

Asymmetric dimethyl arginine levels in chronic hemodialysis patients

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Background/aim: To investigate serum asymmetric dimethyl arginine (ADMA) levels in maintenance hemodialysis (HD) patients and to assess their potential correlations with C-reactive protein (CRP), albumin, and cholesterol levels as the established cardiovascular and nutritional parameters.

Materials and methods: Forty-nine patients on maintenance HD treatment and 22 healthy volunteers with similar age and sex characteristics were recruited into the study. Serum albumin, CRP, creatinine, calcium, phosphate, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, hemoglobin, white blood cell counts, and serum ADMA levels were measured.

Results: HD patients had significantly higher ADMA levels compared with healthy controls (0.51 ± 0.25 vs. 0.35 ± 0.15 , $P = 0.002$). While white blood cell counts and body mass index values were similar between the 2 groups, CRP and LDL cholesterol were significantly higher and albumin and HDL cholesterol were significantly lower in HD patients compared with healthy controls. ADMA concentrations were positively correlated with mean age ($P = 0.02$, $r = 0.360$), LDL cholesterol levels ($P = 0.006$, $r = 0.325$), and CRP levels ($P = 0.02$, $r = 0.268$) and negatively correlated with serum albumin levels ($P = 0.005$, $r = -0.331$).

Conclusion: ADMA levels were found to be higher in HD patients and were shown to be correlated with preestablished inflammatory and nutritional biomarkers.

Key words: Chronic kidney disease, asymmetric dimethyl arginine, ADMA, endothelial dysfunction, atherosclerosis, hemodialysis

1. Introduction

Patients with chronic kidney disease (CKD) of any stage have significantly increased risk of accelerated atherosclerosis and increased cardiovascular mortality rate (1). In end-stage renal disease (ESRD) patients, more than half of all deaths are related to cardiac events and this rate is extremely higher than the rate among the general population (1). The high prevalence of cardiovascular mortality in this group of patients could not be easily explained by traditional risk factors such as diabetes, hypertension, and dyslipidemia. Recently, there have been considerable data supporting the idea that chronic inflammation is a nontraditional risk factor contributing to the accelerated atherosclerosis in CKD patients (2,3). Predicting the cardiovascular risk earlier in CKD patients is crucial in order to decrease the prevalence of cardiovascular events (4).

Endothelium-derived nitric oxide (NO) is a major regulator of vascular dilatation and is derived from

arginine by nitric oxide synthase (5). Via the vasodilator effects, NO reduces both afterload and preload, resulting in prevention of left ventricular hypertrophy (6). Besides being an endothelial-based strong vasodilator, it also inhibits leukocyte adhesion to vascular walls, thrombocyte aggregation, and vascular smooth muscle cell proliferation (5). Asymmetric dimethyl arginine (ADMA) is an endogenous competitive inhibitor of endothelial-based NO synthase and by reducing NO production it may promote endothelial dysfunction, hypertension, and atherosclerosis (7).

ADMA is partially eliminated by the kidneys; therefore, renal failure increases its level (7). In previous studies, ADMA levels were found to be higher in ESRD patients both on peritoneal dialysis and hemodialysis (HD) compared with healthy controls (8,9). Increased levels of ADMA are thought to be associated with the increased frequency of cardiovascular events seen in ESRD patients (10,11), and it is suggested that ADMA might be a novel

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cardiovascular risk factor for both ESRD patients and the general population (7,12). Additionally, its relation with nutritional status in maintenance HD patients was assessed in a recent trial and a negative correlation was found between ADMA levels and nutritional parameters (13).

The objective of our study was to investigate serum ADMA levels in patients with ESRD who were on chronic HD treatment and to assess their potential correlations with C-reactive protein (CRP), albumin, and cholesterol levels as the established cardiovascular and nutritional parameters.

