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INVESTIGATION OF THE EFFECT OF N-ACETYLCYSTEINE ON HEMOGLOBIN IN CHRONIC HEMODIALYSIS PATIENTS

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ABSTRACT

The prevalence of oxidative stress and pro-inflammatory situations is higher in patients with chronic kidney disease. Having contacted the person's blood with heterogeneous membranes of the dialysis machine these situations worsen and causes several reactive oxygen species to be produced which result in inflammatory responses, which in turn exacerbate the uremic, atherosclerosis and sclerosis, cardiovascular events and events such as worsening anemia in hemodialysis patients. In summary, oxidative stress is regarded as a danger factor for exacerbation of atherosclerosis and sclerosis, anemia followed by exacerbation of cardiovascular events and mortality among these patients, to which attention has been given recently.

78 non-hospitalized patients in an age range of 18 to 100 years old with chronic hemodialysis entered the study. They were accidentally divided into two groups of main and control. 39 patients took 600 mg oral Acetylcysteine, two times a day for six weeks. The other 39 patients were given placebo with the same dosage and duration. Then, in the beginning and at the end of the study, hemoglobin level of both groups was measured before hemodialysis.

Findings showed that taking NAC causes Hb level to increase in hemodialysis patients through reduction of oxidative stress. Therefore N-Acetylcysteine can be regarded as a promising treatment to improve anemia of chronic hemodialysis patients and lessen the need to Erythropoietin injection, which can in turn be useful for patients from financial load points of view.

Keywords: N-Acetylcysteine, Hemodialysis, Hemoglobin

INTRODUCTION

The incidence of oxidative stress and pro inflammatory situation in Patients with chronic kidney disease is more than healthy people (1) and as soon as patients with chronic kidney disease reach the end kidney stage and begin their hemodialysis, such situation even worsens. The blood contact with outside body path non biologic materials in hemodialysis can activate blood Leukocytes and produce reactive oxygen species (2,3). ROS cause damage to adjacent tissue cells and result in inflammatory response and stimulation of various inflammatory cytokines (4,6) and thereby exacerbate pro inflammatory responses and result in hemolysis caused by oxidative damage to the membrane of red blood cells (7,8). ROS can exacerbate the anemia of uremia and decrease responsiveness to human recombinant erythropoietin. On the other hand, increase of ROS can produce oxidized LDL, which in turn can lead to atherosclerosis and then cardiovascular events. These controls suggest that treatment

with antioxidants can have a potential role in the treatment of anemia and atherosclerosis in hemodialysis patients (9).

Various studies have been conducted on the efficacy of antioxidant therapy using different materials and methods such as the effect of low electrolyte dialysis fluid (4,10), therapy with vitamin C (8), membranes coated with vitamin E, green tea products (5) and N Acetylcysteine (an oral non-toxic derivative of amino acid which has been used so far as an antioxidant in various clinical situations) (11,13).

The main characteristic of antioxidants is their ability to trap free radicals. Free radicals are highly reactive and are produced from various sources in biological reactions of body together with reactive oxygen species and can oxidize protein, lipids and DNA in the body and cause damage. Antioxidant compounds can inhibit these mechanisms and prevent tissue damage (9,10,11,12).

N Acetylcysteine is a precursor of the antioxidant glutathione. The N

Acetylcysteine has a rail sulfide group with strong antioxidants (14) and improves blood flow to the coronary arteries and peripheral arteries (15). The oxidative stress process is detected with the change in the level of oxidative biomarkers in blood. Since there is an agreement between stress oxidative biomarkers like MDA, Homocysteine CRP, Total Antioxidant, Asymmetric di methyl Arginine and … in blood and inflammation, anemia and the severity of atherosclerosis and cardiovascular disease (16,17), we decided to investigate the effect of oral N Acetylcysteine on Hemoglobin and HCT of patients with chronic hemodialysis.

The target of this project is to investigate the effect of N Acetylcysteine on Hemoglobin of patients with chronic hemodialysis. If the drug results in the increase of Hb of the patients, it can be used to help remedy the anemia caused by inflammation and oxidative stress, whose effects can be sought through the reduction of Eprex dosage in patients who respond to NAC.

