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Assessment of serum levels of asymmetric dimethylarginine, symmetric dimethylarginine and L-arginine in coronary artery disease

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Abstract

Serum asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), L-arginine, and C-reactive protein (hsCRP) levels were assessed in 100 Egyptian male 35–50-year-old patients with coronary artery disease (CAD), classified into: patients under conservative medical treatment, patients directed for percutaneous coronary interventions, patients directed for coronary artery bypass graft operation and patients suffering from acute myocardial infarction. Age- and sex-matched controls ($n = 100$) were included. Correlation between serum levels of biomarkers and dimethylarginine dimethylaminohydrolase-2 (DDAH-2) genotypes was studied. No association between biomarkers and carriage of the specific *DDAH2* SNP2 (-449C/G, rs805305) genotype was detected. Further studies are required to confirm the contribution of the biomarkers in the predisposition of CAD.

Keywords: *ADMA; L-arginine; coronary artery disease; DDAH2 gene polymorphism; Egyptians; SDMA*

Introduction

Coronary artery disease (CAD) remains one of the leading causes of death all over the world. In the early 1990s, an endogenous inhibitor of the nitric oxide synthase (NOS) pathway was identified, namely asymmetric dimethylarginine (ADMA) (Vallance et al. 1992a). Ever since, its role in regulating NO production has attracted increasing attention. Elevated levels of ADMA capable of inhibiting endothelial nitric oxide synthase (eNOS) have been found in several disorders including CAD (Boger 2004). Currently, ADMA is regarded as a novel cardiovascular risk factor.

ADMA is one among three methylarginines physiologically found in all human tissues and biological fluids. The other two are *N*-monomethylarginine (l-NMMA) and symmetric dimethylarginine (SDMA). Methylarginines are generated by the post-translational methylation of arginine residues in proteins. Following proteolysis, free methylarginines are released into the cytosol where they accumulate before being removed to the plasma and cleared into the urine by the kidney (Tran et al. 2003). MacAllister et al. (1996) demonstrated a modest (threefold) but definite increase in plasma ADMA concentration in uremic patients compared with healthy controls. SDMA accumulates to a greater degree (eightfold increase) and more closely parallels creatinine concentration than ADMA.

ADMA is degraded mainly by an intracellular enzyme termed dimethylarginine dimethylaminohydrolase (DDAH), after uptake from the circulation. DDAH degrades ADMA to citrulline and dimethylamine (Dayoub et al. 2003). Two isoforms of DDAH, with different tissue distributions, have been identified, *DDAH1* and *DDAH2*, which regulate to a great extent the levels of ADMA in blood and tissues (Leiper et al. 1999).

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DDAH1 and *DDAH2* are located on chromosomes 1p22 and 6p21.3, respectively (Tran et al. 2000). From gene-silencing studies in rats, it is apparent that *DDAH1* plays an important role in regulating serum ADMA levels, whereas *DDAH2* appears to control NO-mediated functions of the endothelium (Wang et al. 2007). The *DDAH2* isoform predominates in tissues expressing eNOS, such as the endothelium (Jones & Hingorani 2005). Thus, *DDAH2*, through catabolism of ADMA, regulates the activity of eNOS. eNOS synthesizes NO from the terminal guanidino nitrogen of L-arginine (Forstermann et al. 1994). In contrast to ADMA, SDMA does not act as an inhibitor of NO synthase (Vallance et al. 1992b). Despite the fact that several factors affect the amount of ADMA in tissues, and consequently in blood, including oxidative stress, hypercholesterolemia, renal function and DDAH activity, evidence has emerged that ADMA might be a novel cardiovascular risk factor (Boger 2003).

Few studies have yet focused on the possibility that DDAH gene polymorphisms may contribute to the inheritable risk for CAD in humans. None have identified specific differences among ethnic groups. In 2003, Vallance's group found a functional insertion/deletion polymorphism in the DDAH-2 enzyme among unrelated individuals (Jones et al. 2003). The relationship of *DDAH2* polymorphism to disease was addressed in another report which concluded that the severity of organ failure, inflammation and presence of early shock in severe sepsis are associated with increased ADMA levels, which may be influenced by a polymorphism in the *DDAH2* gene (O'Dwyer et al. 2006). Recently, Maas et al. (2009) have indicated that -449 G/C (SNP2) polymorphisms in the *DDAH2* promoter region are not related to serum ADMA levels or measures of cardiac structure and function but are associated with an increased prevalence of hypertension.

