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Original Investigation

Serum Asymmetric and Symmetric Dimethylarginine and Morbidity and Mortality in Hemodialysis Patients

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Background: Asymmetric (ADMA) and symmetric dimethylarginine (SDMA) are putative uremic toxins that may exert toxicity by a number of mechanisms, including impaired nitric oxide synthesis and generation of reactive oxygen species. The study goal was to determine the association between these metabolites and cardiovascular outcomes in hemodialysis patients.

Study Design: Post hoc analysis of the Hemodialysis (HEMO) Study.

Setting & Participants: 1,276 prevalent hemodialysis patients with available samples 3 to 6 months after randomization.

Predictor: ADMA and SDMA measured in stored specimens.

Outcomes: Cardiac death, sudden cardiac death, first cardiovascular event, and any-cause death. Association with predictors analyzed using Cox regression adjusted for potential confounders (including demographics, clinical characteristics, comorbid conditions, albumin level, and residual kidney function).

Results: Mean age of patients was 57 ± 14 (SD) years, 63% were black, and 57% were women. Mean ADMA (0.9 ± 0.2 μmol/L) and SDMA levels (4.3 ± 1.4 μmol/L) were moderately correlated ($r = 0.418$). Higher dialysis dose or longer session length were not associated with lower predialysis ADMA or SDMA concentrations. In fully adjusted models, each doubling of ADMA level was associated with higher risk (HR per 2-fold higher concentration; 95% CI) of cardiac death (1.83; 1.29-2.58), sudden cardiac death (1.79; 1.19-2.69), first cardiovascular event (1.50; 1.20-1.87), and any-cause death (1.44; 1.13-1.83). Compared to the lowest ADMA quintile (<0.745 μmol/L), the highest ADMA quintile (≥1.07 μmol/L) was associated with higher risk (HR; 95% CI) of cardiac death (2.10; 1.44-3.05), sudden cardiac death (2.06; 1.46-2.90), first cardiovascular event (1.75; 1.35-2.27), and any-cause death (1.56; 1.21-2.00). SDMA level was associated with higher risk for cardiac death (HR, 1.40; 95% CI, 1.03-1.92), but this was no longer statistically significant after adjusting for ADMA level (HR, 1.20; 95% CI, 0.86-1.68).

Limitations: Single time-point measurement of ADMA and SDMA.

Conclusions: ADMA and, to a lesser extent, SDMA levels are associated with cardiovascular outcomes in hemodialysis patients.

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INDEX WORDS: Cardiovascular mortality; dialysis outcomes; asymmetric dimethylarginine (ADMA); symmetric dimethylarginine (SDMA); hemodialysis; end-stage renal disease (ESRD); uremic toxins; cardiovascular morbidity; cardiac death; sudden cardiac death.

Patients undergoing dialysis continue to have excessive morbidity and mortality despite many advances in care.[1] Much of this excessive risk is due to cardiovascular disease; however, the underlying mechanisms for the accelerated cardiovascular disease phenotype in dialysis patients are undefined.[2,3] Some of this risk may be due to solutes that accumulate in the body in kidney failure.[4] The identity of these uremic toxins is incompletely known.

Asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) are produced endogenously by the metabolism of arginine-containing proteins.[5] Both metabolites accumulate in patients with kidney failure.[6] A body of evidence

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supports the toxicity of ADMA and, to a lesser extent, SDMA.[5,7,8] Whereas ADMA inhibits nitric oxide (NO) synthesis, SDMA may have vasculotoxic and proatherogenic effects. Both ADMA and SDMA have been associated with cardiovascular mortality in large general population studies,[9-12] and previous studies also suggest associations with cardiovascular outcomes in dialysis patients.[13,14]

We measured ADMA and SDMA in specimens of the Hemodialysis (HEMO) Study, a US multicenter trial of hemodialysis dose and flux. Our aim was to examine the longitudinal association between these solutes and cardiovascular morbidity and mortality in patients receiving dialysis. The design of the HEMO Study,[15,16] including its large sample size, national multicenter design, inclusion of patients without significant residual kidney function, and physician-adjudicated outcomes, provided a unique opportunity to examine associations between the dimethylarginines and outcomes in hemodialysis patients.

METHODS

Study Design

The HEMO Study was a clinical trial that randomly assigned 1,846 prevalent hemodialysis patients to standard or high dialyzer urea clearance (assessed by Kt/V_{urea}, an index of urea clearance by dialysis) and to low-flux or high-flux dialysis membranes (assessed by β_2-microglobulin clearance).[16,17] Patients were enrolled May 1995 to February 2001 from 15 clinical centers in the United States comprising 72 dialysis units and followed up for outcomes until death, kidney transplantation, or end of study in December 2001. Major exclusion criteria included residual urea clearance $>$ 1.5 mL/min/35 L urea volume of distribution, unstable angina, active systemic infection, New York Heart Association class IV congestive heart failure, and severe hypoalbuminemia (albumin $<$ 2.6 g/dL). Our study sample included all HEMO Study participants who had available stored sera collected 3 to 6 months postrandomization (N = 1,276), a time point allowing adequate separation between the trial intervention arms. The participating institutions' institutional review boards reviewed and approved the HEMO Study and all participants provided informed consent. The Johns Hopkins Medicine Institutional Review Board reviewed and approved this study (IRB00081893).

