Therapeutic Apheresis and Dialysis

Therapeutic Apheresis and Dialysis 2018; 22(2):109–117
doi: 10.1111/1744-9987.12613

Relation of Serum ADMA, Apelin-13 and LOX-1 Levels with Inflammatory and Echocardiographic Parameters in Hemodialysis Patients

Ibrahim Dogan,[1] Tolga Dogan,[2] Mucahit Yetim,[2] Huseyin Kayadibi,[3] Mehmet B Yilmaz,[4] Baris Eser,[1] Macit Kalcik[2], and Yusuf Karavelioglu[2]

[1]Nephrology Department, [2]Cardiology Department, [3]Biochemistry Department, Hitit University Corum Training and Research Hospital, Corum, and [4]Biochemistry Department, Cukurova University Medical Faculty, Adana, Turkey

Abstract: Cardiovascular diseases are the leading causes of mortality in patients with chronic kidney disease. Nitric oxide has a critical role in both endothelial dysfunction and the atherosclerosis process. We aimed to investigate the relationships between serum asymmetric dimethyl arginine (ADMA), LOX-1, and Apelin-13 levels, which are known to act over nitric oxide with endothelial dysfunction and cardiac morphology as well as with each other in hemodialysis patients. The study comprised a total of 120 patients (53 females and 67 males) receiving hemodialysis three times a week for at least 6 months and an age-gender matched control group (55 females and 58 males). Serum ADMA, LOX-1, and Apelin-13 levels were measured using the ELISA technique. Echocardiography, 24-h blood pressure monitoring by the Holter and carotid artery intima-media thickness (CIMT) measurement was performed on all of the included subjects. The associations between serum ADMA, LOX-1, and Apelin-13 levels with CIMT, echocardiographic parameters [left ventricular mass (LVM) and left ventricular mass index (LVMI)], and inflammatory markers [high sensitive C-reactive protein (hsCRP) and neutrophil lymphocyte ratio (NLR)] were evaluated by correlation analysis. Serum ADMA, Apelin-13, and LOX-1 levels were significantly higher in the hemodialysis group than the controls ($P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively). CIMT, hsCRP, and NLR levels were also significantly higher in the hemodialysis group ($P < 0.05$, $P < 0.001$, $P < 0.001$, respectively). Significant correlations were observed among the serum ADMA, Apelin-13, and LOX-1 levels. Moreover, notably positive correlations were found between these three biochemical markers and LVM, LVMI, hsCRP, and CIMT. Serum ADMA, Apelin-13, and LOX-1 levels can be indicators not only for the inflammatory process but also for the pathogenesis of cardiovascular diseases in hemodialysis patients. **Key Words:** Apelin-13, Asymmetric dimethyl arginine, Endothelial dysfunction, Hemodialysis, LOX-1.

Cardiovascular diseases are the leading cause of mortality in patients with chronic kidney disease (CKD) (1). The risk of cardiovascular diseases increases linearly until the glomerular filtration rate (GFR) decreases below the 15 mL/min mark. Cardiovascular mortality rate is 7–8 times higher in patients with end-stage renal disease (ESRD) than in a normal population (2). This increased risk is associated not only with the traditional cardiovascular risk factors but also with the CKD-specific hypervolemia, left ventricular hypertrophy (LVH), heart failure, anemia, high calcium-phosphorus metabolism disorder, hyperhomocysteinemia, and endothelial dysfunction (ED), which occurs due to elevated nitric oxide (NO) synthesis inhibitors (3,4).

It was observed that asymmetric dimethyl arginine (ADMA), which inhibits the synthesis of NO known as anti-atherogenic molecule, is increased in CKD. Increased ADMA may also be an inaccurate prognostic marker for renal diseases (5). Moreover, a relationship was reported between elevated ADMA and the risk of cardiovascular events in patients with ESRD (6). The relationship of ADMA with flow-mediated dilation (FMD), which is the indicator of ED, hyperlipidemia, and insulin resistance was also demonstrated (7).

Received May 2017; revised August 2017; accepted August 2017.
Address correspondence and reprint requests to Dr Yusuf Karavelioglu, Hitit University Corum Training and Research Hospital, Department of Cardiology, Corum, TR 19100, Turkey. Email: drcomtr@gmail.com

The other molecule that functions over NO is the oxidized low-density lipoprotein (ox-LDL), which is considered among the risk factors of atherosclerosis. It was demonstrated that ox-LDL causes monocyte entry into the endothelium, endothelial cell dysfunction, and additionally plays a role in the transformation of macrophages into the foam cells in the atherosclerotic plaque. Ox-LDL leads to ED by inhibiting NO production (8). Lectin-like oxidized LDL receptor-1(LOX-1), the ox-LDL receptor, is responsible for the entry of ox-LDL into the endothelial cells and for its numerous effects. Interaction between LOX-1 and ox-LDL causes proinflammatory activation and foam cell formation, as well as decreased intracellular NO concentration. This process ends with atherosclerosis and CV events (9). Increased LOX-1 level was also found in hemodialysis (HD) patients and reported after the inhibition of NO synthesis by ADMA (10). It was determined that deposition of ADMA in CKD patients increases the LOX-1 receptor count and may cause foam cell formation by increasing ox-LDL concentrations (11). In addition, it has also been suggested that LOX-1 might be playing a role in the pathophysiology of cardiorenal syndrome in CKD (12).

