Asymmetric Dimethylarginine and Mortality in Stages 3 to 4 Chronic Kidney Disease

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Background and objectives: Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, reduces bioavailability of nitric oxide and induces endothelial dysfunction. This dimethylated amino acid accumulates in chronic kidney disease and may be involved in the pathophysiology of cardiovascular disease (CVD) in this population.

Design, settings, participants, & methods: The Modification of Diet in Renal Disease Study was a randomized, controlled trial conducted between 1989 and 1993. We measured ADMA in frozen samples collected at baseline (n = 820) and obtained survival status, up to December 31, 2000, from the National Death Index. We examined the relationship of ADMA with prevalent CVD and performed multivariable Cox models to examine the relationship of ADMA with all-cause and CVD mortality.

Results: Mean (SD) age was 52 (12) yr, GFR was 32 ± 12 ml/min per 1.73 m^2, and ADMA was 0.70 ± 0.25 μmol/L. A 1-SD increase in ADMA was associated with a 31% increased odds of prevalent CVD in an adjusted logistic regression model. During the 10-yr follow-up period, 202 (25%) participants died of any cause, 122 (15%) from CVD, and 545 (66%) reached kidney failure. In multivariable Cox models, a 1-SD increase in ADMA was associated with a 9% increased risk for all-cause and 19% increased risk for CVD mortality.

Conclusions: In this cohort of patients with predominantly nondiabetic, stages 3 to 4 chronic kidney disease, there was a strong association of ADMA with prevalent CVD and a modest association with all-cause and CVD mortality.

Clin J Am Soc Nephrol 4: 1115–1120, 2009. doi: 10.2215/CJN.06671208

Endothelium-derived nitric oxide, an important mediator of vascular tone and BP regulation, is produced *via* a reaction catalyzed by nitric oxide synthase (1). Asymmetric dimethylarginine (ADMA), a byproduct of the breakdown of arginine methylated proteins, is an endogenous inhibitor of this reaction (2). Increased ADMA levels lead to nitric oxide depletion, impaired endothelium-dependent vasodilation, reduced free radical scavenging, and plaque rupture with thrombus formation (3–5). Plasma concentrations of ADMA are elevated in cardiovascular high-risk states such as hypertension (6,7), obesity (8), and diabetes (9) and seem to be related to endothelial dysfunction in patients with these conditions. High ADMA levels were an index of carotid intima-media thickness and were associated with future acute coronary events in general population studies (10,11).

Levels of ADMA are elevated in chronic kidney disease (CKD) (12,13). This 202-Da amino acid is eliminated unchanged in the urine but is also taken up and degraded in the kidney by the enzyme dimethylarginine dimethylaminohydrolase (DDAH); ADMA accumulation in kidney failure is due to both decreased elimination and reduced DDAH activity (14). High ADMA was an independent risk factor for cardiovascular disease (CVD) and all-cause mortality in a cohort of patients who were on hemodialysis (13,15) and was associated with faster rates of kidney disease progression in patients in the earlier stages of CKD (16); however, data are limited on the relationship between ADMA levels and CVD in patients with CKD before reaching kidney failure (17,18). We therefore examined the relationship of ADMA with prevalent CVD and with all-cause and CVD mortality during long-term follow-up of a cohort of patients with stages 3 to 4 CKD.

Materials and Methods

Study Population

Details of the Modification of Diet in Renal Disease (MDRD) Study have been published previously (19). In brief, the MDRD study was a randomized, controlled trial of 840 patients with predominantly nondiabetic kidney disease and reduced GFR, conducted between 1989 and 1993, to study the effects of dietary protein restriction and strict BP control on the progression of kidney disease. All patients at baseline had mean arterial pressures ≤125 mmHg, were 18 to 70 yr of age, and had stages 3 to 4 CKD with serum creatinine 1.4 to 7.0 mg/dl in men and 1.2 to 7.0 mg/dl in women. Exclusion criteria were a history of

Received December 24, 2008. Accepted March 23, 2009.

Published online ahead of print. Publication date available at www.cjasn.org.

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ISSN: 1555-9041/406–1115

insulin-requiring diabetes, class III or IV congestive heart failure, renal artery stenosis, kidney transplantation, and frequent hospitalizations. In study A (GFR 25 to 55 ml/min per 1.73 m^2), patients were prescribed a usual or low-protein diet. In study B, (GFR 13 to 24 ml/min per 1.73 m^2), patients were prescribed one of two low-protein diets: The same low-protein diet as in study A or a very-low-protein diet supplemented with a mixture of ketoacids and amino acids. Studies A and B were combined for this analysis. GFR was measured using iothalamate clearance.

