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Oral N-acetyl cysteine lowered IL 6 level among stage V chronic kidney disease patients on continuous ambulatory peritoneal dialysis (CAPD)

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ABSTRACT

Background: IL 6 level is increased in stage V CKD patients on CAPD. N-Acetyl Cysteine is a compound containing thiol with antioxidant and anti-inflammatory effects.
Objective: To examine the effect of oral NAC on IL 6 level in stage V CKD patients on CAPD.
Methods: An experimental research with Randomized Double Blind Control Trial, involving 30 CKD patients on CAPD in Dr Moewardi Hospital. They were divided into control group/placebo (15 patients) and oral NAS treatment group (15 patients). IL 6 level was measured before and after 8 weeks of treatment.
Results : Level of IL 6 before and after treatment in control group were (8.13 + 7.62 vs 11.16 + 8.32 , p= 0,001), significantly (p<0,001). Level of IL 6 before and after treatment in treatment group were (9.72 + 7.29 vs 6.09 + 3.82, p = 0,002); lowered significantly (p<0,05).
Conclusion: Oral NAC lowered the level of IL 6 in stage V CKD patients on CAPD.

Latar Belakang: *Kadar IL 6 meningkat pada pasien CKD stadium V yang menjalani CAPD. N-Acetyl Cysteine adalah senyawa yang mengandung tiol dengan efek antioksidan dan anti-inflamasi.*
Tujuan: *Untuk mengetahui efek NAC oral terhadap kadar IL 6 pada pasien CKD stadium V yang menjalani CAPD.*
Metode: *Penelitian eksperimental dengan Randomized Double Blind Control Trial, melibatkan 30 pasien CKD yang menjalani CAPD di RSUD Dr Moewardi. Mereka dibagi menjadi kelompok kontrol/plasebo (15 pasien) dan kelompok pemberian NAS oral (15 pasien). Kadar IL 6 diukur sebelum dan sesudah 8 minggu pengobatan.*
Hasil: *Kadar IL 6 sebelum dan sesudah perlakuan pada kelompok kontrol adalah (8,13 + 7,62 vs 11,16 + 8,32, p = 0,001), secara signifikan (p <0,001). Kadar IL 6 sebelum dan sesudah pengobatan pada kelompok perlakuan adalah (9,72 + 7,29 vs 6,09 + 3,82, p = 0,002); Menurun secara signifikan (p <0,05).*
Kesimpulan: *Oral NAC menurunkan kadar IL 6 pada pasien CKD stadium V yang menjalani CAPD.*

INTRODUCTION

Increased oxidative stress and chronic inflammation in patients with chronic kidney disease (CKD) on dialysis have many talked about.[1-4] Both conditions are associated with increased morbidity and mortality from vascular heart disease in patients with CKD on dialysis through its involvement in the initiation and progression of the process Atherogenesis.[2,3,5] CKD patient mortality reached 23% with the cause of vascular heart disease about 40-45% of all causes of death.[3,6]

Dialysis is very beneficial for patients with CKD. Across the world there are 2.164 million CKD patients on dialysis and 235,000 of them undergoing dialysis, especially peritoneal dialysis peritoneal.[7] Continuous Ambulatory Peritoneal Dialysis (CAPD) is the one choice, but CAPD in the long run also increase cardiovascular risk in CKD.[8] The CAPD induces inflammation and oxidative stress such as chronic infections and related factors dialisat fluid.[5] Related factors dialysis such as bioinkompatibilitas can lead to release of proinflammatory cytokines and endothelial dysfunction that leads to acceleration aterosklerosis.[9,10] inflammatory markers such as Tumor Necrosis Factor - Alpha (TNF-α) and CRP increased with decreased renal function, suggesting that CKD is a chronic inflammatory process. Several factors may be involved in triggering the inflammatory process including oxidative stress.[11]

C-Reactive Protein, which is an acute phase reactant, is produced in the liver that is activated by various cytokines. In dialysis patients, elevated CRP levels indicate an inflammatory process. High sensitivity C-Reactive Protein (hsCRP) is an acknowledged inflammatory marker and may be a predictor of vascular heart disease events. hsCRP is also a powerful factor for predicting complications and death from vascular heart disease.[12] In 25% of patients with CKD increased hsCRP and IL-6, and there is an inverse relationship between the levels of hsCRP, IL-6 with a function of kidney.[13]

N-acetylcysteine (NAS) is a thiol-containing compounds with antioxidant effects and antioxidant.[14] NAS can occur directly through interaction with ROS electrophilic or as precursors of glutathione.[15] A vital antioxidant that protects cells from oxidative stress is known to decline in CKD.[16] We will investigate the effect of oral NAS supplementation on levels IL in stage V CKD patients on CAPD.