Another NO-associated molecule, Apelin-13, plays a critical role in cardiovascular, gastrointestinal, immune systems, and fluid hemostasis. Apelin-13 is effective in the development of both endothelium-dependent NO-associated vasodilation and endothelium-independent vasoconstriction (13). Serum Apelin level was found to be significantly decreased in the HD patients with non-diabetic coronary artery disease (CAD) (14). It has been postulated that Apelin may be the target molecule in slowing down the atherosclerotic process in pediatric HD patients (15).

The concentrations of these molecules and their effects on cardiac functions have been subjects of interest. The present study aimed to determine the relationships between serum ADMA, LOX-1, and Apelin-13 levels with each other and their effects on ED and cardiac morphology in HD patients.

PATIENTS AND METHODS

A total of 120 patients (53 females; mean age of 57 $\pm$ 13.6 years) receiving HD three times a week for at least 6 months, and 113 controls (55 females; mean age of 56 $\pm$ 11.3 years) were enrolled in this study.

The study was initiated after obtaining the approval of Erciyes University, Kayseri, Local Ethics Committee (Ethics committee no: 215/292 and date: 5 June 2015) and written informed consents of the participants.

Patients with an average Kt/V value of less than 1.2 in the last 6 months, patients with an active infection, malignancy, coronary heart disease, cerebrovascular disease, decompensated liver or heart disease or having a history of surgery, burns or trauma within the last 1 month were excluded from the study.

Duration of hemodialysis therapy and smoking status were recorded for each patient. All patients had been receiving standard HD therapy with HCO_3 (Na: 138–140 mmol/L, K: 1.5 mmol/L, Ca: 1.25–1.50 mmol/L, HCO_3: 32–35 mmol/L, and low-medium flux 1.4–2.1 m^2 dialyzer) for 4 h three times a week. A-V fistula was the route of HD in all patients. Patients with A-V grafts and catheters were not included. All patients were clinically euvolemic during the study enrollment. System examinations revealed no signs of congestion (dyspnea, increased jugular pressure, rales in the basal segments of the lungs), pretibial edema, or abdominal ascites. The ejection fraction was (EF) >35% in all patients. Concurrent medications that the patients have been receiving (ACE inhibitors, angiotensin receptor blockers (ARB), aldosterone antagonists, calcium channel blockers (CCB), alpha and beta blockers (BB), nitrates, statins, digoxin, antiplatelet agents, phosphate binders, erythropoiesis-stimulating agents (ESA), vitamin D preparations, cinacalcet, anticoagulants, antidiabetics, vitamin preparations) were recorded. The mean value of the 24-h blood pressure monitoring by Holter analyses was recorded as the BP value of each patient. Body Mass Index of each patient was calculated using the following formula: body weight (kg)/height^2 (m^2).

Laboratory analysis

Blood samples were obtained from the antecubital vein after fasting for a 12-h session in the morning of a weekday without dialysis. The samples were centrifuged at 4000 $\times$ *g* for 5 min and used to analyze the laboratory parameters [sodium (Na), potassium (K), calcium (Ca), phosphorus, glucose, blood urea nitrogen (BUN), creatinine (Cr), uric acid, total cholesterol, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), triglycerides (TG), albumin, total protein, alkaline phosphatase, parathyroid hormone (PTH), ferritin, high-sensitivity C-reactive protein (hsCRP)]. Blood samples collected to analyze ADMA, Apelin-13, and LOX-1 were stored at –80°C immediately

after centrifugation. Complete blood count was performed by the K2EDTA containing tubes. The ratio of neutrophils to lymphocytes (NLR) was then calculated.

Serum ADMA levels were measured by enzyme-linked immunosorbent assay (ELISA) method (Cat. No: YHB0416Hu, Shanghai YeHua Biological Technology Co., Ltd., Shanghai, China). Intra-assay and inter-assay coefficient of variations (CV) were <10% and <12%, respectively. The range of test was between 200 ng/L and 60 000 ng/L with a sensitivity of 100.21 ng/L.

Serum Apelin-13 level was measured by ELISA method (Cat. No. YHB0364Hu, Shanghai YeHua Biological Technology Co.). Intra-assay and inter-assay CVs were <10% and <12%, respectively. The test range was between 0.5 ng/L and 200 ng/L with a sensitivity of 0.27 ng/L. Samples of 12 patients exceeding 200 ng/L, the upper limit of the test range, were reanalyzed after diluting by one-third.

Serum LOX-1 levels were measured by ELISA method (Cat. No: YHB2228Hu, Shanghai YeHua Biological Technology Co.). Intra-assay and inter-assay CVs were <10% and <12%, respectively. The test range was between 40 ng/L and 10 000 ng/L with a sensitivity of 20.02 ng/L.

Cardiac assessment

Echocardiography was performed in all participants by Acuson-Siemens (Sequoia-C256) device using 3.5 MHz transducer. The patients were examined in the left lateral decubitus position in accordance with the standards of American Society of Echocardiography. Standard images were obtained for M-mode and cross-sectional examinations. The left ventricle ejection fraction was measured using biplane Simpson's and Teicholz methods. As the eligibility criteria, patients with good quality echocardiographic images were included in the study. Ejection fraction (EF) was higher than 35% in all patients. Left ventricle posterior wall thickness (PWT), interventricular septum thickness (IVST), left atrium diameter (LAD), left ventricle end-systolic diameter (LVESD) and left ventricle end-diastolic diameter (LVEDD) were measured in all patients. Left ventricular mass (LVM) was calculated by the Devereux formula [left ventricular mass = 0.8 (1.04 (interventricular septum thickness + left ventricle end-diastolic diameter + posterior wall thickness)^3 – (left ventricle end-diastolic diameter)^3) + 0.6] and the body surface area was calculated by the Mosteller formula [body surface area = (body height (cm) × body weight (kg)/3600)$^{1/2}$]. Left ventricular mass index (LVMI) was calculated dividing the left ventricle mass by the body surface area.

