ORIGINAL ARTICLE

The effect of N-acetylcysteine on oxidative serum biomarkers of hemodialysis patients

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Abstract

Background: The aim of the study was to determine the effect of oral N-acetylcysteine (NAC) on levels of serum oxidative stress biomarkers in hemodialysis patients.

Methods: Forty eight hemodialysis patients were administered NAC orally for six months. Hematological, biochemical parameters and levels of asymmetric dimethylarginine (ADMA), malondialdehyde (MDA), myeloperoxidase (MPO) and nitrogen oxide (NO) were determined prior to and upon completion of the study period.

Results: At the end of the study period white blood cells, neutrophil percentage and C-reactive protein levels were significantly lower. Uric acid, albumin and hemoglobin were significantly higher compared to pre-treatment values. Statistically significant increase in NO, and decrease in MDA and ADMA levels were observed. Serum MPO demonstrated a measurable decrease trend, though not significant.

Conclusion It is suggested that treatment with NAC appears to be associated with restoration of important parameters of antioxidant defence and reduction in the levels of mediators of oxidative cellular damage. Hippokratia 2015; 19 (2):131-135.

Keywords: N-acetylcysteine, oxidative stress, hemodialysis

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Introduction

The uremic milieu is an environment of high oxidative stress, in which increased generation of oxidant radicals is coupled to impaired antioxidant neutralization. This is correlated to major complications of the uremic syndrome namely inflammation, endothelial dysfunction, anaemia and accelerated atherosclerosis. The interruption in redox balance appears early in chronic kidney disease (CKD) and is progressively enhanced as it advances to end-stage (ESRD)[1].

Studies concerning the effects of dialysis on oxidative stress have produced conflicting results, since dialysis-related factors promote the formation of free radicals. One of the principal parameters is biocompatibility of hemodialysis materials, while oxidant neutralization is impaired, due to intradialytic loss of water-soluble antioxidants exacerbating the antioxidant defence deficit.

The need to develop strategies to tackle the burden of oxidative stress in haemodialysis is highlighted. Administration of antioxidants is considered a promising approach. N-Acetylcysteine (NAC) is a thiol-molecule that demonstrated a positive effect on oxidative biomarkers[2-6] and a reduction in the incidence of primary cardiovascular events[7], in limited clinical trials involving dialysis patients.

Subjects and Methods

We examined 48 patients, aged 56.9 ± 9.3 years, under dialysis for 43 ± 31 months (Table 1). Inclusion criteria were: thrice-weekly, four-hour hemodialysis session, for a minimum of 12 months, efficacy of single-pool Kt/V (K is the dialyzer blood urea clearance in liters/hour, t is the dialysis session length in hours, V is the urea distribution volume in liters), (spKt/V) ≥1.2-1.4 and compliance with the prescribed treatment. Patients with: inflammatory diseases, peptic ulcer, vascular access failure at any time throughout the study or hypersensitivity to NAC, were excluded. The study protocol was approved by the Institution Scientific Committee, (Ilion Medical Nursery Scientific and Ethics Committe 12/05-10-2013), patients were informed and written consent was obtained.

The study period was set at 6 months, during which time patients received oral 600 mg of NAC, twice daily before meals. Alterations, involving patient therapeutic regimen or hemodialysis parameters, were not permitted during this period. Hematological and biochemical parameters, as well as levels of asymmetric dimethylarginine (ADMA), malondialdehyde (MDA), myeloperoxidase (MPO) and nitrogen oxide (NO), were determined prior to and upon completion of the study period, from samples obtained before the second dialysis session of the week.

Serum ADMA and MDA were quantified via competitive enzyme-linked immunosorbent assay (ELISA) (USNC Life Science Inc., Wuhan, China). Serum NO and MPO via sandwich ELISA, using the Human NO

Table 1: Demographics, hemodialysis parameters, comorbidity and therapeutic regimen of the 48 hemodialysis patients who participated in the study.

