

# DIALYSIS AND TRANSPLANTATION

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ROBERT KRAWCZYK<sup>1,3</sup>, WACŁAW WEYDE<sup>1</sup>, MAGDALENA KRAJEWSKA<sup>1</sup>, KATARZYNA MADZIARSKA<sup>1</sup>, ALICJA PIASECKA-GRZESZEK<sup>2</sup>, URSZULA SOLSKA<sup>2</sup>, MARIAN KLINGER<sup>3</sup>

## Elevated ADMA Levels Are an Early Event in Chronic Hemodialysis Patients

### Podwyższone stężenie ADMA wczesnym zjawiskiem w czasie leczenia hemodializami

<sup>1</sup> Department of Nephrology and Transplantation Medicine, Silesian Piasts University of Medicine in Wrocław, Poland

<sup>2</sup> Department of Internal Medicine, Occupational Diseases and Hypertension, Silesian Piasts University of Medicine in Wrocław, Poland

<sup>3</sup> International Dialysis Center, Ostrów Wlkp., Poland

#### Abstract

**Background.** Elevated ADMA concentration appears as a novel cardiovascular risk factor in chronic HD patients. However, no data exist in the literature on a potential relationship between ADMA levels and duration of maintenance HD treatment.

**Objectives.** The aim of this study was to analyze the connections between ADMA levels and the period of chronic hemodialysis treatment (HD), the efficiency of the procedure, severity of hypertension, intensity of inflammatory reaction, and nutritional indices.

**Material and Methods.** The study was performed on 37 healthy volunteers (18 female and 19 male, mean age:  $48.1 \pm 11.3$  years) and in two groups of chronic HD patients: 68 patients on maintenance HD for 6–24 months (31 female and 37 male, age:  $52.4 \pm 10.3$  years) and 24 patients on HD for over 60 months (10 female and 14 male, age:  $56.8 \pm 10.3$  years). The ADMA and L-arginine concentrations were measured by the HPLC method.

**Results.** ADMA serum levels were significantly elevated in the HD patients independent of the duration of maintenance HD treatment. ADMA concentrations in the HD patients were also not affected by age, smoking habit, or the class of antihypertensive drugs. A paradoxical positive correlation between serum albumin and ADMA concentration appeared in the HD patients treated for less than two years. This can be explained by the fact that higher protein intake supports ADMA generation. Significant correlation was found between ADMA and total serum cholesterol only in the patients on HD for over five years. In addition, elevated L-arginine occurred in well-nourished HD patients, possibly due to inhibition of the degradation process.

**Conclusions.** The duration of maintenance HD treatment, age, smoking habit, and class of antihypertensive drugs do not exert an impact on elevated ADMA concentration. In adequately nourished HD patients, a deficiency of L-arginine (substrate for nitric oxide synthesis) does not occur (*Adv Clin Exp Med* 2008, 17, 2, 191–196).

**Key words:** ADMA, L-arginine concentrations, HD patients, period of maintenance HD, hypertension, CRP, albumin, cholesterol levels.

#### Streszczenie

**Wprowadzenie.** Podwyższone stężenie ADMA uznaje się za nowy czynnik zagrożenia sercowo-naczyniowego u przewlekle dializowanych chorych. W piśmiennictwie nie ma jednak danych na temat zależności między stężeniem ADMA a długością leczenia dializami.

**Cel pracy.** Analiza powiązań między stężeniem ADMA a długością okresu dializoterapii, skutecznością zabiegów hemodializy, ciężkością nadciśnienia, nasileniem procesu zapalnego i stanem odżywienia.

**Materiał i metody.** Badania zostały przeprowadzone u 37 zdrowych ochotników (18 kobiet i 19 mężczyzn w wieku  $48.1 \pm 11.3$  roku) i w 2 grupach przewlekle hemodializowanych chorych: a) 68 chorych leczonych dializami 6–24 miesiące (31 kobiet i 37 mężczyzn w wieku  $52.4 \pm 10.3$  roku) oraz b) 24 chorych leczonych hemodializami powyżej 60 miesięcy (10 kobiet i 14 mężczyzn w wieku  $56.8 \pm 10.3$  roku). Stężenia ADMA i L-argininy oznaczono metodą HPLC.

