

# The relationship between L-arginine/ADMA ratio and coronary collateral development in patients with low glomerular filtration rate

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## Abstract

**Background:** *It is yet to be established which factors are responsible for differences among patients with the same degree of coronary artery disease in terms of coronary collateral development (CCD).*

**Methods:** *Patients who had a greater than or equal to 95% stenosis in at least one epicardial coronary artery were classified into two groups according to their glomerular filtration rate (GFR) level. Afterwards, the degree of CCD was evaluated according to their plasma concentration of asymmetric dimethylarginine (ADMA) and GFR levels.*

**Results:** *Rentrop grade 2–3 was found more frequently in patients with GFR > 60 mL/min than in patients with GFR < 60 mL/min (68.6% vs 41.4%,  $p = 0.032$ ). Then we divided patients into four groups according to their GFR levels and Rentrop grades; whereas we did not find any significant difference for L-arginine or ADMA levels (respectively  $p = 0.629$  and  $p = 0.076$ ), we did find a statistically significant difference between groups for L-arginine/ADMA ratio ( $p = 0.003$ ) and this statistically significant difference was evident between patients with GFR < 60 mL/min and Rentrop 0–1 and patients with GFR > 60 mL/min and Rentrop 2–3 (1.23 vs 1.69,  $p < 0.001$ ). Multivariate logistic regression analysis revealed that L-arginine/ADMA ratio was the only variable which had a significant effect on CCD (OR = 1.016; 95% CI 1.001–1.031, Wald = 4.565;  $p = 0.033$ ).*

**Conclusions:** *These results showed that CCD was poor in patients with GFR < 60 mL/min, presumably because of the adverse effect of decreased L-arginine/ADMA ratio on endothelial cells and angiogenesis. (Cardiol J 2011; 18, X: xx–xx)*

**Key words:** coronary collateral, glomerular filtration rate, plasma ADMA level

## Introduction

The prognosis and severity of coronary artery disease (CAD) is not uniform among patients with similar traditional risk factors. Therefore, condi-

tions other than traditional cardiovascular (CV) risk factors should always be kept in mind.

One of these is the presence of coronary collateral development (CCD). Sufficient collateral network limits infarct size, preserves myocardial

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function and improves the prognosis. Thus, patients become less symptomatic despite the presence of chronic total coronary occlusion. A negative correlation has been shown in studies examining the effect of CV risk factors on CCD [1, 2]. It is still uncertain why there are differences among patients in terms of developing coronary collaterals and which factors can affect this process.

Endothelium plays a crucial role in the maintenance of vascular structure, control of vascular tone, homeostasis, and inflammation. Also, endothelium plays a pivotal role in all steps of collateral development. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthesis (NOS) and competes with L-arginine for the active site of endothelial NOS. ADMA decreases the production and bioavailability of nitric oxide (NO), and thus decreases the vessel compliance, increases vascular resistance and limits the blood flow [3].

CCD may be influenced by plasma ADMA level and endothelial dysfunction resulting from impaired NOS. Although there are animal studies investigating the relationship between CCD and plasma ADMA level in the literature, few clinical studies have examined this relationship in humans and in patients with chronic kidney disease. CCD can be influenced by the existence of multiple potential anti-angiogenic factors and impaired angiogenesis in patients with chronic kidney disease.

In the current study, we aimed to investigate the relationship between glomerular filtration rate (GFR) and plasma ADMA level and CCD.

## Methods

### Study population

Between June 2008 and July 2009, patients in whom coronary angiography (CAG) was performed due to a high clinical suspicion of CAD, and a greater than or equal to 95% stenosis in at least one epicardial coronary artery, were enrolled in this study. Refusal to participate, acute myocardial infarction (AMI) in the past month, clinically unstable coronary syndrome, type 1 diabetes mellitus, uncontrolled hypertension (systolic blood pressure > 190 mm Hg), previous coronary artery bypass graft (CABG) operation, severe systemic inflammatory disease and hepatic failure were regarded as exclusion criteria. Seventy eight patients in all were assessed, of whom 17 were excluded due to the exclusion criteria (two patients because of acute myocardial infarction in the past month, two patients because of CABG surgery and 13 patients because

of the absence of significant CAD at CAG). The remaining 61 patients constituted the study population.

