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Dimethylarginine levels and nutritional status in hemodialysis patients

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Abstract

Background: **Asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) originate from hydrolysis of methylated proteins, including dietary proteins, and are retained in end-stage renal disease (ESRD). This study aimed to detect the correlation of ADMA and SDMA to nutritional parameters in dialysis patients.**

Methods: **Before and after a single dialysis session, L-arginine, ADMA and SDMA plasma levels were measured in 38 hemodialysis patients by HPLC–tandem mass spectrometry. Biochemistry, protein intake, anthropometry and bioelectric impedance analysis were evaluated**

Results: **Predialysis plasma levels of ADMA were higher than in normal controls (n=20) (1.14 ± 0.27 µmol/L vs. 0.56 ± 0.09 µmol/L, p<0.001), as were SDMA levels (3.49 ± 1.00 µmol/L vs. 0.44 ± 0.13 µmol/L, p<0.001). On univariate analysis, predialysis ADMA levels were inversely related to BMI and albumin levels, whereas SDMA was directly related to nPNA, phase angle, prealbumin and creatinine serum levels. ADMA/SDMA ratio was inversely related to prealbumin and albumin, creatinine, urea and phosphorus serum levels, as well as nPNA, but positively to C-reactive protein. On multiple regression analysis, serum albumin and BMI were the stronger predictors of ADMA, whereas prealbumin serum levels followed by dietary protein intake were the stronger predictors of SDMA. Prealbumin followed by C-reactive protein was predictive of the ADMA/SDMA molar ratio.**

Conclusions: **Our results confirm that ADMA and SDMA levels are increased in ESRD patients and suggest that a link may exist with inflammation and nutritional status. High ADMA levels associated with reduced SDMA may be a predictive marker of malnutrition-inflammation-atherosclerosis syndrome.**

Key words: *ADMA, ESRD, Hemodialysis, Mass spectrometry, Nutrition, SDMA*

Introduction

Asymmetric dimethylarginine (ADMA) is a nitric oxide (NO) synthase inhibitor considered to be a major cardiovascular risk factor in the general population (1) and in renal disease patients (2), whereas symmetric dimethylarginine (SDMA) is thought to be a biologically inactive substance, although it competes with L-arginine in its intracellular transport (3). Both ADMA and SDMA originate from proteolysis of methylated proteins (including dietary proteins). They are normally cleared by urine, but ADMA is also metabolized by dimethylarginine dimethylaminohydrolase (DDAH) (4). Thus the circulating levels are increased in uremic patients (5, 6), and the ADMA/SDMA molar ratio may reflect DDAH activity, which seems to be sensitive to oxidative stress and possibly to inflammation. Thereby several factors related to renal function, nutrition and inflammation may be important in the variability of dimethylarginine serum levels in end-stage renal disease (ESRD) patients. Protein-energy malnutrition

is considered a common feature of hemodialysis patients. Several cohort studies have revealed that protein-energy malnutrition is associated with inflammation and increased cardiovascular disease that negatively affect the clinical outcome of the hemodialysis population (7).

Zoccali et al described an inverse relationship between serum albumin and ADMA (2), but to our knowledge, no study exists dealing with the relationships between ADMA or SDMA and a panel of different parameters of nutritional status assessment. This cross-sectional study aimed to detect the relations between ADMA and SDMA plasma levels and several nutritional parameters in a cohort of stable hemodialysis patients.

Subjects and methods

Patients

Thirty-eight patients (25 men, 13 women, mean age 63 ± 13 years) affected by chronic renal failure stage V, who had been on maintenance hemodialysis treatment for at least 6 months, were selected for this observational study. The exclusion criteria were the following: malignancy, inflammatory or infectious disease, cachexia, anorexia, severe congestive heart failure (NYHA class IV or V), liver failure, psychiatric disease, lower limb critical ischemia, parenteral nutrition or use of steroids.

The underlying renal disease was chronic glomerulonephritis in 10 cases, ischemic nephropathy in 8 cases, diabetic nephropathy in 6 cases, polycystic renal disease in 3 cases, tubulointerstitial nephritis in 3 cases and unknown in 8 cases. No patient was being treated with angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers, whereas nitroglycerine was used in 10 patients and calcium channel blockers in 10 cases. Thirty-five out of the 38 patients were on erythropoiesis-stimulating agents. Some clinical features of the studied population are depicted in Table I.

