Asymmetric Dimethylarginine and Homocysteine Levels in Dialysis Patients

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ABSTRACT

Cardiovascular diseases and endothelial disfunction are major causes of mortality in patients with end stage renal disease (ESRD). Treatment strategies like continuous ambulatory peritoneal dialysis (CAPD) and hemodialysis (HD) have different effects on different parameters. Asymmetric dimethylarginine (ADMA) is an endogenous nitric oxide (NO) synthase inhibitor and it has been reported to be a novel marker for the progression of chronic kidney disease (CKD). Homocysteine is believed to cause atherogenesis and thrombogenesis via endothelial damage, vascular smooth muscle proliferation and coagulation abnormalities. In previous studies, conflicting findings have been reported about the effect of HD and CAPD on oxidant and antioxidant systems. In this study, we aimed to investigate ADMA, homocysteine and C- reactive protein (CRP) levels in patients with ESRD having HD and CAPD treatment and healthy individuals. This study was performed on 44 (23M, 21F) CAPD patients, 26 (13M, 13F) HD patients and 29 (15M, 14F) age and sex matched healthy control subjects. The lipid profile, ADMA, homocysteine, arginine and CRP levels were measured. Serum ADMA, homocysteine and CRP levels of the ESRD patients were significantly higher, whereas serum arginine levels were significantly lower in both HD and CAPD patients compared to control subjects. No differences were found between serum ADMA, homocysteine and CRP levels of the CAPD and HD patients. Our results suggest that ADMA, homocysteine and CRP levels were increased in HD and CAPD patients compared to the control subjects. These findings suggest that ESRD patients are prone to inflammation, oxidative stress and endothelial dysfunction. We conclude that endothelial dysfunction, inflammation and oxidative stress are increased in dialysis patients and ADMA concentrations are not affected by the modality of dialysis treatment.

Key words: *Asymmetric dimethylarginine, homocysteine, hemodialysis*

Diyaliz Hastalarında Asimetrik dimetilarjinin ve Homosistein Düzeyleri

ÖZET

Kardiyovasküler hastalıklar ve endotelyal disfonksiyon son dönem böbrek yetmezliği hastalarında en önemli ölüm nedenleridir. Periton diyalizi ve hemodiyaliz gibi tedavi yöntemlerinin farklı parametreler üzerine farklı etkileri vardır. Asimetrik dimetilarjinin endojen nitrik oksit sentaz inhibitörüdür ve kronik böbrek yetmezliğinin progresyonunda yeni bir belirteç olduğu gösterilmiştir. Homosistein endotel hasarı, damar düz kas hücre proliferasyonu ve koagülasyon anormallikleri yoluyla trombogenez ve aterogeneze neden olur. Daha önceki çalışmalarda periton diyalizi (PD) ve hemodiyalizin (HD) oksidan ve antioksidan sistemler üzerine etkisiyle ilgili çelişkili bulgular vardır. Bu çalışmada HD, PD ve sağlıklı kişilerde ADMA, homosistein ve CRP düzeylerini belirlemeyi amaçladık. Çalışmaya 44 PD (23E,21K), 26HD (13E,13K) hastası ve 29 (15E,14K) sağlıklı kişi katıldı. ADMA, homosistein, arjinin ve CRP düzeyleri ölçüldü. Son dönem böbrek yetmezliği (SDBY) hastalarının ADMA, homosistein ve CRP düzeyleri sağlıklı kontrol grubuna göre yüksek (p<0.001), arjinin düzeyleri düşük olarak bulundu(p<0.001). HD ve PD gruplarında ADMA, homosistein ve CRP seviyeleri açısından farklılık bulunamadı (sırasıyla p:0.287, p: 0.587, p: 0.835) Bizim sonuçlarımız HD,PD hastalarında sağlıklı kontrole göre ADMA ve homosistein düzeylerinin yüseldiğini gösterdi. Bu bulgular; diyaliz hastalarında endotel disfonksiyonu, inflamasyon ve oksidatif stresin arttığını ve ADMA ve homosistein düzeylerinin diyaliz tedavi yönteminden etkilenmediğini göstermektedir

Anahtar kelimeler: *Asimetrik dimetilarjinin, homosistein, hemodiyaliz*

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INTRODUCTION

Patients with chronic kidney disease (CKD) have increased risk of cardiovascular diseases. Cardiovascular events are the major causes of mortality and morbidity in patients with advanced renal failure causing more than 40% to 50% of the mortalities in these patients (1). Cardiovascular mortality is 10 to 30 fold higher in end-stage renal disease (ESRD) patients than general population (2). The number of patients requiring renal replacement therapy (RRT) due to ESRD is increasing worldwide. It has been reported that both CKD and inflammation give rise to the endothelial dysfunction through increased levels of asymmetric dimethylarginine (ADMA), an endogenous nitric oxide (NO) synthase inhibitor (3-6).