Demographics	**Patients (n=48)**
Sex (Male/Female)	25/23
Age (years)	56.9 ± 9.3
Therapy Duration (months)	43 ± 31
Residual Diuresis (ml/day)	n=15, 160 ± 50
Renal Replacement Therapy Parameters	**Patients (n=48)**
Mode (Hemodialysis/Hemodiafiltration)	31/17
Vascular Access (Fistula/Graft/Catheter)	32/4/12
Dialysis Membrane (Polysulfone/Polyethersulfone)	26/22
Membrane Surface (m^2)	1.7 ± 0.1
Dialysis Anticoagulation-Low Molecular Weight Heparin (IU)	4,300 ± 1,800
Comorbidity	**Patients (n=48)**
Diabetes Melitus	40
Coronary Heart Disease	8
Arterial Hypertension	32
Peripheral Vascular Disease	11
Pulmonary Disease	2
Renal Transplantation	4
Drug Therapy	**Patients (n=48)**
B-blockers	19
Statins/Ω_3 Fatty Acids	25/13
Angiotensin Converting Enzyme inhibitors/ Angiotensin II Receptor blockers	19
Vitamin D (Alphacalcidol/Paracalcitol) (μg/week)	14 (1μg) / 5 (7.5μg)
Cinacalcet (mg/day)	9 (15 ± 22.5mg)
Epoetin β (IU/week)	14,440 ± 8,370
Iron Sucrose (mg/week)	143 ± 83
Vitamin B complex (B_1, B_6, B_{12})	48
Carnitine	48
Folate	31

All values are mean ± standard deviation.

Kit (Cusabio, Wuhan, China) and MPO kit (USNC Life Science Inc., Wuhan, China). The delivered dose of hemodialysis was measured using Urea Reduction Ratio (URR) from predialysis and postdialysis serum urea samples [(predialysis Urea-postdialysis Urea)/predialysis Urea] and the single-pool urea kinetic volume model i.e. spKt/V [-ln(R-0.008×t)+(4-3.5×R)×UF/W, where R=1-URR, UF is the fluid volume removed during dialysis, t is session length and W is the postdialysis body weight].

Results

Administration of NAC was correlated with significant changes in haemoglobin levels (p=0.029), a decrease in leukocyte count (p=0.002), in particular, neutrophil percentage (p=0.001) while lymphocytes rose (p=0.008). Changes in serum iron (p=0.376) and ferritin (p=0.647) were not significant, while uric acid (p=0.008), phosphate (p=0.009) and calcium-phosphate product (p=0.014) differed significantly. Decrease in levels of C-reactive protein (CRP) (p=0.002) and increase in serum total protein (p=0.003), which was attributed to the increase in serum albumin (p=0.001), were also noted. The decrease in intact parathyroid hormone (iPTH) was statistically significant (p=0.001). A comparison of the values for spKt/V (p=0.96) and URR (p=0.73) revealed no significant changes in adequacy markers, presuming thus that the state of oxidative stress was not affected by changes in hemodialysis efficacy (Table 2).

Significant increases in levels of NO (p=0.001) and decreases in MDA (p=0.001) and ADMA levels (p=0.032) were observed. Moreover, MPO levels demonstrated a decrease trend though not shown to be significant (p=0.385) (Table 3).

Discussion

A correlation exists between indicators of antioxidant capacity, markers of inflammation and oxidative stress in uraemia. It seems that persistent inflammation depletes

Table 2: Values of serum biomarkers before and after treatment with N-acetylcysteine of the 48 hemodialysis patients, who participated in the study.

Marker	Before	After	p Value
Hemoglobin (g/dL)	**11.7 ± 1.0**	**12.0 ± 0.7**	**0.029**
White Blood Cells (/μL)	**8,003 ± 2,223**	**7,408 ± 1,800**	**0.002**
Neutrophil (%)	**67.6 ± 7.9**	**64.3 ± 6.8**	**0.001**
Lymphocytes (%)	**21.7 ± 6.4**	**23.0 ± 6.3**	**0.008**
Serum Urea (mg/dL)	165 ± 38	161 ± 34	0.532
Serum creatinine (mg/dL)	9.5 ± 1.9	9.8 ± 2.0	0.311
Uric Acid (mg/dL)	**5.8 ± 1.0**	**6.3 ± 1.5**	**0.008**
Serum Calcium (mg/dL)	9.0 ± 0.7	9.1 ± 0.5	0.971
Serum Phosphate (mg/dL)	**6.3 ± 1.9**	**5.7 ± 1.3**	**0.009**
Calcium-phosphate Product (mg^2/dL^2)	**56.8 ± 16.5**	**51.3 ± 10.1**	**0.014**
CRP(mg/dL)	**0.79 ± 0.6**	**0.56 ± 0.4**	**0.002**
Serum Total Protein (g/dL)	**6.55 ±0.6**	**6.71 ± 0.4**	**0.003**
Serum Albumin (g/dL)	**3.80 ± 0.4**	**3.98 ± 0.3**	**0.001**
ALP(IU/L)	116 ± 56	106 ± 43	0.108
Serum Iron (μg/dL)	86.3 ± 43.7	86.1 ± 41	0.647
Serum Ferritin (ng/mL)	743.5 ± 277.4	776.3 ± 320.2	0.376
iPTH (pg/ml)	**379 ± 362**	**314 ± 297**	**0.001**
URR (%)	69.9 ± 5.1	69.5 ± 5.5	0.068
spKt/V	1.44 ± 0.19	1.44 ± 0.21	0.509