Dialysis treatment was performed on a thrice-a-week schedule, for 210-270 minutes. The dialysis vintage was 70 ± 58 months. A native arteriovenous fistula was used as vascular access in 33 patients, arteriovenous graft in 3 cases and a permanent central vein catheter in 2 cases. All of the patients followed their usual dialysis technique chosen on a clinical basis: standard hemodialysis in 12 cases, online hemodiafiltration in 16 cases and acetate-free biofiltration in 10 cases; high-flux or low-flux synthetic membranes (polyamine, polysulphone or AN69) were used.

Twenty healthy subjects, comparable for sex and age, were recruited to form a normal control group regarding ADMA and SDMA plasma levels.

Methods

Before and after a single dialysis session, L-arginine, ADMA and SDMA plasma levels were measured. Predialysis biochemistry examinations included serum albumin, prealbumin, urea, creatinine, calcium, phosphorus, sodium bicarbonate, hematocrit, hemoglobin, C-reactive protein (CRP), Kt/V and normalized Protein Nitrogen Appearance (nPNA). Body mass index (BMI) was calculated by height and postdialysis body weight. Erythropoietin index was calculated by the following formula: weekly dose of erythropoiesis-stimulating agent (ESA) / weight / hemoglobin; where weight was in kilograms and hemoglobin in grams per deciliter. Values <10 are desirable or normal. Bioelectric impedance vectorial analysis (BIVA) was performed 30 minutes after the end of the hemodialysis session (8, 9), using a Bioelectrical Impedance Analyzer (BIA/STA; Akern, Florence, Italy) with a distal, tetrapolar technique, delivering an excitation current at 50 kHz; BIVA parameters were measured in duplicate.

BIVA gives 2 bioelectric parameters: body resistance (R) and reactance (Xc), and the impedance vector (Z) is a combination of R and Xc across tissues. The arc tangent of Xc/R is called the phase angle (PA), which is a derived measure obtained from the relation between the direct measures of resistance and reactance reflecting hydration status and soft tissue cellular mass. Reduced phase angle reflects an increased extracellular to intracellular water ratio as well as a decrease in body cell mass. It is a predictor of survival in a number of diseases and also in the dialysis population (10), where phase angle values lower than 4.0° are associated with increased mortality risk (11).

Blood samples were drawn from the arterial line, before the beginning of the first dialysis of the week, and at the end of the dialysis session using the stop-flow method. Serum samples were cooled within 1 hour and then stored at -20°C until analysis was performed.

Serum levels of L-arginine, ADMA and SDMA were detected by high-performance liquid chromatography (HPLC)–tandem mass spectrometry, using a method strictly derived from that proposed by Schwedhelm et al (12). Sample preparation was carried out as follows: 12 µL of a water solution containing ADMA-D_6 and L-arginine-D_7, 2 µM and 200 µM, respectively, was added to a 20-µL aliquot of plasma sample, in a 1.5-mL 12x32 conical vial. After vigorous vortex mixing, 868 µL of pure methanol was added, and the mixture was shaken again by vortex. The protein matter was

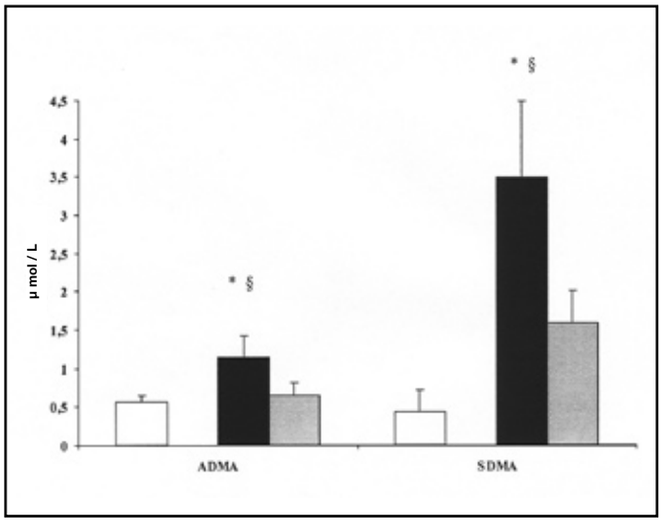

Fig. 1 - Asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) plasma levels in the end-stage renal disease patients studied, as recorded before (black column) and after (grey column) a hemodialysis session, compared with normal control values (empty column); *p<0.001, vs. normal controls; §p<0.001, vs. after dialysis.