This study examines the association of serum ADMA, SDMA and L-arginine with the incidence and severity of CAD in young Egyptians, in addition to testing the correlation of these biomarkers with *DDAH2* SNP2 genotypes.

Methods

Study population

One hundred random, unrelated male patients with CAD, aged 35–50 years, were recruited for the study from in- and outpatient settings of the National Heart Institute (NHI), Imbaba, Cairo, Egypt. Patients were included if they had a diagnosis of single or multivessel CAD verified by history of myocardial infarction, percutaneous coronary interventions (PCI) or coronary angiography. Age- and sex-matched volunteer controls ($n = 100$) were included from the general population if they had no clinical or diagnostic evidence for CAD and having controlled blood pressure below 140/90 mmHg. Exclusion criteria for both patients and controls included any concomitant acute or chronic severe diseases such as renal failure, hepatic insufficiency, severe chronic heart failure, diabetes mellitus and age >50 years.

CAD patients were further subclassified according to severity of coronary insufficiency, as verified by coronary angiography into the following groups: (1) patients under conservative medical treatment (Med, $n = 12$); (2) patients directed for PCI (PCI, $n = 41$); (3) patients advised to have a coronary artery bypass graft operation (CABG, $n = 36$); and (4) patients suffering from acute myocardial infarction (AMI, $n = 11$). An additional control group of subjects who were admitted to NHI after complaints of chest pain but after inspection were found to have normal coronary angiography was also included in the study (Normal angio, $n = 12$). All recruited subjects gave written informed consent that complied with the principles of the Helsinki declaration. The study protocol was approved by the local ethics committee.

Blood sampling

Blood samples were collected, allowed to clot at room temperature for 30 min and centrifuged at 2500 rpm for 10 min at 4°C. The serum was kept frozen at –80°C until analysis.

Analysis of L-arginine, ADMA and SDMA

L-Arginine, ADMA and SDMA were measured by a liquid chromatography/mass spectroscopy (LC/MS) method (Shwedhelm et al. 2005, 2007) as described below.

Sample preparation

For protein precipitation, 96-well 0.20-μm microfiltration plates were precoated with 800 pmol of L-[$^2H_7$]-arginine (reference standard) and 40 pmol of [$^2H_6$]-ADMA (internal standard) dissolved in 20 μl of acetone–water (50:50, v/v), corresponding to a concentration of 40 and 2 μM, respectively. The microfiltration plates were then loaded with 100 μl of methanol and 25 μl of serum in each well. Proteins were precipitated by shaking the microfiltration plates on top of multiple-polymerase chain reaction (PCR) polypropylene plates for 5 min using an orbital shaker (Heidolph, Schwabach, Germany). To separate analytes from precipitated proteins, the combined plates were centrifuged for 15 min at 2000 rpm (Eppendorf, Hamburg, Germany). Then, the two plates were separated and the methanol was evaporated by heating in an incubator at 70°C for 60 min. Three calibrators were used; L-arginine, ADMA and SDMA at six different aqueous concentrations: 0, 25,

50, 100, 250 and 500 µmol l^{-1} for L-arginine and 0, 0.25, 0.5, 1, 2 and 4 µmol l^{-1} for ADMA and SDMA.

Derivatization

All compounds were analysed as their butyl ester derivatives. Derivatization was performed in 96-well polypropylene plates. After addition of 100 µl of 1 M HCl in 1-butanol, plates were sealed with the sealing mat. The plates were allowed to stand for 30 min at 65°C. The sealing mat was removed cautiously and the derivatization reagent was evaporated by putting the open plate under the hood for 60–90 min until dry. Samples were then reconstituted in 100 µl of HPLC solvent (50 ml H_20, 50 ml methanol, 250 µl 25% ammonia and 100 µl formic acid). The pH was adjusted to 5 with formic acid. The plates were put on a shaker for 30 min. Before starting HPLC, the solution in each well was transferred to another 96-well polypropylene plate and centrifuged for 5 min at 2000 rpm. Afterwards, the polypropylene plates were transferred to a Prostar Autosampler (Varian, Palo Alto, CA, USA), and 10-µl aliquots were injected onto the chromatographic column from each sample.