NO, synthesised from L-arginine by a family of NO synthases, is involved in many functions including regulation of vascular tone, neurotransmission, and host defence (7). ADMA has been reported to be a novel marker for the progression of CKD with ADMA accumulation triggering peritubular capillary loss that contributes to tubulointerstitial ischemia and fibrosis (8). In addition, ADMA is strongly associated with carotid artery intima media thickness (9).

Homocysteine is derived from the metabolic conversion of the essential amino acid methionine. Homocysteine is believed to cause atherogenesis and thrombogenesis via endothelial damage, vascular smooth muscle proliferation and coagulation abnormalities (10, 11). The prevalence of hyperhomocysteinemia is 85% to 100%, when ESRD is developed (12).

C reactive proteine (CRP) is considered as the best characterized inflammation biomarker, solidly associated with adverse cardiovascular outcome in the general population. Current data suggest that the addition of lipid screening to CRP can im¬prove detection of absolute coronary risk (13).

Treatment strategies like continuous ambulatory peritoneal dialysis (CAPD) and hemodialysis (HD) have different effects on different parameters. In previous studies, conflicting findings have been reported about the effect of HD and CAPD on oxidant and antioxidant systems. In this study, we have investigated ADMA, arginine, homocysteine and CRP levels in HD and CAPD patients, compared to control subjects. We grouped the patients according to the treatment and aimed to determine the effect of dialysis treatment.

MATERIAL AND METHODS

Patients

This study was performed on 44 (23M, 21F) CAPD patients (four exchanges of 2 L dialysate / day containing varying glucose concentrations to obtain adequate ultrafiltration) at least 6 months under dialysis, aged 38-65 years, 26 (13M,13F) HD patients at least 6 months under dialysis, aged 35-68 years and 29 (15M, 14F) healthy control subjects, aged 35-64 years. HD session duration was approximately four hours and it was applied three times a week using unfractionated heparin for anticoagulation. Exclusion criteria for the study included malignant disease, chronic liver disease, infectious disease, chronic respiratory insufficiency, rheumatoid arthritis, pregnancy, alcohol and smoking habit. All patients maintained the usual dialysis diet. Control subjects were on a free mediterranean diet. All anthropometric measurements were made with participants wearing light clothing and no shoes. Body mass index (BMI) was measured in all participants. The study protocol was approved by the Ethics Committee of Meram Medical School, University of Selcuk, Konya, Turkey. All patients were informed of the details of the study and the written consent of each patient was received.

Laboratory investigations

Blood samples were taken immediately before the first dialysis session of the week. Venous blood samples were obtained in the morning after a 12 h fasting. Serum samples were obtained after centrifugation (4000 rpm for 10 min at 4 oC) and samples were stored frozen at -80 oC until ADMA, arginine, homocysteine analysis. Serum lipids and CRP levels were measured immediately.

Measurements of ADMA and Arginine

Measurement of ADMA was accomplished by high performance liquid chromatography (HPLC), using the method described by Chen and associates (14). Serum levels of ADMA and arginine were measured by HPLC (HP Agilent 1100; Agilent Technologies, Palo Alto, CA, USA) with fluorescence detection. The areas of the peaks detected by the fluorescent detector (excitation, 338 nm; emission, 425 nm) were used for quantification. The serum ADMA and arginine values were expressed as micromol per liter (μmol / L).

Homocysteine measurements

Serum homocysteine concentrations were measured us-

ing the Chromsystems (Munich, Germany) reagent kit for HPLC technique with fluorescence-detector. The serum homocysteine values were expressed as micromol per liter (μmol / L).

Measurements of other analyses

Serum total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-cholesterol) and CRP were measured by commercially available kits based on routine methods on the Synchron LX System (Beckman Coulter, Fullerton CA). Low density lipoprotein cholesterol (LDL-cholesterol) was calculated using the formula by Friedewald et al (15).

Statistical analyses

All data are expressed as mean ± standart deviations (SD). Statistical analyses were done using SPSS v. 16.0 (SPSS Inc., IL, USA). The normality of the variables was evaluated using the one-sample Kolmogorov-Smirnov test. The normal distribution of variables (serum lipids and BMI) were examined with one-way ANOVA; tukey test. To compare the ratio of categorical variables, we used the chi-squared test. Pearson's correlation coefficient was used to evaluate the associations between variables. Differences were considered significant at a probability level of $p<0.05$.