then separated from the liquid solution by centrifugation at 6,000 rpm, and 60 µL of supernatant was placed into a 96-well plate, each tube containing 200 µL. The plate content was then evaporated to dryness by a gentle stream of nitrogen, heating at 65°C. The solid matter was then reconstituted with 3N HCl in n-butanol (Regis Technologies, Morton Grove, IL, USA) used as a derivatization reagent, vortexed, incubated 25 minutes in a oven at 65°C and evaporated again under nitrogen. The successive reconstitution was carried out by 125 µL of a mixture methanol and water 1/1 (v/v). The calibration curves were built by 7 standard solutions, prepared in the same way as the samples, containing the analytes in the following concentrations: 375, 750, 1,500, 3,000 6,000, 12,000 and 24,000 nM for L-arginine; 3.75, 7.5, 15, 30, 60, 120 and 240 nM for ADMA and SDMA. One microliter of either sample and standard solution was injected in the HPLC–tandem mass spectrometry system, which was constituted of autosampler, column oven, binary micro-HPLC pump system (all components; Perkin Elmer, Boston, MA, USA) Series 200, and an Applied Biosystems-MDS Sciex API 4000 triple quadrupole mass spectrometer (Concord, Ontario, Canada), equipped with a Turbo-V IonSpray source. HPLC column and conditions were the same as described by Schwedhelm et al (12), as well as the mass spectrometry selected reaction monitoring transitions. The operative parameters resulted as follows: IonSpray voltage, 5.0 kV; gas source 1, 50; gas source 2, 30; turbo temperature, 650°C; entrance potential, 10 V; collision gas, nitrogen; CAD gas pressure, 3.5 mPa. In addition, declustering potentials, collision energies and collision exit potentials were optimized for either analytes or standards in order to get the best sensitivity.

Statistics

Descriptive statistics are given as means ± standard deviation. Statistical analysis was performed by Student's *t*-test for unpaired and paired data. The Spearman rank correlations of plasma ADMA and SDMA were identified by univariate and multivariate analysis; forward and backward elimination was used in identifying the most important prognostic factors. The statistical package StatView 5 version 5.0.1 for personal computer was utilized for processing data. Differences were considered to be statistically significant when $p<0.05$.

Results

Figure 1 shows that predialysis plasma levels of ADMA were higher in ESRD patients than in controls (1.14 ± 0.27 µmol/L vs. 0.56 ± 0.09 µmol/L, $p<0.001$), as were levels of SDMA (3.49 ± 1.00 µmol/L vs. 0.44 ± 0.13 µmol/L, $p<0.001$) and L-arginine (127 ± 55 µmol/L vs. 56 ± 15 µmol/L, $p<0.001$). ADMA levels were significantly lower than SDMA levels ($p<0.001$) (Fig. 1). Following a single dialysis treatment, serum levels of L-arginine (73 ± 32 µmol/L), ADMA (0.65 ± 0.17 µmol/L) and SDMA (1.58 ± 0.43 µmol/L) were significantly reduced (Fig. 1).

Table I shows clinical and nutritional features of the studied population. Signs of protein-energy malnutrition emerged in a small proportion of patients. Namely, BMI <20 kg/m^2 was detected in 10 out the 38 patients (18%), serum albumin <3.5 g/dL in 10%, phase angle <4.0° in 26%, nPNA <0.8 g/kg per day in 18% and <1.0 g/kg per day in 60%. Finally, a proinflammatory status (CRP >5.0 mg/L) was detected in 55% of the studied patients.

On univariate analysis, predialysis ADMA levels were inversely related to albumin levels, BMI and body weight after dialysis (Tab. II). A stepwise regression model, with ADMA as dependent variable, including body weight before and after dialysis, BMI and albumin levels showed albumin as the strongest independent predictor of ADMA ($p=0.01$; $F=6.602$), followed by BMI ($p<0.01$; $F=5.633$).