### Glomerular filtration rate

Patients were classified into two groups according to their estimated GFR values using the six variable modifications of diet in renal disease (MDRD) equation. Group 1 consisted of 29 patients with CAD and estimated GFR < 60 mL/min/1.73 m<sup>2</sup> and Group 2 consisted of 32 patients with CAD and estimated GFR > 60 mL/min/1.73 m<sup>2</sup>.

### Coronary angiography

Standard selective CAG with at least four views of the left coronary system and two views of the right coronary artery using the Judkins technique were performed. Coronary artery stenosis greater than or equal to 95% in at least one epicardial coronary artery was determined as the lesion which could make possible the development of a coronary collateral. CCD was graded according to the Rentrop classification [4]. Rentrop grade (RG) 0–1 was regarded as poor CCD, and RG 2–3 was regarded as good CCD.

### Measurement of plasma ADMA level

Before CAG, venous blood samples for ADMA measurement were collected into tubes which included EDTA as an anticoagulant. The collected blood samples were centrifuged for 10 min at 200 rpm to separate the plasma. All plasma samples were stored frozen at –80°C before use. Plasma concentrations of ADMA and L-arginine were measured by high-performance liquid chromatography (HPLC) using the technique of Chen et al. [5]. An Agilent 1100 series HPLC device and fluorescence detector and 150 × 4.6 mm THERMO Hypersil ODS 5 μm analytic column were used. Total analysis time was 40 min.

All the patients enrolled in the study gave written informed consent. This study was carried out in accordance with the Declaration of Helsinki and approved by the local Ethics Committee of our Institute.

### Statistical analysis

Data gained from patients was transferred to the computer. The error control and corrections were done. Data was tested graphically and statistically for normal distribution using Shapiro-Wilk analysis. All results for continuous variables with normal distribution were expressed as mean ± standard deviation (SD) and skewed variables as median (interquartile range — IQR). Categorical variables were defined as percentages. Differences

**Table 1.** Basal demographic and clinical features.

	Group 1 (n = 29)	Group 2 (n = 32)	P
	N (%) / mean $\pm$ SD / median (IQR)		
Age (years)	70.86 $\pm$ 9.41	67.90 $\pm$ 3.31	0.101
Male/female	18/11; 62.1%/37.9%	28/4; 87.5%/12.5%	0.021
Heart rate [bpm]	76.82 $\pm$ 16.27	74.46 $\pm$ 12.71	0.529
Systolic blood pressure [mm Hg]	131.96 $\pm$ 24.39	124.28 $\pm$ 20.16	0.184
Diastolic blood pressure [mm Hg]	70.65 $\pm$ 13.35	71.25 $\pm$ 12.82	0.860
Stable angina pectoris	22 (75.9%)	26 (81.3%)	0.608
Risk factors:			
Hypertension	21 (72.4%)	20 (62.5%)	0.414
Diabetes mellitus	9 (31%)	12 (37.5%)	0.596
Hyperlipidemia	5 (17.2%)	11 (34.4%)	0.129
Smoking	10 (34.5%)	18 (56.3%)	0.088
Previous CAD history	19 (65.5%)	21 (65.6%)	0.993
Family history	12 (41.4%)	15 (46.9%)	0.666
Chronic kidney disease	11 (37.9%)	0 (0%)	0.001
Laboratory findings:			
Fast blood glucose [mg/dL]	97 (40%)	104.50 (33.8%)	0.222
Total cholesterol [mg/dL]	198.24 $\pm$ 52.21	178.46 $\pm$ 40.41	0.102
LDL-cholesterol [mg/dL]	124.00 $\pm$ 41.35	103.96 $\pm$ 35.92	0.047
HDL-cholesterol [mg/dL]	38 (14%)	41 (14.5%)	0.478
Triglycerides [mg/dL]	128.00 (136.5%)	156.00 (107.8%)	0.729
Glomerular filtration rate [mL/min]	37.32 $\pm$ 16.35	83.66 $\pm$ 16.37	< 0.001
Ejection fraction (%)	45 (16.3%)	54 (18%)	0.125

CAD — coronary artery disease; IQR — interquartile range; SD — standard deviation

between categorical variables (e.g. group, sex, hypertension, diabetes) were analyzed using  $\chi^2$  test and  $\chi^2$  likelihood ratio. Students' t test was used to compare the continuous variables with normal distribution, and Mann-Whitney U test was used for skewed variables between two groups. Spearman's and Pearson's correlation coefficients were used for correlation analysis. A linear regression analysis model was used to explain the parameters which may effect the plasma ADMA level. Backward stepwise multivariate logistic regression model was performed to determine the effect of independent risk factors for coronary collateral development. A  $p \leq 0.05$  was considered statistically significant. MS-Excel and SPSS 15.0 Statistical Package Program for Windows (SPSS Inc., Chicago, IL, USA) were used for all statistical analysis and calculations.