Measurement of ADMA

ADMA was measured in 821 frozen fasting serum samples drawn at baseline from the randomized cohort of the MDRD Study. Samples underwent four freeze/thaw cycles before being assayed for ADMA. These assays were performed at the University of Vermont using a commercially available ELISA kit (DLD Diagnostika GMBH, Hamburg, Germany) with sensitivity of 0.05 μmol/L, intra-assay variation of 7.5%, and interassay variation of 10.3%. No cross-reactivity with arginine, monomethylarginine, or symmetric dimethylarginine was noted (<1.5%).

Outcomes

A history of CVD (defined as a composite of coronary heart disease, cerebrovascular disease, and peripheral vascular disease) at baseline was obtained through patient self-report and medical record review. We obtained survival status and cause of death from the National Death Index and ascribed deaths to CVD when the primary cause of death was *International Classification of Diseases, Ninth Revision* (ICD-9) codes 390 to 459 (n = 98) or when kidney disease was listed as the primary cause of death and CVD was the secondary cause (n = 25). Diabetes was defined as ICD-9 codes 250.0 through 250.9. Death as a result of kidney disease was defined as ICD-9 codes 580 through 599 and 753.1. We defined survival time as time from randomization to death or end of follow-up (December 31, 2000). The Cleveland Clinic and Tufts-New England Medical Center institutional review boards approved the data collection procedures.

Statistical Analysis

We evaluated the distribution and normality of variables of interest using box plots, histograms, and normal probability plots. Summary statistics according to tertiles of ADMA are presented as percentages for categorical data, mean ±SD for approximately normally distributed continuous variables, and median (interquartile range) for skewed continuous variables. We tested for differences in baseline characteristics between the ADMA groups using the χ^2 test, one-way ANOVA, and the Kruskal-Wallis test as appropriate.

Factors associated with ADMA at baseline were identified in a multivariable linear regression model. We performed logistic regression models to assess the association between ADMA and history of CVD at baseline as the outcome of interest, initially without adjustment, and subsequently adjusting for several *a priori* defined confounding variables including age, gender, race, history of diabetes, body mass index, diastolic BP (DBP), LDL cholesterol, HDL cholesterol, C-reactive protein (CRP), GFR, and proteinuria. Odd ratios (ORs) and 95% confidence intervals (CIs) are presented per 1-SD change (0.25 μmol/L) in ADMA levels.

We performed Cox proportional hazards models to evaluate the relationship between ADMA and all-cause and CVD mortality initially without adjustment and subsequently adjusting for several groups of *a priori* defined confounding variables. Model 1 adjusted for age, gender, race, and randomization assignments to diet and BP groups; model 2 adjusted for traditional CVD risk factors including history of diabetes, DBP, LDL cholesterol, HDL cholesterol, CRP, and body mass index in addition to the variables in model 2; and model 3 adjusted for variables in model 2 as well as the following kidney disease factors: Cause of kidney disease, GFR, and proteinuria. Hazard ratios (HRs) and 95% CIs are presented per 1-SD change in ADMA levels.

Additional Analyses

Use of angiotensin-converting enzyme inhibitors and aspirin may be potential confounders of the association between ADMA and mortality. We therefore repeated the final regression model with the addition of baseline medication use. Because ADMA is inversely correlated with GFR, it may be related to progression of kidney disease, and kidney failure may modify its relationship with the mortality outcomes; therefore, Cox models (corresponding to model 3) were repeated with kidney failure (defined as dialysis or transplantation) and a composite outcome of kidney failure and all-cause mortality as outcomes.

Results

Baseline Characteristics of the Study Cohort

The study cohort had mean ± SD age of 52 ± 12 yr and GFR of 32.5 ± 12.0 ml/min per 1.73 m^2. Forty percent of the sample was female, 5% had a history of diabetes, and 13% had a history of CVD at baseline. Mean ± SD of ADMA was 0.73 ± 0.25 μmol/L (range of 0.19 to 1.80 μmol/L).

Baseline Characteristics According to Tertiles of ADMA

Patients in the higher ADMA tertiles were more likely to have a history of CVD and have a lower DBP and lower GFR (Table 1). There was no statistically significant difference between tertiles in age, race, history of diabetes, current tobacco use, systolic BP, LDL cholesterol, HDL cholesterol, or CRP.

Cross-Sectional Analyses for Factors that Are Associated with ADMA

In a multivariable linear regression model that included age, gender, race, history of diabetes, DBP, and GFR, only GFR was independently associated with ADMA. A 10-ml/min per 1.73 m^2 lower GFR was associated with a 0.04-μmol/L higher ADMA level. This model explained 4.6% of the variability in ADMA levels.

