
ORIGINAL ARTICLE

The effects of renal replacement therapy on plasma, asymmetric dimethylarginine, nitric oxide and C-reactive protein levels

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Abstract

Purpose: Asymmetric dimethylarginine (ADMA), nitric oxide (NOx), and C-reactive protein (CRP) are important risk factors for endothelial dysfunction and mortality in the end stage renal diseases population. The aim of the study was to investigate the relationship between renal replacement therapy and endothelial dysfunction.

Methods: Plasma NOx, ADMA and CRP levels were examined in randomized selected 30 patients with chronic kidney diseases (CKD), 28 patients receiving continuous ambulatory peritoneal dialysis (PD) and 30 patients receiving regular hemodialysis (HD) and age-matched 20 healthy controls. The duration of dialysis was from 4, 5 to 11, and 6 years, respectively.

Results: CKD patients had higher plasma ADMA ($1.26 \pm 0.53 \mu\text{mol/L}$) and CRP levels ($1.02 \pm 0.25 \text{mg/L}$) and lower NOx levels ($28.6 \pm 5.4 \mu\text{mol/L}$) than controls (0.45 ± 0.20 ; 0.65 ± 0.45 ; 32.5 ± 3.7 respectively, $P < 0.001$). Plasma NOx and CRP levels were higher in HD patients ($32.9 \pm 5.5 \mu\text{mol/L}$, $P < 0.05$ and $4.59 \pm 3.18 \text{mg/L}$, $P < 0.001$) and plasma ADMA and CRP levels were higher in PD patients ($1.82 \pm 0.98 \mu\text{mol/L}$, $P < 0.001$ and $2.40 \pm 1.53 \text{mg/L}$, $P < 0.001$) than in CKD patients. PD patients had higher plasma ADMA levels ($P < 0.05$) and lower plasma NOx and CRP levels than HD patients ($P < 0.001$ and $P < 0.001$). Plasma ADMA levels were negatively correlated with NOx levels in all patient groups

($P < 0.001$). Plasma CRP levels in CKD and HD patients were positively correlated with plasma urea levels ($r: 0.437$, $P < 0.001$) and duration of dialysis ($r: 0.370$, $P < 0.01$), respectively.

Conclusion: CRP and ADMA may be emerging as important risk factors for atherosclerosis in dialysis patients. Reduced NO elaboration secondary to accumulation of ADMA and elevated inflammation may be important pathogenic factors for endothelial dysfunction in both dialysis treatment strategies.

The replacement of renal function by dialysis is one of the major achievements of modern medicine. However, given the fact that renal diseases share common causes with cardiovascular diseases (CVD). Also, imperfect clearance of known and unknown ureamic toxins, dialysis patients comprise a population with a risk profile of unique severity.¹ The CV risk has emerged as the central problem limiting the rehabilitation and survival of end-stage renal disease (ESRD) patients. One pathogenic mechanism that might contribute the CV risk is inflammation related-endothelial dysfunction²

Nitric oxide (NO) is a potent chemical mediator synthesized from L-arginine by a family of NO synthases. Under normal conditions, NO is continuously generated in the endothelium and, it has a protective role for the cardiovascular system because it inhibits vascular muscle cell proliferation, platelet aggregability, and the adhesion of monocytes to the endothelium.³ A reduction in NO availability is a common feature during ESRD.^{4, 5} Vallance reported for the first time that the dysfunction of the L-arginine/NO pathway could be secondary to the accumulation of asymmetrical dimethyl-L-arginine (ADMA) in ESRD patients.⁶ ADMA is an endogenous inhibitor of NO synthase. By competitively displacing L-arginine from the substrate binding site of NO synthase, ADMA interferes with many of the physiological functions of NO, like endothelium-dependent vasodilatation and leukocyte adhesion.⁷ It has been suggested that the accumulation of ADMA in renal failure might cause inhibition of NO synthase and that; this might explain some of the features of renal failure dysfunction, such as cardiovascular morbidity and mortality.⁸ There is disagreement about the ADMA levels and the effects of dialysis on plasma ADMA levels.

