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Asymmetric dimethylarginine (ADMA) as a novel independent risk factor for cardiovascular disease in haemodialysis patients

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SUMMARY
Objective: Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthase and is regarded as a novel risk factor for cardiovascular disease. ADMA concentrations are increased in blood of haemodialysis (HD) patients and may contribute to endothelial dysfunction in them. It was published that the metabolic pathways of ADMA are connected to the metabolic cycle of homocysteine. The aim of our study was to test a relation of ADMA to other cardiovascular risk factors in HD patients.
Design: Observational study.
Settings: Institute of Clinical Biochemistry and Haematology and 1st Department of Internal Medicine of Charles University Medical Faculty and Faculty Hospital, Pilsen, Czech Republic.
Material and Methods: We analysed ADMA levels by ELISA method, total and HDL cholesterols, apolipoproteins AI (apoA) and B (apoB), triglycerides (TG), oxidized LDL (oxLDL), C-reactive protein measured by ultrasensitive method (CRP), lipoprotein (a), thiobarbituric acid reactive substances (TBARS) and homocysteine (Hcy) in plasma of 176 chronically HD patients (70 females, 106 males; mean age ± SD = 66.4 ± 10.63 years). ADMA levels were determined also in 73 healthy men (mean age ± SD = 48.15 ± 5.78). Comparison of ADMA concentration between the two studied groups was performed by the Wilcoxon unpaired test. Relations among variables in the group of HD patients were evaluated using the Spearman correlation. Independence of ADMA was assessed by multiple regression analysis.
Results: Average ADMA concentration in HD patients was significantly higher than control values (1.13 ± 0.22, median = 1.14 μmol/l vs. 0.75 ± 0.15, median = 0.72 μmol/l; CI = 0.33 to 0.44, $p < 0.001$). Interesting correlations were found between BMI and oxLDL ($r = 0.33$, $p < 0.001$), apoA and CRP ($r = -0.28$, $p < 0.001$), apoB and oxLDL ($r = 0.75$, $p < 0.001$) and finally TG and oxLDL ($r = 0.45$, $p < 0.001$). No correlation was found between ADMA and Hcy ($r = -0.07$, $p = 0.39$). Multiple regression analysis revealed that ADMA is independent from BMI, age and all other measured parameters.
Conclusion: ADMA is independent of all other measured cardiovascular risk factors including homocysteine in HD patients.
Key words: asymmetric dimethylarginine, haemodialysis, cardiovascular risk factors, homocysteine, nitric oxide.

SOUHRN
Cibulka R., Široká R., Rajdl D., Racek J., Trefil L., Eiselt J.: Asymetrický dimethylarginin (ADMA) jako rizikový faktor u hemodialyzovaných pacientů
Cíl studie: Asymetrický dimethylarginin (ADMA) působí jako endogenní inhibitor syntázy oxidu dusnatého a je považován za nový kardiovaskulární rizikový faktor. Plazmatická koncentrace ADMA je zvýšená u nemocných léčených hemodialýzou (HD) a může u nich přispívat k endotelové dysfunkci. V literatuře bylo publikováno, že metabolismus ADMA je úzce spojen s metabolismem homocysteinu. Cílem naší studie bylo otestovat vztah ADMA a dalších kardiovaskulárních rizikových faktorů u HD nemocných.
Typ studie: Observační.
Pracoviště: Ústav klinické biochemie a hematologie a 1. interní klinika LF UK a FN v Plzni, Česká republika.
Materiál a metody: Stanovili jsme hladiny ADMA metodou ELISA, celkový cholesterol a HDL-cholesterol, apolipoproteiny AI (apoA) a B (apoB), triglyceridy (TG), oxidované LDL částice (oxLDL), C-reaktivní protein (CRP) měřením ultrasenzitivní metodou, lipoprotein (a), látky reagující s kyselinou thiobarbiturovou (TBARS) a homocystein (Hcy) v plazmě u 176 dlouhodobě HD nemocných (70 žen, 106 mužů; průměrný věk ± SD = 66,4 ± 10,63 roků). Plazmatické hladiny ADMA jsme stanovili navíc u 73 zdravých mužů (průměrný věk ± SD = 48,15 ± 5,78 roků), kteří tvořili kontrolní skupinu. Pro porovnání koncentrace ADMA mezi těmito dvěma skupinami byl použit Wilcoxonův nepárový test. Vztahy mezi proměnnými ve skupině HD nemocných byly vyhodnoceny použitím korelačního koeficientu podle Spearmana. Nezávislost ADMA byla hodnocena mnohočetnou regresní analýzou.
Výsledky: Průměrná koncentrace ADMA u skupiny HD nemocných byla statisticky významně vyšší než u kontrolní skupiny (1,13 ± 0,22; medián = 1,14 μmol/l vs 0,75 ± 0,15; median = 0,72 μmol/l; CI = 0,33 – 0,44; $p < 0,001$). Významné korelace byly nalezeny mezi BMI a oxLDL ($r = 0,33$; $p < 0,001$), apoA a CRP ($r = -0,28$; $p < 0,001$); apoB a oxLDL ($r = 0,75$; $p < 0,001$) a mezi TG a oxLDL ($r = 0,45$; $p < 0,001$). Žádný vztah nebyl nalezen mezi ADMA a Hcy ($r = -0,07$; $p = 0,39$). Mnohočetná regrese prokázala, že hladina ADMA je nezávislá na BMI, věku, i na všech ostatních měřených parametrech.
Závěr: Hladina ADMA je nezávislá na všech ostatních kardiovaskulárních rizikových faktorech včetně homocysteinu u HD nemocných.
Klíčová slova: asymetrický dimethylarginin, hemodialýza, kardiovaskulární rizikové faktory, homocystein, oxid dusnatý.