METHODS

Types and Research Design

This type of research is an experiment, Randomized Double Blind Controlled Trial.

Place of Research

The study was conducted at Hypertension Kidney Installation of Dr. Moewardi Surakarta, Indonesia.

Sample Research

Samples were randomly drawn from all CKD stage V patients who had undergone CAPD for 3 months to 5 years at the Hypertension Kidney Dr.Moewardi Hospital Surakarta, fulfilling the inclusion and exclusion criteria and willing to take blood for the study.

Sample Size

The determination of sample size involves the parameter of the error rate (Error term) or α and power test level (power test) or 1 - β.

The large sample formulation in this study is as follows 17,18:

$$n = \frac{(Z_{1-\alpha} + Z_{1-\beta})^2 \sigma^2}{\delta^2}$$

Where:
N: sample size.
Z1-α: normal standard value of error rate, if α = 0,05 then:
Z1-α = 1.96.
Z1-β: normal standard power test value, if 1 - β = 0.90 then:
Z1-β = 1,282.
Δ: desired difference (difference of interest)
Σ: the amount of deviation (standard deviation) that can be tolerated.

Due to the paired sample groups apply: δ2 = σ2 = 1, so: $n = (Z_{1-\alpha} + Z_{1-\beta})^2$

Then with the above conditions, this study uses a minimum sample size is:

N = (1.96 + 1.282) 2 = 10.51 rounded to 11.

Thus the minimum sample in this study is 11 respondents in each group, so the use of the number of samples n = 15 respondents in this study is sufficient and meet the formulation of the large sample used. In this study a sample of 30 people with a division of 15 people received treatment with oral NAS and 15 people with placebo for 2 months. A sample of 30 people, 15

people received treatment with oral NAS and 15 people with placebo for 2 months.

Inclusion criteria:

1. Patients with CKD stage V;
2. Age of 20-59 years;
3. Have undergone CAPD for 3 months - 5 years.

Exclusion criteria:

1. CKD patients with diabetic nephropathy stage V;
2. CKD patients undergoing steroid therapy;
3. CKD patients with stage V with malignancy;
4. CKD stage V patients with obstructive uropathy;
5. Patients in the condition of infection/ sepsis;
6. Patients suffering from hepatitis B and or C.;
7. Patients drinking alcohol, smokers, obese, in the treatment of folic acid.

Table 1. Complaints Subjects After Obtaining Oral and Plasebo NAS Drugs.

No	Type of Complaint	NAS	%	Plasebo	%
1	Stomachache	2	13,3	1	6,6
2	Nausea	1	6,6	1	6,6
3	Vomiting	2	13,3	0	0
4	Bloated	0	0	1	0
5	Frequent wind	1	6,6	1	6,6
6	Diarrhea	0	0	0	0
7	Hot body	0	0	0	0
8	Dizziness	0	0	0	0
9	Appetite reduced	1	13,3	1	93,3
10	Appetite equal	14	93,3	14	93,3
11	Appetite increases	0	0	0	0
12	The frequency / number of urine is reduced	0	0	0	0
13	Frequency / amount of urine equal 15	15	100	14	93,3
14	Frequency / number of urine increased	0	0	1	6,6
15	Frequency / number of defecation reduced	0	0	0	0
16	Frequency / number of defecation equal	15	100	15	100
17	The number of defecation increases	0	0	0	0
18	The CAPD liquid result is reduced	0	0	0	0
19	CAPD liquid yields equal	14	93,3	15	100
20	CAPD fluid yield increased	1	6,6	0	0
21	Body condition deteriorates	0	0	0	0
22	Body condition is same	2	13,3	14	93,3
23	Body condition improved	13	86,6	1	6,6
24	Feel the loss after therapy	0	0	0	0
25	Do not feel the benefit of medicine	2	13,3	14	93,3
26	Feel the benefits of medicine	13	86,6	1	6,6

Research Flow

Research subjects were asked for informed consent. The subjects were divided into two groups by drawing on a roll of paper inscribed with numbers 1-30. One group (even-numbered) received oral NAS 600 mg 2x/day and another group (odd numbered) received placebo treatment. Before the treatment, all subjects were taken blood samples for IL-6 levels. The examination was repeated after eight weeks of treatment.

Statistic analysis

Data were analyzed statistically using SPSS.15 for windows.

