

Evaluation of serum Asymmetric Dimethyl Arginine concentrations in coronary artery disease patients without traditional cardiovascular risk factors

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Summary. *Background:* Previous studies have shown that Asymmetric Dimethyl Arginine (ADMA) is increased significantly during coronary artery diseases (CAD). However it is not clear either this increase is due to cardiovascular disease (CVD) risk factors or ADMA is increased independently in CAD. The aim of this study is to evaluate ADMA's plasma level as an independent biomarker in CADs. *Patients and methods:* In current study a total of 165 subjects with no traditional CVD's RFs, who fulfilled the inclusion and exclusion criteria, were recruited; 55 CAD+ patients which had more than 50% stenosis (CAD+); 55 CAD- patients which had less than 50% stenosis in their coronary arteries (CAD-), based on their angiography record and 55 healthy individuals as controls. CAD+ patients were divided into three groups: single (SVD), double (2VD), and triple vessel (3VD) disease. Plasma level of soluble ADMA was measured with an enzyme-linked immuno sorbent assay (ELISA) kit. *Results:* No significant difference between ADMA's plasma levels was found between CAD+, CAD- and healthy groups. In addition ADMA's plasma levels was not significantly different between CAD+'s subgroups. *Conclusions:* The result of this study indicates no significant relation between ADMA's plasma levels and either presence or severity of coronary artery stenosis. Therefore, it is presumed that ADMA may not be an independent biomarker for CADs. (www.actabiomedica.it)

Key words: coronary artery disease, atherosclerosis, Asymmetric Dimethyl Arginine, biomarker

Introduction

The leading global cause of morbidity and mortality is atherosclerotic cardiovascular disease (CVD), which is commonly accompanied by stenosis in one or more of coronary arteries (1).

Endothelial dysfunction plays an important role in the pathophysiology of atherosclerosis (2). Normal endothelium can maintain vessel's tone and structure

by producing hemostatic regulatory factors such as nitric oxide (NO). NO is synthesized by nitric oxide synthesizer (NOS) and it maintains tone and structure of blood vessels. It also performs a series of anti-atherosclerotic functions such as vasodilation, inhibition of coherence and accumulation of platelets, inhibition of coherence of monocytes and leukocytes to endothelium and inducing the apoptosis of smooth muscle cells (1, 3).

Asymmetric dimethyl arginine (ADMA) is L-arginine's natural analog. ADMA inhibits NOS (1). It is synthesized by the action of an enzyme called protein arginine methyl transferase (PRMT) (4). It is eliminated via two ways, renal excretion and by the action of an enzyme called dimethyl arginine dimethyl hydroxylase (DDAH) (3-5). ADMA can enhance the process of atherosclerosis in many ways. It inhibits all of the isoforms of NOS (6) and it could also produce super oxides by uncoupling NOS. ADMA can revert the effects of NO, restrict blood flow and increase vascular wall resistance (7).

There have been studies suggesting a positive correlation between ADMA's level and the degree of coronary artery stenosis (1, 8-10). In addition, many studies have been performed in order to discover the association between ADMA's levels and traditional risk factors (RFs) of CVD, including smoking (6, 11-13), diabetes (2, 14-16), dyslipidemia (13, 17, 18), hypertension (9, 13, 19, 20), obesity (21-23). However, not all findings have been consistent.

Even though the association between ADMA's plasma concentration and the extent of coronary artery diseases (CAD) has been indicated, it is still unclear whether ADMA is considered as an independent biomarker or is it related to other CVD RFs. This study is the first which aimed to investigate ADMA's role as an independent biomarker in CAD.

Patients and methods

This study has been approved by the Ethics Commission of MUMS (Mashhad University of Medical Sciences) and patients signed a consent form before joining the study. The study was performed in year 2014 in Ghaem hospital, Mashhad, Iran.