RESULTS

Clinical characteristics and biochemical parameters of the subjects are presented in Table 1. As seen from the table, serum triglycerides, total-cholesterol, LDL-cholesterol levels were found to be significantly higher in CAPD and HD patients compared to control subjects ($p<0.001$). There were no significant differences between the groups in terms of BMI.

The serum levels of ADMA, arginine, arginine/ADMA ratio, homocysteine and CRP of the groups are presented in Table 2. As seen from the Table 2, serum ADMA, CRP and homocysteine levels were significantly higher in HD and CAPD patients compared to controls ($p<0.001$). There were no significant differences between serum ADMA, CRP and homocysteine levels of the HD and CAPD groups (p: 0.287, p: 0.587, p: 0.835 respectively). Arginine levels and arginine/ADMA ratio of the HD and CAPD patients were significantly lower compared to controls ($p<0.001$, $p<0.001$ respectively). There were no significant differences between serum arginine levels and arginine/ADMA ratio of the HD and CAPD groups (p: 0.924, p: 0.711 respectively).

Correlation analyses are presented in Table 3. ADMA levels were positively correlated with triglyceride (r:0.315), total-cholesterol (r:0.332) and homocysteine (r:0.335). Arginine levels were negatively correlated with total-cholesterol (r:-0.244) and homocysteine (r:-0.260).

DISCUSSION

We demonstrated that ADMA, homocysteine and CRP levels were increased in HD and CAPD patients compared to the control subjects. In our study, there were no significant difference in ADMA, homocysteine and CRP levels between HD and CAPD patients. Plasma homocysteine is an important risk factor of atherosclerosis in patients on dialysis. Several studies have reported prevalent severe hyperhomocysteinemia in patients on hemodialysis (16, 17). Higher homocysteine levels in our study group were in accord with previous studies. There were no significant differences between our CAPD and HD groups for homocysteine levels. Previous clinical data indicate that

***Table 1**. Clinical characteristics and biochemical variables of the study population*

	Control (n:29)	*CAPD patients (n:44)*	*HD patients (n:26)*
Gender (M/F)	*(15 / 14)*	*(23 / 21)*	*(13 / 13)*
Age (years)	*49,0 ± 8,0*	*53,0 ± 7,6*	*52,6 ± 6,9*
Duration of ESRD (years)	*-*	*4,7 ± 2.0*	*4,2 ± 1,7*
BMI (kg/m2)	*24,5 ± 3.5*	*26,2 ± 4,6*	*25,8 ± 3,5*
Hypertension (yes/no)	*-*	*(13 / 31)*	*(7 / 19)*
Diabetes (yes/no)	*(- / 29)*	*(12 / 32)*	*(9 / 17)*
Total-cholesterol (mg/dL)	*115,8 ± 19,2*	*184,4 ± 46,1**	*169,5 ± 41,9**
Triglycerides (mg/dL)	*119,1 ± 20,7*	*196,0 ± 101,9**	*182,1 ± 73,5**
LDL-cholesterol (mg/dL)	*82,4 ± 15,4*	*152,6 ± 21,4**	*139,3 ± 19,2**
HDL-cholesterol (mg/dL)	*45,6 ± 8,2*	*33,2 ± 14,5**	*30,9 ± 13,8**

*CAPD: continuous ambulatory peritoneal dialysis, HD: hemodialysis, ESRD: end stage renal disease, BMI: body mass index, LDL-cholesterol: low density lipoprotein- cholesterol, HDL-cholesterol: high density lipoprotein- cholesterol, All values (except gender, hypertension, diabetes) are mean ± standart deviations. *p<0.001*

***Table 2**. ADMA, arginine, homocysteine and CRP levels in HD and CAPD patients and controls*

	Control	CAPD	HD
ADMA (µmol/L)	0,72 ± 0,12	1.84 ± 0,82*	1,58 ± 0.81*
Arginine (µmol/L)	116,0 ± 20,1	96,3 ± 21,5*	94,3 ± 23,1*
Arginine/ADMA	165,4 ± 44,1	64,3 ± 35,1*	71,5 ± 32,2*
Homocysteine (µmol/L)	8,0 ± 2,7	37,9 ± 15,6*	40,0 ± 20,4*
CRP (mg/L)	1,4 ± 0,9	11,2 ± 10,8*	14,0 ± 17,8*

CAPD: continuous ambulatory peritoneal dialysis, HD: hemodialysis, ADMA: asymmetric dimethylarginine, CRP: C-reactive proteine
*All values are mean ± standart deviations. *p<0.001*

hyperhomocysteinemia is associated with an increased left ventricular mass and increased incidence of heart failure (18). It has been reported that possible causes of high serum homocysteine levels in chronic renal failure may include reduced renal clearance, impaired degradation by the kidney and deficiency of, or abnormally high requirement for folic acid and vitamin B12 (19).

