Effect of Oral N-acetylcysteine Treatment on Immune System in Continuous Ambulatory Peritoneal Dialysis Patients

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ABSTRAK

Tujuan: mengevaluasi efek N-asetilsistein (NAS) oral terhadap kadar petanda inflamasi pada pasien Continuous Ambulatory Peritoneal Dialysis (CAPD). **Metode:** *32 pasien yang menjalani regular CAPD, dibagi menjadi dua kelompok masing-masing 16 pasien. Sistem imun ditentukan dari rerata kadar Prokalsitonin, IL-6, IL-1, C3, sICAM, hsCRP, dan TNF-α sebelum dan sesudah pemberian NAS 2x600 mg/hari selama 8 minggu. T-test digunakan untuk membedakan rerata kelompok NAS dengan kontrol, Semua uji statistik menggunakan SPSS software (SPSS Ver 16.0).* **Hasil:** *pemberian NAS, secara bermakna menurunkan kadar petanda inflamasi dibandingkan kelompok kontrol PCT (-0,38±0,57 vs 0,09±0,14; p=0,004), IL-6 (-1,94±3,03 vs 1,19±1,99; p=0,002), IL-1 (-0,14±0,21 vs 0,01±0,04; p=0,010), C3 (-7,40±12,04 vs 4,60±8,12; p=0,002), sICAM (-80,59±29,18 vs -35,02±46,99; p=0,007), hsCRP (-1,50±1,32 vs 0,81±1,17; p<0.001) and TNF-α (-0,73±0,47 vs 0,14±0,74; p<0.001).* **Kesimpulan:** *pemberian NAS peroral mampu menurunkan kadar PCT, IL-6, IL-1, C3, sICAM, hsCRP, dan TNF-α pasien CAPD.*

Kata kunci: CAPD, prokalsitonin, IL-6, IL-1, C3, sICAM, hsCRP, TNF-α, N-asetilsistein.

ABSTRACT

Aim: to determine the effect of oral N-acetylcysteine (NAC) on plasma levels of inflammatory markers in Continuous Ambulatory Peritoneal Dialysis (CAPD) patients. **Methods:** *we performed a placebo-controlled study over 8 weeks in 32 patients on regular CAPD. The patients were divided into 2 groups of 16 patients matched for age and gender. The first group was given NAC 2x600 mg/day for 8 weeks and inflammatory parameter was compared with control group. The immune system is determined from the average levels of Procalcitonin, IL-6, IL-1, C3, SICAM, hsCRP, and TNF-α before and after treatment with NAC. Student t-test was performed to compare the means between NAC receiving and control groups. All statistics were done using SPSS software (SPSS Ver 16.0).* **Results:** *administration of NAC, significantly diminished PCT (-0.38±0.57 vs 0.09±0.14; p=0.004), IL-6 (-1.94±3.03 vs 1.19±1.99; p=0.002), IL-1 (-0.14±0.21 vs 0.01±0.04; p=0.010), C3 (-7.40±12.04 vs 4.60±8.12; p=0.002), sICAM (-80.59±29.18 vs -35.02±46.99; p=0.007), hsCRP (-1.50±1.32 vs 0.81±1.17; p<0.001) and TNF-α (-0.73±0.47 vs 0.14±0.74; p<0.001) levels compared control to group.* **Conclusion:** *short-term oral NAC treatment resulted in reduction of circulating PCT, IL-6, IL-1, C3, sICAM, hsCRP, and TNF-α in CAPD patients.*

Key words: CAPD, procalcitonin, IL-6, IL-1, C3, sICAM, hsCRP, TNF-α, N-acetylcysteine.