Introduction

Asymmetrically methylated arginine metabolites including N^G-monomethyl-L-arginine (L-NMMA) and N^G, N'^G-dimethyl-L-arginine (ADMA) act as competitive inhibitors of the nitric oxide synthase (NOS) and therefore negatively influence the nitric oxide (NO) production. As the ADMA blood concentration is about ten-fold higher than that of L-NMMA, it is considered to be the predominant endogenous NOS inhibitor [1]. It is well known that NO is an important vasodilator and an inhibitor of platelet function, proliferation and migration of vascular smooth muscle cells. That's just it, the decreased NO production leads to endothelial dysfunction and can result in a variety of conditions affecting the health of the cardiovascular system [2].

Patients with end-stage renal disease treated with haemodialysis (HD) face a particularly high risk of a cardiovascular disease and cardiovascular mortality. Part of their increased risk is due to a higher prevalence of the established risk factors, such as arterial hypertension, diabetes mellitus, lipid metabolism disorders and anaemia. Inflammatory processes, high sympathetic activity, and the accumulation of endogenous inhibitors of NOS (especially ADMA) have recently emerged as the cardiovascular risk factors of paramount importance [3]. Furthermore, it was published that the metabolic pathways of ADMA are connected to the metabolic cycle of homocysteine, another risk factor of cardiovascular diseases [4, 5].

The methods of determining ADMA concentration in plasma applying different detection techniques have already been published [4, 6] and until quite recently occurred an immunochemical method based on ELISA technique [7].

The aim of our study was to test a relation of ADMA to other cardiovascular risk factors in HD patients.

Material and Methods

We collected serum and plasma of 176 chronically HD patients (70 females and 106 males with mean age ± SD = 66.4 ± 10.63 years) and 73 men with normal kidney function (mean age ± SD = 48.15 ± 5.78) who served as controls. Basic characteristic of the group of HD patients is shown in Table 1. It is necessary to point out that blood samples were not strictly fasting in our HD patients because of different day-time of the blood taking in individual patients on dialysis.

The sampling procedure was standardised, blood samples were collected into EDTA tubes and centrifuged at 3600 g for 5 minutes within half an hour from the blood taking. Plasma was separated and stored frozen at -70 ^{0}C until the analysis. All samples were subsequently assayed in one series. We measured levels of the following parameters in the group of HD patients: ADMA, total and HDL cholesterols, apolipoproteins AI (apoA) and B (apoB), triglycerides (TG), oxidized LDL (oxLDL), C-reactive protein measured by ultrasensitive method (CRP), lipoprotein (a) - Lp(a), malondialdehyde measured as thiobarbituric acid reactive substances (TBARS) and homocysteine (Hcy). In the control group, ADMA concentrations were measured and compared with values in HD patients.

For ADMA immunochemical quantification, the ELISA method from DLD Diagnostika GmbH (Hamburg, Germany) and the AUTO-EIA II microplate reader from Labsystems Oy (Espoo, Finland) were used. Hcy was measured by enzymatic photometric test from Carolina Liquid Chemistries (Brea, California, USA), CRP by ultrasensitive method using kits from Orion Diagnostica (Espoo, Finland). Total and HDL cholesterols, apoA, apoB and TG were measured by routine photometric tests on the Olympus AU 400 analyser (Mishima, Japan), oxLDL using ELISA method from Mercodia (Uppsala, Sweden) and Lp(a) immunoturbidimetrically by kits form Roche Diagnostics (Mannheim, Germany) on the Olympus AU 400 analyser. TBARS were measured by photometric method with modification by Jentzsch [8].

