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Case Report

ACTION OF N-ACETYLCYSTEINE ON ASYMMETRIC DIMETHYLARGININE AND ALBUMINURIA IN STAGE 1-4 NON-DIABETIC CHRONIC KIDNEY DISEASE PATIENTS

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ABSTRACT

Background: Uremic patients are in a pro-oxidant state and show an increased level of asymmetric dimethylarginine (ADMA), which is due to increased PRMT1 activity and reduced dimethylarginine dimethylaminohydrolase (DDAH) as degradation enzymes. Reactive oxidant species may play an important role in increasing the action of PRMT1 and in inhibiting the action of DDAH. Albuminuria and ADMA are closely correlated with progression of cardiovascular disease in chronic kidney disease (CKD) patients as well as indicators for decreasing renal function. Although ACEIs and/or ARBs reduced albuminuria in CKD patients, the results are still conflicting. Several factors in these patients may play important roles in the mechanism of albuminuria such as oxidative stress. The antioxidant N-acetylcysteine may prove to have beneficial therapeutic effect, because it can reduce oxidative stress as shown by evidence in humans, and subsequently increase ADMA. The objective of the present study is to explore the contribution of the antioxidant N-acetylcysteine (NAC) to the decrease of ADMA and albuminuria in non-diabetic CKD patients. Material and Methods: Patients with non-DM CKD stage 1–4 with albuminuria were randomized to receive ACEI and/or ARB alone (control group) or with antioxidant NAC 600 mg orally twice a day (treatment group). Observations were performed for 3 months to measure ADMA and albuminuria before and after-treatment. 80 patients in total 40 in the control group and 40 in the treatment group were used. Results: After oral treatment with NAC, the plasma level of ADMA in the treatment group increased from 0.604 µmol/l to 0.689 µmol/l, whereas ADMA level in the control group exhibited a higher increase from 0.561 µmol/l to 0.743 µmol/l. The increases in these groups were significantly different ($p < 0.02$). Moreover, the level of albuminuria was reduced from 148.12 µg/mg • cr to 132.7 µg/mg • cr in the treatment group, and from 75.25 µg/mg • cr to 71.85 µg/mg • cr in the control group. The difference was significant ($p < 0.001$). Conclusion: The anti-oxidant N-acetylcysteine can be used as adjuvant therapy to inhibit the progression of CKD in patients by decreasing the ADMA level and albuminuria.

Key words: *Chronic kidney disease, reactive oxidant species, asymmetric dimethylarginine, albuminuria, N-acetylcysteine*

INTRODUCTION

Chronic kidney disease (CKD) is highly prevalent with an estimated world wide prevalence of 10%. In CKD patients the main cause of death is cardiovascular disease (CVD).[1] The mechanism underlying this relationship is the occurrence of endothelial dysfunction due to reduced nitric oxide (NO) bioavaibility associated with atherosclerosis.[2–5] Impairment of NO synthesis in CKD might be due to decreased substrates L-arginine or tetrahydrobiopterin (BH4) and/or inhibition of nitric oxide synthase (NOS), which is required for synthesis of NO. Currently, several studies have revealed that the main cause of NOS pathway disturbance is the presence of asymmetric dimethylarginin (ADMA).[6,7] ADMA is suspected to be a predictor risk of CVD in CKD. It is known that ADMA increases in CKD, even in CKD stage 1.[8-10] There are at least four mechanisms of ADMA increase as follows: i) increased protein methylation by PRMT, ii) increase protein turnover, iii) decreased metabolism by dimethylarginine dimethylaminohydrolase (DDAH) and iv) decreased kidney excretion, but it is assamed that increased protein methylation by PRMT is the main mechanism.

Although the molecular mechanisms of increased activity of PRMT and DDAH down-regulation remain unclear various studies indicate that oxidative stress is the main cause.[11-14] Researchers showed that increased oxidative stress in patients with CKD is caused by increased ROS (reactive oxygen species) and decreased antioxidants.[15-16] Release of NO a potent vasodilator, into the circulation from endothelial cells, regulates vascular resistance and blood flow into organ tissue. NO can also inhibit the process of monocyte adhesion to endothelial cells, platelet aggregation and vascular smooth muscle cell proliferation.[17] If there is a decrease of NO, endothelial dysfunction and glomerular damage characterized by proteinuria will occur. Persistent proteinuria is generally a marker of kidney damage.