SDMA was directly related to nPNA, phase angle, hemoglobin, hematocrit, prealbumin and urea serum levels (Tab. II). A stepwise regression model, with SDMA as dependent variable, including nPNA, prealbumin, hematocrit and hemoglobin levels, showed prealbumin as the strongest inde-

TABLE I
CLINICAL AND BIOCHEMICAL FEATURES OF THE STUDIED PATIENTS

	Mean ± SD	Median	Range
Age, years	66 ± 14	71	32-85
Dialysis vintage, months	72 ± 59	50	6-216
Body weight postdialysis, kg	63.2 ± 11.4	62.2	43.4-89.5
BMI, Kg/m2	24.1 ± 4.8	22.9	17.7-37.2
Systolic BP, mm Hg	120 ± 27	120	72-170
Diastolic BP, mm Hg	65 ± 12	65	38-88
Mean BP, mm Hg	83 ± 16	84	49-106
Pulse BP, mm Hg	45 ± 24	52	15-95
Kt/V	1.55 ± 0.29	1.53	1.0-2.3
nPNA, g/kg per day	0.95 ± 0.21	0.91	0.52-1.63
Serum calcium, mg/dL	8.9 ± 0.6	8.9	7.2-9.9
Serum phosphorus, mg/dL	4.8 ± 1.7	4.3	2-9
Serum albumin, g/dL	4.2 ± 0.7	4.2	2.7-5.0
Serum prealbumin, mg/dL	33 ± 10	32	14-53
Serum sodium bicarbonate, mM	23.2 ± 1.8	23.5	19-30
Hemoglobin, g/dL	11.4 ± 1.7	11.6	7.4-13.9
EPO resistance	8.5 ± 5.3	9.4	2.3-22.5
Serum urea, mg/dL	133 ± 39	125	63-249
Serum creatinine, mg/dL	9.2 ± 3.1	8.6	2.0-16.0
C-reactive protein, mg/L	8.9 ± 8.1	6.0	1.2-39.1
Phase angle, °	5.3 ± 1.3	5.2	2.9-7.7
Body cell mass index, kg/m^2	7.7 ± 2.6	7.6	3.6-12.5

BMI = body mass index; BP = blood pressure; EPO = erythropoietin ; nPNR = normalized Protein Nitrogen Appearance.

pendent predictor of SDMA ($p<0.01$; $F=8.775$), followed by nPNA ($p<0.01$; $F=5.595$).

Finally, predialysis ADMA/SDMA molar ratio was inversely related to prealbumin and albumin serum levels, predialysis creatinine, urea and phosphorus serum levels, as well as nPNA, but positively to CRP (Tab. II).

A stepwise regression model, with ADMA/SDMA as dependent variable, including prealbumin levels, nPNA levels, creatinine levels and CRP, showed prealbumin as the strongest independent predictor of ADMA/SDMA ($p<0.01$; $F=17.813$) followed by CRP ($p<0.01$; $F=13.968$).

The subgroup of hemodialysis patients with CRP ≥5.0 mg/L have higher ADMA/SDMA ratio than patients with CRP <5.0 mg/L (0.39 ± 0.09 vs. 0.29 ± 0.08, $p=0.01$).

Discussion

In ESRD patients, the risk of cardiovascular morbidity and mortality is greatly increased (13), due to the coexistence in the uremic patient of a number of cardiovascular risk factors, both traditional and emerging ones, including abnormalities of the NO metabolism and endothelial dysfunction (14-16). ADMA is an inhibitor of nitric oxide synthase (NOS), and increased circulating levels of ADMA are believed to

TABLE II
SIGNIFICANT CORRELATIONS OF ADMA, SDMA AND ADMA/SDMA MOLAR RATIO

	r	p Value
ADMA		
BMI	-0.420	<0.01
Serum albumin	-0.440	<0.01
Body weight, postdialysis	-0.340	<0.05
SDMA		
Hematocrit	0.476	<0.01
Hemoglobin	0.472	<0.01
Serum urea	0.402	<0.01
Phase angle	0.448	<0.01
nPNA	0.497	<0.01
Serum prealbumin	0.568	<0.001
ADMA/SDMA		
Serum albumin	-0.446	<0.01
Serum urea	-0.466	<0.05
Serum phosphorus	-0.434	<0.01
Serum creatinine	-0.470	<0.01
nPNA	-0.485	<0.01
Serum prealbumin	-0.617	<0.001
C-reactive protein	0.526	<0.001

ADMA = asymmetric dimethylarginine; BMI = body mass index; BP = blood pressure; nPNA = normalized Protein Nitrogen Appearance; SDMA = symmetric dimethylarginine.

reduce production and action of NO on vascular endothelium, and thus ADMA is considered to be a novel risk factor for vascular disease.