One hundred and sixty five individuals which were candidate for angiography and had fulfilled the exclusion and inclusion criteria were selected. The inclusion criteria of the study were men and women, Candidate for angiography. And the exclusion criteria were liver, kidney or heart failure, activated infection, chronic inflammation, any discovered malignancy, statin usage, supplement drug usage, brain stroke, any CVD RFs (defined by American heart association: obesity, hyper-

tension (defined by JNC8), dyslipidemia (defined by AHA/ACC), diabetes (defined by American diabetic association), age, current Tabaco usage, family history of diabetes, HTN and dyslipidemia) and finally any CVD in control group. The study group was divided into three groups of fifty five individuals. First group had more than 50% stenosis in their coronary arteries (CAD⁺). Second group had less than 50% coronary artery stenosis (CAD⁻). And the third group had no sign of stenosis in their coronary arteries (control), as it was claimed in their angiography record.

Blood sampling was performed in the morning of angiography in a fasting condition from the femoral artery (which is the entrance of angiography catheters). The blood samples were kept in -80 degree centigrade freezer until 10 minutes before the onset of experiment. Angiography was performed by two cardiologists, who were unaware of patient's data, independently to assess the extent and severity of coronary artery stenosis.

ADMA plasma level concentration was measured by Elisa kit (LDL, S.N.: REA 201/96). This assay is based on the method of competitive enzyme linked immunoassays. Statistical analyzes was performed by SPSS (Statistical Package for the Social Science) version 21 for windows.

Results

Patients Data

The study population consisted of 165 individuals (55 CAD⁺, 55 CAD⁻ and 55 Healthy). Demographic data, base line details and biochemical tests, are summarized in Table 1.

The comparison between ADMA's plasma levels and the extent of coronary artery stenosis

ADMA's concentration was compared between CAD⁺, CAD⁻ and the control group. Then inter-group comparison was performed, and there was no significant difference between 3 groups. The results are shown in Table 2.

Table 1. Characteristics of study population

Parameters	CAD+ (Mean±SD)	CAD- (Mean±SD)	Control (Mean±SD)	CAD- + CAD+ (Mean± SD)
Demographic data				
Patients (n)	55	55	55	110
Age (year)	56.63±12.48	49.6±12.46	48.81±10.79	53.08±12.84
Body Mass Index (BMI)	23.88±3.55	24.97±3.21	23.86±3.21	24.43±3.43
Female/Male ratio	0.33	1.82	1.25	0.79
Biochemical data				
Fasting blood sugar (FBS) ⁽¹⁾	93.67±13.87	101.12± 17.35	81.24±12.14	97.43±16.03
Total cholesterol (TC) ⁽¹⁾	149.12±31.18	156.62±23.84	161.24 ±24.01	152.9±27.83
Triglyceride (TG) ⁽¹⁾	106.96±32.93	97.68±33.06	97.49±29.73	102.27±33.16
High density lipoprotein cholesterol (HDL-C) ⁽¹⁾	38.02±11.24	41.94±9.67	43.70±7.65	40±10.61
Low density lipoprotein cholesterol (LDL-C) ⁽¹⁾	86.73±23.80	91.08±17.95	99.19±20.58	88.92±21.05
Systolic Blood Pressure (SBP)	117.24±10.51	112.98±11.90	114.67±14.22	115.09±11.38
Diastolic Blood Pressure (DBP)	75.71±6.29	71.94±8.22	67.04±8.88	73.8±7.53
Red blood cell (RBC) ⁽²⁾	5.18±4.67	4.86 ±1.45	4.66±0.54	5.02±3.43
Wight blood cell (WBC) ⁽³⁾	6.63±2.11	6.55±2.49	5.47±1.33	6.59±2.3
% of ex-smokers	19%	9%	16%	14%

⁽¹⁾Unit: mg/dl⁽²⁾Unit: 10¹²/L⁽³⁾Unit: 10⁹/L**Table 2.** Relation between ADMA's plasma levels and the extent of artery stenosis