Data Collection

Laboratory Measurements

We measured ADMA/SDMA by liquid chromatography–tandem mass spectrometry using ADMA-d7 (Cambridge Isotope Laboratories) and SDMA-d6 (Toronto Research Chemicals) as internal standards. Plasma was deproteinized by mixture with an internal standard solution and methanol (2:1:20 vol:vol:vol). Five microliters of each sample supernatant was injected in a Shimadzu Prominence LC-20A system, and analytes were separated on a silica column (150 × 2.1 mm, 3 Om Luna silica; Phenomenex) at room temperature. The mobile phase was 90% methanol containing 10 mmol/L of ammonium formate and 0.2% formic acid (vol/vol) at a flow rate of 0.2 mL/min. Mass spectrometry was performed on an API 4000 triple quadrupole mass spectrometer (AB Sciex) with electrospray ionization in the positive mode. Ion transitions used for quantitation were m/z 203→70 for both ADMA and SDMA with corresponding transitions for the internal standards. For ADMA and SDMA, recoveries averaged 102%±8% and 115%±6%, respectively. Intraday coefficients of variation for ADMA were 0.3% at 0.461 μmol/L, 2.1% at 1.71 μmol/L, and 1.0% at 3 μmol/L, and for SDMA, 1.4% at 0.737 μmol/L, 3.7% at 2.1 μmol/L, and 0.3% at 3.7 μmol/L. Interday data were similar. When measured values were $<$80% of the lowest standard, a value halfway between zero and the low end of the standard curve was imputed. For other laboratory tests, including urea, albumin, and β_2-microglobulin, we used data collected as part of the HEMO Study.

Outcomes

The primary outcomes for our analyses were cardiac death, sudden cardiac death, and first cardiovascular event (composite of first cardiovascular hospitalization or death from any cause). The secondary outcome was all-cause mortality. Cardiac death was defined as death due to coronary events, congestive heart failure, arrhythmias, and other heart diseases and conditions. Sudden cardiac death was defined as a witnessed death with preceding duration of symptoms less than 24 hours or unwitnessed unexpected death with symptom duration less than the interval since the last dialysis session.[16] Cardiovascular hospitalization was defined as hospitalization for ischemic heart disease, heart failure, arrhythmias, other cardiac conditions, hypertension, and peripheral vascular disease. Causes for death and hospitalizations in the HEMO Study were determined locally and then adjudicated by an outcomes committee that was unaware of treatment group assignments.[18]

Other Covariates

Demographics and clinical information were available for all participants at baseline. We used the Index of Coexisting Disease (ICED) score, assessed by chart abstraction by trained nurses at baseline and then annually, for comorbid condition assessment. The final ICED score ranges from 0 to 3, with higher numbers indicating greater comorbid conditions. We assessed dietary information that was collected at baseline and then annually using 2-day assisted recall. We assessed residual kidney function at baseline from a timed urine collection with measurement of urinary urea clearance. Other baseline data included self-reported appetite[19,20] and the mental health index subscale of the 36-Item Short-Form Health Survey (SF-36) questionnaire, which correlates with depressive symptoms in dialysis patients.[21] We used data for systolic blood pressure, weight, and volume removed on dialysis collected as per the dialysis unit routine and recorded on the monthly HEMO kinetic modeling day, the same date as blood sample collection. We calculated relative volume removed as predialysis weight minus postdialysis weight divided by predialysis weight, and body mass index, as target weight (in kilograms) divided by height (in meters) squared. We used data for Kt/V_{urea} and normalized protein catabolic rate (an index of protein intake) provided in the HEMO database.

Statistical Analysis

We analyzed baseline characteristics of participants overall and compared differences in included and excluded participants using χ^2 test for categorical variables and linear regression for continuous variables. Covariates with missing values included race (0.1%), cause of end-stage renal disease (2.3%), systolic blood pressure (0.1%), albumin level (0.5%), and residual kidney function (0.1%). To avoid listwise deletion,[22] we imputed missing data with 10 data replicates using multiple imputation by chained equations method implemented by the "proc mi" procedure in SAS (SAS Institute Inc) and used "proc mianalyze" to combine results. We censored participants at kidney transplantation or end of the study for mortality analyses and also for transfer to nonparticipating clinical centers for hospitalization analyses because the