The carotid artery intima media thickness (CIMT) was measured by the same device [Toshiba SSA – 240 Ultrasound (Toshiba, Tokyo, Japan)] in all participants using the 7.5 MHz linear array transducer. The measurement was performed bilaterally through the 1 cm proximal of the bifurcation of the two main carotid arteries while the patient was in a supine position with the head in slight extension. Three measurements were obtained when the intima layer was seen in the anterior and posterior walls, and the arithmetical mean of these three random measurements was used. Presence of atherosclerotic plaque was recorded.

Twenty four-hour ambulatory blood pressure monitoring were performed by the device Mobil-O-Graph PWA, I.E.M. GmbH, Stolberg, Germany. The appropriate cuff size was chosen for each patient. Blood pressure measurements were performed every 15 min during the daytime (between 06:00 and 22:00) and every 30 min at night (between 22:00 and 06:00). A daytime mean systolic BP >135 mm Hg and/or diastolic BP >85 mm Hg, and a night time mean systolic BP >120 mm Hg and/or diastolic BP >70 mm Hg were considered hypertension.

Statistical analysis

SPSS for Windows 15.0 package program (Chicago, IL, USA) was used for the statistical analyses. Normality distribution of the variables was analyzed by the Shapiro–Wilk test. The variables distributed normally were presented as mean ± standard deviation, whereas the variables not distributed normally were presented as a median (25th–75th interquartile range). For normally distributed variables, comparisons between the two independent groups were performed using the Student's *t*-test. For the variables not distributed normally, comparison of the two groups was performed using the Mann–Whitney *U*-Test. Categorical variables were compared using the χ^2 test. The relationship between the variables was evaluated with Spearman's or Pearson's correlation analysis as appropriate. All of the reported *P*-values were two-tailed, and those less than 0.05 were considered to be statistically significant.

RESULTS

Efficiency of the patients' hemodialysis therapy was evaluated by calculating the mean Kt/V as 1.67 ± 0.33. CKD occurred due to HT in 55, DM in 31, chronic glomerulonephritis in 16, polycystic kidney disease in eight, urological reasons in five, amyloidosis in three patients, and idiopathic in two patients. The patients were asked for the presence of DM, HT, peripheral artery disease (PAD), coronary artery disease (CAD), heart failure, arrhythmia, lung disease (chronic obstructive pulmonary disease, bronchial asthma, asbestosis, occupational lung disease), cerebrovascular disease (transient ischemic attack, stroke, etc.), and endocrine disease. Demographic characteristics, medications and co-morbidities of the study groups are shown in Table 1.

Systolic and diastolic blood pressures of the HD group were significantly higher than those of the controls ($P < 0.001$ and $P < 0.01$, respectively). Body mass index was higher in the control group as compared with the HD group ($P < 0.001$). There was no significant difference between the groups in terms of smoking habit.

Comparison of the biochemical data between the patient and the control groups is demonstrated in Table 2. The hemoglobin (Hb) and hematocrit (Hct) levels were lower but ferritin and transferrin saturations were higher in the patient group as compared to the control group ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.01$, respectively). There was no significant difference between the groups in terms of uric acid, glucose and total protein values. Na, Ca, albumin, and HCO_3 values were significantly lower ($P < 0.001$, $P < 0.001$, $P < 0,001$, $P < 0.001$, respectively), whereas K, phosphorus, and PTH values were significantly higher ($P < 0.001$, $P < 0.001$, $P < 0.001$, respectively) in the patient group.

Total cholesterol, LDL-C, and HDL-C were lower but triglyceride level was higher in the patient group ($P < 0.001$, $P < 0.001$, $P < 0.01$, $P < 0.01$, respectively). NLR and hsCRP, which are the inflammatory markers, were higher in the patient group ($P < 0.001$, $P < 0.001$, respectively). CIMT was higher in the hemodialysis group ($P < 0.05$).

ADMA, Apelin-13, and LOX-1 levels were found to be significantly higher in the patient than the control group ($P < 0.001$, $P < 0.001$, $P < 0.001$, respectively) (Table 2).

Echocardiographic parameters in the patient and the control group are shown in Table 3.

A comparison of the echocardiographic parameters between the patient and the control group revealed no difference in terms of LVESD and EF. LVEDD, LAD, IVST, PWT, LVM, and LVMI were significantly higher in the patient group as compared with the control group ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, respectively).

A correlation of ADMA, Apelin-13 and LOX-1 with echocardiographic parameters is demonstrated in Table 4. A positive correlation was determined between ADMA and IVST, LVM, LVMI; between Apelin-13 and LVEDD, LAD, IVST, LVM, LVMI;

TABLE 1. *The demographic characteristics, medications and co-morbidities of the studied population*