Liquid chromatography–tandem mass spectrometry

Analyses were performed on a Varian 1200L Triple Quadruple MS equipped with two Varian ProStar model 210 HPLC pumps and a Varian analytical column (50 × 2.0 mm) packed with Polaris C18-Ether (3 µM bead size). The mobile phase consisted of methanol/formic acid 1000:1 (mobile phase A) and water/formic acid 1000:1 (mobile phase B). Chromatography was performed at 25°C with a flow rate of 0.4 ml min^{-1}. The gradient started with 2% A for 0.5 min and increased linearly to 50% A over 1.5 min, with subsequent re-equilibration with 2% A for 2 min. Nitrogen was used as the nebulising and drying gas (380°C) at 90 and 180 l h^{-1}, respectively. For positive electrospray ionisation (ESI) the needle and shield voltage were set at 5600 and 400 V, respectively. The following transitions were used for quantitative analyses in the multiple reaction monitoring (MRM) mode after fragmentation with argon (2 Pa): *m/z* 231–70 for L-arginine; *m/z* 238–77 for L-[$^2H_7$]-arginine; *m/z* 259–214 for ADMA; *m/z* 259–228 for SDMA; and *m/z* 265–220 for [$^2H_6$]-ADMA. Dwell time was set to 190 ms for each ion; interscan time was 20 ms in the MRM mode. Thus, a total scan cycle time of 0.97 s resulted (Figure 1).

Analysis of other biochemical parameters

Commercially available kits for serum cholesterol and creatinine (BioMed Diagnostics, Singapore) and serum hsCRP (ELISA kit; DRG instruments GmbH, Marburg, Germany) were used.