All values are mean ± standard deviation. CRP: C-reactive protein, ALP: Alkaline phosphatase, iPTH: intact parathyroid hormone, URR: Urea Reduction Ratio, spKt/V: single-pool Kt/V (K, dialyzer blood urea clearance; t, session length; V, urea distribution volume equal to 55% of body weight).

Table 3: Values of serum oxidative stress-specific biomarkers before and after treatment with N-Acetylcysteine of the 48 hemodialysis patients, who participated in the study.

Marker	Before	After	p Value
NO (ng/mL)	**9.05 ± 4.57**	**13.48 ± 4.97**	**0.001**
MDA (ng/mL)	**20.50 ± 14.91**	**11.84 ± 8.03**	**0.001**
ADMA (ng/mL)	**5.20 ± 3.39**	**3.91 ± 2.09**	**0.032**
MPO (ng/mL)	87.30 ± 27.15	82.37 ± 35.27	0.385

All values are mean ± standard deviation. NO: nitrogen oxide, MDA: malondialdehyde, ADMA: asymmetric dimethylarginine, MPO: myeloperoxidase.

antioxidant defense mechanisms, disrupts redox potential, leading to and amplifying oxidative stress.

In our study, NAC appeared to have a positive effect on hemoglobin levels without modifications to either erythropoietin or iron dosage. Data suggests a correlation between erythrocytic lipid peroxidation products and resistance to erythropoietin, on one hand, and levels of oxidative stress biomarkers and hemoglobin, on the other. It seems that NAC acts as an adjuvant in the treatment of CKD anaemia[8].

The relationship between oxidative stress, inflammation and endothelial dysfunction is observed early in CKD becoming prominent in ESRD. Oxidative stress biomarkers have been shown to be surrogate markers of cardiovascular morbidity and mortality of equal prognostic power to proinflammatory cytokines[9]. A positive correlation between CRP and lipid peroxidation by-products and a negative correlation between CRP and antioxidants has been described in ESRD[10]. In a recent study, treatment with NAC was associated with a significant reduction in inflammatory markers[11].

Low albumin levels have been observed in CKD, being correlated to disease stage and are considered to be an independent risk factor of mortality[12]. The ratio of oxidized albumin is higher in ESRD and is attributed to high oxidative stress burden[13], uremic inflammation and malnutrition, resulting in attenuation of total antioxidant plasma capacity[14].

Considering the significant reduction in the number of leukocytes, neutrophils, CRP levels and the increase in serum albumin, it could be argued that the reduction of oxidative stress was likely associated with a decrease in uremic inflammation and restoration of key antioxidant mechanisms. Interpreting the increase in blood lymphocytes, it could be attributed to a potential decreased oxidative-mediated lymphocytic apoptotic rate, a collateral positive antioxidant effect of NAC therapy.

Uric acid is an essential component of plasma antioxidant capacity. A 'J-shaped' association is observed between urate and mortality in ESRD. Extremely high (>8.9mg/dL) and low (<6.3mg/dL) levels are associated with increased mortality[15]. The administration of NAC was possibly correlated to increase of urate to levels without negative outcome on end-points, attributed to reduction of oxidative stress accompanied by partial balancing of redox potential due to uric acid recovery.

Data analysis revealed a reduction in iPTH levels that could be attributed to NAC-mediated reduction in oxidative stress-induced osteoclastic activity and bone resorption. It has been reported that antioxidant treatment has had a beneficial effect on bone mass loss in experimental models. It is associated with osteoclastic inhibition, activation of transcription factor NF-κB and expression of TNF-a, inhibiting thus bone loss.