Parameters	CAD+					CAD-	Healthy	P-value ⁽¹⁾
	SVD	2VD	3VD	Total	P-value ⁽¹⁾			
Patients (n)	15	17	23	55		55	55	
ADMA serum levels ⁽²⁾ (mmol/L)	1.09±0.44	1.07±0.34	1.10±0.40	1.09±0.39	0.97	1.05±0.39	1.05±0.34	0.43

⁽¹⁾ One-way ANOVA test, (P-value<0.05 considered as significant)⁽²⁾ Mean ± SD

The comparison between ADMA's plasma levels in CAD+ subgroups

ADMA's concentration was compared between CAD+ subgroups according to the number of stenosed vessels; i.e. single-vessel disease (SVD), two-vessel disease (2VD) and three-vessel disease (3VD). Between-group comparison did not suggest any significant difference between the three groups. The results are shown in Table 2.

Discussion

Results of this study showed no significant association between plasma ADMA levels and either pres-

ence or severity of coronary artery stenosis according to the number of narrowed vessels in angiography.

During biochemical or mechanical damage to endothelium, production of hemostatic mediators, such as NO, is compromised. This is in part due to ADMA-related reduction in endothelial NO production and an increase in production of reactive oxygen species (ROS) that could cause NO inactivation (9). Also, LDL oxidation may inhibit DDAH and cause further elevation of ADMA levels (24).

Bai et al. reported a positive association of circulating ADMA levels with intima-media thickness (IMT) in a meta-analysis research including 6168 participants from 22 studies, investigating the relation between ADMA's level and carotid IMT. It was shown that this association is stronger in the subgroup

of patients with chronic kidney disease (CKD) (25). Shivkar et al.'s study showed that serum ADMA/NO balance can be a valuable predictor for severity of CAD and the extent of coronary atherosclerotic stenosis. Lu et al.'s study on 997 consecutive individuals referred for coronary angiography, showed that plasma ADMA levels increases significantly in CAD⁺ patients, compared with CAD⁻ and healthy individuals and it can predict long-term clinical outcomes in CVD; however after adjusting for CV RFs, ADMA level remained independent except for eGFR, which is not consistent with the present results (9). Gurel. et al. studied plasma ADMA, hs-CRP and homocysteine as predictive biomarkers of CAD. They claimed that Plasma ADMA is a predictor and risk factor of CVD, whereas homocysteine and hs-CRP are not (8). Kruszelnicka et al.'s study on 151 non-diabetic men with stable angina and obstructive CAD, to assess the association of angiographic extent and severity of coronary artery disease with ADMA's level, indicated that ADMA is associated with diffuse but not focal coronary atherosclerosis (10).

There has been several studies that delineated the correlation between the existence of CVD's RFs and ADMA's plasma levels; however there are conflicting results in this area. Some of them are mentioned below:

ADMA & Smoking: Jiang et al.'s study showed that a period of nicotine treatment can damage endothelial functions, decrease DDAH activity and elevate circulating ADMA levels (6). A study by Alkan et al. shows that smokers have higher plasma viscosity, a biophysical mechanical marker, that can lead to endothelial dysfunction and an increase in ADMA plasma levels (11). Wang et al.'s study shows that smoking is associated with a decrease in nitrate and nitrite levels, an increase in ADMA plasma levels and impaired endothelium-mediated vessel dilatation (12). In contrast, Korandji et al.'s study showed that smoking was not associated with ADMA's level elevation, in fact their study showed that ADMA is independent of all the conventional CV RFs which is in contrast with our findings (13).

ADMA & Diabetes: Krzyzanowska et al. and Celik et al.'s study shows that ADMA tends to increase in

type 1 and 2 diabetes (2, 14). In addition Bestermann et al.'s study shows a positive correlation between ADMA levels and insulin resistance and suggests that ADMA levels were not increased in hypertensive individuals unless they were insulin resistance (26); However an study by Pitocco et al. on subjects with type 1 diabetes with no diabetic complications shows that ADMA plasma levels is decreased in diabetic patients compared to non-diabetics (15). An other study by Heilman et al. on children with type 1 diabetes showed that paradoxically they have a lower blood levels of homocysteine and ADMA (16). Studies by Böger et al and Lu et al. and Korandji et al. show no difference in ADMA plasma levels between diabetic and non-diabetic groups (9, 13, 27).