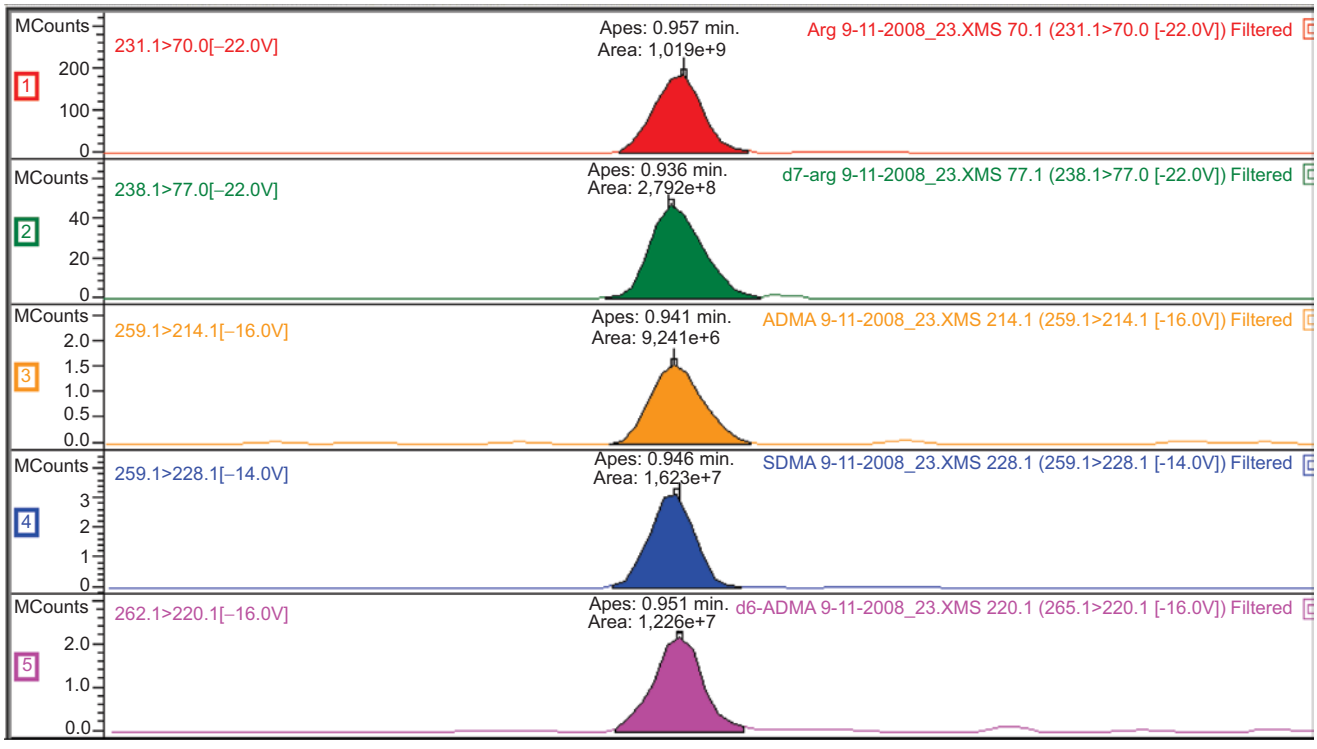

Figure 1. Chromatogram from the analysis of the human serum sample (50 µl) to which 50 µmol l^{-1} L-[$^2H_7$]-arginine and 2 µmol l^{-1} [$^2H_6$]-ADMA have been added. Trace 1 (top), L-arginine; trace 2, L-[$^2H_7$]-arginine; trace 3, ADMA; trace 4, SDMA; trace 5 (bottom), [$^2H_6$]-ADMA. ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine.

DDAH2 genotyping

Genomic DNA was extracted from whole blood samples using QiAmp DNA minikit (Qiagen, Hilden, Germany). DNA was checked for concentration and purity by measuring the absorbance at wavelengths 260 and 280 nm using the NanoDrop® ND-1000 Spectrophotometer. Genotyping was performed using ABI Prism Sequence Detection System (Real-Time qPCR System). For PCR amplification, in a 384-well plate, 3 µl of genomic DNA was added to 5 µl of SNP reaction mixture (Applied Biosystems P/N 4371355) made up of 2.5 µl of TaqMan Universal PCR Master Mix, 0.125 µl of 40x working stock of SNP2 (-449 C/G, rs805305, assay ID: C___3233673_10), and 2.375 µl DNase-free water. The initial denaturation temperature for AmpliTaq Gold Enzyme Activation was 95°C for 10 min. The settings included 40 cycles of denaturation at 92°C for 15 s and annealing/extension temperature at 60° C for 1 min. Allelic discrimination assays were performed using two TaqMan MGB probes (FAM/VIC dye) that target the SNP sites (Afonina et al. 1997, Kutyavin et al. 1997). Genotyping data in this study are presented according to the HapMap notation and NCBI SNP cluster reports.

Statistical analysis

Statistical analyses were performed using the STATISTICA (data analysis software system, www.statsoft.com) statistical package (StatSoft Inc., 2007), version 8.0. The two-sided *p*-values for odds ratios were calculated using the Fisher's exact test, and confidence intervals were calculated using the approximation of Woolf. Means of biomarker levels were compared using Student's *t*-test. Due to small number of patients in the acute group, normality of values was assessed using quantile–quantile plots.

Results

Biochemical analysis of CAD patients vs control

Table 1 reveals significant differences between CAD patients and controls in all biochemical parameters except ADMA, L-arginine and the ADMA/L-arginine ratio. Levels of SDMA, hsCRP and creatinine were higher in CAD patients reaching 113%, 390% and 120%, respectively, of control levels. In contrast, the mean cholesterol level was lower by 23%.

Biochemical analysis of the individual CAD groups

Serum levels of biomarkers in individual groups are displayed in Table 2. Subjects in the Normal angio group had results comparable to the controls. The only significant difference was in ADMA and cholesterol levels. None of the results of CAD patients under medical treatment were significantly different from controls. A specific pattern that copes with the severity of CAD can be seen when comparing the results of the less severe PCI to the more severe CABG and most severe AMI patients. A gradual increase in ADMA (from 0.57 for PCI – through 0.60 for CABG – to 0.75 for AMI), and in SDMA (0.43 for PCI – 0.56 for CABG – 0.85 for AMI), a decrease in L-arginine (102.0 for PCI – 101.2 for CABG – 64.3 for AMI), an increase in creatinine (1.78 for PCI – 1.92 for CABG – 2.1 for AMI), and an increase in hsCRP (11.5 for PCI – 36.1 for CABG – 39.6 for AMI) can be recognized in the Table. However, the limited number of patients and variability of the data affected the statistical significance of some biomarkers. One noticeable observation is that levels of cholesterol in all CAD groups were lower than those of controls. Most patients were under dietary and/or therapeutic regimens after verification of CAD.

