IJNU Original Article

Int J Nephrol Urol, 2009; 1(1): 27 - 32

Does N-Acetyl Cysteine Have a Dose-Dependent Effect on Plasma Homocysteine Concentration in Patients Undergoing Hemodialysis?

Mehdi Khosravi[1], Majid Shohrati[2*], Kiyanush Falaknazi[3]

[1]Islamic Azad University Pharmaceutical Branch, Tehran, Iran

[2]Research Center of Chemical Injuries, Baqiyatallah University of Medical Sciences, Tehran, Iran

[3]Department of Nephrology, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract

Background and Aims: High homocysteine (Hcy) levels in hemodialyzed patients are associated with coronary disease. On the other hand, different effects were reported for impact of N-Acetyl Cysteine (NAC) on plasma Hcy levels in patients undergoing hemodialysis. The current study was conducted to evaluate the effect of oral NAC on plasma Hcy values in hemodialysis patients, comparing to placebo.

Methods: This double blind randomized clinical trial study was done on 99 ESRD subjects undergoing hemodialysis with hyperhomocysteinemia at three dialysis centers in 2008. The effect of one month consumption of 3 different doses of oral NAC (600, 1200 and 1800 mg daily) were assessed on plasma Hcy concentration. Each group was randomly divided into two sub-groups of drug (n=15, 19 and 14, respectively) and placebo (n=18, 19 and 14, respectively).

Results: There were significant differences between groups 2 (1200 mg daily) and 3 (1800 mg daily) of NAC within the study (P=0.000 and 0.004, respectively). Furthermore, there was a relation between the rate of reducing plasma Hcy concentrations and the higher doses of NAC (P=0.000), it means that the effect of drug was dose-dependent. On the other hand, highest dose of drug (1800 mg per day) caused GI disterbance in 5 patients without further effect than 1200 mg daily dose of NAC (P=0.6).

Conclusions: The current study showed that oral NAC in dialysis patients for normalization of the plasma Hcy level has been successful and appeared to be dose-dependent.

Keywords: Homocysteine, N-Acetylcysteine, ESRD, Hemodialysis

Introduction

Homocysteine (Hcy) is a sulphur-containing amino acid produced in the body with the metabolism of the amino acid of methionine. Studies in animal models have shown that high levels of Hcy can lead to increased oxidative stress, impaired endothelial function and increased thrombogenicity, that act together to promote atherosclerosis (1). In addition, several cross-sectional and case-control studies have shown a significant relationship between plasma Hcy concentration and the increase of atherosclerosis as well as coronary artery disease (2-6). Because high Hcy levels are associated with coronary complications, many studies were done on the effect of various agents on Hcy concentrations, and N-Acetyl Cysteine (NAC) is one of them (7). Furthermore, there are several reports that have shown that NAC has antioxidant

*Correspondence:
Majid Shohrati, Ph.D.
Research Center of Chemical Injuries, Baqiyatallah University of Medical Sciences
Molla Sadra Ave. Vanak Sq. Tehran – Iran
Tel.: +98-21-88211524
Email: shohrati@bmsu.ac.ir
Received: 2 Mar 2009
Revised: 28 Mar 2009
Accepted: 6 Apr 2009

properties and also reduced the plasma Hcy levels (8-13).

Although in the healthy individuals, normal range of plasma Hcy concentration is 5–15 µmol/L and plasma Hcy concentration more than 50µmol/l is considered as a severe hyperhomocysteinemia (14); in end stage renal disease (ESRD) patients, however, the plasma Hcy level is higher than healthy persons. The normal plasma Hcy level has been variously reported in ESRD patients such as 22.46 µmol/l (9), 20.9 µmol/l (10) and 17.3 µmol/l (15).

