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The effect of oral N-acetylcysteine on serum high sensitive CRP and plasma hemoglobin levels in end-stage renal disease patients under routine hemodialysis; a randomized placebo-controlled clinical trial

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ABSTRACT

Background: Oxidative stress and systemic inflammation is increased in end-stage renal disease (ESRD) patients. Due to the various effects of oxidative stress in ESRD patients, different antioxidants have been evaluated.

Objectives: In this study, we evaluated the effect of oral N-acetylcysteine (NAC) as an antioxidant on the serum high-sensitive C-reactive protein (hs-CRP) and plasma hemoglobin levels in the ESRD patients who were under routine hemodialysis.

Patients and Methods: In this randomized placebo-controlled clinical trial, 51 ESRD patients under routine hemodialysis were randomly assigned to receive NAC 1200 mg daily for 1 month (n=26) or placebo (n=25). Laboratory findings including hemoglobin, ferritin, hs-CRP were measured in patients before and after treatment.

Results: NAC group compared to placebo group had significantly higher ferritin levels before treatment (p=0.02) and lower phosphorus levels after treatment (p=0.03). Comparing the results before and after treatment in each group, a significant reduction in hematocrit (p=0.002), ferritin (p=0.006), hs-CRP (p=0.02) and an increase in alkaline phosphatase levels (p=0.005) in NAC group and significant reduction in calcium levels (p<0.001) in placebo group was detected. No major side effects were seen.

Conclusions: One month treatment with oral NAC resulted in reduced levels of hematocrit, ferritin and Hs-CRP, indicative of role of NAC in controlling inflammation in ESRD patients under hemodialysis. However, NAC was not effective in treatment of anemia, although the treatment duration was low.

Implication for health policy/practice/research/medical education:
Oxidative stress and systemic inflammation is increased in end-stage renal disease patients. Treatment with oral N-acetylcysteine 1200 mg daily could reduce levels of hematocrit, ferritin and Hs-CRP, indicative of role of N-acetylcysteine in controlling inflammation in end-stage renal disease patients under hemodialysis.

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1. Background

End-stage renal disease (ESRD) patients represent a high cardiovascular risk population (1), where the extent of cardiovascular risk is unexplained by traditional risk factors. ESRD and hemodialysis accompany with high oxidative stress and increased generation of free oxidant radicals with impaired antioxidant neutralization. This yields to inflammation, endothelial dysfunction, anemia and accelerated atherosclerosis (1-3).

Previous studies have evaluated various antioxidants ef-

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fects in reducing oxidative stress in ESRD patients (1,4). Considering low cost, safety, with its beneficial effects, N-acetylcysteine (NAC) has been recommended as a potential anti-inflammatory and antioxidant drug in dialysis patients (5). NAC is a free radical scavenger that increases glutathione and inhibits inducible nitric oxide synthase, suppresses cytokine expression/release, and inhibits the expression of adhesion molecules (6-8). It has been used extensively as a mucolytic agent, in the treatment of acetaminophen toxicity, as a cytoprotective agent during cancer chemotherapy, and in the prevention of contrast-induced nephropathy (9,10). Also it is indicated that NAC acts as an adjuvant in the treatment of chronic kidney disease (CKD) anemia (11).

2. Objectives

In this study, we aimed to evaluate the effects of NAC as an antioxidant on inflammation by measuring high-sensitive C-reactive protein (hs-CRP) and on anemia in hemodialysis patients.

3. Patients and Methods

3.1. Patients

In this randomized placebo-controlled clinical trial, 51 ESRD patients on regular hemodialysis 3 times weekly for at least 3 months in Bou-Ali dialysis center, Ardabil were recruited. Patients with vasculitis, cardiac disease, respiratory disorders, uncontrolled diabetes (HbA_{1C} >7.5%) and those who receiving lipid-lowering agents and immunosuppressive drugs in the last 3 months were excluded from the study. Patients with primary hs-CRP below 6 mg/L, a history of smoking, a known allergy to NAC, chronic inflammatory disease (e.g., rheumatic diseases), evidence of malignancy, cardiac disease or hematologic diseases (e.g., leukemia or lymphoma), active infection, HIV or hepatitis B or C, abnormal liver function tests, use of vitamin E or vitamin C or any antioxidant, were excluded from the study too. Additionally, all study patients were strongly advised to not consume any nutritional material with known anti-oxidant effects. The trial is powered to detect an effect size of d ≥0.70 as statistically significant in a two-tailed test with α = 0.05 and power of 0.80 with N = 25 per condition. As there was possibility that some patients do not complete the study, we included 30 patients in each group. Using RandList 1.2 software, random numbers were produced and according to sample size, patients were enrolled into the study. During the study period, four patients in NAC group (one with infection and three for personal issues) and five patients from placebo group (one died, two with infection and two for personal issues) were lost to follow-up and were excluded (Figure 1).