**Wyniki.** Stężenia ADMA były istotnie większe w obu grupach hemodializowanych chorych. Na ich wartości nie miały również wpływu: wiek chorych, palenie papierosów oraz rodzaj przyjmowanych leków hipotensyjnych. Zaskakująca, dodatnia korelacja między zawartością albumin i stężeniem ADMA w surowicy, pojawiająca się w grupie chorych będących w programie hemodializ mniej niż 2 lata, może być wytłumaczona zwiększonym wytwarzaniem ADMA u chorych spożywających dietę z większą zawartością białka. Istotna korelacja między stężeniami ADMA i cholesterolu ujawniła się tylko u chorych leczonych hemodializami powyżej 5 lat. U dobrze odżywionych przewlekle hemodializowanych wykryto ponadto zwiększenie stężeń L-argininy, prawdopodobnie na skutek zahamowania procesu jej rozkładu.

**Wnioski.** Długość okresu dializoterapii, wiek, nikotynizm oraz rodzaj leczenia hipotensyjnego nie wywierają wpływu na zwiększone stężenie ADMA. U prawidłowo odżywionych, przewlekle dializowanych chorych nie występuje niedobór L-argininy, substratu do syntezy tlenku azotu (*Adv Clin Exp Med* 2008, 17, 2, 191–196).

**Słowa kluczowe:** ADMA, stężenie L-argininy, pacjenci leczeni hemodializami, czas leczenia hemodializami, nadciśnienie, CRP, albumina, stężenie cholesterolu.

It is widely documented that chronic kidney disease patients comprise a group at particular risk of cardiovascular morbidity and mortality. This is connected with the appearance of specific cardiovascular risk factors in patients with diminished kidney function. Among these additional factors which negatively affect the cardiovascular system in chronic kidney disease patients is asymmetric dimethylarginine (ADMA). In recent years, data concerning elevated ADMA levels in chronic hemodialysis patients have been published. ADMA is a strong endogenous inhibitor of nitric oxide synthase and, by reducing nitric oxide availability, may trigger pro-atherogenic effects. Increased plasma ADMA concentration has been associated with intima-media thickening, left ventricular hypertrophy, and cardiovascular mortality in patients with stage 5 chronic kidney disease [1–13]. However, no studies have been reported on a potential relationship between elevated ADMA and the duration of maintenance hemodialysis and its efficiency. Therefore, the aim of this study was to analyze the connections between ADMA levels and the period of chronic hemodialysis treatment (HD), the efficiency of the procedure, the severity of hypertension, the intensity of inflammatory reaction, and nutritional indices.

## Material and Methods

The study was performed in 37 healthy volunteers (18 female and 19 male, mean age:  $48.1 \pm 11.3$  years) and in two groups of chronic HD patients: 68 patients on maintenance HD for 6–24 months (31 female and 37 male, age:  $52.4 \pm 10.3$  years) and 24 patients treated by HD for over 60 months (10 female and 14 male, age:  $56.8 \pm 10.3$  years). The tested groups were matched with respect to gender. The HD patients were older than the healthy controls, with the difference on the borderline of statistical significance in the group treated for less than two years ( $p = 0.06$ ) and sta-

tistically significant in the group treated for more than five years ( $p < 0.01$ ). The difference in age between the two HD groups was on the borderline of statistical significance ( $p = 0.08$ ).

Plasma concentrations of asymmetric dimethylarginine (ADMA) and of L-arginine were measured by high-performance liquid chromatography (HPLC) and precolumn derivatization with o-phthalaldehyde (OPA). HPLC was performed on a computer-controlled Varian Star chromatography system (Star Chromatography Workstation ver. 6.3) consisting of a ternary gradient HPLC pump (Pro Star 240), an automatic injector with automated sample-reagent mixing capabilities (Pro Star 410), and a fluorescence detector (Pro Star 363) according to [14, 15]. The other laboratory measurements were conducted by standard analytical methods at the Central Laboratory of the University Hospital.