Cross-Sectional Analyses for Association Between ADMA and Prevalent CVD

At baseline, 13% (n = 107) of the cohort had a history of prevalent CVD. In unadjusted analysis, a 1-SD (0.25 μmol/L) increase in ADMA was associated with a 41% increase in the odds of prevalent CVD at baseline (Table 2). Adjustment for demographic risk factors slightly attenuated this relationship such that the adjusted odds of having prevalent CVD were 34% higher for every 0.25-μmol/L increase in ADMA. Further adjustment for CVD risk factors and kidney disease factors did not appreciably alter this relationship.

ADMA and All-Cause and CVD Mortality

Median follow-up for survival analyses was 9.5 yr (range 0.25 to 11.60 yr). Of the 821 patients in the study, 202 (25%) died from any cause, and 122 (15%) died from CVD. Rates for death and kidney failure increased from the lowest to the highest tertiles of ADMA (Figure 1).

Table 1. Baseline characteristics by tertiles of ADMA[a]

Characteristic	Tertile 1 (0.19 to 0.62 μmol/L; $n = 273$)	Tertile 2 (0.62 to 0.81 μmol/L; $n = 274$)	Tertile 3 (0.81 to 1.80 μmol/L; $n = 274$)	*P*
Demographic factors				
age (yr; mean ± SD)	51.0 ± 11.9	52.0 ± 0.51	52.0 ± 12.7	0.5100
female (%)	40	41	38	0.8300
white (%)	82	85	88	0.1500
CVD risk factors				
CVD history (%)	10	12	18	0.0200
current tobacco use (%)	10	7	12	0.2200
history of diabetes (%)	5	6	4	0.6300
SBP (mmHg; mean ± SD)	132.0 ± 16.9	133.0 ± 18.2	130.0 ± 17.7	0.3600
DBP (mmHg; mean ± SD)	82.0 ± 10.4	80.0 ± 10.1	80.0 ± 9.6	0.0200
total cholesterol (mg/dl; mean ± SD)	221.0 ± 48.8	215.0 ± 42.2	215.0 ± 44.9	0.2300
LDL (mg/dl; mean ± SD)	150.0 ± 44.2	144.0 ± 39.1	149.0 ± 40.7	0.2600
HDL (mg/dl; mean ± SD)	40.0 ± 14.4	40.0 ± 14.1	39.0 ± 14.2	0.4600
CRP (mg/L; median [IQR])	2.50 (5.00)	2.20 (5.10)	2.40 (5.30)	0.7900
BMI (kg/m^2; mean ± SD)	27.0 ± 4.3	27.0 ± 4.5	27.0 ± 4.5	0.7700
ACEI (%)	34	40	36	0.3000
aspirin (%)	11	9	8	0.3100
Kidney disease factors				
GFR (ml/min per 1.73 m^2; mean ± SD)	35.0 ± 12.2	33.0 ± 11.8	30.0 ± 11.3	<0.0001
proteinuria (g/d; median [IQR])	0.30 (1.46)	0.30 (1.10)	0.40 (1.70)	0.2800
cause of kidney disease				
polycystic kidney disease (%)	24	26	22	0.4800
glomerular disease (%)	32	33	30	0.6800
other (%)	43	41	49	0.1700
albumin (g/dl; mean ± SD)	4.0 ± 0.4	4.0 ± 0.3	4.0 ± 0.3	0.1400

[a]ACEI, angiotensin-converting enzyme inhibitor; ADMA, asymmetric dimethylarginine; BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular disease; DBP, diastolic BP; IQR, interquartile range; SBP, systolic BP.

Table 2. Logistic regression model examining association between ADMA and prevalent CVD[a]

Parameter	OR[b]	95% CI	*P*
Unadjusted	1.41	1.16 to 1.70	0.0005
Adjusted			
model 1	1.34	1.09 to 1.64	0.0060
model 2	1.34	1.08 to 1.66	0.0080
model 3	1.31	1.05 to 1.62	0.0170

[a]Model 1 adjusted for age, gender, and race; model 2 adjusted for tobacco, history of diabetes, DBP, LDL, HDL, CRP, and BMI in addition to variables in model 1; model 3 adjusted for GFR and proteinuria in addition to variables in model 2. CI, confidence interval; OR, odds ratio.
[b]ORs are per 1 SD of ADMA (0.25 μmol/L).