Table 1. Serum levels of biochemical parameters in patients with coronary artery disease and controls.

	Controls ($n=100$)	Patients ($n=100$)	*p*-Value
ADMA (µmol l^{-1})	0.65 ± 0.018	0.61 ± 0.016	0.098
SDMA (µmol l^{-1})	0.47 ± 0.010	0.53 ± 0.026	**0.033**
L-Arginine (µmol l^{-1})	87.4 ± 3.95	97.3 ± 4.31	0.098
Arginine/ADMA	136.5 ± 6.90	156.8 ± 8.2	0.06
hsCRP (mg l^{-1})	6.0 ± 1.06	23.4 ± 2.87	$\mathbf{8\times10^{-7}}$
Cholesterol (mg dl^{-1})	238.6 ± 9.63	185.4 ± 6.02	$\mathbf{6\times10^{-5}}$
Creatinine (mg dl^{-1})	1.57 ± 0.059	1.89 ± 0.127	**0.024**

Results are expressed as means ± SEM; *p*-values ≤0.05 are in bold. ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; hsCRP, C-reactive protein.

Comparative analysis of biochemical parameters in chronic vs acute patients

The results of the Med, PCI and CABG groups were combined into a collective chronic patient group, and compared with the acute patients in the AMI group as illustrated in Table 3. The comparison reveal that acute patients showed higher serum levels of ADMA ($p=0.011$), SDMA ($p=0.037$) and hsCRP ($p=0.04$), and lower serum L-arginine ($p=0.008$) and L-arginine/ADMA ratio ($p=0.006$) than the chronic patients.

Association between genotypes and biochemical markers

No significant correlation was perceived between the serum levels of studied biomarkers and carriage of the specific *DDAH2* SNP2 genotype, except for ADMA levels in the AA/GG group (Table 4). A trend of higher, but not significant, SDMA, creatinine and hsCRP and lower

L-arginine and L-arginine/ADMA ratio was observed in the AA/GG group compared with the other two genotypes.

Discussion

The inter-relationship and regulatory mechanisms that control DDAH activity and ADMA, SDMA, L-arginine and NO levels are quite complex. ADMA is a potent inhibitor of eNOS (Vallance et al. 1992b). eNOS co-localizes with DDAH-2 in the cytosol of endothelial cells of blood vessels. This supports the hypothesis that DDAH-2 may regulate NOS activity by controlling the metabolism of ADMA (Tran et al. 2000). Overexpression of *DDAH2*, but not its isoform *DDAH1*, reverses the impaired NO production of endothelial cells exposed to glycated haemoglobin (Lu et al. 2007). This observation indicates that *DDAH2* is the predominant isoform expressed in blood vessels and endothelium. Studies by Birdsey et al. (2000) and Murray-Rust et al. (2001) established that DDAH metabolizes ADMA intracellularly, whereas SDMA is not a substrate for DDAH. Thus serum ADMA will be dependent primarily on factors that affect DDAH expression and activity, whereas serum SDMA will depend on the rate of renal excretion (Palm et al. 2007).

The discovery of a functional polymorphism within the *DDAH2* gene that might promote individual differences in the ability to metabolise ADMA *in vivo*, and in turn, underlie susceptibility to CAD has been previously addressed by Jones et al. (2003). In this study, the researchers identified several *DDAH2* gene polymorphisms, one of them is the subject of our study: SNP2 (-499 C/G, rs805305) localized within intron 2 of the gene. In another study, O'Dwyer et al. (2006) observed that carriage of a G allele at position -449 in the promoter region of the *DDAH2* gene is associated with increased ADMA levels, which suggests that the *DDAH2* gene expression with a G allele at this position is lower than that with a C. In fact, most studies on DDAH polymorphisms focused on two sites: SNP2 and SNP1 (-1151 C/A, rs805304) polymorphisms. We did find in a separate study in our lab that the allele frequency and genotype distribution for the two sites are exactly the same for both normal and CAD patients (in press). Therefore, we used here the data for SNP2 only.