RESULTS

Description of Demographic and Clinical Characteristics

This study was intended to determine the effect of oral N-acetyl cysteine (NAS) on the decrease in IL-6 levels in CKD stage V patients undergoing CAPD. The 30 patients were divided into two groups: control group and treatment group, each of which was 15 people. The treatment group received treatment with NAS, while the control group was given a placebo. Before performing further analysis, a granular anamnesa emerged in the treatment group receiving oral NAS and in the control group receiving a placebo for eight weeks (Table 1).

Further described the characteristics of research objects for each sample group (Table 2). Based on table 3, in the treatment group of 15 people the sample consisted of 10 men (66.67 percent) and 5 women (33.33 percent), while in the control group with 15 people the sample also consisted of 10 Men (67.67 percent) and 5 women (33.33 percent).

The age characteristic variable of the

Table 2. Comparison of Early Clinical Characteristic Variables in Control Groups and Treatment Groups

Variables	Treatment groups		Control Groups		T test	
	Mean	SD	Mean	SD	Statistical values	P value
Hemoglobin	10,27	1,89	10,99	4,00	t = -0,636	0,530
Leukocytes	7,53	2,13	7,66	1,53	t = -0,197	0,845
Platelets	282,47	115,79	315,53	102,60	t = -0,828	0,415
Albumin	3,79	0,39	3,66	0,77	t = 0,602	0,552
Ureum	115,00	33,13	85,53	32,00	t = 2,378	0,019**
Creatinine	13,34	4,54	12,70	3,46	t = 0,434	0,667
Glucosa	82,40	14,81	100,87	19,63	t = -2,909	0,007**
LDL	100,67	27,26	109,20	24,52	t = -0,902	0,375
HDL	39,13	10,59	37,87	14,32	t = 0,275	0,785
Triglycerides	116,67	48,21	152,40	54,33	t = -1,905	0,067
Potassium	3,47	0,64	3,55	0,96	t = -0,269	0,790
BMI	22,29	3.64	22,09	3,90	t = 0,146	0,885
Systole	154,67	22,00	164,00	21,31	t = -1,180	0,248
Dyastole	95,33	7,43	102,67	12,23	t = -1,985	0,057
Duration of CAPD	26,60	16,23	28,20	18,56	t = -0,251	0,803

Description: ** Significant at 5 percent significance level (p <0.05).

Characteristics of sex and homogeneity test of gender characteristics variable to show that gender variable was homogenous between control and treatment sample group. The value of Chi Square was obtained at 0.000 with probability of 0.650 (p> 0.05) which means there was no difference in the proportion of male or female sex between the control group and the treatment group (table 3)

treatment group showed an average value of 45.27 ± 7.58 years and for the control group averaging 43.80 ± 6.75 years. Distribution of respondent age variable data is normal both in treatment and control group, so homogeneity test for age variable used statistical test parametric t test for difference 2 mean independent sample. Description and test results of age characteristics are at table 4.

Table 3. Comparison of Sex of Control Group and Treatment Group

Sex	Treatment groups		Control Groups		Chi Square Test	
	n	%	n	%	X2	P value
Male	10	66,67	10	66,77		
Female	5	33,33	5	33,33	0,000	0,650
Total	15	100,00	15	100,00		

Table 4. Comparison of Age of Control Group and Treatment Group

Variable	Treatment groups		Control Groups		T test	
	Mean	SD	Mean	SD	Nilai t	P value
Age (year)	45,27	7,58	43,80	6,75	0,559	0,580

Description: ** Significant at 5 percent significance level (p <0.05).

Effect of Oral N-Acetyl Cysteine on IL-6 level

IL-6 variables before treatment in both control and treatment groups were not normally distributed. After treatment the condition was also the same is IL-6 control and IL-6 treatment were equally distributed abnormally. Therefore, the test of difference of 2 mean between control group and treatment was decided using non parametric statistic that is Mann-Whitney test. The result of the test of difference of 2 mean of control group and treatment before and after treatment is as follows:

Table 5. Comparison of IL-6 Levels of Control and Treatment Groups

Group	IL-6			
	Pretest mean±SD	Postest mean±SD	p	Mean±SD Difference
Experiment	9.72±7.29	6.09±3.82	0,002*	-3.62±4.18 (Decrease)
Control	8.13±7.62	11.16±8.32	0,001*	3.03±3.02 (Increase)
P	0.310	0.040*		0.000**

Description: * Significant at 5 percent significance level (p <0.05).
Description: ** Significant at 1 percent significance level (p <0.01).