## Results

A total of 61 patients (15 women and 46 men) aged 41–88 years (mean age  $69.31 \pm 7.01$  years) were enrolled in this study. The mean age of pa-

tients was  $70.86 \pm 9.41$  years in Group 1 and  $67.90 \pm 3.31$  years in Group 2 ( $p = 0.101$ ). There was no statistically significant difference for basal demographic and clinical features between the two groups, except gender ( $p = 0.021$ ). Whereas there was no diagnosis of CKD in Group 2, 11 (37.9%) of 29 patients had CKD in Group 1 ( $p < 0.001$ ). The mean estimated GFR value was  $37.32 \pm 16.35$  mL/min for Group 1 and  $83.67 \pm 16.37$  mL/min for Group 2 ( $p < 0.001$ ). LDL-cholesterol levels were higher in Group 1 than in Group 2 (respectively,  $124.00 \pm 41.35$  mg/dL vs  $103.96 \pm 35.93$  mg/dL,  $p = 0.047$ ; Table 1).

The severity of CAD was similar between the two groups. There was no statistically significant difference in the number of disease vessels, number of total occluded vessels or type of occluded vessel between the two groups ( $p > 0.05$  for all; Table 2). The relationship between GFR levels and RG was evaluated by  $\chi^2$  test. A good coronary collateral development (RG 2–3) was present in 41.4% of patients with  $GFR < 60$  mL/min/1.73 m<sup>2</sup> and in 68.8% of patients with  $GFR > 60$  mL/min/1.73 m<sup>2</sup> ( $p = 0.032$ ).

**Table 2.** Coronary angiography findings.

	Group 1 (n = 29)	Group 2 (n = 32)	P
Number of diseased vessels — median (IQR)	3.00 (IQR = 0.00)	3.00 (IQR = 0.00)	0.051
Number of total occluded vessels			
0	3 (10.3%)	1 (3.1%)	0.132
1	12 (41.4%)	21 (65.6%)	
2	14 (48.3%)	10 (31.3%)	

IQR — interquartile range

**Table 3.** Relationship between glomerular filtration rate and plasma asymmetric dimethylarginine (ADMA) levels.

	Group 1 (n = 29)	Group 2 (n = 32)	P
	Mean ± SD/median (IQR)		
L-arginine [mmol/L]	17.74 ± 6.96	17.59 ± 7.54	0.935
ADMA [mmol/L]	14.97 (11.1)	10.03 (9.8)	0.043
L-arginine/ADMA ratio	1.23 (0.7)	1.64 (0.9)	0.008

IQR — interquartile range; SD — standard deviation

**Table 4.** Relationship between Rentrop grades and plasma asymmetric dimethylarginine (ADMA) levels.

	RG 0–1 (n = 27)	RG 2–3 (n = 34)	P
	Mean ± SD/median (IQR)		
L-arginine [mmol/L]	16.56 ± 7.03	18.54 ± 7.33	0.291
ADMA [mmol/L]	13.01 (11.9)	12.01 (9.9)	0.089
L-arginine/ADMA ratio	1.23 (0.7)	1.68 (0.8)	0.001

RG — Rentrop grade; IQR — interquartile range; SD — standard deviation

There was no statistically significant difference between patients with GFR < 60 mL/min/1.73 m<sup>2</sup> and patients with GFR > 60 mL/min/1.73 m<sup>2</sup> with regard to L-arginine levels. However, the difference was statistically significant for plasma ADMA levels and L-arginine/ADMA ratio (respectively, p = 0.043 and p = 0.008; Table 3). Higher plasma ADMA level and lower L-arginine/ADMA ratio were found in patients with GFR < 60 mL/min/1.73 m<sup>2</sup> than those of GFR > 60 mL/min/1.73 m<sup>2</sup>.

In linear regression analysis, GFR and the number of diseased vessels were found to be factors responsible for the increase of plasma ADMA level (respectively, beta = -0.336; p = 0.010 and beta = 1.182; p < 0.001). When all patients enrolled in the study were evaluated together, we found a significant negative correlation between plasma ADMA level and GFR level (r = -0.405, p = 0.001).