In the present study, we did not find a significant difference between serum levels of ADMA in controls and CAD patients. This might be attributed to inherent variability in serum ADMA among the different study groups, in addition to the elevated levels of ADMA in controls relative to those reported in the literature (Meinitzer et al. 2007) and in a previous study from our lab (El-Mesallamy et al. 2008). A similar finding was reported by Wang et al. (2005) who did not find a significant difference in plasma

Table 3. Serum levels of biochemical parameters in chronic (Med + PCI + CABG) vs acute (AMI) patients.

	Chronic ($n=89$)	Acute ($n=11$)	p-Value
ADMA (μmol l^{-1})	0.59 ± 0.016	0.75 ± 0.051	**0.011**
SDMA (μmol l^{-1})	0.5 ± 0.018	0.85 ± 0.145	**0.037**
L-Arginine (μmol l^{-1})	100.6 ± 4.52	64.3 ± 10.7	**0.008**
Arginine/ADMA	178.7 ± 8.56	104.1 ± 21.1	**0.006**
hsCRP (mg l^{-1})	22.3 ± 3.06	39.6 ± 7.0	**0.04**
Cholesterol (mg dl^{-1})	188.4 ± 6.52	160.8 ± 13.0	0.077
Creatinine (mg dl^{-1})	1.86 ± 0.14	2.1 ± 0.238	0.4

Results are expressed as means ± SEM; p-values ≤0.05 are in bold. Med, conservative medical treatment; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; AMI, acute myocardial infarction; ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; hsCRP, C-reactive protein.

Table 2. Serum levels of biochemical parameters in individual coronary artery disease groups and controls.

	Controls ($n=100$)	Normal angio ($n=12$)	Med ($n=12$)	PCI ($n=41$)	CABG ($n=36$)	AMI ($n=11$)
ADMA (μmol l^{-1})	0.65 ± 0.018	0.59 ± 0.003 (**$p=0.0013$**)	0.6 ± 0.064 ($p=0.46$)	0.57 ± 0.019 (**$p=0.0026$**)	0.6 ± 0.027 ($p=0.124$)	0.75 ± 0.051 ($p=0.09$)
SDMA (μmol l^{-1})	0.47 ± 0.010	0.46 ± 0.02 ($p=0.64$)	0.48 ± 0.052 ($p=0.85$)	0.43 ± 0.017 (**$p=0.048$**)	0.56 ± 0.033 (**$p=0.013$**)	0.85 ± 0.18 ($p=0.062$)
L-Arginine (μmol l^{-1})	87.4 ± 3.95	71.4 ± 7.43 ($p=0.074$)	95.2 ± 13.9 ($p=0.60$)	102.0 ± 6.28 ($p=0.053$)	101.2 ± 0.167 ($p=0.10$)	64.3 ± 10.7 ($p=0.065$)
Arginine/ADMA	136.5 ± 6.90	128.2 ± 16.2 ($p=0.23$)	164.2 ± 23.2 ($p=0.27$)	181.5 ± 11.2 (**$p=0.001$**)	181.0 ± 15.0 (**$p=0.0096$**)	104.1 ± 21.1 ($p=0.17$)
hsCRP (mg l^{-1})	6.0 ± 1.06	12.6 ± 7.63 ($p=0.40$)	15.1 ± 8.50 ($p=0.30$)	11.5 ± 2.89 ($p=0.08$)	36.1 ± 5.47 (**$p=4\text{x}10^{-6}$**)	39.6 ± 6.99 (**$p=7\text{x}10^{-4}$**)
Cholesterol (mg dl^{-1})	238.6 ± 9.63	182.5 ± 12.1 (**$p=0.001$**)	199.7 ± 19.0 ($p=0.086$)	246.3 ± 8.38 ($p=0.54$)	182.8 ± 11.5 (**$p=4\times10^{-4}$**)	160.8 ± 13.0 (**$p=7\times10^{-5}$**)
Creatinine (mg dl^{-1})	1.57 ± 0.059	2.64 ± 0.53 ($p=0.068$)	1.9 ± 0.46 ($p=0.49$)	1.78 ± 0.21 ($p=0.34$)	1.92 ± 0.203 ($p=0.10$)	2.1 ± 0.238 ($p=0.054$)