On the other hand, patients with ESRD are in a higher risk for cardiovascular disease. Moreover, the risk factors for atherosclerosis in dialysis patients are hyperlipidemia, hyperhomocysteinemia, abnormalities of glucose and calcium metabolism, hypertension, hyperuricemia and mytogenic factors that can stimulate smooth muscle proliferation (16). Furthermore, in ESRD patients on maintenance dialysis, event rates for myocardial infarction and stroke are 5- to 10- fold higher when compared to the general population (17).

Specifically, it was shown that Hcy is a relevant risk factor for death and cardiovascular sequels in ESRD patients (18). Efforts to return the elevated serum Hcy levels to the normal ranges in dialysis patients using pharmacological-dose vitamin therapy or other agents generally have failed (19).

There were controversial reports about the effect of NAC on Hcy concentrations in ESRD patients on maintenance dialysis (19-22). Therefore, the aim of this study was to appraise the effect of oral NAC on plasma Hcy levels in ESRD patients on maintenance hemodialysis compared to placebo.

Materials and Methods

This double blind randomized clinical trial was done on 99 dialyzed patients with hyperhomocysteinemia at three dialysis centers of Tehran in 2008. In the current study, the effect of oral NAC on plasma Hcy levels was assessed. Patients were randomly divided into 3 groups, according to exposure to various doses of NAC (600, 1200 and 1800 mg per day).

Each group was randomly separated into two sub-groups of drug and placebo (Table 1). We enrolled patients who were on hemodialysis thrice a week (4 hours for every session) for more than 3 months. The ESRD patients who participated in this study did not give history of using any drugs which had interaction with NAC. The exclusion criteria were hypersensitivity to NAC, appearance of severe side effects of NAC and non compliant patients.

Plasma levels of Hcy were determined before and after study with HPLC (Diazyme Hcy Enzymatic Assay Kit). We defined hyperhomocysteinemia as a plasma Hcy concentration greater than 15.0 µmol/l; Thus moderate and severe hyperhomocysteinemia were considered as its levels 25-50 and > 50µmol/l, respectively. All patients were on folic acid. Neither patients nor physicians were informed of the tablet contents, except the statistics technician.

The ethical aspects of the present study were approved by the ethical committee of Baqiyatallah University of Medical Sciences. In addition, the Helsinki Protocol was respected as part of the study design.

Statistics:

Data analysis was done by SPSS version 15.0 software. In all cases, the Kolmogorov-Smirnov test was applied to test for a normal distribution and because the data was not normally distributed non parametric tests were used such as the Mann Whitney and the Kruskal Wallis tests. Comparisons were carried out using the Mann Whitney test for two independent samples or the Kruskal Wallis test for multiple comparisons. Furthermore, differences between paired (dependent) samples were analyzed using the Wilcoxon test. A P-value of less than 0.05 was considered statistically significant for all analyses.

Table 1: Mean age, gender and frequency of severe hyperhomocysteinemia in various groups

Groups	Group 1 (600 mg)		Group 2 (1200 mg)		Group 3 (1800 mg)	
	NAC N=15	Placebo N=18	NAC N=19	Placebo N=19	NAC N=14	Placebo N=14
Mean age- Yrs ± SD	53.0±17.9	54.4±20.6	52.6±17.0	49.8±16.1	55.8±10.8	60.4±11.7
Gender (M/F)-%	67/33	55/45	74/26	68 /32	71/29	57/43
Severe HHcy, N	11	12	14	15	9	9

NAC, N-Acetyl Cysteine; **mg**, milligram; **Yrs**, years; **SD**, Standard Deviation; **M**, Male; **F**, Female; **HHcy**, hyperhomocysteinemia; **N**, Number.

Results

In the present study, 48 dialysis cases had taken NAC and 51 dialysis patients received placebo. Mean age of case and placebo groups had no significant differences (Table 1). Moderate and severe hyperhomocysteinemia were seen in 29.3% and 70.7% of all cases, respectively.