INTRODUCTION

Cardiovascular diseases are important main causes of mortality and morbidity in chronic kidney disease (CKD).[1,2] Non classical factors such as inflammation and oxidative stress, played an important role.[3] Patients on dialysis endure persistent oxidative and inflammation stresses. The properties of Reactive Oxygen Species (ROS) that impaired and destroy cells contribute to atherogenesis.[4]

Patients with CKD, show an increase on blood uremic level that will amplify pro-oxidant and pro-inflammation responses which will lead to progressivity of atherosclerosis processes and the rise of mortality number.[5]

Epidemiological studies have reported the association between circulating inflammation marker (includes hsCRP, IL-6 and TNF-α) with the risk of chronic diseases.[6] Ureum decreases cellular immunity, fagocytosis ability and antibody production, that will increase the risk of infection.[7] C3 plays a central role in complement system activation through clasiccal and alternative pathway, meaning that C3 deficiency will lead to the increase of the body susceptibility to infection.[8] sICAM is a biomarker for inflammation processes that involves activation or desruction on cells such as trombocytes and endothelial cells.[9]

Antioxidant supplementation is one strategy to reduce oxidative stress in CKD patients. Low cost, safety, with its aptitude good effects, N-acetylcysteine (NAC) becomes a potential antioxidant and anti-infllamation drug for dialysis patients. NAC is a thiol containing subtance with antioxidative properties that eradicate free radicals. In its reactive sulfhidril form, NAS shows its capability to increase its reduction capacity.[10] NAC has been used as mucolytic in chronic bronchitis therapy and as antidotes in paracetamol overdose. In vitro studies have showed that NAS raise a number of T cell functions.[4]

In the field of nephrology, NAC has been used to prevent contrast induced nephropathy. In hemodialysis patients, NAC has been showed to markedly reduce cardiovascular events (Tepel, 2007). In peritoneal dialysis, studies showed that NAC has the antioxidant capacity to protect peritoneal membrane and to reduce intraperitoneal advanced glyclation end products (AGEs) production in the laboratory animals.

Despite some evidences, the antioxidant and antiinflammatory effects of NAC on CAPD patients remain unclear. In this study, we would like to evaluate, whether NAC's supplementation antioxidant or antiinflammation properties, could reduce the level of PCT, IL-6, IL-1, C3, sICAM, hsCRP and TNF-α in CAPD subjects.

METHODS

Study, Design, and Participants

This is a prospective randomized control trial study, held in nephrology and hypertension division in the Moewardi public hospital, Surakarta, Indonesia.

From August to September 2011, we enrolled 32 consecutive CKD stage V patients that were undergoing CAPD therapy for at least 3 months to 5 years in the hospiltal's CAPD unit and those who agreed to join the study had to sign an informed consent.

Thirty two eligible patients joined the study, were randomly divided into 2 groups, the NAC and control groups each consisted of 16 subjects. The treatment group received NAC (2x600 mg/day) for 8 weeks and the other group received placebo.

Patients with CKD stage V fulfilling the following criteria were recruited:

Those who had renal damage for at least 3 months with structural or functional impairment, with or without decreased glomerular filtration rate, with GFR less than 60 ml/minutes/1.73m². and with signs of renal impairment, including blood and urine marker, or imaging test findings.

Those who had stage V CKD marked by GFR of <15 mL/minutes without considering the ethiology of the impairment and has undergoing at least 3 months of CAPD therapy.

Before a patient could undergo CAPD therapy, he/she must fulfill prerequisite conditions.

Inclusion criteria included patient who had been diagnosed with stage V CKD proved by renal ultrasonography, blood laboratory and urinalysis that are in concordance with K/DOQI 2002 criteria, 20-59 years of age, underwent CAPD for at least 3 months and less than 5 years, not suffering from any form of infection, not in sepsis, systolic blood pressure more than 100 mmHg, hemoglobin level more than 6 mg/dL, not suffering from heart arrhythmya or chronic hepatitis B and C. Exclusion criteria included

patients with stage V Diabetic Nephropathy, on steroid therapy, malignancy, or obstructive uropathy.

Study Parameters

PCT, IL-6, IL-1, C3, sICAM, hsCRP, and TNF-α level were measured from 5 cc venous blood sample taken before and after 8 weeks of NAC therapy. ELISA method was used for the blood examination conducted by PRODIA laboratory.