We compared the L-arginine level, ADMA level and L-arginine/ADMA ratio between the good co-

ronary collateral group (RG 2–3) and the poor collateral group (RG 0–1). Whereas there was no statistically significant difference in plasma L-arginine or ADMA level, a statistically significant difference for L-arginine/ADMA ratio was found (p = 0.001; Table 4).

After we categorized the patients according to their GFRs and RGs into four groups, we evaluated L-arginine (with One-Way ANOVA test) level, plasma ADMA level and L-arginine/ADMA ratio (with Kruskal Wallis test) between the groups. We did not find a significant difference for L-arginine or ADMA concentration (respectively, p = 0.629 and p = 0.076). However, we found a statistically significant difference for L-arginine/ADMA ratio (p = 0.003) between groups, and this statistically significant difference was prominent between patients with GFR < 60 mL/min/1.73 m<sup>2</sup> and RG 0–1 and patients with GFR > 60 mL/min/1.73 m<sup>2</sup> and RG 2–3 (1.23 vs 1.69, p < 0.001; Table 5).

**Table 5.** L-arginine and ADMA levels of patients according to their GFR levels and Rentrop grades.

	L-arginine [ $\mu\text{mol/L}$ ] Mean $\pm$ SD	ADMA [ $\mu\text{mol/L}$ ] Median (IQR)	L-arginine/ADMA ratio Median (IQR)
GFR < 60 mL/min/1.73 m <sup>2</sup> , RG 0–1	16.28 $\pm$ 6.04	13.01 (11.0)	1.23 (0.7)
GFR > 60 mL/min/1.73 m <sup>2</sup> , RG 0–1	17.03 $\pm$ 8.81	14.83 (19.1)	1.20 (1.0)
GFR < 60 mL/min/1.73 m <sup>2</sup> , RG 2–3	19.81 $\pm$ 7.88	16.04 (11.5)	1.30 (1.0)
GFR > 60 mL/min/1.73 m <sup>2</sup> , RG 2–3	17.84 $\pm$ 7.10	8.55 (8.1)	1.69 (0.7)

ADMA — asymmetric dimethylarginine; GFR — glomerular filtration rate; RG — Rentrop grade; IQR — interquartile range; SD — standard deviation

Backward stepwise multivariate logistic regression analysis including GFR, diabetes mellitus, hypertension, LDL-cholesterol, smoking, fasting blood glucose, ADMA and L-arginine/ADMA ratio was performed in order to elucidate the CCD, and revealed that L-arginine/ADMA ratio (OR = 1.016, 95% CI 1.001–1.031) was the only independent variable which had a significant effect on CCD (Wald = 4.565;  $p = 0.033$ ).

## Discussion

In this study, we found that CCD was better in patients with GFR > 60 mL/min/1.73 m<sup>2</sup> and occlusive CAD compared to those with GFR < 60 mL/min/1.73 m<sup>2</sup> and occlusive CAD. L-arginine/ADMA ratio might have an effect on CCD in those patients.

CCD has a pivotal role in the continuation of myocardial viability. Coronary collateral vessels protect the myocardium by limiting the border of myocardial ischemia in case of coronary occlusion, and symptoms of CAD appear less in the presence of sufficient coronary blood flow [6]. Pressure gradient and growth factors play an important role in CCD [7]. At the same time, there is a discrepancy among patients with occlusive CAD in view of CCD, and this suggests that a lot of other factors may have an effect on CCD.

Studies looking at the effect of CV risk factor on CCD have shown that CV risk factors associated with endothelial dysfunction such as diabetes mellitus, hypertension, and hyperlipidemia had an adverse effect on CCD [1, 8–10]. There was no statistically significant difference with regard to these risk factors between the two groups in our study. Also, there was no statistically significant difference in terms of frequency or duration of stable angina pectoris, which has an effect on the development of coronary collateral [11], between the two groups in our study ( $p = 0.608$ ).

Angiogenic adaptation (formation of new vessels) and arteriogenic adaptation (maturation of these new vessels) are pivotal steps towards CCD.

The stimulation of angiogenesis starts as a physiologic response to ischemia and hypoxia, and blood flow increases to ischemic tissue [12]. Endothelial cells are an important influence on the development and maturation of collateral vessels [7].