In univariate analysis, a 1-SD increase in ADMA increased the risk for all-cause mortality by 18% (Table 3). After adjustment for demographic, randomization, CVD risk factors, and kidney disease factors, the HR decreased to 1.09 with a trend toward statistical significance. In unadjusted analysis, a 1-SD increase in ADMA increased the risk for CVD mortality by 25%, and this relationship remained significant after adjustment for previously described covariates (Table 3).

Additional Analyses

The HR for ADMA remained relatively unchanged with the addition of baseline aspirin and angiotensin-converting en-

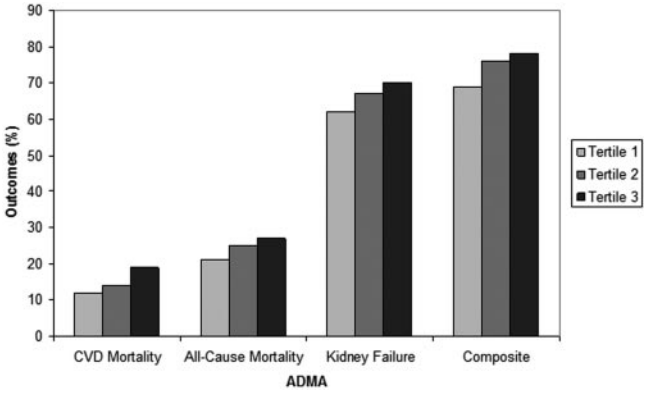

Figure 1. Percentage of outcome based on tertiles of asymmetric dimethylarginine.

zyme inhibitor use for both all-cause (HR 1.12; 95% CI 0.99 to 1.30) and CVD mortality (HR 1.23; 95% CI 1.02 to 1.47). A total of 545 (66%) participants progressed to kidney failure and 611 (74%) reached a composite end point of death or kidney failure with a median follow-up time of 6.40 yr (range 0.25 to 11.60 yr). A 1-SD increase in ADMA increased the risk for both kidney failure (HR 1.13; 95% CI 1.04 to 1.22) and the composite outcome (HR 1.13; 95% CI 1.05 to 1.23) in unadjusted analysis. There was no relationship between ADMA and kidney failure (HR 0.99; 95% CI 0.91 to 1.08) or the composite outcome (HR 0.99; 95% CI 0.90 to 1.08) after adjustment for the variables described in model 3.

Discussion

In this cohort of patients with stages 3 to 4 CKD, in cross-sectional analyses, reduced GFR was associated with higher baseline ADMA levels, which in turn was associated with increased odds of prevalent CVD. In prospective analyses, higher ADMA levels seemed to increase risk for all-cause and CVD mortality and to a lesser degree kidney failure in unadjusted models; after adjustment for covariates, higher ADMA levels seemed to confer increased risk for all-cause and CVD mortality but not kidney failure or the composite outcome.

The mean ADMA level in our study (0.73 ± 0.25 μmol/L) was similar to that reported in a previous study of 131 patients with stages 2 to 5 CKD with mean GFR 31 ml/min per 1.73 m^2 and mean ADMA of 0.78 ± 0.17 μmol/L that also used an ELISA assay (17). In this study cohort of patients with stages 3 to 4 CKD, GFR was the only independent baseline factor associated with ADMA. This is consistent with the previously described role of the kidney in ADMA clearance (12,14).

Baseline ADMA levels in the MDRD Study cohort were related to prevalent CVD after adjustment for traditional CVD risk factors. Other studies have demonstrated a similar association (15,20). A study by Tarnow *et al.* (21) of 408 patients with type 1 diabetes and diabetic nephropathy (mean age 43 yr) found a significant association between ADMA levels and presence of CVD.

Several prospective studies have suggested an association between ADMA and increased risk for CVD in non–kidney disease populations (22,23). There are limited data on the relationship between ADMA levels and CVD outcomes in patients with CKD. In a cohort of 225 hemodialysis patients, higher levels of ADMA were associated with increased risk for cardiovascular events during a follow-up period of 33 mo (13). In a study of patients with diabetic nephropathy, with mean age of 43 yr and mean serum creatinine of 1.06 mg/dl, a 0.01-μmol/L increase in ADMA levels was associated with increased risk for fatal and nonfatal CVD events (21). In a study of 131 patients with mean GFR of 31 ml/min per 1.73 m^2, higher ADMA levels were associated with increased risk for all-cause mortality (17).