Various meta-analyse indicated that angiotensin converting enzyme inhibitors (ACEIs) and/or angiotensin II receptor blockers (ARBs) are able to inhibit serum creatinine increases, proteinuria and the progression to ESKD. Therefore, they are recommended as standard renoprotective and antiproteinuric therapy.[18] However, both ACEIs and/or ARBs reduce proteinuria by only 23–32% within 1 to 4 months. Thus, most CKD patients are still in a proteinuric state. Since proteinuria reduces the GFR (ml/minute/10.54, 73m^2/year) patients quickly become worse.[19] Therefore, efforts are needed to discover alternative adjunctive therapies.

The antioxidant N-acetylcysteine (NAC) contains thiol groups as synthetic precursors of cysteine and glutathione. NAC is officially indicated for prevention of mucolytic, acetaminophen poisoning and contrast-induced nephropathy. Several studies have examined the effect of NAC in patients with CKD. Antioxidant NAC when given to experimental animals was able to reduce homocysteine and pulse pressure, to increase NO availability, and to decrease ADMA levels. Hermansyah *et al*. (2008) reported that NAC use in hemodialysis patients decreased ADMA levels by 31.9%.[20-23] Based on those findings, the clinical trial in the present study was conducted to determine the effect of oral NAC 600 mg bid for 3 months on plasma ADMA levels and albuminuria in non-diabetic CKD stage 1–4 patients with albuminuria who were receiving ACEIs/ARBs.

SUBJECTS AND METHODS

This study complies with the principles outlined in the Declaration of Helsinki. It was approved by the local ethics committee and all participants gave written informed consent. The study was an open-labelled randomized clinical trial for determining the effect of oral NAC on serum asymetric dimethylarginine (ADMA) and albuminuria in patients who received ACEI or ARB at Dr. Soetomo's Nephrology Outpatient in Surabaya Clinic, Indonesia.

Patients in this study showed albuminuria > 30mg/day, age of 21–65 years, Hb > 10 g/dl, albumin > 2.5 g/dl and controlled hypertension. They had received an ACEI or ARB for at least one month. Research subjects were selected by a simple-random test to receive oral NAC and ACE inhibitor or ARB for 3 months. Serum ADMA levels and albuminuria were checked before and after administration of oral NAC for 3 months. The exclusion criteria were as follows: (i) trigger factors of proteinuria; pregnant women, heart failure class II-IV (NYHA); (ii) trigger factors of ROS; dyslipidemia, nephrotic syndrome, diabetes mellitus and smoking (in the past 2 weeks and during the study), (iii) risk factors for CKD, use of NSAIDs (more than 2 doses per week), (iv) folate therapy, vitamin B6, B12 or other antioxidants, (v) urinary tract infection (UTI), (vi) steroid therapy or other immunosuppressive theraphy in at least the past 6 months, (vii) serum potassium > 5.1 mEq/L, (viii) cardiac valvular disease and AV block II or III without a pacemaker, (ix) history of hypertensive encephalopathy, cerebrovascular accident or transient ischemic cerebral attacks, (x) history of myocardial infarction, unstable angina pectoris, coronary bypass surgery or percutaneous coronary intervention, (xi) history of malignancy, including leukemia or lymphoma in the last 5 years, (xii) known or suspected contraindications or allergy to ACEI, ARB or NAC, (xiii) consumption of alcohol (last 2 weeks and during the study). The drop-out criteria we as follows: (i) uncontrolled blood pressure; systolic pressure > 130 mmHg, (ii) drug discontination, (iii) died during the study period or (iv) stopped participation in the study.