In previous studies, conflicting findings have been reported about the effect of CAPD and HD on ADMA levels (20-23). Anderstam et al. (20) reported that ADMA concentrations were not affected by the different regimens of dialysis treatment. Our ADMA results are in a accordance with Anderstam et al., whereas Oner-Iyidogan et al. (21), Eiselt et al. (22) and Kielstein et al. (23) reported that ADMA concentrations were higher in patients undergoing HD than those having CAPD. Iyidogan et al. reported that ADMA concentrations of HD and CAPD groups were higher than those of controls and HD group had significantly higher levels of ADMA than those of CAPD group. Kielstein et al. reported that ADMA concentrations in HD group were higher than those in control and CAPD groups whereas ADMA levels in CAPD patients were similar to those of control subjects. Zoccali et al. (24) reported that nontraditional risk factors were far more prevalent in ESRD patients than in the general population and ADMA might play a greater role in cardiovascular risk in CAPD than in HD patients.

In our study ADMA concentrations were significantly higher in HD and CAPD patients compared to the control subjects. In our study, there were no significant differences in ADMA levels between HD and CAPD patients.

In our study, we have found that plasma arginine concentrations were significantly lower in both dialysis groups. Our arginine results were in accord with Oner-Iyidogan et al. (21). It has been demonstrated that CKD patients lose L-arginine during dialysis (25). Iyidogan et al. reported that the decline in plasma arginine levels might result from the dialysis-mediated losses of this amino acid (21). Arginine loss might be involved in the mechanism of endothelial damage seen after a long-term dialysis treatment (21).

CRP is a general marker for inflammation and infection. It has been reported that a level above 2.4 mg/L has been associated with a doubled risk of a coronary event compared to levels below 1 mg/L (26). Elevated CRP levels correlate with an increased relative risk of mortality in HD patients. Chronic inflammatory conditions, such as rheumatoid arthritis and HD for end-stage renal failure, those are characterized by persistently elevated CRP concentrations in some individuals, are associated with premature cardiovascular disease (26).

Current data suggest that the addition of lipid screening to CRP can improve detection of absolute coronary risk (13). Risk estimates using CRP in combination with lipid screening have been reported to be superior to assesments that evaluate just CRP, homocysteine or lipoprotein a (13). Our study demonstrated that CRP levels were increased in HD and CAPD patients compared to the control subjects.

It has been reported that patients on CAPD are exposed to a high glucose intake and its possible metabolic side

***Table 3**. Pearson's correlation analyses between variables.*

	Total-cholesterol	Triglycerides	Homocysteine
ADMA	0,332**	0,315**	0,335**
Arginine	-0,244*	-0,062	-0,260*
CRP	0,225*	0,004	0,238*

*ADMA: asymmetric dimethylarginine, CRP: C-reactive proteine, **. Correlation is significant at the 0,01 level. *. Correlation is significant at the 0,05 level.*

effects, such as obesity, hyperglycemia-diabetes or hyperlipidemia. Patients on HD are exposed to significant hemodynamic stresses (27). In our study, cardiovascular risk factors such as ADMA, CRP and homocysteine levels were higher and modality of dialysis did not affect to cardiovascular risk factors. Locatelli et al. compared the risk of developing de novo cardiovascular disease (CVD) in HD and CAPD patients who showed no signs of CVD at the time they began RRT and, after adjusting for age, gender and stratifying by diabetic status, found no statistical difference between the two modalities (27).

In summary, our results suggest that serum ADMA, CRP and homocysteine levels of the CAPD and HD patients were significantly higher than those of the control subjects. There were no significant differences between serum ADMA and homocysteine levels of the CAPD and HD groups. Serum ADMA levels in dialysis patients are potentially affected by other factors besides the modality of dialysis treatment. We conclude that endothelial dysfunction, and increased inflammation and oxidative stress are seen in dialysis patients, serum ADMA and homocysteine concentrations are not affected by the modality of dialysis treatment. Further randomized controlled trials to determine the clinical significance of this result are needed.