Statistical Analysis

Data (before and after 8 weeks of oral NAC supplementation) were analyzed using unpaired t-test. All statistical analyses were done by software SPSS 16 for windows.

RESULTS

The basal clinical characteristics of the two groups of patients are given in **Table 1**. Age, gender, hemoglobin, ureum, creatinin, kalium and albumin status before the intervention did not differ significantly between the groups.

This study shows that 8 weeks of oral NAC supplementation, significantly reduced the level of PCT (p=0.004), IL-6 (p=0.002), IL-1 (p=0.010), C3 (p=0.002), sICAM (p=0.007), hsCRP (p<0.001) and TNF-α (p<0.001) compare to the control group.

Table 1. Baseline characteristics of patients receiving N-acetylcysteine (NAC) or placebo

	NAC group	Placebo group
Age (years)	45,79±7.59	42.54±6.79
Male gender (%)	64,28	69,23
Hemoglobin (g/dL)	10.44±2.01	9.49±2.44
Serum Ureum (mg/dL)	128.6±63.9	121.1±39.5
Serum creatinin (mg/dL)	15.01±4.64	21.71±29.73
Serum Kalium (mEq/L)	3.49±0.79	3.40±1.05
Serum albumin (g/dL)	3.33±0.51	3.25±0.59

DISCUSSION

Chronic kidney disease patients are 10 to 20 times more prone to cardiovascular diseases compared to others in general population. Endothelial dysfunction plays a major role in the progressivity of atherosclerosis process. CKD patients shows endothelial depended

Table 2. Difference in cytokine levels (before and after 8 weeks of NAC therapy) in group treated with NAC and in control group. Values are presented as mean±SD

Variables	NAC	Control	p value
PCT	-0.38±0.57	0.09±0.14	0.004
TNF	-0.73±0.47	0.14±0.74	<0.001
hsCRP	-1.50±1.32	0.81±1.17	<0.001
C3	-7.40±12.04	4.60±8.12	0.002
sICAM	-80.59±29.18	-35.02±46.99	0.007
IL-6	-1.94±3.03	1.19±1.99	0.002
IL-1	-0.14±0.21	0.01±0.04	0.010

vasodilatation impairment, increase level of endothelial dysfunction marker, and the escalation of oxidative stress.

Endhotelial damage induced by uremic environment indicates the involvement of specific uremic factor. A number of uremic toxins such as asymmetric dimethylarginine (ADMA), homocystein, advanced glycation end products (AGE), p-cresyl sulfate and indoxyl sulfate, that most of which protein bounded, expresses toxicity to endhotelial cells. The amount of those toxin that could be reduced through hemodialysis is negligible or not at all. The toxin will then cause endhotelial toxicity by a number of mechanism which will lead to augmentation of pro-oxidant and pro-inflammation responses and also impairment in endothelial repairment.[12]

Chronic Kidney disease patients show an increase of blood uremic level. Uremic toxin such as guanidin, AGE, p-cresyl sulfate, platelet diadenosine polyphosphates, and indoxyl sulfate causes vascular dysfunction through (i) the increase of atherosclerosis process, (ii) uremia which involves ADMA, AGE, and oxidative stress will stiffen the artery and will cause left ventricle hypertrophy and decrease coronary perfussion, (iii) vascular calsification and, (iv) abnormality in the vascular repairment ability and neointimal hyperplasia will intensify vascular narrowing. Atherosclerosis and cardiovascular diseases are the most important cause for CKD patients; morbidity and mortality.[13] There is correlation between uremic toxin and cardiovascular death.[14]