Results are expressed as means ± SEM; p-values ≤0.05 are in bold. Bold numbers indicate significant difference from healthy controls. Normal angio, normal coronary angiogram; Med, conservative medical treatment; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; AMI, acute myocardial infarction; ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; hsCRP, C-reactive protein

Table 4. Biochemical parameters according to *DDAH2* SNP1 and SNP2 genotypes (all subjects in study).

SNP2	ADMA (μmol l^{-1})	SDMA (μmol l^{-1})	L-Arginine (μmol l^{-1})	Arginine/ADMA	hsCRP (mg l^{-1})	Cholesterol (mg dl^{-1})	Creatinine (mg dl^{-1})
CC ($n=46$)	0.67 ± 0.03	0.50 ± 0.02	94.5 ± 6.25	156.2 ± 12.6	11.3 ± 2.76	223.0 ± 14.2	1.76 ± 0.13
CG ($n=114$)	0.60 ± 0.014	0.48 ± 0.019	92.5 ± 4.11	161.0 ± 7.57	14.6 ± 2.33	193.5 ± 6.25	1.89 ± 0.15
GG ($n=52$)	0.66[a] ± 0.022	0.53 ± 0.029	88.3 ± 4.26	141.9 ± 7.59	15.9 ± 3.27	215.3 ± 11.5	1.92 ± 0.17

Results are expressed as means ± SEM. [a]Significant difference between GG and CG genotypes at $p=0.05$. ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; hsCRP, C-reactive protein.

ADMA levels between patients with triple vessel disease and subjects with no detectable coronary disease. Also, levels of SDMA, L-arginine and L-arginine/ADMA did not differ.

Moreover, while a study has reported increased plasma ADMA levels in patients with CAD (Boger 2003), Jonasson et al. (2003) did not observe an increase. They suggested that the previously reported increase in ADMA in CAD patients could be due to a concomitant subtle renal dysfunction. It is worth mentioning that in the large multicentre case–control study by Schulze et al. (2006) the conclusion derived from this study about ADMA was based on a small, but significant, increase in serum ADMA, from 0.60 μmol l^{-1} in controls to 0.70 μmol l^{-1} in CHD patients. This could explain the discrepancy in serum ADMA levels among study groups, which might be influenced by a vast array of factors that control serum ADMA levels, such as hypercholesterolemia, smoking, diet, drugs and insulin resistance. Also we did not find a significant change in the L-arginine/ADMA ratio that might be correlated to the CAD. However, the observation that the lowest L-arginine/ADMA ratio was in AMI directs the attention to its link to the severity of coronary insufficiency. In contrast, mean serum SDMA levels were found to be significantly higher in CAD patients ($p=0.033$). In agreement with previous reports of it being a marker of CAD, the difference in mean serum hsCRP level in CAD patients was highly significant ($p=8\times10^{-7}$) compared with the controls. No association between genotype and biochemical data was observed other than lower ADMA levels in the heterozygous subjects compared with the homozygous subjects.

Among the notable observations in this study is the explicit difference in the levels of biomarkers between chronic CAD patients (Med, PCI and CABG groups) and the AMI patients. Acute patients showed higher serum levels of ADMA, SDMA, hsCRP and lower serum L-arginine and L-arginine/ADMA ratio. The positive association of SDMA with ADMA in AMI was previously noticed by Korandji et al. (2007), who addressed the suggestion that SDMA could be a good risk indicator for CAD in AMI patients. The elevated level of serum hsCRP in AMI agrees with previous reports (Wong et al. 2004).

In conclusion, the most noticeable findings of this study are: (1) serum ADMA, SDMA, L-arginine and hsCRP levels are correlated with the severity and incidence of CAD; (2) there is no direct association between *DDAH2* SNP2 genotypes and serum levels of the studied biomarkers.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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