3.2. Intervention and biochemical measurement

For a period of month, patients received NAC 1200 mg daily (intervention group) or placebo (with the similar size, color and shape). Patients and the physician evaluating the treatment outcome were blinded to the regimens. To control the appropriate use of medications, tablets were delivered to the patients weekly and participants gave back the used tablet sheets to be checked. Before and after intervention, blood samples were taken from each patient to measure complete blood cell count, ferritin, hs-CRP, Ca, P and alkaline phosphatase (ALP). The blood samples for measuring all the above-mentioned variables were taken just before the initiation of dialysis.

3.3. Ethical considerations

The research followed the tenets of the Declaration of Helsinki; the study protocol was approved by the ethics committee of the Ardabil University of Medical Sciences and all participants gave written informed consent before enrolling into the study. Besides that, the study protocol was registered in the Iranian Registry of Clinical Trials website (identifier: IRCT2016111030821N1; http://www.en.search.irct.ir/view/33908).

3.4. Statistical analysis

Results are expressed as mean ± standard deviation (SD) or percentage. The chi-squared and Fisher's exact tests were used to compare categorical variables and the

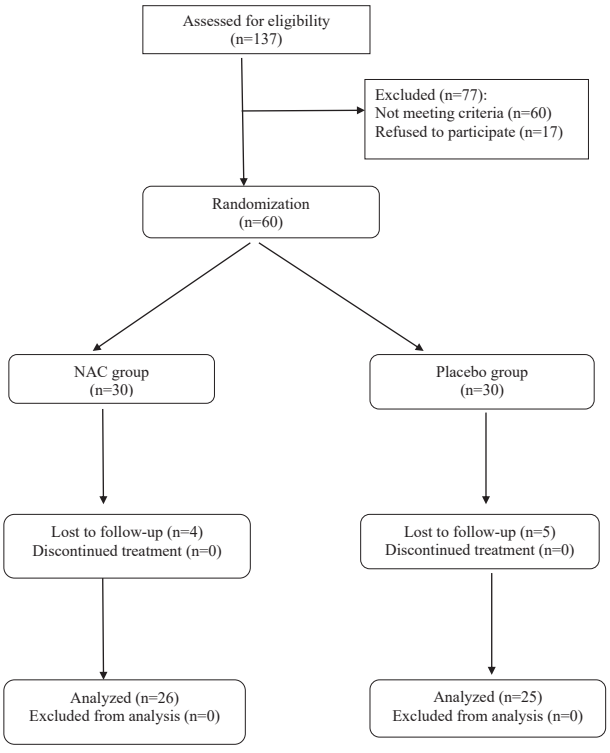

Figure 1. Flow diagram of the study protocol.

Mann–Whitney U test and independent *t* test to compare continuous variables. Paired *t* test was used to compare post treatment results with baseline in each group; *P* values of less than 0.05 were considered statistically significant. All data were analyzed using SPSS 17 (version 17; SPSS Inc., Chicago, IL).

4. Results

Fifty-one patients were studied in NAC (n = 26) and placebo (n = 25) group. There was no significant difference between groups regarding baseline findings (Table 1).

Laboratory findings before and after treatment in NAC and placebo group are shown in Table 2. Among variables, only ferritin levels before and phosphorus levels after treatment were significantly different between groups. Comparing the results before and after treatment in each group, we observed significant changes in hematocrit ($P = 0.002$), ferritin ($P = 0.006$), hs-CRP ($P = 0.02$) and ALP levels ($P = 0.005$) in NAC group and increase in calcium levels ($P < 0.001$) in placebo group.

In order to better evaluate the efficacy of treatment, we compared the percent of changes in laboratory variables between groups and found only significant difference in calcium changes (Table 2). No major side-effects were reported following administration of NAC.

Table 1. Baseline findings between NAC and placebo group

	NAC group	Placebo group	P
Age (y)	65.50±11.05	62.76±14.47	0.45
Gender, No. (%)			0.88
Male	13 (50)	13 (52)	
Female	13 (50)	12 (48)	
Time on dialysis (mon)	41.09±16.15	38.29±14.80	0.052
Underlying cause, No. (%)			0.9
Hypertension	7 (26.9)	6 (24)	
Diabetes mellitus	9 (34.6)	9 (36)	
Glumerulonephritis	4 (15.4)	3 (12)	
Unknown	6 (23.1)	7 (28)	

5. Discussion

In this randomized clinical trial, we observed that oral NAC 1200 mg daily for one month can significantly improve ferritin and hs-CRP levels. However, the percentage of changes were not significantly different between NAC and placebo.