Our data confirm that dimethylarginines are increased in dialysis patients, but ADMA is lower than SDMA, because of the additional metabolism by DDAH.

Some contrasting data exist about the effect of the dialysis procedure on ADMA plasma levels. Namely, some authors claim low dialysance of ADMA, probably due to significant protein binding (17), leading to negligible changes following dialysis (5). Some authors suggest that mixed diffusive-convective dialysis techniques are more efficient than standard diffusive dialysis in lowering ADMA plasma levels, but others have failed to demonstrate any benefit (18).

Our results demonstrate that a significant decrease of L-arginine and dimethylarginine plasma levels occur following a single hemodialysis treatment, and these are well in keeping with Bergamini et al (19). Additionally, other authors have found an average decrease in ADMA and SDMA levels of 32% and 44%, respectively, with all hemodialysis modalities (20, 21).

A number of relationships between dimethylarginine plasma levels and parameters of nutritional status and inflammation were shown in the present study.

ADMA plasma levels were inversely related to albumin levels. Our data are quite different from those of Busch et al (22), but they are well in keeping with those of Zoccali et al (2), who found a negative association between ADMA and serum albumin in a cohort of 250 hemodialysis patients. Serum albumin and BMI were strong predictors of ADMA serum levels, so it may be speculated that poor nutritional status, in particular abnormal protein metabolism, is associated with high ADMA levels.

In contrast, low levels of SDMA are associated with several markers of poor nutritional status, namely low protein intake, low prealbumin and creatinine serum levels and low phase angle. The reason may be that SDMA production is derived from hydrolysis of methylated proteins, including dietary proteins, and its metabolism is not affected by DDAH, but depends on renal excretion or dialysis removal. Serum prealbumin and nPNA were strong predictors of SDMA serum levels; thus, it is likely that the predialysis SDMA levels may be related to protein catabolic rate and then to dietary protein intake, in the stable patient at least.

ADMA/SDMA ratio may give indirect information about the rate of DDAH activity, since only ADMA is metabolized by the enzyme. Our data show that ADMA/SDMA ratio is associated with inflammation status and signs of malnutrition. A direct relationship was found between ADMA/SDMA ratio and CRP levels, and patients indicating increased inflammation have significantly higher ADMA/SDMA ratios. This is well in keeping with the findings that inflammation is able to inhibit DDAH activity. In addition, higher ADMA/SDMA ratios were associated with lower protein intake, lower prealbumin and albumin serum levels and lower predialysis levels of creatinine, urea and phosphorus. As a whole, these data suggest that ADMA/SDMA ratio is strictly related to nutritional and inflammation in dialysis patients; that is to say, Increased ADMA/SDMA is suggestive of malnutrition and inflammation. Actually, serum prealbumin and CRP were the stronger predictors of ADMA/SDMA molar ratio. It could be speculated that high ADMA levels together with high ADMA/SDMA ratio

may be markers or mediators of the malnutrition-inflammation-atherosclerosis syndrome in uremic patients (7, 23). Therefore, in addition to reporting ADMA as a potent inhibitor of NO synthase and a major factor affecting NO metabolism and endothelial functioning, it is important also to report SDMA levels.

The method we used (HPLC–tandem mass spectrometry) is the most reliable one, and it allows the measurement of ADMA and SDMA at the same time and in the same run. However, the cross-sectional design and the small sample size were limitations of this study, which prevent us from drawing conclusions.

In summary, ADMA and SDMA plasma levels are increased in hemodialysis patients, and ADMA levels are lower than SDMA levels, because of additional metabolism by DDAH. Several associations have been found between plasma levels of dimethylarginines and inflammation and nutritional indicators. Increased ADMA levels are associated with low BMI and low albumin levels – both markers of poor nutrition and possibly of inflammation. Higher SDMA levels are associated with markers of good nutritional status, namely with higher protein intake, and with higher prealbumin serum levels. Serum prealbumin and CRP are strong predictors of the ADMA/SDMA molar ratio. As a whole ADMA/SDMA seems to be a good predictor of poor nutrition and inflammation – that is, high levels of ADMA coupled with low SDMA levels in uremic patients may be linked to enhanced risk of the malnutrition-inflammation-atherosclerosis syndrome. This hypothesis needs to be confirmed by further investigations.

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