ADMA & Oxidized LDL: In Fabian et al.'s study increase in Ox-LDL was positively correlated with increase in ADMA (17). Päivä et al.'s study showed a bivalent effect of ADMA, it can have a protective role by slowing LDL oxidation and also a damaging role by decreasing NO availability (18). In contrast, Korandji et al.'s study showed that ADMA's concentration was independent of dyslipidemia; however, as mentioned before, their study conclusion is inconsistent with ours (13).

ADMA & Renal impairment: Renal failure in which metabolism and exertion of different substances is disturbed is an example of united and combined risk factor in CV physiology. Renke et al.'s study confirms the positive relation between ADMA and renal failure (28). A study performed by Marra et al. showed that in diabetic renal impairment, DDAH's polymorphisms, which metabolizes ADMA, plays an important role in increasing plasma ADMA's levels; and it could lead to a greater risk of CVD (5). Another study by Aysegul et al showed that after renal transplant, ADMA will decrease significantly and it can be a predictor of atherosclerosis in RT patients (29). Lu et al. and Korandji et al.'s studies showed that ADMA's concentration was negatively correlated with eGFR (9, 13). In addition, Jacobi et al.'s study shows that inducing CKD in mice does not affect the levels of ADMA (4).

ADMA & Hypertension: Lu et al. and Mamatha et al.'s study showed that patients with hypertension had

a higher level of ADMA (9, 19). Perticone et al.'s study suggests that endothelium-dependent vasodilation would be compromised in hypertensive patients and they would have a significantly higher concentration of ADMA and L-arginine than normotensive individuals (20). In contrast to previous studies, in Korandji et al.'s study, hypertension had no influence on ADMA's plasma levels. In fact an inverse relation was showed between SBP and L-arginine/ADMA ratio (13).

ADMA & obesity: Mauricio et al.'s study showed that morbid obesity is associated with endothelial dysfunction. This is due to decreased level of NO, increased level of ADMA, chronic inflammatory process, oxidative stress and increasing prostanoid-dependent vasoconstriction (21). Gruber et al.'s study showed that ADMA is slightly increased in obese adolescents, but it is not associated with any of obesity related conditions such as dyslipidemia and hypertension (22). In contrast, a study by Rudofsky showed that a weight reduction program can improve endothelial function measured by pulse wave velocity, however it is not associated with ADMA level reduction (23).

According to previously mentioned studies, ADMA's association with CVD's RFs is unclear and there has been no study which investigate ADMA's role as an independent biomarker in CADs.

In current study, individuals of all three groups had no CVD's RFs (age, diabetes, HTN, dyslipidemia, obesity, Tabaco usage, family history of diabetes, HTN and dyslipidemia) and they had no history of renal and hepatic failure. The result of this study indicates no significant relation between ADMA's plasma levels and either presence or severity of coronary artery stenosis. Therefore, it is presumed that ADMA may not be an independent biomarker for CADs; however it is necessary to carry out a better evaluation with more studies and larger samples volumes.

New knowledge gained

Based on the present results, serum ADMA concentrations may not be associated with the presence of CAD in subjects free from traditional CV RFs.

Limitations

A major limitation of this study is that the groups were only matched for traditional CV risk factors, whilst non-traditional factors, particularly inflammation, might have been different between the groups.

Another limitation is lack of information on the NO status between the study groups, it is important to know if the NO levels of studied subjects were depressed or normal, and if the groups were comparable regarding NO levels at baseline. Finally, the present study cannot answer if serum ADMA levels are associated with the severity of CAD, as number of stenosed vessels is not an ideal indicator for the severity of CAD.