Data Analysis and Statistics

The Kolmogorov Smirnov normality test was used to determine differences in distribution of albuminuria and ADMA in the treatment and control groups. Correlation between glomerular filtration rate (GFR) and ADMA level was examined using the Pearson correlation test. If the significance was greater than 0.05, the distribution was normal and *vice versa*. For normal group distribution, the parametric t-test was used, and the Wilcoxon non-parametric test was used for the abnormal group. In the control and treatment groups, the Wilcoxon test was used to determine whether there were differences in levels of albuminuria after 3 months treatment. In both tests, to determine whether differences were significant or not, table asymp. Sig (2-tailed) was used. If the value was less than 0.05, differences was significant. If the value was more than 0.05, there was no significant difference.

RESULTS

Characteristics of the sample data are listed in Table 1. Figure 1 shows the graphic correlation. From Figure 1, the inverse correlation between the glomerular filtration rate (GFR) and ADMA level was moderate (r = –0.537). After 3 months of observation, the ADMA level in the control group was elevated by 0.182 µmol/l (p values; 0.001). ADMA levels in the NAC treatement group were elevated by 0.086 µmol/l (p value; 0.001). ADMA level pre- and post- treatment comparisons in both groups are shown in

Table 2. ADMA level elevation in both groups is shown in Figure 2.

Table 1. Clinical and demographic characteristics of treatme and control groups

	Treatment	Control	p
Number of samples	40	40	
Male/Female	9/31	15/25	
Age	54.2 ±	52.5 ±	0.000**)
Staging of CKD I-II/III-IV	20/20	20/20	
ADMA			0.000**)
Systolic blood pressure			0.000**)
Diastolic blood pressure			0.000**)

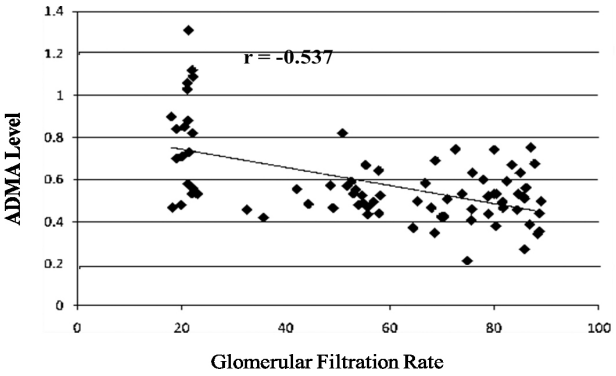

Figure 1. Graphic correlation of GFR and ADMA level in CKD patients (n = 80).

Levels of albuminuria were examined before and after NAC treatment. Albuminuria was significantly decreased by 3 µg/mg • cr during 3 months of treatment in the control group (p value; 0.016), while it decreased by 15 μg/mg • cr in the treatment group (p value 0.000) Figure 3.

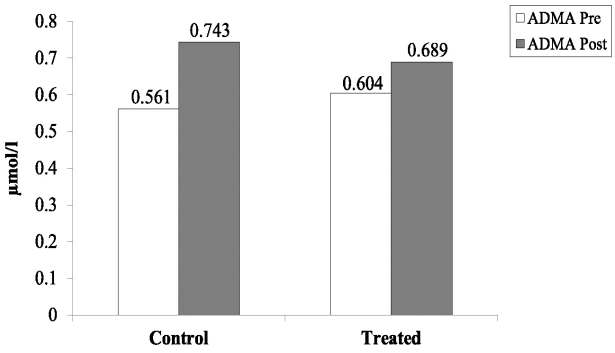

Figure 2. Elevated levels of ADMA in the control group and treatment group.

A comparison of pre and post treatment albuminuria in both groups is shown in Table 3.

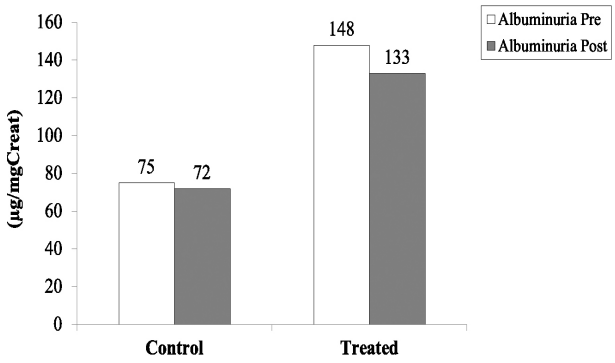

Figure 3. Albuminuria lowering in both groups

DISCUSSION

In Table 1, the distribution of CKD stages between the treatment group and control group did not differ significantly. Every 10ml/min/1, 73 m^2 decrease of GFR, will accelerate a GFR decline of 0.38 ± 0.08 ml/min/year,[19] so that if there is a difference among the groups, it can