We found an independent association between ADMA and all-cause and CVD mortality in our cohort of patients with stages 3 to 4 CKD. High levels of ADMA may increase risk for CVD *via* its actions on the endothelium, impaired vasodilation, reduced free radical scavenging, and plaque rupture and thrombus formation (3–5). Alternatively, ADMA may be a marker for preexisting CVD. The strong cross-sectional association between ADMA and prevalent CVD supports this latter hypothesis; however, in multivariable Cox models, adjustment for history of CVD and traditional CVD risk factors did not greatly attenuate the relationship between ADMA and mortality. It therefore is unlikely that ADMA is solely a marker of preexisting CVD. Second, in patients with preexisting CKD, ADMA may be a measure of the severity of kidney disease and reflect reduced kidney function, a widely known risk factor for

Table 3. Association of ADMA with all-cause and CVD mortality[a]

Parameter	All-Cause Mortality		CVD Mortality	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Unadjusted	1.18 (1.04 to 1.35)	0.012	1.25 (1.06 to 1.47)	0.007
Adjusted				
model 1	1.13 (0.99 to 1.29)	0.067	1.21 (1.02 to 1.29)	0.023
model 2	1.15 (1.00 to 1.31)	0.062	1.25 (1.05 to 1.48)	0.027
model 3	1.09 (0.99 to 1.26)	0.083	1.19 (1.00 to 1.42)	0.034

[a]Hazard ratio (HR) presented per 1-SD (0.25 μmol) increase in ADMA. Model 1 adjusted for age, gender, race, and randomization assignments; model 2 adjusted for history of CVD, history of diabetes, smoking, DBP, LDL, HDL, CRP, and BMI in addition to variables in model 1; model 3 adjusted for GFR, proteinuria, and cause of kidney disease in addition to variables in model 2.

CVD (24). Whereas adjustment for GFR, cause of kidney disease, and proteinuria slightly attenuated the relationship between ADMA and all-cause and CVD mortality, it remained significant for CVD mortality and showed a trend toward significance for all-cause mortality. These data suggest that the association between ADMA and CVD is independent of level of kidney function.

Two studies examined ADMA as a risk factor for progression of kidney disease. In the study by Fliser *et al.* (16), higher baseline ADMA levels were associated with increased risk for a composite outcome of either the need for renal replacement therapy or doubling of serum creatinine. In the study by Ravani *et al.* (17), there was an association between ADMA and increased risk for a composite outcome of death or progression defined as needing dialysis or halving of baseline GFR. This study used estimated GFR as a measure of kidney function rather than measured GFR. In our study, high ADMA level was associated with a moderate increase in the risk for developing kidney failure; however, this was attenuated with adjustment for GFR and other kidney disease risk factors. Population differences in age, stage of CKD, comorbid conditions, and prevalence of diabetes may account for the differences in results.

There are a few limitations to our study, including the use of a single baseline measurement of ADMA to predict events several years in the future. Another limitation is the use of frozen samples for the measurement of ADMA; however, there are several precedents for the use of frozen samples to measure ADMA levels (17,25,26). Valtonen *et al.* (27) demonstrated that ADMA levels were stable in frozen samples and unaltered after four freeze/thaw cycles. Finally, the observed results may be unique to this study population. The MDRD Study represents a predominantly nondiabetic, relatively young, and healthy CKD population. This cohort is different from the preponderance of patients with CKD in that a majority of the participants progress to kidney failure; however, the low prevalence of preexisting CVD, diabetes, and malnutrition reduces confounding by these powerful risk factors. Finally, we lack information on symmetric dimethylarginine. The strengths of this study include the large number of patients with nondiabetic kidney disease, long-term follow-up, low prevalence of CVD at baseline, detailed ascertainment of potentially confounding variables, and precise measurement of GFR as a marker of kidney function.

Conclusions

In this cohort of patients with predominantly nondiabetic, stages 3 to 4 CKD, there was a strong association between ADMA levels and prevalent CVD, a modest association of ADMA with all-cause and CVD mortality, and no association with kidney failure or the composite outcome. These findings may have diagnostic and therapeutic implications. ADMA levels may be used in risk stratification and identification of patients at high risk for CVD. ADMA may also be a potentially modifiable risk factor as studies have shown that rosiglitazone, pioglitazone, amlodipine, valsartan, and targeted gene therapies decrease ADMA levels in animal models and patients with kidney failure (28–30).

Acknowledgments

This study was supported by grants K23 DK067303, K23 DK02904, and K24 DK078204 from the National Institute of Diabetes and Digestive and Kidney Diseases.

Parts of this article were presented in abstract form at the annual meetings of the American Society of Nephrology; ASN Renal Week, November 14 through 19, 2006, San Diego, CA, and ASN Renal Week, October 31 through November 5, 2007, San Francisco, CA.

Disclosures

None.

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