Statistical analysis was performed using the program R 2.2.0. The comparison of ADMA concentration between the two studied groups was performed by the

Table 1. Basic characteristic and results of ascertained parameters in haemodialysis patients

	Results	r_{ADMA} (p)
Age (years)	68 (60 to 75)	-0.08 (0.33)
Sex (male/female)	106/70	–
Systolic blood pressure (mm Hg)	145 (130 to 159.75)	0.08 (0.32)
Diastolic blood pressure (mm Hg)	75 (68 to 82.75)	-0.05 (0.51)
Body mass index (kg/m^2)	25.52 (22.86 to 29.56)	-0.11 (0.14)
Months on HD	21.5 (8 to 46); 56	0.21 (0.02)
Creatinine (μmol/l)	622.5 (524 to 740.75)	0.03 (0.69)
Glucose (mmol/l)	6.09 (5.26 to 7.87)	0.03 (0.68)
Albumin (g/l)	38.7 (35.5 to 41.07)	-0.09 (0.23)
C-reactive protein (mg/l)	6.32 (2.58 to 19.09)	-0.11 (0.17)
ADMA (μmol/l)	1.14 (0.98 to 1.3)	–
Homocysteine (μmol/l)	34.05 (25.6 to 41.88)	-0.07 (0.39)
Total cholesterol (mmol/l)	4.51 (3.73 to 5.15)	-0.04 (0.61)
HDL-cholesterol (mmol/l)	1.09 (0.94 to 1.29)	-0.07 (0.35)
Apolipoprotein A I (g/l)	1.03 (0.93 to 1.19)	-0.01 (0.86)
Apolipoprotein B (g/l)	0.8 (0.66 to 0.92)	-0.08 (0.28)
Lipoprotein (a) (g/l)	0.18 (0.09 to 0.36)	-0.13 (0.11)
Triglycerides (mmol/l)	1.77 (1.32 to 2.43)	-0.07 (0.35)
Malondialdehyde – TBARS (μmol/l)	1.82 (1.58 to 2.06)	-0.06 (0.42)
Oxidized LDL-cholesterol (IU/l)	58.39 (42.65 to 74.3)	-0.12 (0.12)

Wilcoxon unpaired test. Relations among observed variables were expressed as Spearman correlation coefficient, an independence of ADMA was assessed by multiple regression analysis. P-value smaller than 0.05 was considered to be statistically significant.

Results

Average ADMA concentration in our group of HD patients was significantly higher than average ADMA concentration of our controls (1.13 ± 0.22, median = 1.14 μmol/l vs. 0.75 ± 0.15, median = 0.72 μmol/l; 95 % CI = 0.33 to 0.44, $p < 0.001$). Nevertheless, it is correct to point out that both groups were not quite comparable in terms of their structure and age.

Results of ascertained parameters in HD patients are summarised in Table 1. We can see that HD patients in our hospital have been cured properly in respect of their blood pressure, body mass index (BMI) and surprisingly also most of measured laboratory parameters. Slightly higher values of glucose and triglycerides were certainly influenced, in part, by a different fasting time in individual patients. Hypoalbuminemia is certainly not a rare phenomenon in HD patients. Low serum albumin concentration, usually used as a marker of malnutrition, is also affected by other factors, such as chronic inflammatory state, which is usually present in HD patients. Concentration of CRP was really higher in some patients in our study and corresponded to lower albumin concentration. An unexpected status of lipid metabolism and oxidative stress (we used traditional markers: TBARS and oxLDL) was found in our HD patients. Most of measured parameters were within reference ranges for healthy population. On the other hand, we observed a markedly increased concentration of Hcy which is a very frequent finding in HD patients. Hyperhomocysteinemia greater than 30 μmol per litre is considered to be an important cardiovascular risk factor [9]. Since metabolic pathways of ADMA and Hcy are strongly intertwined, many authors began to find relationships among them and cardiovascular risk with ambiguous conclusions [6, 10, 11]. Some investigators described a relation between metabolism of ADMA and cholesterol [12, 13] and even between ADMA and blood pressure levels [14].

Our goal was to test a relation of ADMA to other cardiovascular risk factors in HD patients. Results are shown in Table 1.