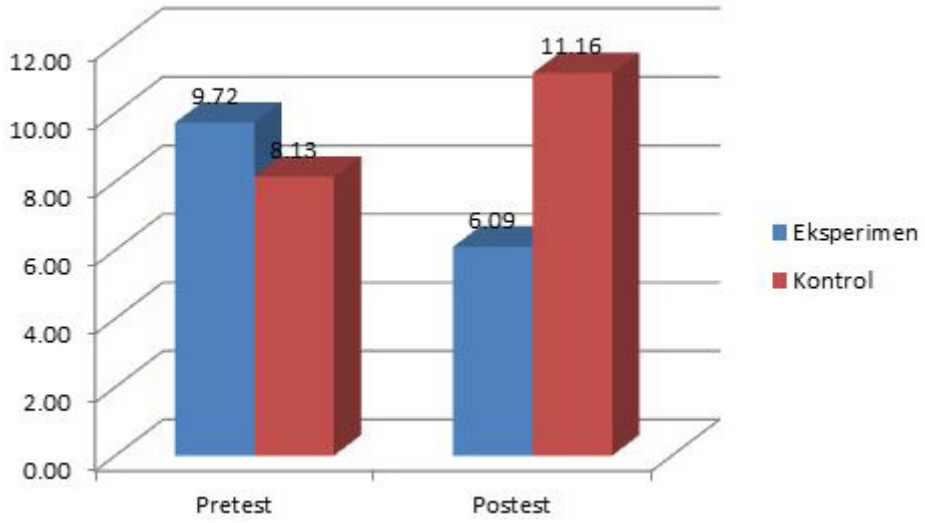

Figure 1. IL-6 levels in Control and Treatment Groups Pretest and posttest

DISCUSSION

The results of this study are consistent with the literature that there are no significant side effects during oral NAS use. It proves the safety of oral NAS in its therapeutic use. Many oral NAS clinical trials with specific indications of using high doses or in long-term treatment have shown that oral NAS drugs are well tolerated. But sometimes gastro-intestinal complaints (nausea, vomitus, dyspepsia) and rarely are urticaria, anorexia, vomitus, meteorism.[19,20,21]

The result of the T test for IL-6 before treatment showed not significant at 5% significance level (p> 0,05). The results of the analysis for the 2 different mean samples showed that the IL-6 variable in the control group, both not significant at 5 percent significance level, can be concluded that IL-6 levels in the control group did not change after placebo for eight weeks. There is even an increase in the average IL-6 levels. These results are consistent with the literature suggesting that inflammatory markers such as IL-6 increase with decreased renal function in CKD. [11]

The result of the difference analysis of 2 mean paired samples showed that the test of the IL-6 concentration variable in the treatment group were both significant at 1% significance level (p <0.01), so it can be concluded that the IL-6 levels in the treatment group actually experienced a convincing decrease . It can be interpreted that the administration of oral NAS has a decisive effect on the decrease in IL-6 levels.

The results of this study are in line with the results of Nascimento et al, 2010 study, in which oral NAS administration may reduce levels of inflammatory cytokines in patients undergoing peritoneal dialysis.

Uremia patients, especially those who undergo long-term CAPD are at high risk of developing oxidative stress caused by free radicals. Various disruptions to the intracellular and extracellular antioxidant systems that serve as a protector against the harmful effects of free radicals play an important role in the progression of oxidative damage to uremia and dialysis.[3]

Based on the literature that NAS is a thiol-containing compound with antioxidant and anti-inflammatory effects, antioxidant effects of NAS can occur directly through interactions with electrophilic ROS as well as glutathione precursor (GSH), a vital antioxidant that protects cells from known oxidative stress decreases in CKD.[15]

Pharmacodynamically, the oral NAS acts as a precursor of Glutathione (GSH) or indirect

antoxidant, direct antioxidant neutralizes oxidants (ROS) removes oxidative stress states and improves cell dysfunction.[22]

NAS serves as an anti-inflammatory in patients with CKD by reducing NFκB activity. NFκB as a transcription factor will cause macrophages to decrease the expression of proinflammatory cytokines in this case IL-6. With this decrease in inflammatory mediators, end-organ stimulation such as the liver to release acute phase proteins decreases, thereby decreasing the stimulation of endothelial dysfunction, ultimately preventing plaque formation and the occurrence of atherosclerosis.[20,23,24]

Overall benefit of this study was 2 x 600 mg oral NAS for eight weeks to reduce IL-6 levels in CKD stage V patients undergoing CAPD, so that the incidence of atherosclerosis can be decreased.

CONCLUSION

Oral NAC lowered the level of IL 6 in stage V CKD patients on CAPD.

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