Chronic infections may also raise circulating levels of pro-inflammatory cytokines, with a chronic low-grade inflammation that may accelerate the progression of atherosclerotic lesions.³ C-reactive protein (CRP) is considered the best-characterized inflammation biomarker, definitely associated with adverse cardiovascular outcomes in the general population.⁹ It has also been reported that dialysis patients have an activated inflammatory response, evidenced by increased serum CRP levels.¹⁰ The relationship between inflammation and endothelial dysfunction in these patients has not been clearly identified.

We investigated the effects of hemodialysis (HD) and peritoneal dialysis (PD) on endothelial dysfunction and inflammation markers in ESRD patients. Plasma ADMA, NO and CRP levels were determined. Since the normalized treatment ratio

(Kt/V= the ratio of the urea (clearance x time) product to total body water) have become widely accepted measures of dialysis doses¹¹, we also determined the relationship between effectiveness of dialysis and plasma markers.

Materials and methods

The study protocol was approved by our local institutional ethics committee. The protocol conforms with the ethical guidelines of the 1975 Helsinki Declaration. Written informed consent was obtained from all of the subjects.

Thirty patients with chronic kidney diseases (CKD; 14 female, 16 male, mean age : 44.8 ± 12.8 yr, estimated Glomerular filtration rate (<30 ml/min), 28 patients receiving continuous ambulatory peritoneal dialysis (PD group, 16 female, 12 male, mean age: 45.2 ± 12.4 yr) and 30 patients receiving regular hemodialysis (HD; 16 female, 14 male, mean age: 47.1 ± 13.5) for ESRD were randomized selected and compared with 20 healthy controls (10 female, 10 male, mean age: 45.5 ± 15.5 yr). The causes of ESRD were chronic glomerulonephritis, chronic tubulointerstitial nephritis and hypertensive nephrosclerosis. Exclusion criteria were acute infection, acute vascular disease (history of stroke, myocardial infarction, or peripheral arterial occlusive disease within 3 month before the study), pregnancy, diabetes and participation in another trial within 4 week before the study day. Between 30 and 50 % of patients in dialysis groups and 80% of the CKD patients were hypertensive despite concurrent treatment with an average of two antihypertensive agents. Smokers did not smoke for 12 hr before or during the study day.

All patients receiving HD had been on regular hemodialysis and were dialyzed three times a week each for 4 hr, using bicarbonate dialysate and hemophan dialyzing membranes. The duration of dialysis was from 4, 5 to 11, and 6 yr, respectively. The diet of the dialysis patients was not modified from that already prescribed for their end stage renal disease.

Blood pressure was measured three times (at 2- to 5-min intervals) with a mercury sphygmomanometer, and the average value was considered for data analysis. Drug prescriptions were recorded. They included the use of calcium channel blockers, beta blockers, diuretics, converting enzyme inhibitors, and angiotensin receptor and aldosterone antagonists. 50% of patients in the PD group and 70 % in the HD groups were taking erythropoietin. None received antioxidant supplements, and no subjects were on nitrates. Studies were conducted in a quiet room with an ambient temperature of 22°C with complete resuscitation facilities. Blood samples were collected from hemodialysis patients immediately before a dialysis session and also from PD and CRF patients during regular outpatient visits. Venous blood samples were taken after an overnight fast (9–12 a.m.) and were collected in vacuum plain tubes containing disodium EDTA. Two milliliters aliquot was removed to determine hemoglobin (Hb), hematocrit (Hct) and erythrocyte counts. Samples were immediately centrifuged (3000 rpm for 10 min. at 4°C) and were used for routine biochemical analysis. 2ml of plasma were stored at - 80°C until the determination of NOx, ADMA and CRP analysis.

NOx was determined as the concentration of nitrate plus nitrite in the plasma. Nitrate was reduced to nitrite by nitrate reductase, the sample was deproteinized with ZnSO₄, and the concentration of nitrite was measured spectrophotometrically at 430 nm using the Griess reaction with a commercial kit (Boehringer Mannheim, GmbH, Germany). The intra- and interassay coefficients of variation for NOx were 4.9% and 5.1%.

Plasma ADMA concentrations were determined by competitive ELISA assay (ADMA-ELISA DLD Gesellschaft für diagnostika und medizinische Geräte mbH, Hamburg). ADMA in samples is acylated and competes with solid phase bound ADMA for a fixed number of rabbit anti-ADMA antiserum binding sites. The intraassay and interassay coefficients of variation were 3.4 % and 4.5% for ADMA, respectively.