Table 2. ADMA level comparison in the control group and treatment group

	ADMA (µmol/l)		Normality	Statistical	Significance
	Before	After	Test	Analysis	(p < 0.05)
Control Group	0.561	0.743	Normal Distribution	Paired-t	0.001 = S
Treatment Group	0.604	0.689	Normal Distribution	Paired-t	0.001 = S

Table 3. Albuminuria comparison in both groups

	Albuminuria (µg/mgCreat)		Statistical	Significance
	Before	After	Analysis	(p < 0.05)
Control Group	75.25	71.85	Wilcoxon	0.016 (Significant)
Treatment Group	148.12	132.7	Wilcoxon	0.000 (Significant)

affect the outcome of therapy. However, since the two groups showed no differences, confounding factors can be minimized.

The results obtained from this study showed a correlation between glomerular filtration rate (GFR) in patients with CKD stage 1-4 and ADMA levels (Figure 1), with a moderate level (r = 0.537) of correlation. This result is similar to several previous studies. Kielstein *et al*.[9,24] showed that ADMA plasma concentrations in non-diabetic CKD patients were significantly difference from those in patients without CKD ($p < 0.001$), even in the early stages of CKD. The study was also consistent with Baylis *et al*.[7] that CKD patients with higher ADMA levels showed a high incidence of CKD progression. In research by Yilmaz *et al*.,[25] there we increased levels of ADMA in non-nephrotic proteinuric patients. An NAC dose of 600 mg bid for 3 months is expected to reduce oxidative stress, which will decrease ADMA levels through reduction of PRMT1 activity, increase DDAH activity and further improve endothelial function reflected by decreased albuminuria. So far, no study has mentioned the effective dose of NAC as an antioxidant in CKD stage I-IV. The NAC dose used in the prevention of contrast induced nephropathy is 1200 mg/day before and after the procedure. Cases of contrast-induced acute kidney injury (CI-AKI) have a similar pathogenesis as CKD. However AKI, mostly occurs transiently, while CKD is a chronic process. Extending the use of NAC for 3 months, will decrease the level of oxidative stress.

The results showed that in the control and treatment groups, increased ADMA levels were 0.182 µmol/l and 0.086 µmol/l. When compared, the ADMA level increase on the treatment group was owen ($p = 0.021$). These results suggest that NAC therapy inhibits the increase of ADMA in CKD stage I-IV with albuminuria. Table 3 revened that NAC at 1200 mg/day for 3 months decrease albuminuria as much as 15.42 μg/mg • cr. In the control group, the decrease was only 3.42 μg/mg • cr. The decrease of albuminuria in the treatment group was significantly greater ($p = 0.02$). This evidence suggests that a decrease in proteinuria was very helpful in slowing the acceleration renal deterioration. A two-fold increase in proteinuria may accelerate a decline in GFR by 0.54 ± 0.05 ml/min/year. The decline in GFR at 10 ml/min/1.73 m^2 will accelerate the decline in GFR by 0.38 ± 0.08 ml/min/year.[19] This study revealed that administration of NAC antioxidant at 1200 mg/day can inhibit ADMA level increases and reduce albuminuria in CKD stages 1-4 patients with albuminuria who have received ACEI/ARBs therapy. Possible mechanisms underlying this correlation are decreased oxidative stress, decreased PRMT activity and increased DDAH activity. Thus, decreased ADMA level improved endothelial function and reduced albuminuria. It appears that administration of NAC at 1200 mg/day for 3 months in CKD stages 1–4 patients with albuminuria may inhibit ADMA level increases and reduced albuminuria.

Conclusion

The anti-oxidant N-acetylcysteine can be used as adjuvant therapy to inhibit the progression of CKD in patients by decreasing the ADMA level and albuminuria.

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