MATERIALS AND METHODS

The investigation is a double-blind randomized clinical trial which was carried out in parallel and with the control of placebo. Subjects include patients, female and male, older than 18 years on chronic hemodialysis in Ahvaz Imam Khomeini hospital who have the inclusion criteria.

Investigating Imam Khomeini hospital various shifts patients profiles, all patients were screened and having clearly explained and getting their informed consent and observation of ethical considerations, 78 patient eligible for inclusion were selected. All patients were satisfied for both placebo and N Acetylcysteine, provided that they don't know which study group (main or control) they would enter. The patients were entered remedy or control group randomly while selecting a numbered card in a lightproof sealed pocket. The drugs giving and randomization was done by someone who is not involved in the conduct of trials and investigation of results. These individuals were classified into two groups of cases and control by the above method. 45 patients (60%) were female and 33 others (40%) were male. 53.3% of the placebo group and 66.7% of the remedy group was female. The average of participants was 53.3±17.8. There is no meaningful difference between the two groups from gender or average age perspectives.

In the remedy group, one patient died and one patient as a result of kidney transplantation and one patient as a result of rejection of the drug left the study and finally

the analysis of the group was conducted on 36 patients. In the placebo group, two patients died under the study and one person had lack of acceptance and the other didn't cooperate and finally the information of 36 patients was analyzed.

The hemoglobin base level was measured immediately before the beginning of hemodialysis by the cell counter machine, type Sysmen, Japanese Kx21-N model. Then the main group took the 600 mg, N Acetylcysteine pill, made by Zambon Company, Swiss, two times a day, every 12 hours, solved in 50 CC water before the meal. The duration of medication is six weeks. In the control group, pills similar to NAC were given as the placebo for six weeks, every 12 hours. The placebo is similar to NAC from appearance, color, stiffness and taste point of view and is prepared at Ahvaz Jundishapur University of medical sciences school of pharmacy and it is composed of micro crystalline cellulose, lactose, starch, corn, sorbitol and Talk (2% of pill weight). Having been solved in water, its physical form and taste is similar to NAC pill.

Having finished the study (six weeks) and immediately before conducting hemodialysis in both groups, Hb is measured again by the above method.

The data collected from the indicator situation examined (Hb) before and after the intervention, are recorded in observation sheets and finally the data are entered into the statistical program of SPSS, version 19 and are analyzed. The sample volume of the project was determined as 78 cases with Med-Calc software with a 5% error level and ability of 80%, where 39 samples shall be put in each groups.

FINDINGS

The investigated indicators of patients of both groups before and after the intervention are shown in tables 1 and 2 respectively. Table 1 shows that there is no meaningful difference between the two groups before conducting the study. According to table 2, it is observed that the serum albumin level, ultra filtration and the adequacy of dialysis and hemoglobin in the two groups have a meaningful difference.

Table 1: Indicators studied in patients of both groups before the intervention

	Placebo	Treatment(NAC)	
p-value	Mean±SD	Mean±SD	
0.057	3.29±0.25	3.43±0.3	Dialysis duration
0.670	4.06±0.49	3.99±0.74	Albumin
0.082	67.7±10.87	72.36±8.94	Ultra filtration

0.184	1.29±0.28	1.38±0.21	Dialysis adequacy
0.615	8.08±2.94	9.15±10.76	Cr
0.667	54.86±18.45	52.7±19.42	BUN
0.068	183.39±25.82	167.2±38.46	Cholesterol
0.102	9.9±0.4	9.7±0.4	Hemoglobin (Hb)

Table 2: Indicators studied in patients of both groups after the intervention

p-value	Placebo Mean±SD	Treatment(NAC) Mean±SD	
0.057	3.29±0.25	3.43±0.3	Dialysis duration
0.002	4.04±0.18	4.18±0.14	Albumin
0.022	69.25±9.17	74.65±7.85	Ultra filtration
0.010	1.34±0.22	1.48±0.17	Dialysis adequacy
0.283	6.98±1.94	29.58±111.24	Cr
0.101	51.57±22.54	61.41±22.01	BUN
0.054	173.82±16.35	159.1±35.65	Cholesterol
0.033	10±0.2	9.9±0.3	Hemoglobin (Hb)

Comparing tables 3 and 4, it can be concluded that the average level of Hb in the control group was 9.9446 before receiving placebo and 10 after receiving placebo.