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References

1. Shivkar RR, Abhang SA. Ratio of Serum Asymmetric Dimethyl Arginine (ADMA)/Nitric Oxide in Coronary Artery Disease patients. *Journal of clinical and diagnostic research : J Clin Diagn Res.* 2014;8:CC04-6.
2. Celik M, Cerrah S, Arabul M, Akalin A. Relation of asymmetric dimethylarginine levels to macrovascular disease and inflammation markers in type 2 diabetic patients. *J Diabetes Res* 2014; 2014: 139215.
3. Nijveldt RJ, Siroen MP, Teerlink T, van Leeuwen PA. Elimination of asymmetric dimethylarginine by the kidney and the liver: a link to the development of multiple organ failure? *J Nutr* 2004; 134: 2848S-52S; discussion 53S.
4. Jacobi J, Maas R, Arend M, Cordasic N, Hilgers KF. Effect of lowering asymmetric dimethylarginine (ADMA) on vascular pathology in atherosclerotic ApoE-deficient mice with reduced renal mass. *Int J Mol Sci* 2014; 15: 5522-35.
5. Marra M, Marchegiani F, Ceriello A, Sirolla C, Boemi M, Franceschi C, et al. Chronic renal impairment and DDAH2-1151 A/C polymorphism determine ADMA levels in type 2 diabetic subjects. *Nephrol Dial Transplant* 2013; 28: 964-71.