2. Materials and methods

This cross-sectional, single-center study enrolled maintenance HD patients and healthy control subjects. Seventy-six prevalent HD patients treated at our institute were screened. Exclusion criteria were history or existence of malignancy, chronic liver diseases, autoimmune diseases, symptoms of active infection or inflammation, hepatitis B and C virus infection, and steroid treatment. Forty-nine adult HD patients (18–80 years of age) who fulfilled the inclusion criteria were recruited into the study. The study protocol was approved by the Medical Ethics Committee of Ankara Education and Research Hospital and was performed in accordance with the principles of the Helsinki Declaration. Informed consent was obtained from all participants.

Forty-nine patients were on maintenance HD treatment 3 times weekly for 4 h with standard bicarbonate dialysate and with synthetic membranes. Twenty-two healthy volunteers, with similar age and sex characteristics and without any clinical signs of chronic disease or laboratory abnormality were recruited as controls from subjects who were admitted to our hospital for routine examination. Demographic and clinical data were recorded for all subjects and body mass index (BMI) was calculated as the ratio of weight (kg) to the square of height (m). Blood samples were drawn after 12 h of fasting, once after the weekend. Serum albumin, CRP, creatinine, calcium, phosphate, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, hemoglobin, and white blood cell counts were measured by using standard methods in a routine biochemistry laboratory. Serum samples that were separated for ADMA measurement were stored at -70°C for a short period of time, and the tests were performed by the enzyme-linked immunosorbent assay (ELISA) method (ADMA ELISA Kit, Immundiagnostik, Bensheim, Germany).

2.1. Statistical analysis

Data were presented as means \pm standard deviations. Comparisons of numeric variables between groups were performed by Student's t-test or the Mann-Whitney U

test, as appropriate. Categorical variables were compared using the chi-squared test and were shown as frequency and percentages. Univariate correlation was established by using either Pearson's or Spearman's correlation coefficient. All statistical calculations were performed using SPSS 13. All P-values were calculated as 2-sided, and $P < 0.05$ was considered significant.

3. Results

The present study involved 49 prevalent HD patients and 22 age- and sex-matched controls. Mean age was 55.3 ± 14.2 years for HD patients and 49.3 ± 15.0 years for the control group ($P = 0.11$). The patients were on HD treatment for 64.1 ± 4.5 months on average. Primary causes of ESRD were diabetic nephropathy ($n = 21, 42.8\%$), hypertension ($n = 14, 28.6\%$), chronic glomerulonephritis ($n = 8, 16.3\%$), and unknown etiology ($n = 6, 12.2\%$). The anthropometric, demographic, and biochemical parameters as compared between the control group and the HD patients are given in Table 1. HD patients had significantly higher ADMA levels compared with healthy controls (0.51 ± 0.25 vs. 0.35 ± 0.15 , $P = 0.002$). While white blood cell counts and BMI values were similar between the 2 groups, CRP and LDL cholesterol were found to be significantly higher and albumin and HDL cholesterol significantly lower in HD patients compared with healthy controls.

ADMA concentrations were positively correlated with mean age ($P = 0.02$, $r = 0.360$), LDL cholesterol levels ($P = 0.006$, $r = 0.325$), and serum CRP levels ($P = 0.02$, $r = 0.268$). Additionally, there was a significant negative correlation between ADMA concentrations and serum albumin levels ($P = 0.005$, $r = -0.331$). Table 2 shows the correlations of ADMA with clinical and laboratory parameters and the Figure illustrates these correlations.

4. Discussion

In our study, serum ADMA levels of chronic HD patients were found to be significantly higher than those of healthy controls. This result is consistent with the previous similar studies in the literature regarding ADMA levels in ESRD patients (9,14,15). Circulating levels of ADMA had a positive correlation with levels of CRP. This finding might suggest that a connection exists between endothelial dysfunction and inflammation in ESRD patients. Recently some studies pointed out the association of ADMA levels with biomarkers of inflammation in some chronic conditions like familial Mediterranean fever, inflammatory bowel disease, and chronic kidney disease (16–18).

Additionally, serum ADMA levels were inversely correlated with serum albumin levels in the present study. As serum albumin level is a marker of nutritional status, we think that this result suggests an association between nutrition and inflammation in ESRD patients. This result

Table 1. Baseline characteristics and laboratory results of study population.