Tables 2 shows that mean plasma Hcy levels were raised after study in group 1 of NAC (600 mg per day) and all placebo groups, but reduction of its levels can be seen in two other groups of NAC (1200 and 1800 mg daily). However, there was no significant differences between plasma Hcy levels before and after study in group 1 of drug (P=0.3). In addition, significant differences in decreasing plasma concentrations of Hcy were observed between groups 2 and 3 of NAC within the study (P=0.000 and 0.004, respectively), it means that two higher doses of drug (1200 and 1800 mg daily) had therapeutic effect on plasma Hcy levels in dialysis patients. Furthermore, there was a relation between the rate of reducing plasma Hcy concentrations and the higher doses of NAC (P=0.000) (Table 3), meaning that the effect of drug was dose dependent. Although, there was a relationship in the mean plasma levels of Hcy differences between group 1 and two other groups with higher doses (P=0.000 and 0.001, respectively) but it was not statistically significant between groups 2 and 3 (P=0.6) (Table 3). On the other hand, Gastrointestinal (GI) discomfort was only seen in 5 patients that had taken NAC with 1800 mg per

Table 2: Comparison of plasma Hcy concentration before and after study in all groups and sub-groups

Groups	Sub-Groups	Before the study ±SD (µmol/l)	After the study ±SD (µmol/l)	P value
Group 1 (600 mg/d)	Drug	69.2± 26.8	70.4 ± 23.2	0.3
	Placebo	73.3 ± 32.1	83.7 ± 39.5	0.002
Group 2 (1200 mg/d)	Drug	70.3± 29.4	63.9 ±28.8	0.000
	Placebo	62.2 ± 18.3	70.5 ± 20.2	0.000
Group 3 (1800 mg/d)	Drug	61.0 ± 26.0	51.2 ± 17.9	0.004
	Placebo	56.5 ± 15.7	56.6 ± 18.8	0.7

SD, Standard Deviation; **mg**, milligram; **d**, day; **µmol/l**, micro mole per liter.

Table 3: Comparison of differences of plasma Hcy concentration in different groups of NAC

Groups	Mean of Hcy differences ± SD (µmol/l)	P value
1, 2 and 3[a]	1.2 ± 6.8, -6.3 ± 4.2 vs -9.8 ± 10.7	0.000
1 and 2[b]	1.2 ± 6.8 vs -6.3 ± 4.2	0.000
1 and 3[b]	1.2 ± 6.8 vs -9.8 ± 10.7	0.001
2 and 3[b]	-6.3 ± 4.2 vs -9.8 ± 10.7	0.6

Group 1, 600 mg daily; **Group 2**, 1200 mg daily; **Group 3**, 1800 mg daily; **SD**, Standard Deviation; **mg**, milligram; **d**, day; **µmol/l**, micro mole per liter.

[a] Kruskal Wallis test

[b] Mann-Whitney test

day within the study, and no other side effects were observed in all other groups. Moreover, comparison of differences of plasma Hcy concentrations in drug and placebo groups showed that there were significant differences in reducing plasma levels of Hcy between different groups of NAC and their placebo (P= 0.01, 0.000 and 0.006, respectively) (Table 4).

Table 4: Comparison of differences of plasma Hcy concentration in drug and Placebo groups in 600, 1200 and 1800 mg groups

	Mean of Hcy differences ± SD (µmol/l)		
Dose (mg/d)	Drug	Placebo	P value
600	1.2 ± 6.8	9.9 ± 10.6	0.01
1200	-6.3 ± 4.2	8.3 ± 5.1	0.000
1800	-9.8 ± 10.7	0.07 ± 4.8	0.006

SD, Standard Deviation; **mg**, milligram; **d**, day; **µmol/l**, micro mole per liter.

Discussion

Our study findings revealed that oral administration of NAC (1200 and 1800 mg per day) leads to reduction of plasma Hcy levels among ESRD patients undergoing hemodialysis. Hultburg in 1994 reported that administration of high amounts of NAC probably displaces from their protein binding sites by disulfide interchange reactions and this leads to the formation of mixed low molecular-weight cysteine and NAC disulfides with high renal clearance and possibly also increased metabolic bio-availability, thereby eliminating Hcy and cysteinylglycine from plasma (8). Intravenous administration of NAC (50 mg/Kg) in a clinical trial study reduced plasma Hcy concentration in ten healthy individuals (9).