We can see that ADMA levels correlated significantly only with the dialysis treatment period (months on HD) – rADMA = 0.21, $p = 0.02$. All rest relationships among ADMA and other observed cardiovascular risk factors were insignificant. No correlation was found between ADMA and Hcy ($r = -0.07$, $p = 0.39$).

Moreover, interesting correlations were found between BMI and oxLDL ($r = 0.33$, $p < 0.001$), apoA and CRP ($r = -0.28$, $p < 0.001$), apoB and oxLDL ($r = 0.75$, $p < 0.001$) and finally TG and oxLDL ($r = 0.45$, $p < 0.001$).

Multiple regression analysis revealed that ADMA is independent from BMI, age and all other measured parameters.

Discussion

Several authors confirmed that ADMA, an endogenous NOS inhibitor, was accumulated in patients with end-stage renal disease (ESRD). Most of them found a two- to six-fold increase of ADMA levels in patients with chronic renal failure as compared to controls, which Kielstein concluded in his review [15]. We did not find so high differences, and our results correspond more to the data reported by Fleck et al. [16]. We think that these lower concentrations were partly caused by relatively good metabolic state of our patients. Furthermore, we paid particular attention to prompt delivery of blood samples to laboratory and early centrifugation. We are convinced that the processing later than 30 minutes from the blood taking leads to the release of ADMA from erythrocytes resulting in spuriously higher concentrations. Nevertheless, we measured highly significantly increased ADMA levels in our HD patients in comparison with controls as well ($p < 0.001$).

High ADMA concentrations in patients with ESRD probably contribute to their excess cardiovascular event rate, as in clinical studies a relationship between ADMA and carotid artery intimal thickening was found. Moreover, Boger and Zoccali demonstrated that determination of ADMA plasma concentration is useful to predict future cardiovascular event rate and total mortality in their patient population [17].

While one group of authors affirms that ADMA concentration depends on other traditional markers of cardiovascular risk, a second strand of literature states that it is quite independent. The most logical connection appears to be between ADMA and Hcy. Hcy is produced during the synthesis of ADMA and can alter ADMA catabolism mainly by inhibiting dimethylarginine dimethylaminohydrolase [5]. Boger et al. found that the hyperhomocyst(e)inemic and also hypercholesterolemic diet produced two to three-fold increases in plasma levels of ADMA and approved a strong correlation between ADMA and Hcy in monkeys [18]. Yoo and Lee observed that elderly patients with stroke had significantly higher plasma ADMA concentrations than controls. ADMA levels were reputedly positively correlated to Hcy levels in them and Hcy should be a significant predictor of elevated ADMA level [10]. Stuhlinger et al. also published a significant correlation between ADMA and Hcy [11]. Studies with opposite findings are for example the following: Jonasson et al. examined patients with hyperhomocysteinemia and found out that plasma ADMA concentration in patients with elevated total Hcy levels was not significantly higher than in patients with low total Hcy levels [19]. Široká et al. came up with interesting explanation that ADMA and Hcy correlated significantly, however this correlation is only apparent due the dependence of both parameters on age [6]. Široká et al. also confirmed that ADMA concentration is partially dependent on renal function. It agrees with Jonasson's observation that plasma ADMA levels correlated with cystatin C levels [19]. It naturally could not pass for patients in ESRD, which we certified as well. We did not confirm any correlation between ADMA and creatinine levels.

In the current study, ADMA levels correlated significantly only with the dialysis treatment period. All rest relationships among ADMA and other observed cardiovascular risk factors were insignificant. Likewise the multiple regression analysis revealed that ADMA is independent of all other measured parameters. Our results represent the most similar data to Fleck's findings [16], who also did not find any significant correlations between concentrations of dimethylarginines and age, blood pressure, cholesterol levels, diabetes mellitus occurrence and other factors in HD patients.

It should be discussed, why our findings differ from findings of some other investigators? There are more possibilities how to explain this discrepancy. Firstly, as mentioned above, preanalytical conditions are very important for precise detection. Earlier studies might have led to mistaken conclusions due to late processing of samples which caused falsely positive ADMA levels. Secondly, positive correlations between ADMA and other biochemical markers were only apparent due the dependence of all these parameters on age. Lastly, there is a possibility to explain this discrepancy by the theory of reverse epidemiology in HD patients [20].

Our future aim is to accept or reject whether ADMA is really independent risk factor in HD patients and whether its concentration could have some prognostic importance for them.

Conclusion

ADMA levels correlated significantly only with the dialysis treatment period. All rest relationships among ADMA and other observed cardiovascular risk factors were insignificant. ADMA showed to be a marker independent of all other measured cardiovascular risk factors including homocysteine in chronically HD patients.

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