Hemodialysis patients exhibit high levels of ADMA, a molecule with inhibitory action on nitric acid synthetase (NOS) and low levels of the endothelium-derived relaxant factor NO[16]. Reports concerning the effect of dialysis on ADMA and NO levels have been inconclusive[17]. A temporary reduction in ADMA, attributed to clearance by dialysis has been reported[18]. The administration of antioxidants appears to be associated with a persistent reduction in ADMA levels having a beneficial effect on outcome[3,18]. This is possibly due to restoration of the balance between enzymes involved in ADMA synthesis and catabolism, whose activity depends on oxidative stress magnitude, leading to partial restoration of the ADMA-NOS pathway, increase in the bioavailability of NO and oxidative stress reduction, via negative feedback.

Our study suggests a beneficial effect on the levels of NO and ADMA, which could be attributed to restoration of redox balance as a result of oxidative stress reduction. NAC has been shown to have a positive effect in the treatment of albuminuria in non-diabetic CKD patients[19] and has been associated with increases in dialysis patients' residual renal function[20,21], attributed to reduction in ADMA and restoration of NO.

In interpreting the reduction in lipid peroxidation marker MDA, it could be argued that the administration of NAC contributed in limiting the extent of peroxidation reactions, due to oxidative stress decline. Available data concerning MDA levels in ESRD patients is limited and conflicting. Dialysis patients present with higher than normal MDA levels, which are negatively correlated with serum albumin and positively correlated with CRP levels[22]. In ESRD, the relationship between lipid peroxidation by-products (F_2-isoprostanes), antioxidant mechanisms and inflammation is confirmed. Peroxidation products have been shown to be negatively correlated with albumin[23] and positively correlated with MDA[24], which appear higher in dialysis patients at high cardiovascular risk.

All the above highlight the negative consequences of oxidative stress (lipid peroxidation) on outcome[25]. The beneficial effect of NAC on MDA levels in hemodialysis patients have been demonstrated[4], whereas in studies evaluating the effect of intradialytic iron therapy on MDA treatment with NAC was shown to have a protective effect[5].

The value of MPO levels in predicting cardiovascular morbidity and endpoints for dialysis patients has been evaluated in recent clinical studies[26]. In the case of renal diseases, MPO appears to be primarily associated with oxidative stress[27]. It constitutes the link between inflammation, oxidative stress and endothelial dysfunction, acting as a NO oxidase, regulating NO availability[27]. In vitro studies[28] have highlighted an inductive effect of advanced oxidation protein products on the MPO-dependent oxidative burst of leucocytes. Hemodialysis per se has been associated with an increase in MPO levels, attributed to leukocyte activation and related to the duration of dialysis, type of membrane and anticoagulant used[26-29]. Administration of heparin mobilizes vascular-bound MPO, suggesting that the observed increase in MPO levels could possibly be attributed, apart from the neutrophilic oxidative burst, to vascular release of MPO[29].

Controlled in vitro studies involving dialysis patients found NAC to have a positive effect. According to certain in vivo studies, administering NAC to diabetic dialysis patients was associated with a significant persistent reduction in MPO levels[30]. In the present study, NAC administration was associated with a non significant reduction in MPO levels. The fact that the levels of advanced oxidation protein products, which are the prime indicators of MPO-dependent activation in ESRD, were not determined, prevents the establishment of conclusions. However, the response exhibited by other MPO-related oxidation markers has provided significant indirect evidence of an ultimately positive effect.

Our study is subject to limitations, such as the relatively small sample of patients, the absence of control group and randomized distribution. Moreover, markers of protein and DNA oxidation were not investigated. Measurement of NAC serum levels were not conducted while those concerning oxidative parameters were performed at two points in time and hence possibly do not reflect variations attributed to disease progression. Therefore, the generalization of the results is limited.

Conclusion

Treatment of hemodialysis patients with NAC appears to be associated with restoration of important parameters of antioxidant defence and reduction in the levels of oxidative cellular damage mediators. The overall effect appears to affect both sides of the ESRD-associated redox imbalance. It appears to re-establish part of the antioxidant defence and to limit the magnitude of oxidation reactions and free radicals formation, leading to at least partial restoration of redox balance. Large-scale research and prospective controlled studies are required.

Conflict of interest

Author reports no conflict of interest.

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