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REFERENCES

1. *United States Renal Data System. VI. Causes of death in ESRD. Am J Kidney Dis 1999;34(2 Suppl 1):87-94.*
2. *Foly RN, Parfrey PS, Sarnak MJ. Clinical epi¬demiology of cardiovascular disease in chronic renal disease. Am J Kidney Dis 1998;32:112-11¬9.*
3. *Guebre-Egziabher F, Fougue D Metabolic consequences of inflammation in kidney failure. Nephrologie 2003;24:383-6.*
4. *Mak RH, Cheung W Adipokines and gut hormones in end-stage renal disease. Perit Dial Int 2007;27(Suppl 2):298-302.*
5. *Galli F Protein damage and inflammation in uremia and dialysis patients. Nephrol Dial Transplant 2007;22(Suppl 5):20-36.*
6. *Locatelli F, Canaud B, Eckardt KU, Stenvinkel P, Wanner C, Zoccali C. Oxidative stress in end-stage renal disease: an emerging threat to patient outcome. Nephrol Dial Transplant 2003;18:1272-80.*
7. *Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med 1994;329:2002-12.*
8. *Ueda S, Yamagishi S, Matsumoto Y, et al. Involvement of asymmetric dimethylarginine (ADMA) in glomerular capillary loss and sclerosis in a rat model of chronic kidney disease (CKD). Life Sci 2009;84:853-6.*
9. *Kielstein JT, Fliser D. The past, presence and future of ADMA in nephrology. Nephrol Ther 2007; 3: 47-54.*
10. *Kalita J, Kumar G, Bansal V, Misra UK. Relationship of homocysteine with other risk factors and outcome of ischemic stroke. Clin Neurol Neurosur 2009;111:364-7.*
11. *Vrentzos G, Papadakis JA, Malliaraki N, Zacharis EA, Katsogridakis K, Margioris AN. Association of serum total homocysteine with the extent of ischemic heart disease in a Mediterranean cohort. Angiology 2004;55:517-24.*
12. *Van Guldener C. Why is homocysteine elevated in renal failure and what can be expected from homocysteinelowering? Nephrol Dial Transplant 2006;21:1161-6.*
13. *Rifai N, Ridker PM. Proposed cardiovascular risk assessment algorithm using high-sensi¬tivity C-reactive protein and lipid screening. Clin Chem 2001;47:28-30.*
14. *Chen BM, Xia LW, Zhao RQ: Determination of N(G),N(G)-dimethylarginine in human plasma by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 1997;692:467-71.*
15. *Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.*
16. *Eikelboom JW, Lonn E, Genest J, Jr., Hankey G, Yusuf S. Homocysteine and cardiovascular disease: a critical review of the epidemiologic evidence. Ann Intern Med. 1999;131:363-75.*
17. *Menon V, Wang X, Greene T, et al. Homocysteine in chronic kidney disease: Effect of low protein diet and repletion with B vitamins. Kidney Int 2005;67:1539-46.*
18. *Sundstrom J, Vasan RS. Homocysteine and heart failure: a review of investigations from the Framingham Heart Study. Clin Chem Lab Med 2005;43:987-92.*
19. *Refsum H,Helland S, Ueland PM. Radioenzymatic determination of homocysteine in plasma and urine Clin Chem 1985;31:424-8.*
20. *Anderstam B, Katzarski K, Bergström J (1997) Serum levels of NG,NG-dimethyl-L-arginine, a potential endogenous nitric oxide inhibitor in dialysis patients. J Am Soc Nephrol 1997;8:1437-42.*
21. *Oner-Iyidogan Y, Oner P, Kocak H, et al. Dimethylarginines and inflammation markers in patients with chronic kidney disease undergoing dialysis. Clin Exp Med 2009; 9: 235-41.*
22. *Eiselt J, Rajdl D, Racek J, Siroká R, Trefil L, Opatrná S. Asymmetric dimethylarginine in hemodialysis, hemodiafiltration, and peritoneal dialysis. Artif Organs 2010;34: 420-5.*
23. *Kielstein JT, Böger RH, Bode-Böger SM, et al. Asymmetric dimethylarginine plasma concentrations differ in patients with end-stage renal disease: relationship to treatment method and atherosclerotic disease. J Am Soc Nephrol 1999;10:594-600.*

24. Zoccali C, Enia G, Tripepi G, Panuccio V, Mallamaci F. Clinical epidemiology of major nontraditional risk factors in peritoneal dialysis patients. Perit Dial Int 2005; 25 Suppl3:84-7.

25. Gutierrez A. Protein catabolism in maintenance haemodialysis: the influence of the dialysis membrane. Nephrol Dial Transplant 1996; 11:108-11.

26. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest. 2003;111:1805-12.

27. Locatelli F, Marcelli D, Conte F, et al. Survival and development of cardiovascular disease by modality of treatment in patients with end-stage renal disease. J Am Soc Nephrol 2001; 12: 2411-7.