	HD group (n = 120)	Controls (n = 113)	P-value
Age (years)		56 ± 11.3	0.564
Gender (female/male)	57 ± 13.6	55/58	0.491
BMI (kg/m^2)	26.2 ± 4.74	28.6 ± 4.32	<0.001
SBP (mm Hg)	134 ± 24	122 ± 12	<0.001
DBP (mm Hg)	83 ± 16	78 ± 10	<0.01
Smoking status, n (%)	14 (11.7)	11 (9.7)	0.677
Kt/V	1.67 ± 0.33		
HD duration (months)	55.5 (22–97.5)	—	—
HT, n (%)	93 (77.5)	58 (51.3)	<0.001
DM, n (%)	37 (30.8)	16 (14.2)	<0.01
CAD, n (%)	76 (63.3)	7 (6.2)	<0.001
PAD, n (%)	14 (11.7)	0 (0)	<0.001
PD, n (%)	10 (8.3)	0 (0)	<0.001
CVD, n (%)	4 (3.3)	0 (0)	<0.001
Vitamin D, n (%)	95 (79.2)	0 (0)	<0.001
Cinacalcet, n (%)	11 (9.2)	0 (0)	<0.001
Statin, n (%)	18 (15)	17 (15)	0.992
ACEi/ARB, n (%)	24 (20)	44 (38.9)	0.001
CCB, n (%)	62 (51.7)	24 (21.2)	<0.001
BB, n (%)	60 (50)	26 (23)	<0.001

ACEi/ARB, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers; BB, beta blockers; BMI, body mass index; CAD, coronary artery disease; CCB, calcium channel blockers; CVD, cerebrovascular disease; DBP, diastolic blood pressure; DM, diabetes mellitus; HT, hypertension; PAD, peripheral artery disease; PD, pulmonary diseases; SBP, systolic blood pressure.

TABLE 2. *Comparison of laboratory parameters between HD group and the controls*

	HD group (N = 120)	Controls (N = 113)	P-value
BUN (mg/dL)	49 (41–62)	14 (11–17)	<0.001
Cr (mg/dL)	6.82 (5.7–8.0)	0.73 (0.62–0.9)	<0.001
GFR (mL/min/1.73 m^2)	7.9 (5.7–8.1)	100 (86–119)	<0.001
Glucose (mg/dL)	114.7 ± 55	110 ± 34	0.436
Uric acid (mg/dL)	4.87 ± 1.36	4.99 ± 1.32	0.477
Hb (g/dL)	12.6 (11.6–13.6)	14.2 (13.1–15.3)	<0.001
Hct (%)	39.5 (36.0–42.5)	43.3 (40.1–46.1)	<0.001
Na (mEq/L)	137 (136–139)	140 (138.5–141)	<0.001
K (mEq/L)	4.95 (4.5–5.5)	4.38 (4.2–4.6)	<0.001
Ca (mg/dL)	9.0 (8.4–9.6)	9.4 (9.1–9.6)	<0.001
Phosphorus (mg/dL)	4.4 (3.8–5.3)	3.3 (3.0–3.7)	<0.001
Total cholesterol (mg/dL)	179 (147–214)	195 (177–235)	<0.001
HDL-C (mg/dL)	40 ± 12	44 ± 13	<0.01
LDL-C (mg/dL)	100 (79–127)	123 (108–155)	<0.001
TG (mg/dL)	155 (111–256)	120 (80–192)	<0.01
Total protein (g/dL)	7.4 (6.8–7.8)	7.3 (7–7.7)	0.781
Albumin (g/dL)	4.1 (3.9–4.3)	4.4 (4.2–4.6)	<0.001
Ferritin (ng/mL)	853 (529–1099)	55 (29–116)	<0.001
Transferrin saturation (%)	27 (20–35)	22 (16–30)	<0.01
HCO_3 (mmol/L)	22.9 (21.36–24.6)	24.0 (23.2–24.8)	<0.001
PTH (pg/mL)	349 (206–539)	49 (36–65)	<0.001
hsCRP (mg/dL)	7 (4.0–12.2)	3.6 (2.2–5.7)	<0.001
NLR	3.07 (2.45–4.44)	2.03 (1.66–2.63)	<0.001
CIMT (mm)	0.71 (0.60–0.85)	0.65 (0.55–0.75)	<0.05
ADMA (ng/L)	39 871 (16473–52 113)	11 992 (8552–18 444)	<0.001
Apelin-13 (ng/L)	176 (103–199)	36 (17–77)	<0.001
LOX-1 (ng/L)	5808 (3413–7529)	2137 (993–3920)	<0.001

ADMA, asymmetric dimethyl arginine; BUN, blood urea nitrogen; CIMT, carotid artery intima media thickness; GFR, glomerular filtration rate; HDL-C, high density lipoprotein; hsCRP, high sensitive C-reactive protein; LDL-C, low density lipoprotein; LOX-1, lectin-like oxidized LDL receptor-1; NLR, ratio of absolute neutrophil count to absolute lymphocyte count; PTH, parathyroid hormone; TG, triglycerides.

TABLE 3. *Comparison of echocardiographic parameters between study groups*

	HD group (N = 120)	Controls (N = 113)	P-value
LVESD (mm)	32 (27–35)	30 (26–33)	0.037
LVEDD (mm)	48 (44–53)	44 (41–48)	<0.001
EF (%)	60 ± 7.6	63 ± 5.1	0.058
LAD (mm)	36 (32–40)	32 (30–35)	<0.001
IVST (mm)	12 (10–13)	11 (9–12)	<0.001
PWT (mm)	12 (10–13)	10 (9–11)	<0.001
LVM (g)	212 (166–264)	147 (102–171)	<0.001
LVMI (g/m^2)	123 (95–146)	75 (63–93)	<0.001

EF, ejection fraction; IVC, inferior vena cava diameter; IVST, interventricular septum thickness; LAD, left atrium diameter; LVEDD, left ventricle end-diastolic diameter; LVESD, left ventricle end-systolic diameter; LVM, left ventricular mass; LVMI, left ventricular mass index; PWT, left ventricle posterior wall thickness.