The results of recent experimental studies have shown that NO plays a critical role in the maintenance of normal endothelial function, angiogenesis and arteriogenesis [13]. NO deficiency and endothelial dysfunction due to the existence of multiple potential anti-angiogenic factors in patients with renal failure has been found to impair the microvascular adaptation and ischemic tolerance of tissues [9]. Few studies have examined the effect of low GFR on CCD. In this study, we pointed out that CCD was impaired by a decrease in GFR. The poor CCD in patients with renal failure can be explained by this impaired ischemic tolerance.

Plasma ADMA level is increased in patients with CAD compared to healthy subjects, and this increase predicts all-cause and CV mortality [14]. In CARDIAC study, plasma ADMA concentration was found to be 20% higher in those with CV disease, and was increased significantly by several traditional risk factors [15]. ADMA is mainly eliminated by renal excretion and begins to accumulate when renal excretion is impaired. Despite the 2- to 3-fold elevation of ADMA plasma concentration in many diseases, plasma concentrations of ADMA are ~ten-fold higher in patients with end stage renal disease. The accumulation of ADMA inhibits NO production. Increased plasma ADMA level (thus a decrease of NO) is associated with oxidative stress and endothelial dysfunction, and impairs CCD in patients with renal failure [16–18].

In literature, a few studies have revealed that there is a relationship between plasma ADMA level and CCD, and CCD is poor in those with increased plasma ADMA level. Similarly, a very few studies have indicated the relationship between impaired renal function and CCD. Our study is the first to investigate the effect of plasma ADMA concentration measured according to GFRs on CCD.

ADMA competes with L-arginine for NOS and L-arginine level is measured rarely. However, the L-arginine/ADMA ratio is more important for NOS function than ADMA alone. When we examined the L-arginine/ADMA ratio of the patients enrolled in our study, we observed that the L-arginine/ADMA ratio was higher in patients with GFR > 60 mL/min/1.73 m<sup>2</sup> and good CCD than in patients with GFR < 60 mL/min/1.73 m<sup>2</sup> and poor CCD (p < 0.001). Furthermore, L-arginine/ADMA ratio was found to be the only independent variable which had a significant effect on CCD in multivariate logistic regression analysis (p = 0.033).

Therapeutic strategies for increasing L-arginine/ADMA ratio may improve CCD. The pharmacological arrangement of plasma ADMA concentration is an interesting area. The effect of several pharmacological agents on ADMA metabolism has been investigated in order to increase CCD, also called 'natural bypass', especially in patients with diffuse and severe CAD who are not good candidates for revascularization (angioplasty or CABG) [19–25]. Theoretically, arginine should be able to displace ADMA and restore NOS activity. Exogenous ADMA competes with ADMA, blocks the inhibition of NO production through ADMA, and also acts as an antioxidant and eliminates the superoxide [16]. Arginine has been reported to improve endothelial function [26] and increase walking distance [27]. Plasma arginine concentration can be increased [28] or decreased [29] by pharmacotherapy.

### Limitations of the study

This study has a number of limitations. The small number of patients is the major limitation, and might disallow the determination of risk factors affecting coronary collateral development. The other limitation of this study is the evaluation of CCD by CAG. As is well-known, angiography only visualizes collateral flow in vessels greater than 100 μm in diameter, and vessels smaller than 100 μm cannot be detected [30]. This might cause an underestimation of the degree of CCD and there might be a collateral vessel in patients considered to have no CCD at CAG. Also, only plasma concentrations of ADMA were measured, and data about the cellular concentration of ADMA, which might be more associated with NOS function, is inadequate.

### Conclusions

Our findings suggest that the L-arginine/ADMA ratio seems to be a more important determinant of NOS function than ADMA alone. Presum-

ably because of the adverse effect of decreased L-arginine/ADMA ratio (relatively increased plasma ADMA level) on endothelial cells and angiogenesis, CCD is worse in patients with GFR < 60 mL/min/1.73 m<sup>2</sup> than in those with GFR < 60 mL/min/1.73 m<sup>2</sup>.

Hence, L-arginine/ADMA ratio may be a novel risk marker for predicting CCD in patients with GFR < 60 mL/min/1.73 m<sup>2</sup>. Therapeutic strategies to increase arginine concentrations or decrease ADMA concentrations may positively influence the endothelium and angiogenesis by improving NOS function, and may contribute to CCD (also called 'biological bypass'), especially in patients not considered to be good candidates for revascularization.

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