The study result shows that 2X600 mg of NAC for 8 weeks significantly downregulates the level of inflammation marker such as PCT, IL-6, IL-1, C3, sICAM, hsCRP and TNF-α. This is probably due to the ability of NAC as antioxidant

to prevent the activation of NF-kB induction and inhibits induction of expression and also secretion of proinflammatory cytokines.[15] NAC also prevented endhotelial mollecular adhesion expression and damage due to peroxinitrit free radicals.[16] In the cytoplasm, NF-kB bounded with IkB protein, but in the event of oxidative stress, the bound break apart causing further degradation and the release of IkB. NAC therapy will block the TNF-α and NF-KB activation. The antioxidant properties of NAC also induced structural modification which will reduce the TNF-α affinity receptor.[17]

Reduction of IL-6 by NAC is in concordance with the study by Nascimento et al (2010).[18] NAC also affectively decreases TNF-α level,[19] which differ from Nascimento's study,[18] and shows that there's no significant effect on TNF-α level in patients on peritoneal dialysis. IL-6 and TNF-α will stimulate the bone marrow to produce more leucocytes. High level of leucocytes will increase the number of PMN and monocytes. Neutrophyl chemotacting factor properties of IL-8 will in turn pull the circulating PMN closer to the endhotelial surface. Number of studies have showed that sICAM production has an important role in arterial plaque process.

The result of a study made by Bambang (2011),[19] shows that NAC supplementation 2X600mg per day for 8 weeks will lower IL-6, serum TNF-α and leucocyte count significantly compare to that of control. This will further support the role of antioxidant and/or antiinfllamation in preventing coronary heart disease and overall reducing the cardiovascular events in patients with CKD.[20]

N-acetylcysteine use rationally in patients with CKD, based on its biological activity being capable of reducing oxidative stress and inflamation. Fact has proved that thiol in NAC will inhibit the production pro-inflammation mediators and stimulates GSH celular system.[4] NAC and its precursor, cystein, work as precursor in GSH biosynthesis, which is important as intracellular and extracellular antioxidant.[21] Thaha et al (2008), reported that NAC administered intravenously during dialysis could reduce ADMA blood concentration, which is a strong predictor for cardiaovascular death on dialysis.[22] High level of uremic toxemia on CKD will impair eritropoetin process as well. It will increase the destruction of erythrocytes

membrane leading to anemia in CKD patients.

N-acetylcysteine supplementation shows the capability of inhibiting cytokines and pro-inflammatory biomarker such as IL-6, TNF-α,[19,23] PCT,[23] hsCRP, IL-1, C3 and sICAM. This in turn, will prevent erythrocytes destruction. Proinflammatory cytokines will induce ROS (reactive oxygen species) which will lead to impairment of eritropoetic receptor. In the study by Bambang (2011), NAC therapy, although not statisically significant, shows the increase of hemoglobin. This probably due to the fact that eritropoetic process needed 120 days (4 week more or less), while the research duration conducted in only 8 weeks (56 days).[23]

Chronic kidney disease marked by a progressive retention of a number of substance which plays a major role in cardiovascular damage. Cardiovascular damage is the main etiology of mortality in CKD. During the last year, especially protein bounded substances (eg. indoxylsulfate and p-cresylsulfate) and/or medium molecules (eg. AGE, sitokin dan polifosfat dinukleosid) have been identified as the primary toxin that involves in the vascular lesion processes affecting endothelial cell functions, leucocytes, trombocytes and/or vascular smooth muscle cell in people with CKD.

Many of these dissolved substances could not be filtered by common dialysis strategy. Eradication of the protein bound dissolved substances are still limited due to only the free fraction of the dissolved substance are filtered by diffusion, while the eradication of the bigger medium molecules (most are larger peptide substance) could be used by increasing the dializer pores diameter and using convective strategy.

Also, the new threpeutic strategy for specific molecules elimination (eg. Adsorbtion) and/or specific effect neutralization of a substance through pharmacological intervention. All of which has with the aim of getting better result for people with CKD.

CONCLUSION

Oral NAC supplementation 2X600 mg perday for eight weeks, could significantly lower PCT, IL-6, IL-1, C3, sICAM, hsCRP dan TNF-α serum level. This result shows NAC administration could be use as a strategy to reduce inflammatory reaction in CAPD patients.

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