



# The influence of renin angiotensin aldosterone system blockers on asymmetric dimethylarginine levels in patients with chronic glomerulonephritis

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## ABSTRACT

Endothelial dysfunction could be related to the limited availability of nitric oxide (NO). NO is synthesized with the participation of an NO synthase whose activity is inhibited by asymmetric dimethylarginine (ADMA). The synthesis of ADMA is exacerbated by oxidative stress, and several studies have shown the efficacy of drugs acting on the renin-angiotensin-aldosterone system (RAAS) (converting enzyme inhibitors and angiotensin II receptor antagonists) in reducing the level of ADMA. The probable mechanism of drug action is a reduction of oxidative stress through a decrease of angiotensin II formation. The aim of this study was to assess the influence of RAAS blockers on the plasma concentration of ADMA in patients with chronic glomerulonephritis (ChGN). The study included 37 patients, placed into group A and group B, depending on the treatment. Both groups were treated with RAAS blockers. In group B, immunosuppressive drugs were additionally administered. The control visits were at the 0, 6 and 12 months of observation. In both the studied groups (A+B), a significant reduction of ADMA (0.77 vs 0.4  $\mu\text{mol/l}$ ;  $p < 0.05$ ) was noticed. In patients suffering from ChGN, the use of RAAS blockers resulted in a significant decrease of plasma ADMA concentration, independently of immunosuppressive treatment.

## INTRODUCTION

Nitric oxide (NO), produced by the endothelial cells of vessels, induces vasodilatation by way of the relaxation of the vascular smooth muscle. In 1992, analogues of L-arginine were first identified in plasma and urine samples. Two of these (L-N-mono-methylarginine (L-NMMA), NG, NG-dimethylarginine (Asymmetric dimethylarginine – ADMA), are endogenous inhibitors of NO synthase. ADMA concentrations in human plasma is 10 times greater than L-NMMA, and ADMA is, therefore, considered a very significant endogenous NO synthase (NOS) inhibitor. The synthesis

of ADMA is exacerbated by oxidative stress, and several studies have shown the efficacy of drugs acting on the renin-angiotensin-aldosterone system (RAAS) in reducing the level of ADMA [4,5]. This phenomenon's mechanism is unclear. The probable action of these drugs is the bringing about of a reduction of oxidative stress, by decreasing the formation of angiotensin II.

The aim of this study was to assess the relationship between plasma concentration of ADMA, proteinuria, some other biochemical parameters and blood pressure, in patients with chronic glomerulonephritis (ChGN) who are undergoing medical treatment including RAAS blockade.

## MATERIALS AND METHODS

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The study group consisted of 37 adult patients (26 M, 11F) at a mean age of  $38.5 \pm 11.5$  (range 22-64) years, suffering from chronic glomerulonephritis (ChGN). The patients presented either a nephrotic (n=14) or a non-nephrotic (n=23) range of proteinuria, and by way of renal biopsy, different types of ChGN were diagnosed. Patients suffering from diabetes mellitus and coronary artery disease were, however, excluded from the study. The control group consisted of 29 healthy volunteers (M=15, F=14) at a mean age  $36.4 \pm 9.5$  (range 19-52) years, who were not taking any (even over-the-counter) medication.

The whole study group of patients has been divided further into two groups. Group A (n=17) consisted of patients who were treated with RAAS blockers. These drugs were used separately or together. Patients from Group B (n=20), in addition to RAAS blockers, received immunosuppressive therapy according to indication (Table 1).

**Table 1.** Treatment schedule

The type of treatment	Total (n=37)	Group A (n=17)	Group B (n=20)
ACE inhibitors	15 (40%)	6 (35%)	9 (45%)
Sartans	4 (11%)	0	4 (20%)
Double blockade of RAA	15 (40%)	11 (65%)	4 (20%)
Statins	9 (24%)	4 (24%)	5 (25%)
Diuretics	16 (43%)	1 (6%)	15 (75%)
Glucocorticosteroids	19 (51%)	0	19 (95%)
Cyclophosphamide	4 (11%)	0	4 (20%)
Cyclosporin	1 (3%)	0	1 (5%)

The project received the approval of the Medical University of Gdansk Bioethical Committee (NKEBN/289/2007). Both volunteers and patients, after hearing the study protocol, signed written informed consent to participate in the study.