Furthermore, plasma Hcy levels were significantly decreased (P<0.000) in a double blind cross over design with 2 weeks wash-out between treatments (11). Ventura et al. have indicated that oral administration of NAC (600 and 1800 mg per day for 1 month) could reduce plasma values of Hcy (12). Moreover, oral NAC induced a quick and highly significant decrease in plasma Hcy levels in 9 young healthy females (13). Scholze et al (2004) reported that intravenous NAC reduced plasma Hcy concentration in a randomized placebo control cross-over study in 20 ESRD patients undergoing hemodialysis (20). In addition, administration of intravenous NAC lowered plasma Hcy concentration in 60 ESRD subjects (P<0.001) (21).

However, according to our data, blood levels of Hcy were not decreased by lower dose of oral NAC administration (600 mg daily). On the contrary, another clinical trial study showed that 600 mg of NAC for eight weeks lowered plasma homocysteine levels (P=0.03) (10). Furthermore, the effects of NAC in our study appeared to be dose-dependent, being more marked in patients treated with higher dosages. Ventura P et al studied the effects of oral NAC therapy in 40 healthy subjects and they observed that the effect of drug was dose dependent (12).

On the other hand, there are contrary reports for

the effect of NAC on lowering the plasma Hcy levels in patients undergoing hemodialysis. Friedman has performed a randomized placebo-controlled trial and they showed that oral NAC (1.2 g twice a day for 4 weeks) could not reduce plasma Hcy concentration in 38 hemodialyzed patients (19).

Furthermore, Bostom et al in a randomized placebo control trial showed that oral NAC (1.2 g daily) had no significant effect on the acute dialysis-related lowering of total plasma Hcy in hemodialysis patients (22).

We found no statistically significant differences in the effect of NAC on reducing the plasma Hcy levels between two oral higher doses (1200 and 1800 mg daily). On the other hands, GI discomfort was only seen in 5 cases (5.5%) who had received 1800 mg per day of NAC. Thus, the administration of 1.2 g daily of drug is preferred due to its safety and efficacy.

Conclusions

The current study indicated that oral NAC was safe and efficient in lowering the plasma Hcy levels among ESRD patients undergoing hemodialysis. However, lower dose of NAC (600 mg daily for one month) could not help to reducing plasma Hcy levels in these patients. On the other hand, highest dose of drug (1800 mg per day) can cause GI complication without further effect than 1200 mg daily dose of NAC.

Acknowledgments

We would like to express our thanks the staff members of Baqiyatallah, Shahid Labbafi-Nejad and Shahid Chamran hospitals for their cooperation.

