addition to the effect of vasodilation, NO also exerts the effect on the inhibition of platelet aggregation, cell adhesion, smooth muscle cells proliferation, and ROS production, which play an importance role in maintaining the integrity of vascular endothelium and prevent atherosclerosis development[4]. There are multiple factors that lead to the reduction of NO. Among them, the reduction in NOS activity is a crucial one. Since ADMA can competitively inhibit NOS activity, We hypothesized that the increase in ADMA level may contribute to the reduction of NOS activity. There is plenty of evidence that the plasma level of ADMA was significantly increased in many cardiovascular diseases such as hypertension, coronary heart disease, heart failure and atherosclerosis, supporting the concept that ADMA is a novel risk factor for cardiovascular diseases[1-2].

In general, CHD patients are classified as SAP and ACS depending on the clinical symptoms. The occurrence of ACS closely correlates with instability and rapture of coronary plaques as well as the formation of secondary thrombus. It has been shown that the plasma levels of ADMA in CHD patients significantly elevated[5], However, most of studies were not involved in clinical classification. Our study showed that the plasma levels of ADMA in the ACS group dramatically elevated compared with the non-CHD and SAP groups. The ADMA levels in the SAP group showed a tendency to increase, suggesting that the change of plasma level of ADMA more closely correlates to the ACS. Cavusoglu, et al.[6] indicated that the plasma level of ADMA had definite value on the prognosis of ACS patients. Therefore, the plasma level of ADMA not only correlates with the occurrence and development of coronary atherosclerosis[7], but also indirectly reflects the stability and degree of the coronary atherosclerosis and accordingly has a certain evaluation value on clinical CHD types and severity of diabetic microangiopathy. We also found the plasma level of NO in the ACS group was significantly reduced in agreement with the reduction of ADMA. Interestingly, there was no significant change in the plasma level of ADMA in the SAP group, but the NO level markedly decreased. These results suggest that there existed other mechanisms besides ADMA, to modulate the activity of NOS and the generation of NO. In addition, the plasma level of vWF in the SAP and ACS groups elevated compared with that in the non-CHD group, but there was no significant difference between the SAP and ACS groups. These results were not inconsistent with the change of ADMA level but in agreement with the change of NO level, supporting that there were additional mechanisms for modulating the NO production. The detailed mechanism needs further investigations.

In summary, we have demonstrated that the change of plasma ADMA level was closely correlated with acute coronary syndrome. Its relation with stable angina, however, needs further investigation before drawing a firm conclusion. ADMA might be a clinical marker for acute coronary syndrome.

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