Before the study and after 6 and 12 months, all patients underwent assessment of ADMA plasma concentration, as well as daily blood pressure measurements (ABPM). Blood samples were also taken for the following tests: total cholesterol, creatinine, sodium, potassium, CRP, glucose, albumin and fibrinogen, and daily urine protein excretion. In the controls, ADMA measurements were done only once. ADMA concentration measurement was performed using ELISA (Enzyme Linked Immunosorbent Assay) kits (Immunodiagnostik).

Standard descriptive statistics (mean, standard deviation) were calculated. Compliance distribution of the variables with normal distribution was evaluated through analyzing histograms. In situations of non-gaussian distribution, appropriate transformation functions were applied. Intergroup comparison of variables' normal distribution or similar, was done with the t-Student test or analysis of variance (supplemented

with a post-hoc test according to Scheffe). If the distribution greatly deviated from the normal, the Mann-Whitney test was applied. Correlations between variables were ascertained using the Pearson or Spearman method. The relationships between qualitative variables were assessed using multi-way tables and chi-square test according to Pearson. Moreover, variability of individual parameters over time was determined using multilevel mixed effects linear regression. All calculations were carried out by way of help software STATA 13.1 StataCorp Company, LP (United States of America, 2013).

## RESULTS

The study groups and the controls did not differ in respect to age: 38.5 vs 36.4, respectively. The gender in the study group was similar, but it differed from the controls where 70% were male (40 vs 70%; in A+B vs controls, respectively). In Group A, more patients were hypertensive (47% vs 35%;  $p < 0.05$ ), and less were smokers, than were those composing Group B (23% vs 45%;  $p < 0.05$ ). The average daily systolic and diastolic blood pressure did not change significantly, and blood pressure was satisfactorily monitored ( $< 140/90$  mmHg).

In the study groups (A+B), statistically significant changes in biochemistry were observed (table 2), however, renal function remained stable during the whole study period. Furthermore, the concentration of ADMA at various points during the observation period did not differ between Group A and B, yet, the relationships between plasma