Plasma high sensitive CRP concentrations were obtained by a method based on the principle of a solid phase enzyme-linked immunosorbent assay (CRP ELISA, Biomerica, Inc. USA). The intraassay and interassay coefficients of variation were 3.2 % and 3.5% for CRP, respectively.

Whole blood analysis for Hb, Hct and erythrocyte counts and plasma urea, creatinine, glucose, albumin and urea concentrations were determined using routine laboratory methods. Dialysis dose was defined by the fractional urea clearance per dialysis determined by the predialysis BUN and the equilibrated post dialysis BUN after urea rebound is completed (Kt/V).¹²

Data are presented as the mean \pm standard deviation (SD). For parametrically distributed data. Comparisons were made using the unpaired t test and ANOVA followed by Bonferroni post hoc test for data between groups. For nonparametrically distributed data, Kruskal-Wallis test were used where appropriate. Correlations between changes in variables were tested using Pearson's correlation. $P < 0.05$ was considered statistically significant.

Results

The general characteristics and plasma test values of CKD and dialysis groups were given in table 1. Plasma levels of urea and creatinine levels were higher, and plasma albumin and Hb and Hct levels were lower in CKD dialysis patients than in healthy controls ($P < 0.001$). CKD patients had higher systolic and diastolic blood pressure than controls ($P < 0.001$ and $P < 0.001$) and both dialysis groups ($P < 0.001$ and $P < 0.001$). Plasma urea and creatinine levels in HD patients were higher than in CKD patients ($P < 0.001$ and $P < 0.001$). PD patients had higher plasma creatinine levels than CKD patients ($P < 0.001$). There were differences in plasma urea and Kt/V urea levels between PD and HD patients ($P < 0.001$ and $P < 0.001$). No differences were found for other routine parameters between the HD and PD groups.

TABLE 1. General characteristics and plasma test values of patients with chronic kidney disease (CKD), hemodialysis (HD) and peritoneal dialysis (PD).

	Healthy Controls	CKD Patients	HD Patients	PD Patients
n	20	30	30	28
Sex(Female/Male)	10/10	14/16	16/14	16/12
Age (years)	45.5 ± 15.5	44.8 ± 12.8	47.1 ± 13.5	45.2 ± 12.4
Systolic Blood Pressure (mmHg)	105.5 ± 9.5	155.1 ± 16.4*	126.1 ± 31.6	125.9 ± 29.9 †
Diastolic Blood Pressure (mmHg)	84.4 ± 5.9	107.8 ± 10.6 *	78.1 ± 22.6 †	77.3 ± 20.1 †
Hypertension (n)	-	26	15	17
Urea (mg/dl)	28.5 ± 8.9	105.6 ± 27.0*	147.5 ± 14.2*, †	109.7 ± 25.6 *, §
Creatinine (mg/dl)	0.75 ± 0.19	3.39 ± 1.32*	9.01 ± 3.97 *, †	10.39 ± 1.95 *, †
Kt/V Urea	-	-	1.11 ± 0.10	2.09 ± 0.16 §
Total Cholesterol (mg/dl)	199.6 ± 21.4	196.1 ± 43.9	205.8 ± 39.6	191.4 ± 46.2
Glucose (mg/dl)	102.5 ± 15.5	95.2 ± 27.7	106.2 ± 19.5	107.7 ± 24.1
Albumin(g/dl)	4.52 ± 0.75	3.52 ± 1.10 *	3.29 ± 1.06 *	3.17 ± 0.69 *
Hemoglobine (g/dl)	13.5 ± 0.9	10.5 ± 1.25 *	10.9 ± 1.6 *	10.9 ± 2.2 *
Hematocrit (%)	42.5 ± 3.6	31.8 ± 3.5 *	32.9 ± 4.8 *	32.6 ± 6.9 *

Statistically different from controls *, patients with CKD † and HD patients §
P < 0.05; statistically significant

TABLE 2. Plasma asymmetric dimethylarginine (ADMA), nitric oxide (NOx) and C-reactive protein (CRP) levels in controls and in patients with chronic kidney disorders (CKD), hemodialysis (HD) and peritoneal dialysis (PD).