Table 3- Investigation of hemoglobin level in the group receiving NAC

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	HBafter	9.7722	36	1.61946	.26991
	HBbefore	9.6064	36	1.61350	.26892

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	HBafter & HBbefore	36	.972	.000

Paired Samples Test

		Paired Differences		
		Mean	Std. Deviation	Std. Error Mean
Pair 1	HBafter - HBbefore	.16583	.38016	.06336

Paired Samples Test

		Paired Differences 95% Confidence Interval of the Difference				
		Lower	Upper	t	df	Sig. (2-tailed)
Pair 1	HBafter - HBbefore	.03721	.29446	2.617	35	.013

Table 4: Investigation of Hb level in the placebo group (control group)

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	HBbefore	9.9446	36	1.77653	.28447
	HBafter	10.0000	36	1.57814	.25270

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	HBbefore & HBafter	36	.693	.000

Paired Samples Test

		Paired Differences		
		Mean	Std. Deviation	Std. Error Mean
Pair 1	HBbefore - HBafter	-.05538	1.32669	.21244

Paired Samples Test

		Paired Differences				
		95% Confidence Interval of the Difference				
		Lower	Upper	t	df	Sig. (2-tailed)
Pair 1	HBbefore - HBafter	-.48545	.37468	-.261	35	.796

DISCUSSION AND CONCLUSION

Oxidative stress is a situation in which the balance between produced free radicals and body defense confronting them is disturbed and results in bio-molecular and construction changes in cells such as damage to RBC and anemia. However the same trend can naturally occur, when free radicals are produced in white blood cells and causing damage to microbes and defense of body against pathogen agents. Anemia in dialysis patients with cardiovascular diseases such as diabetes, blood pressure and atherosclerosis and …, can be followed by the increased intensity of mortality as a result of cardiovascular diseases as the most important death cause of dialysis patients (4). In the first stage, the anemia is a result of lack of erythropoietin production and in the next levels a result of factors such as high production of cytokines especially IL1. In addition, factors such as oxidative stress, intensification of production of which occurs as a result of contact with dialysis membrane, while damaging cells such as RBC can also cause anemia (1, 2, 3 and 4).

It was shown in this study that taking 600 mg N Acetylcysteine every 12 hours for six weeks is followed by improvement of anemia and better modification of Hb, while in the study conducted by Marie Lazarove and et al in the year 2004 in Czech Republic, N

Acetylcysteine was shown to be effective on the reduction of oxidative stress with no effect on anemia. The difference in conclusions can originate from the difference in duration of taking NAC, when the duration in our study was 42 days while in Lazarove's study the period was 20 days.

In a study conducted by Shin-Plug Heu and et al in Taiwan in the year 2009 on the effectiveness of NAC on the treatment of anemia in hemodialysis patients, a conclusion similar to ours was obtained and the effect on the improvement of patients' anemia was proved. The difference between the two studies was in the period and dosage of taking NAC. In our study, 600 mg of NAC was taken every 12 hours for 1.5 months (six weeks) and in Mr. Shin's study the amount was 200 mg, three times a day for three months. However, finally the effectiveness result of NAC on hemodialysis patients' anemia was similar in the two studies. In other studies, the effect of NAC on oxidative stresses was mostly investigated and in most cases the effect of NAC on oxidative stresses has had a role which may indirectly affect the anemia of those people.

The study showed that taking NAC by hemodialysis patients can be effective in the improvement of Hb situation, which is one of the factors causing intensification of cardiovascular diseases and mortality of these patients. In addition, improvement of their anemia situation and reduction of their need to injection of Eprex can financially help them and reduce the financial burden on them.

According to the duration and dosage of NAC, it may be possible to conduct more studies on the change of dosage (less than 600 mg, two times a day) or reduction of duration of consumption (less than six weeks) and evaluate the responsiveness or irresponsiveness of the drug for the shorter or lesser dosage conditions.

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