Variable	HD patients (n = 49)	Healthy controls (n = 22)	P-value
Mean age (years)	55.3 ± 14.2	49.3 ± 15.0	0.11
Sex (male/female)	26/23	7/15	0.09
Body mass index (kg/m ²)	24.7 ± 3.8	25.6 ± 3.6	0.32
Time on dialysis (months)	64.1 ± 4.5	-	-
Creatinine (mg/dL)	7.50 ± 1.78	0.79 ± 0.25	<0.001
Albumin (g/dL)	3.6 ± 0.3	4.2 ± 0.3	<0.001
Hemoglobin (g/dL)	10.8 ± 1.5	13.4 ± 1.9	<0.001
WBC (U/L)	6810 ± 2019	6787 ± 1384	0.96
LDL cholesterol (mg/dL)	147.7 ± 24.3	132.8 ± 23.7	0.02
HDL cholesterol(mg/dL)	31.1 ± 6.2	46.1 ± 13.4	<0.001
ADMA (µmol/L)	0.51 ± 0.25	0.35 ± 0.15	0.002
CRP (mg/dL)	15.2 ± 17.2	2.7 ± 1.2	<0.001

HD: Hemodialysis, WBC: white blood cell count, LDL: low-density lipoprotein, HDL: high-density lipoprotein, ADMA: asymmetric dimethyl arginine, CRP: C-reactive protein.

Table 2. Correlations of ADMA with clinical and laboratory parameters.

Variable	P-value	r
Mean age	0.02	0.360
Body mass index (kg/m ²)	0.64	0.056
Time on dialysis (months)	0.67	-0.062
Creatinine (mg/dL)	0.07	0.210
Albumin (g/dL)	0.005	-0.331
Hemoglobin (g/dL)	0.06	-0.220
WBC (U/L)	0.17	-0.162
LDL cholesterol (mg/dL)	0.006	0.325
HDL cholesterol(mg/dL)	0.10	-0.197
CRP (mg/dL)	0.02	0.268

ADMA: Asymmetric dimethyl arginine, WBC: white blood cell count, LDL: low-density lipoprotein, HDL: high-density lipoprotein, CRP: C-reactive protein.

is consistent with the findings of a recent trial studying nutritional status in HD patients (13). Unlike the results of similar studies, we could not find any significant relation between ADMA and BMI (13,19). This may be due to the small number of patients in our study.

Findings on the relation between circulating ADMA concentrations and serum cholesterol levels are conflicting. In one study, ADMA levels were found to be higher in patients with metabolic syndrome compared with normal subjects, but no correlation was found between ADMA and cholesterol levels (20). In another study of ESRD patients, ADMA showed significant correlation with serum cholesterol levels (19). Consistent with the latter report, in our study ADMA showed a positive correlation with serum LDL cholesterol levels. LDL cholesterol may have a role in the pathway of the atherosclerotic process of ADMA.

It is reported that there is a positive and significant correlation between ADMA levels and carotid intima media thickness in chronic HD patients (21). In another study, inflammation and endothelial dysfunction as assessed by circulating levels of ADMA were found to be an independent factor for explaining all-cause mortality and cardiovascular events in ESRD patients (18). Similar