	Healthy Controls	CKD patients	HD Patients	PD Patients
N	20	30	30	28
ADMA(μmol/L)	0.45 ± 0.20	1.26 ± 0.53 *	1.05 ± 0.61*	1.82 ± 0.98 *, †, §
NOx (μmol/L)	32.5 ± 3.7	28.0 ± 5.4 *	32.9 ± 5.5 †	28.1 ± 4.5 *, §
CRP (mg/L)	0.65 ± 0.45	1.02 ± 0.25*	4.59 ± 3.18 *, †	2.40 ± 1.53 *, †, §

Statistically different from controls *, patients with CKD † and HD patients §
P < 0.05; statistically significant

Plasma ADMA, NOx and CRP levels were given in table 2. CKD patients had higher plasma ADMA and CRP levels and lower plasma NOx levels than controls (*P* < 0.001, *P* < 0.001, *P* < 0.001, respectively). Plasma CRP levels were higher in both dialysis groups than in CKD patients (*P* < 0.001 and *P* < 0.001). HD and PD patients had higher plasma NOx and ADMA levels than CKD patients, respectively (*P* < 0.05 and *P* < 0.05). Elevated plasma ADMA levels and decreased plasma NOx and CRP levels were obtained from PD patients than from HD patients (*P* < 0.05, *P* < 0.001 and *P* < 0.001, respectively).

Plasma ADMA levels were negatively correlated with NOx levels in patients with CKD (*r* = -0.814, *P* < 0.001), in HD (*r* = -0.888, *P* < 0.001) or PD patients (*r* = -0.703, *P* < 0.001). The correlation coefficient, *r*, was

-0.838 in sum of the ESRD patient groups (figure). Plasma CRP levels in CKD

PLACE FIGURE NEAR HERE

patients were positively correlated with plasma urea (*r* = 0.437, *P* < 0.001). In HD patients, plasma CRP levels in HD patients were negatively correlated with Kt/V (*r* = -0.398, *P* < 0.001) and positively correlated with duration of dialysis (*r* = 0.370, *P* < 0.01). Blood pressure levels were not related to studied parameters.

Discussion

Several studies have reported a high prevalence of cardiovascular disease in patients with end-stage renal disease. This population usually presents risk factors for atherosclerosis. Mechanisms that participate in the

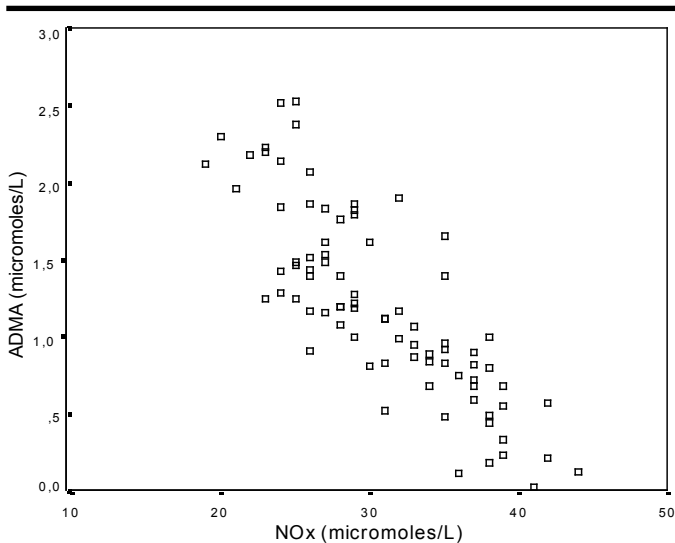


FIGURE 1. Correlation between plasma Asymmetrical dimethyl-L-arginine (ADMA) and nitric oxide (NOx) levels in patients with end stage renal diseases, $r: -0.833$, $P < 0.001$

reduced vasodilator responses in endothelial dysfunction include reduced NO generation.¹³ However, the relationship between endothelial dysfunction of uremia has reached contradictory conclusions. Some authors found to be increased NO levels in uremia^{14,15}, but contrary results have been reported which is explained by increase in ADMA levels.^{16,17} Recently, whole-body NO production has been determined by radionuclide-based measurement of arginine to citrullin conversion and reduced release of NO has been reported in uremic patients compared with matched control subjects.¹⁸ Our results suggest that CKD patients have higher plasma ADMA levels and lower plasma NOx levels than healthy controls and plasma NOx levels were related with ADMA levels. These findings may be due to poor elimination of ADMA by kidney in CKD patients, or accumulation of ADMA has the potential to inhibit NO synthesis.¹⁹ These results may also explain the elevated blood pressure in ESRD patients relative to the healthy controls. On the other hand, inflammation may have resulted in decreased NO bioavailability. It has been suggested that CRP decreases eNOS activity²⁰ and, in

particular, CRP levels rise as renal function declines.^{21,22} Elevated plasma CRP levels were positively correlated with plasma urea levels in our patients with CKD. This finding suggests that uremia itself might be associated with elevated inflammation which may contribute to the development and progression of atherosclerosis.