TABLE 4. *The results of correlation analyses observed between ADMA, Apelin-13 and LOX-1, and echocardiographic parameters*

	ADMA	Apelin-13	LOX-1
LVEDD	r = 0.118	r = 0.203	r = 0.139
	P = 0.086	P < 0.05	P < 0.05
EF	r = –0.029	r = –0.035	r = –0.059
	P = 0.671	P = 0.607	P = 0.390
LAD	r = 0.134	r = 0.153	r = 0.172
	P = 0.050	P < 0.01	P < 0.05
IVST	r = 0.225	r = 0.220	r = 0.212
	P < 0.001	P < 0.01	P < 0.01
LVM	r = 0.285	r = 0.307	r = 246
	P < 0.001	P < 0.001	P < 0.001
LVMI	r = 0.330	r = 0.359	r = 0.296
	P < 0.001	P < 0.001	P < 0.001

EF, ejection fraction; IVC, inferior vena cava diameter; IVST, interventricular septum thickness; LAD, left atrium diameter; LVEDD, left ventricle end-diastolic diameter; LVESD, left ventricle end-systolic diameter; LVM, left ventricular mass; LVMI, left ventricular mass index.

and between LOX-1 and LVEDD, LAD, IVST, LVM, LVMI.

A correlation of serum ADMA, Apelin-13 and LOX-1 levels with each other is demonstrated in Table 5. A positive correlation was determined.

A significant correlation was determined between ADMA and TG; between Apelin-13 and LDL and TG; and between LOX-1 and TG. Significant correlations were also determined among ADMA, Apelin-13, LOX-1, hsCRP, and NLR. While there was a significant correlation between ADMA and CIMT, no correlation was determined between Apelin-13, LOX-1, and CIMT. Moreover, there was a correlation between the presence of atherosclerotic plaque and Apelin-13, whereas a slightly less significant

correlation was determined between ADMA, LOX-1 and presence of plaque (Table 5).

No correlation was determined between ADMA, Apelin-13, LOX-1 and Ca, albumin, glucose, and HCO_3. There were negative correlations between these three markers and Hb and GFR, whereas significantly positive correlations were determined with phosphorus, ferritin, PTH, HD duration and SBP. In addition, a negative correlation was determined between ADMA and BMI but a positive correlation was determined with DBP. Apelin-13 showed a negative correlation with BMI but a positive correlation with transferrin saturation. A positive correlation was determined between LOX-1, transferrin saturation, and SBP (Table 6).

DISCUSSION

The present study revealed higher serum ADMA, Apelin-13, and LOX-1 levels in HD patients as compared to the control group with normal renal functions. Moreover, these markers showed significantly positive strong correlations both with each other and with renal function, HD duration, inflammation, anemia, high phosphorus levels, and left ventricular hypertrophy.

Since cardiovascular diseases are the leading causes of mortality in CKD patients, many studies are being conducted to investigate the molecules that may be the cardiovascular disease biomarkers and their impacts on cardiac events. The relationship of these biomarkers with atherosclerosis, ED and cardiac functions, and morphology are being scrutinized. NO is one of the molecules with the most critical role in endothelial functions. ADMA, Apelin-13, and LOX-1 are also biomarkers that have an impact on NO.

Consistent with the literature, the present study observed serum ADMA level 3.3 times higher in HD patients. Earlier studies have emphasized that elevated serum ADMA is associated with decreased renal excretion due to the decreased GFR, increased binding to proteins, and inadequate removal with dialysis due to the redistribution of ADMA during HD (16). In the present study as well, serum ADMA concentration was high although it was measured after HD. Again, in another study, significant correlation was determined between serum ADMA, serum creatinine, and GFR levels as was in the present study (17).

The literature comprises numerous studies investigating the relation of ADMA, which is a NO inhibitor, with ED and atherosclerosis. Experimental studies have demonstrated the relationship between the chronic ADMA increase and development of atherosclerosis and nephropathy (18). In addition, elevated ADMA concentrations were found to be associated with cardiovascular diseases and all-cause mortality in ESRD (19) and non-diabetic CKD patients (5).

TABLE 5. *The results of correlation analyses observed between ADMA, Apelin-13, LOX-1, lipids, and inflammatory parameters*

	ADMA	Apelin-13	LOX-1
ADMA	1	r = 0.805 $P \leq 0.001$	r = 0.858 $P < 0.001$
Apelin-13	r = 0.805 $P \leq 0.001$	1	r = 0.891 $P < 0.001$
LOX-1	r = 0.858 $P < 0.001$	r = 0.891 $P < 0.001$	1
LDL	r = –0.127 $P = 0.051$	r = –0.151 $P < 0.05$	r = –0.106 $P = 0.104$
TG	r = 0.149 $P < 0.05$	r = 0.226 $P < 0.001$	r = 0.197 $P < 0.01$
hsCRP	r = 0.252 $P < 0.001$	r = 0.277 $P < 0.001$	r = 0.208 $P < 0.01$
NLR	r = 0.259 $P < 0.001$	r = 0.240 $P < 0.001$	r = 0.207 $P < 0.01$
CIMT	r = 0.153 $P < 0.05$	r = 0.094 $P = 0.176$	r = 0.132 $P = 0.056$
Plaque	r = 0.131 $P = 0.059$	r = 0.151 $P < 0.05$	r = 0.131 $P = 0.059$

CIMT, carotid artery intima media thickness; HDL-c, high density lipoprotein; hsCRP, high sensitivec c-reactive protein; LDL-c, low density lipoprotein; NLR, absolute neutrophil count to absolute lymphocyte count; Plaque, carotid artery atherosclerotic plaque; TG, triglyceride.