The statistical analysis was done using the STATISTICA 2006 software package. The ANOVA test, Wilcoxon's test,  $\chi$ -squared test, Pearson's correlation, and Spearman's rank correlation were applied where appropriate.

## Results

The ADMA serum concentrations in the three study groups are shown in Table 1. The ADMA levels in each of the two HD groups were very significantly higher than in the healthy controls ( $p = 0.0003$  for those treated  $< 2$  years and  $p = 0.0005$  for those treated  $> 5$  years). There was no significant difference between HD patients with the shorter and longer periods of maintenance hemodialysis.

Age and smoking habit did not exhibit any significant influence on the ADMA concentration in the healthy controls (age) or HD patients. No smoking person was included to the control group. There were also no significant associations between ADMA levels and hemodialysis efficiency determined by Kt/V values.

**Table 1.** ADMA serum levels in the control and two HD groups**Tabela 1.** Stężenie ADMA w surowicy w grupie kontrolnej i 2 grupach pacjentów leczonych hemodializami

	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.	SD	NA's
Controls (Grupa kontrolna)	0.28	0.59	0.82	0.84	1.06	1.58	0.36	0.00
HD < 2 yrs	0.17	0.87	1.15	1.40	1.46	7.30	1.10	0.00
HD > 5 yrs	0.22	0.99	1.25	1.55	1.71	4.83	1.08	0.00

**Table 2.** Statistical differences in ADMA serum levels in the two groups of HD patients vs. controls**Tabela 2.** Różnice statystyczne stężeń ADMA w surowicy w 2 grupach pacjentów leczonych hemodializami i w grupie kontrolnej

	Controls (Grupa kontrolna)	HD < 2 yrs	HD > 5 yrs
Controls (Grupa kontrolna)	–	difference = –0.33 <i>p</i> = 0.00031	difference = –0.43 <i>p</i> = 0.00051
HD < 2 yrs	difference = 0.33 <i>p</i> = 0.00031	–	difference = –0.098 <i>p</i> = 0.37
HD > 5 yrs	difference = 0.43 <i>p</i> = 0.00051	difference = 0.098 <i>p</i> = 0.37	–

**Table 3.** The relationship between systolic blood pressure and ADMA levels in the two groups of HD patients**Tabela 3.** Zależność ciśnienia skurczowego krwi i stężenia ADMA w 2 grupach pacjentów leczonych hemodializami

	Estimate (Spearman)	<i>p</i> value
Controls (Grupa kontrolna)	–	–
HD < 2 yrs	0.0597	0.629
HD > 5 yrs	0.355	0.0887

**Table 4.** The relationship between diastolic blood pressure and ADMA levels in the two groups of HD patients**Tabela 4.** Zależność ciśnienia rozkurczowego krwi i stężenia ADMA w 2 grupach pacjentów leczonych hemodializami

	Estimate (Spearman)	<i>p</i> value
Controls (Grupa kontrolna)	–	–
HD < 2 yrs	0.178	0.147
HD > 5 yrs	0.377	0.0696

The relationship between the severity of hypertension in the HD patients and ADMA elevation was analyzed by Spearman's rank correlation. The data for systolic blood pressure are depicted in Table 3. The only association (on the borderline of statistical significance, *p* = 0.09) occurred in HD patients treated for over 5 years.

A similar tendency appeared in the HD patients with the longer treatment period in the evaluation of diastolic blood pressure (*p* = 0.07). The class of antihypertensive drug, especially the use of angiotensin-converting enzyme inhibitors and angiotensin II receptor type 1 blockers, did not affect the ADMA concentrations.

In the evaluation of the connections between ADMA concentration and CRP level, serum albumin content, and total serum cholesterol, the following correlations were found. In patients on maintenance HD for less than two years, positive correlation was found between albumin and ADMA concentration (*p* < 0.02). In the group on HD for over five years, there was significant positive correlation between ADMA and total cholesterol level (*p* < 0.02) and, on the borderline of statistical significance, CRP level (*p* = 0.08).

As already mentioned, elevated ADMA levels are strong inhibitors of nitric oxide synthase. In addition to nitric oxide synthase activity, the generation of nitric oxide is also dependent on the availability of substrate L-arginine. The results of L-Arg serum concentration are presented in Table 5.