Dialysis relieves the symptoms of uremia removing low to middle molecular weight molecules and the therapeutic effect of renal replacement therapy indicates that these substances contribute to the pathophysiology of chronic renal failure. Although transplantation is preferred therapy, organ availability is limited: HD continues to be the most common modality of kidney replacement. PD is an alternative modality but it is currently applied to 10% of prevalent dialysis-supported patients.²³ Nonetheless, the dialysis population continues to carry an excess mortality from fatal cardiovascular events.²⁴ We found that plasma ADMA levels were increased by both dialysis treatment and that plasma ADMA levels were negatively correlated with NOx levels. However, the effects of dialysis models on both plasma ADMA and NO are controversial. Our results suggest that ADMA levels were higher and NOx levels were lower in PD patients than in HD patients. Consistent with our results Hon et al.²⁵ demonstrated that HD led to a decrease in plasma NOx level, but Fayed et al.²⁶ suggested that plasma NO levels were increased in uremic patients compared with normal controls, and hemodialysis led to further increases. Schmidt et al.²⁷ demonstrated that NO production was low in ESRD patients on peritoneal dialysis. Zoccali et al.²⁸ also been reported that ADMA was more elevated in PD patients than in HD. Rysz et al.²⁹ evaluated the NO release in peripheral blood during HD. Their results indicated that during HD, NO is released in the peripheral blood. The mechanisms of these results were not clear. We considered that HD may be a more effective process to clean the plasma from ADMA than PD and that the removed ADMA may be responsible for the elevated plasma NOx levels in HD.

On the other hand, renal replacement therapy is known to increase inflammation.²² Our findings suggest that systemic inflammation was elevated in ESRD patient on dialysis and that dialysis-induced inflammation levels were higher in HD than in PD patients. Elevated CRP levels have been described in a significant proportion of ESRD patients undergoing HD or PD.³⁰ Stenvinkel et al.²¹ and Panichi et al.³¹ reported that CRP levels are high even in predialysis patients. Oflaz H et al.³², measuring plasma CRP concentrations, suggested that in patients with end-stage renal disease endothelial dysfunction and endothelial function is more impaired in HD patients than in renal transplant recipients. It has also been indicated that extracorporeal circulation of blood during HD may act as a repeated stimulus for an inflammatory response.³³ Wanner et al. followed a cohort of 280 HD patients for four years the baseline CRP levels were powerful predictors of all-cause and cardiovascular death.³⁴ We considered that the high plasma uremia levels of HD patients may be involved in increased systemic inflammation when compared with PD patients. However, other factors probably exist in uremia that are unrelated to the dialysis procedure *per se* and may contribute to inflammation.

The normalized treatment ratio was measured for the determination of dialysis doses. The single-pool Kt/V, used widely in clinical practice, represents a quantitative measure of the dialysis doses. Several studies have demonstrated a positive correlation between dialysis delivered and patients' survival.³⁵ Although attempts have been made to increase the delivered doses of dialysis over the past few years, the effect of increased dialysis on the Kt/V survival relationship remains unclear. Our results suggest that dialysis doses may be related with increase in cardiovascular risk of ESRD patients. An interesting aspect of our study is the finding that increased dialysis duration was related with elevated CRP levels.

In conclusion, reduced NO elaboration secondary to accumulation of ADMA and elevated systemic inflammation may be an important pathogenic factor for

atherosclerosis in ESRD patients. CRP and ADMA are emerging as important risk factors for CVD in ESRD, and might help to explain the "atherosclerosis" observed in ESRD patients. The dialysis process may increase the cardiovascular risk in ESRD patients and different dialysis treatment strategies were related with elevation in plasma ADMA levels or CRP levels. Further studies will be also necessary to be found of causes of the differences between peritoneal and hemodialysis patients.

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