**Table 2.** The change of biochemical parameters in 12 months of observation in patients with non-diabetic proteinuria

parameter	Mean±SD 1 visit	Mean±SD 2 visit	Mean±SD 3 visit	p
ADMA (μmol/l)	n-37 0.77±0.26 A 0.76±0.21 B 0.78±0.30	n-37 0.56±0.20 A 0.54±0.18 B 0.59±0.21	n-37 0.40±0.14 A 0.42±0.14 B 0.38±0.15	<0.001* <0.001* <0.001*
CRP (mg/l)	n-37 1.66±1.53 A 2.13±1.85 B 1.27±0.95	n-37 1.26±1.02 A 1.17±0.6 B 1.32±1.10	n-37 1.19±0.89 A 1.46±0.95 B 0.98±1.21	0.020* 0.058* 0.121
Albumin (g/dl)	n-37 37.13±9.16 A 43.88±4.43 B 31.40±8.19	n-37 42.88±7.19 A 45.8±5.48 B 40.57±7.66	n-37 41.23±5.45 A 43.75±3.60 B 39.00±5.91	<0.001** 0.346 <0.001**
Fibrinogen (g/l)	n-37 4.93±1.90 A 4.26±1.85 B 5.51±1.79	n-37 3.83±1.10 A 3.83±1.0 B 3.82±1.20	n-37 3.79±1.18 A 3.61±0.89 B 3.94±1.39	<0.001* 0.028** <0.001**
Creatinine (mg/dl)	n-37 1.14±0.37 A 1.20±0.45 B 1.09±0.29	n-37 1.17±0.44 A 1.28±0.59 B 1.09±0.28	n-37 1.15±0.52 A 1.25±0.65 B 1.06±0.36	0.793 0.500 0.376
eGFR (MDRD) ml/min/1.73 m <sup>2</sup>	n-37 80.85±26.57 A 75.12±26.47 B 81.4±28.65	n-37 80.74±26.85 A 73.80±27.61 B 80.95±23.56	n-37 81.47±27.17 A 77.56±29.70 B 85.56±28.45	0.126 0.328 0.230
eGFR (CKD-EPI) ml/min/1.73 m <sup>2</sup>	n-37 83.80±26.1 A 77.82±24.97 B 84±27.55	n-37 83.68±26.78 A 76.47±27.99 B 84.84±24.76	n-37 84.44±27.03 A 80.38±29.81 B 88.11±28	0.055* 0.302 0.102
Total cholesterol (mg/dl)	n-37 285.24±110.79 A 239.47±45.77 B 324.15±134.25	n-37 219.00±49.0 A 222.53±34.53 B 216.21±58.80	n-37 210.79±52.71 A 222.53±34.53 B 209.94±68.96	<0.001* 0.012** <0.001**
Triglycerides (mg/dl)	n-37 238.81±247.46 A 192.12±166.79 B 278.50±298.32	n-37 195.73±217.22 A 267.67±315.27 B 138.95±38.47	n-37 154.53±107.74 A 177.22±145.05 B 134.33±55.23	0.001* 0.114 0.001*
HDL Cholesterol (mg/dl)	n-37 51.73±16.29 A 45.82±11.24 B 56.75±18.40	n-37 56.29±24.25 A 44.86±14.26 B 65.31±26.93	n-37 52.68±21.68 A 47.87±15.57 B 56.94±25.65	0.143 0.445 0.053**
Daily protein urinary excretion (g/d)	n-37 4.13±4.34 A 1.53±0.96 B 6.3±4.87	n-37 1.76±3.23 A 1.23±0.88 B 2.19±4.26	n-37 1.55±1.91 A 1.01±0.86 B 2.04±2.44	<0.001* 0.005* <0.001*

Abbreviations: CRP – C-reactive protein, eGFR – estimated glomerular filtration rate, HDL – high density lipoprotein. Conversion factors to SI units are as follows: for total cholesterol, LDL cholesterol 0.0259, for creatinine 88.4, for triglycerides 0.0114

\* method of analysis of variance - model assuming a linear variation

\*\* method of analysis of variance - model assuming a quadratic function variation

ADMA and TCh, daily urine protein excretion, CRP and fibrinogen were statistically significant.

## DISCUSSION

The risk of cardiovascular events in patients with chronic kidney disease (CKD) is higher than in the general population, and is enhanced in patients in the early stages of CKD. Several potential factors have been found which could explain this phenomenon. These include: dysfunction of the endothelium, vascular calcification, oxidative stress and inflammation [5]. Endothelial dysfunction is now considered a basic process responsible for the development of atherosclerosis [1]. In contrast, albuminuria is also a marker of renal damage and vascular injury [13]. Paisley *et al.* demonstrated that endothelial dysfunction is present in patients with asymptomatic proteinuria, and attributed it to its impact on the process of nitrogen oxide formation in the endothelium [11]. On the other hand, in patients with nephrotic syndrome, lipids disorders coexist which can potentiate endothelial dysfunction.

ADMA level can increase in CKD because of the impaired glomerular filtration rate. The growth comes primarily due to the reduced activity of the enzyme dimethylarginine dimethylaminohydrolase (DDAH), and increased protein arginine N-methyltransferase (PRMT) activity. In patients with severe proteinuria, the increase of ADMA is associated with a higher turnover of the protein [15]. Data on the relationship of proteinuria with ADMA, and ADMA impact on cardiovascular risk is scarce. Elevated ADMA levels seem to be a "link" between proteinuria, endothelium dysfunction and atherosclerosis complications.