The data show that the L-arginine concentrations were very significantly elevated compared with the healthy control (*p* < 0.0001 for both HD groups).

## Discussion

These data confirm previous results [16–24] that ADMA serum levels are significantly elevated

**Table 5.** L-arginine serum levels in controls and the two HD groups**Tabela 5.** Stężenie L-argininy w surowicy w grupie kontrolnej i 2 grupach pacjentów leczonych hemodializami

	Min.	1st Qu.	Median	Mean	3rd Qu.	Max.	SD	NA's
Controls (Grupa kontrolna)	8.71	25.41	31.75	32.71	40.96	60.46	11.18	0.00
HD < 2 yrs	0.17	0.87	1.15	1.40	1.46	7.30	1.10	0.00
HD > 5 yrs	0.22	0.99	1.25	1.55	1.71	4.83	1.08	0.00

**Table 6.** Statistical differences in L-arginine serum levels in the two groups of HD patients vs. controls**Tabela 6.** Statystyczna różnica stężenia L-argininy w surowicy w 2 grupach pacjentów leczonych hemodializami

	Controls (Grupa kontrolna)	HD < 2 yrs	HD > 5 yrs
Controls (Grupa kontrolna)	–	difference = –22 $p < 0.0001$	difference = –23 $p < 0.0001$
HD < 2 yrs	difference = 22 $p < 0.0001$	–	difference = –1.3 $p = 0.57$
HD > 5 yrs	difference = 23 $p < 0.0001$	difference = 1.3 $p = 0.57$	–

in chronic HD patients. In addition, the present study found for the first time that this increase in ADMA level is an early event of HD treatment which is not significantly affected by the duration of maintenance HD. There was no significant difference between HD patients with shorter and longer periods of chronic hemodialysis. In the further analysis it was shown that age and smoking habit do not exert a significant impact on the ADMA concentration in HD patients. Some features of a more detrimental effect of ADMA serum elevation appeared in HD patients treated for over five years. This is suggested by the positive correlation on the borderline of statistical significance between ADMA level and systolic ( $p = 0.09$ ) and diastolic ( $p = 0.07$ ) blood pressure. The paradoxical positive correlation ( $p < 0.02$ ) between serum albumin and ADMA concentration in the HD patients treated for less than two years can be explained by the fact that higher protein intake supports the generation of ADMA. Hyperlipidemia, reflected by total serum cholesterol, remained in the significant correlation ( $p < 0.02$ ) only in patients on HD for over five years, in whom an association also appeared on the borderline of the statistical significance between CRP and ADMA levels ( $p = 0.08$ ). The observation concerning the correlation between ADMA and

cholesterol levels is in accordance with reports on studies [14, 25] in healthy men, in whom correlation between cholesterol and ADMA was noted.

To discern whether elevated ADMA concentration is the only cause of impaired nitric oxide synthase activity in HD patients, L-arginine (the substrate for the enzyme) level was also measured. The L-arginine concentrations were very significantly higher in the HD patients than in the healthy controls. The data concerning L-arginine levels in CKD patients are conflicting, but our results allow excluding a deficiency in the substrate as the cause of impaired nitric oxide synthase activity in well-nourished HD patients. The median values of the albumin concentrations in both HD groups were 40 g/l in the present study. In such adequately nourished patients, elevated L-arginine concentration may arise as a consequence of diminished arginase activity, the key enzyme in the process of L-arginine degradation [26].

The authors conclude that ADMA serum levels are significantly elevated in HD patients independent of the duration of maintenance HD treatment, ADMA concentrations in HD patients were also not affected by age, smoking habit, and class of antihypertensive drugs, in well-nourished HD patients an elevation of L-arginine occurs, possibly due to inhibition of the degradation process.

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**Address for correspondence:**

Robert Krawczyk  
Department of Nephrology and Transplantation Medicine  
Silesian Piasts University of Medicine  
Traugutta 57/59  
50-417 Wrocław  
Poland  
Tel.: +48 71 733 25 02

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