Previous studies have indicated that oxidative stress may cause systemic inflammation and antioxidant neutralization in hemodialysis patients (1-3). As an antioxidant, the effect of NAC on oxidative stress has been evaluated in different studies (5,12-15). Swarnalatha et al (12) showed that NAC can decrease oxidative stress in hemodialysis patients. Shahbazian et al (13) also reported, NAC administration would reduce oxidative stress in hemodialysis patients. Giannikouris (14) explained that NAC

Table 2. Laboratory findings before and after treatment in NAC and placebo group

		NAC group	Placebo group	P value
Hemoglobin (g/dL)	Before	12.34±1.47	11.89±1.71	0.31
	After	12.06±1.74	11.63±1.36	0.33
Hematocrit (%)	Before	39.70±4.61	38.32±4.13	0.32
	After	37.94±5.27	37.04±4.04	0.49
Ferritin (ng/mL)	Before	1317.04±675.08	867.04±743.03	0.021*
	After	1089.96±642.102	734.32±542.46	0.07
Platelets ($\times 10^3/\mu L$)	Before	172.30±48.90	176.01±74.65	0.83
	After	166.81±69.98	185.56±72.64	0.35
hs-CRP (mg/L)	Before	15.51±14.71	17.64±14.60	0.72
	After	9.60±2.10	13.57±2.31	0.22
ALP (IU/L)	Before	510.57±301.23	457.36±220.67	0.47
	After	544.42±302.15	491.72±232.14	0.48
P (mg/dL)	Before	5.34±1.12	5.93±1.42	0.12
	After	5.09±1.23	5.90±1.45	0.03*
Ca (mg/dL)	Before	9.54±0.82	9.75±1.12	0.45
	After	9.51±0.80	9.25±1.13	0.35
Hemoglobin change (%)		-2.40±6.35	-1.44±9.54	0.67
Hematocrit change (%)		-4.46±6.47	-2.59±9.81	0.42
Ferritin change (%)		-16.60±23.32	5.30±71.95	0.14
Platelet change (%)		-3.48±24.93	51.42±240.00	0.25
hs-CRP change (%)		-10.33±108.32	18.24±139.98	0.41
ALP change (%)		9.20±2.73	8.77±21.12	0.93
P change (%)		-3.2±16.83	2.26±28.36	0.40
Ca change (%)		0.19±1.77	-5.09±1.05	0.01*

Abbreviations: P, phosphorus; Ca, calcium; ALP, Alkaline phosphatase; hs-CRP, high-sensitive C-reactive protein.

significantly decreased oxidative stress and inflammatory markers such as hs-CRP in dialysis patients. Purwanto and Prasetyo (15) also concluded that NAC administration for 8 weeks would result in reduction of different inflammatory markers including hs-CRP in patients with continuous ambulatory peritoneal dialysis.

NAC as a supplement therapy may be encouraging in ESRD patients, considering the role of oxidative stress in anemia, inflammation and atherosclerosis in these patients (1). NAC supplementation shows the capability of inhibiting cytokines and pro-inflammatory biomarker such as hs-CRP. Giannikouris (14) suggested that treating hemodialysis patients with NAC could be associated with restoration of important parameters of antioxidant defense and reduction in the levels of oxidative cellular damage mediators. This is probably due to the ability of NAC as an antioxidant to prevent the activation of NF-kB induction and inhibits induction of expression and also secretion of pro-inflammatory cytokines (16). Also, thiol in NAC inhibited the production pro-inflammation mediators and stimulated GSH cellular system (17).

Oxidative stress on one hand and uremic toxins on the other hand could impair erythropoietin process in ESRD patients. It will increase the destruction of erythrocytes membrane leading to anemia in ESRD patients (18). In this study we did not observe a significant change in hemoglobin levels following treatment with NAC. Numerus studies recommended that NAC could improve anemia in hemodialysis patients. Swarnalatha et al (12) showed that NAC as an oral supplement may improve the treatment of anemia. Giannikouris (14) also reported higher hemoglobin levels compared to pre-treatment values.

Inhibiting the inflammatory markers and oxidative stress will prevent erythrocytes destruction. In fact, NAC supplementation may preserve membranous RBC reductases, prevent oxidative damage to the RBC membrane, prolong RBC survival, and improve uremic anemia (11). The difference between studies could be due to the duration of treatment. We only treated patients for 1 month, but other studies duration were 2 to 3 months, while hemoglobin levels depend on erythropoietic process which needs 120 days.

Besides the efficacy of any treatment, its safety is also an issue. Following treatment with NAC we observed no major side effects. Previous studies have also reported very few side effects (11).

6. Conclusions

One month treatment with oral NAC resulted in reduced levels of hematocrit, ferritin and hs-CRP, indicative of role of NAC in controlling inflammation in ESRD patients under hemodialysis. However, NAC was not effective in treatment of anemia, although the treatment duration was low.

Study limitations

Our study is subject to limitations, such as the relatively small sample of patients and short duration of treatment. Also, in this trial, we did not measure biomarkers of oxidative stress.

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Authors' contribution

BB contributed to study design, data collection, drafting and clinical revision of article. RA contributed to data collection, data analysis, and final approval of the version to be published. SMK participated in study design and critical revision of the article. SH contributed to data collection, data analysis and drafting the article. AH performed data analysis and critical revision of the article as well as confirming final approval of the version. All authors read and signed the final paper.

Conflicts of interest

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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