TABLE 6. *The results of correlation analyses observed between ADMA, Apelin-13, LOX-1, blood pressures, and laboratory parameters*

	ADMA	Apelin-13	LOX-1
GFR	r = –0.489 $P < 0.001$	r = –0.533 $P < 0.001$	r = –0.0471 $P < 0.001$
P	r = 0.0369 $P < 0.001$	r = 0.414 $P < 0.01$	r = 0.407 $P < 0.001$
Glucose	r = 0.065 $P = 0.320$	r = –0.000 $P = 0.989$	r = 0.077 $P = 0.240$
Hb	r = –0.154 $P < 0.05$	r = –0.218 $P < 0.001$	r = –0.157 $P < 0.05$
Ferritin	r = 0.420 $P < 0.001$	r = 0.523 $P < 0.001$	r = 0.417 $P < 0.001$
PTH	r = 0.418 $P < 0.001$	r = 0.475 $P < 0.001$	r = 0.398 $P < 0.001$
SBP	r = 0.213 $P < 0.05$	r = 0.178 $P < 0.05$	r = 0.163 $P < 0.05$
DBP	r = 0.154 $P < 0.05$	r = 0.090 $P = 0.234$	r = 0.056 $P = 0.459$
HD duration	r = 0.195 $P < 0.05$	r = 0.272 $P < 0.01$	r = 0.303 $P < 0.01$

BMI, body mass index; DBP, diastolic blood pressure; GFR, glomerular filtration rate; Mean BP, mean blood pressure; SBP, systolic blood pressure.

Increased CIMT has been demonstrated to be a marker of prognostic value for cardiovascular diseases (20). In a population-based study, the relationship between CIMT and ADMA was demonstrated at the end of a 6-year follow-up (20). A study carried out in type 2 DM patients demonstrated a relationship between high serum ADMA levels, development of cardiovascular events, and increased CIMT after a 5-year follow-up period (21). In the present study as well, CIMT was found to be high in hemodialysis patient group, and consistent with the literature. In addition, a positive correlation was determined between CIMT and ADMA.

The present study determined no relationship between serum ADMA levels and age, gender, and LDL levels but found a significant relationship with smoking and blood pressure, which are among the traditional cardiovascular risk factors. In hemodialysis patients, many chronic renal failure-specific conditions, as well as traditional cardiovascular risk factors, are considered as risk factors for atherosclerosis. Hyperparathyroidism and hyperphosphatemia, which are also defined as atherosclerotic risk factors in CKD patients, contribute to ED and atherosclerotic disease (22). In the present study, a positive correlation was demonstrated among hyperparathyroidism, hyperphosphatemia and serum ADMA level.

Inflammation, which is a significant cause of morbidity and mortality in CKD, leads to vascular injury via various pathophysiological pathways, and contributes to the development of atherosclerosis. The relationship between CRP and cardiovascular events, and increased risk of mortality has been demonstrated in ESRD patients (23). In the present study, the correlation analysis between hsCRP, NLR (24), and serum ADMA level revealed significantly positive correlation with hsCRP and NLR in HD patients.

Nitric oxide is a critical molecule in preventing cardiac remodeling. In addition, it was demonstrated that ADMA causes cardiac hypertrophy via NO inhibition and results in myocardial fibrosis by activating fibroblast growth factor receptors in the cardiomyocytes (25). Napora et al. conducted a study in HD patients and determined that high serum ADMA levels are correlated with LVM and LVMI but not with systolic or diastolic functions (26). Various studies determined a relationship between serum ADMA levels and LVH, LVM, and systolic dysfunction in the patients with CKD (27,28). In the present study as well, significant correlations were determined between ADMA and LVM, LVMI, but not systolic functions.

Apelin and ADMA are two molecules that inversely effect the NO production over NO synthase activity. However, contradictory outcomes have been obtained concerning Apelin-13 levels in those with CKD. In a study conducted in 159 HD patients, both ADMA and Apelin-13 levels were found significantly higher as compared with the healthy controls. Serum concentrations of both molecules decreased after HD therapy (29). In another study, serum Apelin-36 level of the HD patients was similar to healthy controls, but serum Apelin-12 level was higher in HD patients than in controls (30). Again, serum Apelin levels were determined to be lower in non-diabetic HD patients as compared with the healthy population (31). Moreover, no relationship was determined between serum Apelin-13 levels and GFR loss in Stage 3–5 CKD patients (32). In the present study, however, serum Apelin-13 levels were higher in the HD patients, and a negative correlation was determined with GFR.

In the present study, consistent with the literature, serum Apelin-13 level showed a positive correlation with smoking, hyperphosphatemia, hyperparathyroidism, hsCRP, and NLR (30). Additionally, a relationship was determined with the presence of plaque in the carotid artery. In a study, it was postulated that Apelin reduces vascular calcification in CKD patients, and accordingly could be the target molecule in the treatment of vascular calcification (33). Although the data of the present study suggest that Apelin-13 increases as a response to atherosclerotic factors such as inflammation, hyperparathyroidism and hypophosphatemia, a negative correlation with LDL-C and BMI is conflicting. The negative correlation between LDL-C, BMI, and ADMA may indicate that the effects of an unhealthy diet and malnutrition should be considered in HD patients. We think that investigating this topic in large population studies would be beneficial.