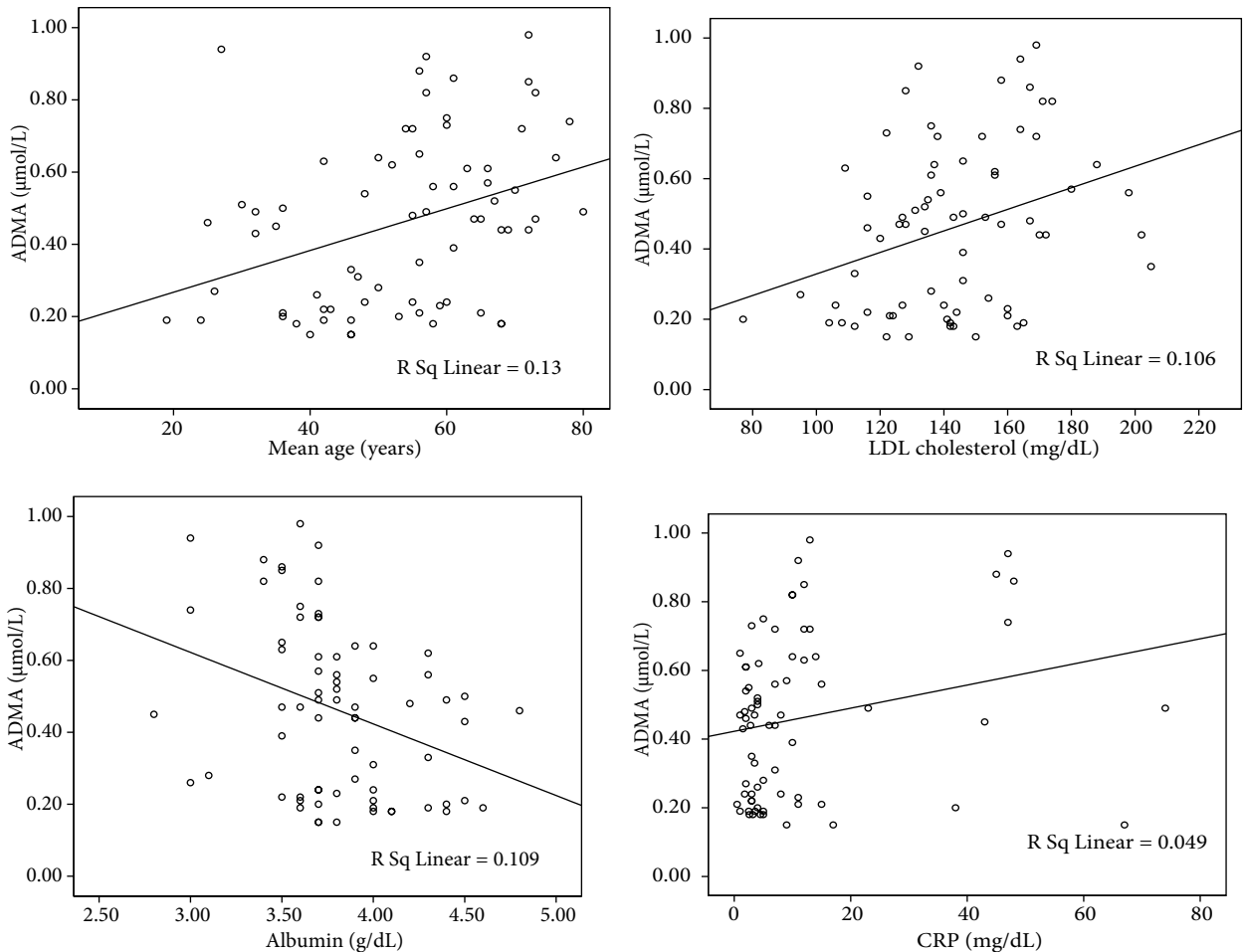


Figure. Correlations of ADMA levels with LDL cholesterol, mean age, albumin, and CRP level in the whole study population.

to this finding, a recent study also reported that ADMA level is a death predictor in ESRD patients (22). These results strengthen the hypothesis that interventions aimed at preventing endothelial dysfunction and inflammation may decrease the high cardiovascular risk for these patients. However, it is still unknown whether lowering the ADMA levels would be of any clinical benefit.

The limitations of our study are the cross-sectional design and small sample size. Additionally, some anthropometric measurements and clinical parameters

representing nutritional status could be added to the study design. A prospective study with a larger sample size would be more helpful for this issue.

In conclusion, in the present study we have found significantly higher serum ADMA levels in chronic HD patients compared with healthy individuals. Our results suggest that ADMA might be a potential novel biomarker of inflammation and atherosclerosis in patients on chronic HD. Measuring ADMA levels may promote awareness of early cardiovascular risk increase in HD patients.

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