6. Jiang DJ, Jia SJ, Yan J, Zhou Z, Yuan Q, Li YJ. Involvement of DDAH/ADMA/NOS pathway in nicotine-induced endothelial dysfunction. *Biochem Biophys Res Commun* 2006; 349: 683-93.
7. Antoniadis C, Shirodaria C, Leeson P, Antonopoulos A, Warrick N, Van-Assche T, et al. Association of plasma asymmetric dimethylarginine (ADMA) with elevated vascular superoxide production and endothelial nitric oxide synthase uncoupling: implications for endothelial function in human atherosclerosis. *Eur Heart J* 2009; 30: 1142-50.
8. Gurel E, Tigen K, Karaahmet T, Gecmen C, Mutlu B, Basaran Y. Predictive value of plasma asymmetric dimethylarginine, homocysteine, and high-sensitive CRP levels in occult coronary artery disease: A multidetector-row computed tomography study. *Herz*, 2013.
9. Lu TM, Chung MY, Lin MW, Hsu CP, Lin SJ. Plasma asymmetric dimethylarginine predicts death and major adverse cardiovascular events in individuals referred for coronary angiography. *Int J Cardiol* 2011; 153: 135-40.
10. Kruszelnicka O, Surdacki A, Golay A. Differential associations of angiographic extent and severity of coronary artery disease with asymmetric dimethylarginine but not insulin resistance in non-diabetic men with stable angina: a cross-sectional study. *Cardiovasc Diabetol* 2013; 12: 145.
11. Alkan FA, Cakmak G, Karis D, Saglam ZA, Saler T, Temiz LU, et al. The evaluation of plasma viscosity and endothelial dysfunction in smoking individuals. *Clin Hemorheol Microcirc* 2014; 58: 403-13.
12. Wang J, Sim AS, Wang XL, Salonikas C, Naidoo D, Wilcken DE. Relations between plasma asymmetric dimethylarginine (ADMA) and risk factors for coronary disease. *Atherosclerosis* 2006; 184: 383-8.
13. Korandji C, Zeller M, Guillard JC, Vergely C, Sicard P, Duvillard L, et al. Asymmetric dimethylarginine (ADMA) and hyperhomocysteinemia in patients with acute myocardial infarction. *Clin Biochem* 2007; 40: 66-72.
14. Krzyzanowska K, Mittermayer F, Wolzt M, Scherthaner G. ADMA, cardiovascular disease and diabetes. *Diabetes Res Clin Pract* 2008; 82 Suppl 2: S122-6.
15. Pitocco D, Zaccardi F, Di Stasio E, Romitelli F, Martini F, Scaglione GL, et al. Role of asymmetric-dimethyl-L-arginine (ADMA) and nitrite/nitrate (NOx) in the pathogenesis of oxidative stress in female subjects with uncomplicated type 1 diabetes mellitus. *Diabetes Res Clin Pract* 2009; 86: 173-6.
16. Heilman K, Zilmer M, Zilmer K, Kool P, Tillmann V. Elevated plasma adiponectin and decreased plasma homocysteine and asymmetric dimethylarginine in children with type 1 diabetes. *Scand J Clin Lab Invest* 2009; 69: 85-91.
17. Fabian E, Bogner M, Elmadfa I. Age-related modification of antioxidant enzyme activities in relation to cardiovascular risk factors. *Eur J Clin Invest* 2012; 42: 42-8.
18. Paiva H, Laakso J, Ruokonen I, Luomala M, Saarela M, Solakivi T, et al. Plasma asymmetric dimethylarginine (ADMA), nitrate and the indices of low-density lipoprotein oxidation. *Clin Chim Acta* 2006; 371: 97-101.
19. Mamatha SN, Nagaraja D, Philip M, Christopher R. Asymmetric dimethylarginine as a risk marker for early-onset ischemic stroke in Indian population. *Clin Chim Acta* 2011; 412: 139-42.
20. Perticone F, Sciacqua A, Maio R, Perticone M, Maas R, Boger RH, et al. Asymmetric dimethylarginine, L-arginine, and endothelial dysfunction in essential hypertension. *J Am Coll Cardiol* 2005; 46: 518-23.
21. Mauricio MD, Aldasoro M, Ortega J, Vila JM. Endothelial dysfunction in morbid obesity. *Curr Pharm Des* 2013; 19: 5718-29.
22. Gruber HJ, Mayer C, Meinitzer A, Almer G, Horejsi R, Moller R, et al. Asymmetric dimethylarginine (ADMA) is tightly correlated with growth in juveniles without correlations to obesity related disorders. *Exp Clin Endocrinol Diabetes* 2008; 116: 520-4.
23. Rudofsky G, Roeder E, Merle T, Hildebrand M, Nawroth PP, Wolfrum C. Weight loss improves endothelial function independently of ADMA reduction in severe obesity. *Horm Metab Res* 2011; 43: 343-8.
24. Jiang DJ, Jiang JL, Zhu HQ, Tan GS, Liu SQ, Xu KP, et al. Demethylbellidifolin preserves endothelial function by reduction of the endogenous nitric oxide synthase inhibitor level. *J Ethnopharmacol* 2004; 93: 295-306.
25. Bai Y, Sun L, Du L, Zhang T, Xin W, Lan X, et al. Association of circulating levels of asymmetric dimethylarginine (ADMA) with carotid intima-media thickness: evidence from 6168 participants. *Ageing Res Rev* 2013; 12: 699-707.
26. Bestermann WH, Jr. The ADMA-Metformin Hypothesis: Linking the Cardiovascular Consequences of the Metabolic Syndrome and Type 2 Diabetes. *Cardiorenal Med* 2011; 1: 211-9.
27. Boger RH, Sullivan LM, Schwedhelm E, Wang TJ, Maas R, Benjamin EJ, et al. Plasma asymmetric dimethylarginine and incidence of cardiovascular disease and death in the community. *Circulation* 2009; 119: 1592-600.
28. Maas R, Boger RH. Old and new cardiovascular risk factors: from unresolved issues to new opportunities. *Atheroscler Suppl* 2003; 4: 5-17.
29. Surdacki A, Zawislak B, Rakowski T, Wiczorek-Surdacka E, Fedak D, Dubiel JS. Parental history of premature coronary artery disease does not affect plasma levels of asymmetric dimethylarginine in young healthy adults. *Pol Arch Med Wewn* 2012; 122: 487-93.

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