Outcomes of the studies investigating the relationship of Apelin with hypertension, LVH, and cardiac functions were also conflicting. In an experimental study of rats, Apelin-13 given after HT and ED created by ADMA decreased the blood pressure in the control group, but not in the CKD group (34). This suggests the probability of variations in the Apelin effect in renal dysfunction. In a study performed in 344 treatment-naive hypertensive patients, plasma Apelin levels were lower in the group with LVH versus without LVH (35). In contrast, the present study determined a positive

correlation between Apelin-13 and LVEDD, LAD, IVST, LVM, and LVMI, which reflects the changes in cardiac morphology. This supports the hypothesis that high serum Apelin-13 level might be associated with LVH in HD patients.

CKD is associated with oxidative stress, inflammation, and lipid metabolism dysregulation. Lipid deposition in the arterial wall with oxidative stress and inflammation in the presence of dyslipidemia is critical for the development of ED and atherosclerosis (36). The inflammatory process in CKD is triggered by the stimulation of LOX-1 by carbamylated-LDL (cLDL), which occurs due to urea-originated cyanate modification; this is thought to result in the increased cardiovascular events associated with ED and atherosclerosis (36). LOX-1 level, which is high in pro-atherogenic situations, was found to be high in HD patients in the present study, and this increment was in line with decreased GFR. In addition, significant correlations were determined with both CIMT and the presence of plaque and levels of hsCRP and NLR. Significantly positive relationships were determined with some of the traditional cardiovascular risk factors such as age, gender, BMI, and smoking.

It is known that LOX-1 is overexpressed in CKD due to the inhibition of NO production by ADMA and which triggers foam cell formation (11). In the present study, positive correlation was determined between ADMA and LOX-1 levels. LDL-C gains more electronegative character in early-stage CKD. This impairs cardiac relaxation by impairing calcium hemostasis via LOX-1 and accordingly, could be important in the pathogenesis of cardiorenal syndrome (12). The present study determined significantly positive correlations between LOX-1 and LVEDD, LAD, IVST, LVM, and LVMI. An evaluation of these outcomes suggests that LOX-1 has an impact on the left ventricle morphology in HD patients as a result of complex interaction between CV risk factors and uremic toxins in CKD.

Although these parameters usually are not studied for routine laboratory analyses in public hospitals, recent studies revealed that they are essential parameters for patient care. This study was conducted by purchasing testing kits for ADMA, Apelin-13, and LOX-1. However, we think that these parameters may be part of routine laboratory analyses in the future.

This cross-sectional study has several limitations. In the present study, cardiac functions, cardiac geometry, carotid artery thickness, and the changes in plaque structure over time were evaluated. However, since the biomarkers were measured once, the changes over time are unknown. Secondly, the limited number of patients and the lack of evaluations regarding racial differences are other limitations of this single-center study. Thirdly, due to the cross-sectional design of this study, only a numerical relationship could be determined between the data, and considerable attention should be paid for causality assessments.

CONCLUSIONS

Serum ADMA, Apelin-13, and LOX-1 levels were higher in hemodialysis patients as compared to the population with normal renal functions. The results suggest that these three biomarkers, as uremic toxins, might be indicative of a complex pathway in the inflammatory process and pathogenesis of cardiovascular diseases in HD patients.

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

Financial Support: None to declare.

REFERENCES

1. Matsushita K, van der Velde M, Astor BC et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet* 2010;375: 2073–81.
2. U.S. Renal Data System. USRDS. *Annual Data Report*. Bethesda: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2013.
3. Charytan D, Kuntz RE, Mauri L, DeFilippi C. Distribution of coronary artery disease and relation to mortality in asymptomatic hemodialysis patients. *Am J Kidney Dis* 2007;49:409–16.
4. Zoccali C. Traditional and emerging cardiovascular and renal risk factors: an epidemiologic perspective. *Kidney Int* 2006;70:26–33.
5. Ravani P, Tripepi G, Malberti F et al. Asymmetrical dimethylarginine predict progression to dialysis and death in patients with chronic kidney disease: a competing risks modeling approach. *J Am Soc Nephrol* 2005;16:2449–55.
6. Shafi T, Hostetter TH, Meyer TW et al. Serum asymmetric and symmetric Dimethylarginine and morbidity and mortality in hemodialysis patients. *Am J Kidney Dis* 2017;70:48–58.
7. Yilmaz MI, Sonmez A, Saglam M et al. ADMA levels correlate with proteinuria, secondary amyloidosis, and endothelial dysfunction. *J Am Soc Nephrol* 2008;19:388–95.
8. Vecchione L, Gargiu E, Borgiani P et al. Genotyping OLR1 gene: a genomic biomarker for cardiovascular diseases. *Recent Pat Cardiovasc Drug Discov* 2007;2:147–51.
9. Chistiakov DA, Orekhov AN, Bobryshev YV. LOX-1-mediated effects on vascular cells in atherosclerosis. *Cell Physiol Biochem* 2016;38:1851–9.
10. Van Tits L, De Graaf J, Hak-Lemmers H et al. Increased levels of low density lipoprotein oxidation in patients with familial hypercholesterolemia and in end-stage renal disease patients on Hemodiaysis. *Lab İnvest* 2003;83:13–21.