References

1. Wierzbicki AS. Homocysteine and cardiovascular disease: a review of the evidence. Diab Vasc Dis Res. 2007 Jun;4(2):143-50.
2. Kazemi MB, Eshraghian K, Omrani GR, Lankarani KB, Hosseini E. Homocysteine level and coronary artery disease. Angiology. 2006 Jan-Feb;57(1):9-14.
3. Verhoef P, Kok FJ, Kruyssen DA, et al. Plasma total homocysteine, B vitamins, and risk of coronary atherosclerosis. Arterioscler Thromb Vasc Biol. 1997 May;17(5):989-95.
4. van den Berg M, Stehouwer CD, Bierdrager E, Rauwerda JA. Plasma homocysteine and severity of atherosclerosis in young patients with lower-limb atherosclerotic disease. Arterioscler Thromb Vasc Biol. 1996 Jan;16(1):165-71.
5. Stampfer MJ, Malinow MR, Willett WC, et al. A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians. JAMA. 1992 Aug 19;268(7):877-81.
6. Bots ML, Launer LJ, Lindemans J, et al. Homocysteine and short-term risk of myocardial infarction and stroke in the elderly: the Rotterdam Study. Arch Intern Med. 1999 Jan 11;159(1):38-44.
7. Dierkes J, Westphal S. Effect of drugs on homocysteine concentrations. Semin Vasc Med. 2005 May;5(2):124-39.
8. Hultberg B, Andersson A, Masson P, Larson M, Tunek A. Plasma homocysteine and thiol compound fractions after oral administration of N-acetylcysteine. Scand J Clin Lab Invest. 1994 Oct;54(6):417-22.
9. Ventura P, Panini R, Pasini MC, Scarpetta G, Salvioli G. N -Acetyl-cysteine reduces homocysteine plasma levels after single intravenous administration by increasing thiols urinary excretion. Pharmacol Res. 1999 Oct;40(4):345-50.
10. Yilmaz H, Sahin S, Sayar N, et al. Effects of folic acid and N-acetylcysteine on plasma homocysteine levels and endothelial function in patients with coronary artery disease. Acta Cardiol. 2007 Dec;62(6):579-85.
11. Wiklund O, Fager G, Andersson A, Lundstam U, Masson P, Hultberg B. N-acetylcysteine treatment lowers plasma Hcye but not serum lipoprotein (a) levels. Atherosclerosis. 1996;119:99-106

12. Ventura P, Panini R, Abbati G, Marchetti G, Salvioli G. Urinary and plasma homocysteine and cysteine levels during prolonged oral N-acetylcysteine therapy. Pharmacology. 2003 Jun;68(2):105-14.

13. Roes EM, Raijmakers MT, Peters WH, Steegers EA. Effects of oral N-acetylcysteine on plasma homocysteine and whole blood glutathione levels in healthy, non-pregnant women. Clin Chem Lab Med. 2002 May;40(5):496-8.

14. Carmel R, Green R, Rosenblatt DS, Watkins D. Update on cobalamin, folate, and homocysteine. Hematology Am Soc Hematol Educ Program. 2003:62-81.

15. Nair AP, Nemirovsky D, Kim M, et al. Elevated homocysteine levels in patients with end-stage renal disease. Mt Sinai J Med. 2005 Nov;72(6):365-73.

16. Soldini D, Zwahlen H, Gabutti L, Marzo A, Marone C. Pharmacokinetics of N-acetylcysteine following repeated intravenous infusion in haemodialysed patients. Eur J Clin Pharmacol. 2005 Feb;60(12):859-64.

17. Kes P. Hyperhomocysteinemia in end-stage renal failure. Acta Med Croatica. 2000;54(4-5):175-81.

18. Zoccali C, Mallamaci F, Tripepi G. It is important to lower homocysteine in dialysis patients. Semin Dial. 2007 Nov-Dec;20(6):530-3.

19. Friedman AN, Bostom AG, Laliberty P, Selhub J, Shemin D. The effect of N-acetylcysteine on plasma total homocysteine levels in hemodialysis: a randomized, controlled study. Am J Kidney Dis. 2003 Feb;41(2):442-6.

20. Scholze A, Rinder C, Beige J, Riezler R, Zidek W, Tepel M. Acetylcysteine reduces plasma homocysteine concentration and improves pulse pressure and endothelial function in patients with end-stage renal failure. Circulation. 2004 Jan 27;109(3):369-74.

21. Thaha M, Yogiantoro M, Tomino Y. Intravenous N-acetylcysteine during haemodialysis reduces the plasma concentration of homocysteine in patients with end-stage renal disease. Clin Drug Investig. 2006;26(4):195-202.

22. Bostom AG, Shemin D, Yoburn D, Fisher DH, Nadeau MR, Selhub J. Lack of effect of oral N-acetylcysteine on the acute dialysis-related lowering of total plasma homocysteine in hemodialysis patients. Atherosclerosis. 1996 Feb;120(1-2):241-4.