In our study, patients suffering from ChGN at early CKD stages (mean eGFR > 80 ml/min/1.73 m<sup>2</sup>; mean proteinuria 4.13 g/day) had elevated levels of ADMA plasma concentration (mean 0.77 µmol/l), in comparison with the control group (mean 0.56 µmol/l). Hence, a significant correlation between the concentration of ADMA and proteinuria has been demonstrated. It is worth mentioning that Fliser *et al.* noticed a significant positive correlation between the concentration of ADMA and proteinuria in CKD patients with both mild impairment of glomerular filtration rate and proteinuria (1 g/day) [6]. Additionally, Caglar *et al.* found a significant relationship between the concentration of ADMA and proteinuria in patients with non-diabetic proteinuria and normal kidney function [3]. These results were similar to that of our study.

Many reports have shown the efficacy of RAAS blockers in reducing proteinuria in renal diseases and in slowing the progression of renal dysfunction [9]. However, only a few studies have demonstrated RAAS blocker efficacy in normotensive patients with CKD. Still, Makino *et al.* reported that sartans reduce the transition to overt nephropathy in normotensive diabetic patients, suggesting that sartans have favorable BP-independent effects on CKD [10]. In addition, Web *et al.* demonstrated that RAAS blockers effectively reduced proteinuria in normotensive children with CKD [14].

Fujii *et al.* published a study that concluded that in patients with ChGN and normal renal function serum, ADMA levels and urinary protein excretion significantly decreased after receiving treatment with RAAS blockers. The drugs, however, did not have any influence on blood pressure. These findings suggest that RAAS blockers improve the NO system in normotensive patients with CKD [7]. In the presented study, after six months of treatment (RAAS blockers alone or with immunosuppressives), a decrease of ADMA to a level comparable with the control group, was evident. What is more, after the following six months, a further reduction of ADMA was observed.

In the present study, we saw a positive correlation between ADMA and serum total cholesterol, as well as a significant reduction of total cholesterol (groups A+B: 285.24 vs 198.5 mg/dl;  $p < 0.001$ ). These results are consistent with those of Böger *et al.*, who, for the first time, showed a significant positive correlation between serum ADMA and hyperlipidemia in patients with asymptomatic hypercholesterolemia and without other comorbidities [2]. Data concerning the relationship between the concentration of ADMA and the indicator of inflammation status (CRP) have also been confirmed in patients with ESRD and chronic cytomegalovirus infection [9]. Furthermore, Caglar *et al.* have shown a relationship between the concentration of ADMA and the level of CRP in patients with normal renal functions and non-diabetic proteinuria [3]. Our study confirms these findings as, among our study participants, a significant correlation between the concentration of ADMA and both CRP and fibrinogen levels was found.

Therefore, it can be assumed that in patients suffering from hypertension, high blood ADMA concentration results from the impaired metabolism of ADMA within various organs, rather than that hypertension being induced by increased ADMA concentration. Fujimi-Hayashida *et al.*, in patients with IgA nephropathy, demonstrated the correlation of ADMA with proteinuria and kidney damage in biopsy, but not with both systolic and diastolic blood pressure [8]. The authors also suggest that ADMA be used as an indicator of glomerular and interstitial damage evaluated in renal biopsy. Fujii *et al.* confirm this, as they saw, in patients with ChGN and normal renal function, no correlation between blood pressure and ADMA level [7]. Similar results in this matter were achieved in our study. At each point of observation, the average blood pressure was in the normal range, both in the whole studied group of patients, as well as in the groups evaluated separately during the 12 months follow-up. Moreover, in all patients, the treatments resulted in a well-regulated blood pressure during the day and night, respectively. Simultaneously, during the observation, a significant decrease in ADMA plasma concentrations was observed. The ADMA level also did not correlate with average daily, systolic or diastolic blood pressure.

## CONCLUSIONS

In summary, even taking into account all the study limitations (a small study population, a short time of observation), in our study, we have demonstrated the presence of

a significant, higher, ADMA concentration in the plasma of patients suffering from ChGN before treatment, when compared with a healthy group. What is more, treatment with RAAS blockers (alone or with immunosuppression) was associated with a significant reduction of both plasma ADMA concentration and urinary protein excretion, without any influence on the blood pressure control.

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