11. Smirnova IV, Kajstura M, Sawamura T, Goligorsky MS. Asymmetric dimethylarginine upregulates LOX-1 in activated macrophages: role in foam cell formation. *Am J Physiol Heart Circ Physiol* 2004;287:H782–90.
12. Chang KC, Lee AS, Chen WY et al. Increased LDL electronegativity in chronic kidney disease disrupts calcium homeostasis resulting in cardiac dysfunction. *J Mol Cell Cardiol* 2015;84:36–44.
13. Foldes G, Harkay F, Szokodi I. Circulating and cardiac levels of Apelin, the novel ligand of the orphan receptor APJ, in patients with heart failure. *Biochem Biophys Res Commun* 2003;308:480–5.
14. El-Wakil H, Abdel-Gawad H, Aboulenein F, Shaath EA, Abdel-Aziz HK. Cardiovascular risks in hemodialyzed patients: could Apelin have a role? *Bull Alex Fac Med* 2007;4:43.
15. Yavuz S, Cetinkaya S, Anarat A, Bayazıt AK. Apelin and nutritional status in children on dialysis. *Ren Fail* 2014;36:1233–8.
16. Kielstein JT, Boger RH, Bode-Boger SM et al. Low dialysance of asymmetric dimethylarginine in vivo and in vitro evidence of significant protein binding. *Clin Nephrol* 2004;62:295–300.
17. Fliser D, Kronenberg F, Kielstein JT et al. Asymmetric dimethylarginine and progression of chronic kidney disease: the mild to moderate kidney disease study. *J Am Soc Nephrol* 2005;16:2456–61.
18. Sitar ME. Asymmetric dimethylarginine and its relation as a biomarker in Nephrologic diseases. *Biomark Insights* 2016;11:131–7.
19. Lu TM, Ding YA, Lin SJ, Lee WS, Tai HC. Plasma levels of asymmetrical dimethylarginine and adverse cardiovascular events after percutaneous coronary intervention. *Eur Heart J* 2003;24:1912–9.
20. Chirinos JA, David R, Bralley JA et al. Endogenous nitric oxide synthase inhibitors, arterial hemodynamics, and subclinical vascular disease: the PREVENCION Study. *Hypertension* 2008;52:1051–9.
21. Konya H, Miuchi M, Satani K et al. Asymmetric dimethylarginine, a biomarker of cardiovascular complications in diabetes mellitus. *World J Exp Med* 2015;5:110–9.
22. Iijima K. Hyperphosphatemia and cardiovascular diseases: impact of vascular calcification and endothelial dysfunction. *Clin Calcium* 2012;22:1505–13.
23. Tripepi G, Mattace Raso F, Sijbrands E et al. Inflammation and asymmetric Dimethylarginine for predicting death and cardiovascular events in ESRD patients. *Clin J Am Soc Nephrol* 2011;6:1714–21.
24. Turkmen K, Ozcicek F, Ozcicek A, Akbas EM, Erdur FM, Tonbul HZ. The relationship between neutrophil-to-lymphocyte ratio and vascular calcification in end-stage renal disease patients. *Hemodial Int* 2014;18:47–53.
25. Luo Z, Teerlink T, Griedling K et al. Angiotensin II and NADPH oxidase increase ADMA in vascular smooth muscle cells. *Hypertension* 2010;56:498–504.
26. Napora M, Graczykowska A, Próchniewska K et al. Relationship between serum asymmetric dimethylarginine and left ventricular structure and function in patients with end stage renal disease treated with hemodialysis. *Pol Arch Med Wewn* 2012;122:226–34.
27. Bartnicki P, Kowalczyk M, Franczyk-Skóra B, Baj Z, Rysz J. Evaluation of endothelial (dys)function, left ventricular structure and function in patients with chronic kidney disease. *Curr Vasc Pharmacol* 2016;14:360–7.
28. Shi B, Ni Z, Zhou W et al. Circulating levels of asymmetric dimethylarginine are an independent risk factor for left ventricular hypertrophy and predict cardiovascular events in predialysis patients with chronic kidney disease. *Eur J Intern Med* 2010;21:444–8.
29. Zhang DL, Liao H, Wei YY, Zhang Y, Zhang QD, Wang ZG. Elevation of serum Apelin-13 is positively correlated with ADMA in patients on maintenance hemodialysis. *Clin Nephrol* 2009;71:405–12.
30. Leal VO, Lobo JC, Stockler-Pinto MB et al. Apelin: a peptide involved in cardiovascular risk in hemodialysis patients? *Ren Fail* 2012;34:577–81.
31. Małyszko J, Małyszko JS, Koźminski P, Myśliwiec M. Apelin and cardiac function in hemodialyzed patients: possible relations? *Am J Nephrol* 2006;26:121–6.
32. Coskun Yavuz Y, Sevinc C, Deniz MS et al. The role of Apelin 13 in progression of chronic kidney disease. *Iran J Kidney Dis* 2015;9:369–73.
33. Han X, Wang LY, Diao ZL, Liu WH. Apelin: a novel inhibitör of vascular calcification in chronic kidney disease. *Atherosclerosis* 2016;244:1–8.
34. Han X, Zhang DL, Yin DX, Zhang QD, Liu WH. Apelin-13 deteriorates hypertension in rats after damage of the vascular endothelium by ADMA. *Can J Physiol Pharmacol* 2013;91:708–14.
35. Ye L, Ding F, Zhang L et al. Serum Apelin is associated with left ventricular hypertrophy in untreated hypertension patients. *J Transl Med* 2015;13:290.
36. Speer T, Owala FO, Holy EW et al. Carbamylated low-density lipoprotein induces endothelial dysfunction. *Eur Heart J* 2014;35:3021–32.