Original Communication

Alpha lipoic acid supplementation improved antioxidant enzyme activities in hemodialysis patients

Reza Mahdavi[1], Tannaz Khabbazi[2], and Javid Safa[1]

[1] Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[2] Nutritional Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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Abstract: *Background:* Cardiovascular disease (CVD) is the main cause of death in hemodialysis (HD) patients and oxidative stress is an important risk factor for CVD. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are primary antioxidant enzymes in human cells acting against toxic reactive oxygen species (ROS) and their reduced activity may contribute to oxidative disorders in HD patients. Alpha lipoic acid (ALA) as a potent strong antioxidant may affect these enzymes. *Objective:* We examined the effects of ALA supplementation on antioxidant enzyme activities in HD patients. *Method:* In this double-blinded, randomized clinical trial, 63 HD patients (43 males and 20 females; age range: 22–79 years) were assigned into the ALA group (n: 31), receiving a daily dose of ALA (600 mg), or a control group (n: 32), receiving placebo for 8 weeks. Body mass index (BMI), antioxidant enzymes, albumin (Alb) and hemoglobin (Hb) were determined before and after intervention.
Results: At baseline, the mean blood activities of SOD, GPx, and CAT in ALA group were 1032±366, 18.9±5.09 and 191±82.7 U/gHb which increased at the end of study to 1149±502, 19.1±7.19 and 208±86.6 U/gHb respectively. However, only the increase of SOD was statistically significant in comparison with placebo group ($P = 0.04$). The mean levels of Alb, Hb, weight and BMI were not significantly changed in study groups ($P>0.05$). *Conclusion:* ALA may be beneficial for HD patients by increasing the activity of antioxidant enzymes; however, further studies are needed to achieve precise results.

Keywords: Hemodialysis, antioxidant enzymes, alpha lipoic acid, cardiovascular disease

Introduction

Chronic kidney disease (CKD) is a worldwide health problem with a high incidence rate that will progressively develop into end-stage renal disease (ESRD) [1]. Cardiovascular disease (CVD) is the main cause of death in patients with ESRD, including patients undergoing hemodialysis (HD). The physiopathology of cardiovascular events in HD is multifactorial [2]; however, accumulated evidence suggests that an increased oxidative stress is an important risk factor for CVD in this population [1, 2].

Reactive oxygen species (ROS) are highly reactive and unstable substrates produced by oxygen metabolite [1]. Oxidative stress is a state of imbalance between excessive oxidant formation and lack of antioxidants as a defense mechanism [1]. Primary antioxidant enzymes in human cells that seem to be necessary for cell protection are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [1]. The reduced activity of SOD [3–6], GPx, and CAT [7], have been described in chronic renal failure that seems to contribute to the occurrence of oxidative disorders [3, 6]. Furthermore, HD patients usually have low intakes of dietary antioxidant micronutrients such as vitamin E, vitamin C, selenium and zinc [3]. Oxidative stress, as a non-traditional risk factor, not only explains the high incidence of CVD in CKD, but also becomes a new target in therapeutic intervention [1]. In this context, various natural compounds with pleiotropic actions such as antioxidants are of interest. Antioxidants are now widely used as a therapy to reduce oxidative stress in many diseases [1]. One antioxidant agent that is usually used in oxidative stress related disease is alpha lipoic acid (ALA) [8–10]. ALA is a naturally occurring compound which is de novo synthesized in mitochondria and acts as an essential cofactor for certain dehydrogenase complexes [8]. In pharmacological doses (200–2400 mg/day) [11], ALA acts as a potent strong antioxidant [9]. It is widely used in diabetes mellitus (DM) [12–14], hypertension [15], Alzheimer's disease [16], Down syndrome [17] and some types of cancer [18]. Furthermore, growing evidence has demonstrated a nephroprotective role for ALA [8]. We have previously shown that ALA supplementation

may have beneficial effects in HD patients by reducing inflammation [19].

Owing to high prevalence of antioxidant disturbance in HD patients, there is an increasing need to investigate the potential of effective antioxidants to be used in addition to routine therapies for improving oxidative stress in these patients. To the best of our knowledge, no adequately powered studies have examined the efficacy of ALA on antioxidant enzymes in HD patients [20]. In keeping with this strategy, we evaluated the effects of ALA supplementation on antioxidant enzyme activities in hemodialysis patients.

Materials and Methods

Subjects

63 patients (43 Males and 20 Females; with age range, 22–79 years) with ESRD were selected for the study (Fig. 1). The patients were clinically stable; and undergoing maintenance HD initiated ≥6 months previously (mean ± SD, 51.7 ± 38.9 months and ranged from 6 to 152 months) at a rate of 3 times/wk for 3.5–4.5 h per session. Exclusion criteria were being <20 years old, having gastrointestinal disorders, hepatitis and any infectious diseases, smokers, kidney transplantation candidates, pregnant and lactating mothers, those receiving any NSAID (Non-Steroidal Anti-Inflammatory Drug), SAID (Steroidal Anti-Inflammatory Drug), valproic acid or ALA, vitamin C and E supplements within the last month. The study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences. It was registered in the Iranian Registry of Clinical Trials website (IRCT138902041197N4). This website is a WHO Primary Registries and conforms to International Clinical Trail Registry Platform (ICTRP). Written informed consent was obtained from all patients.

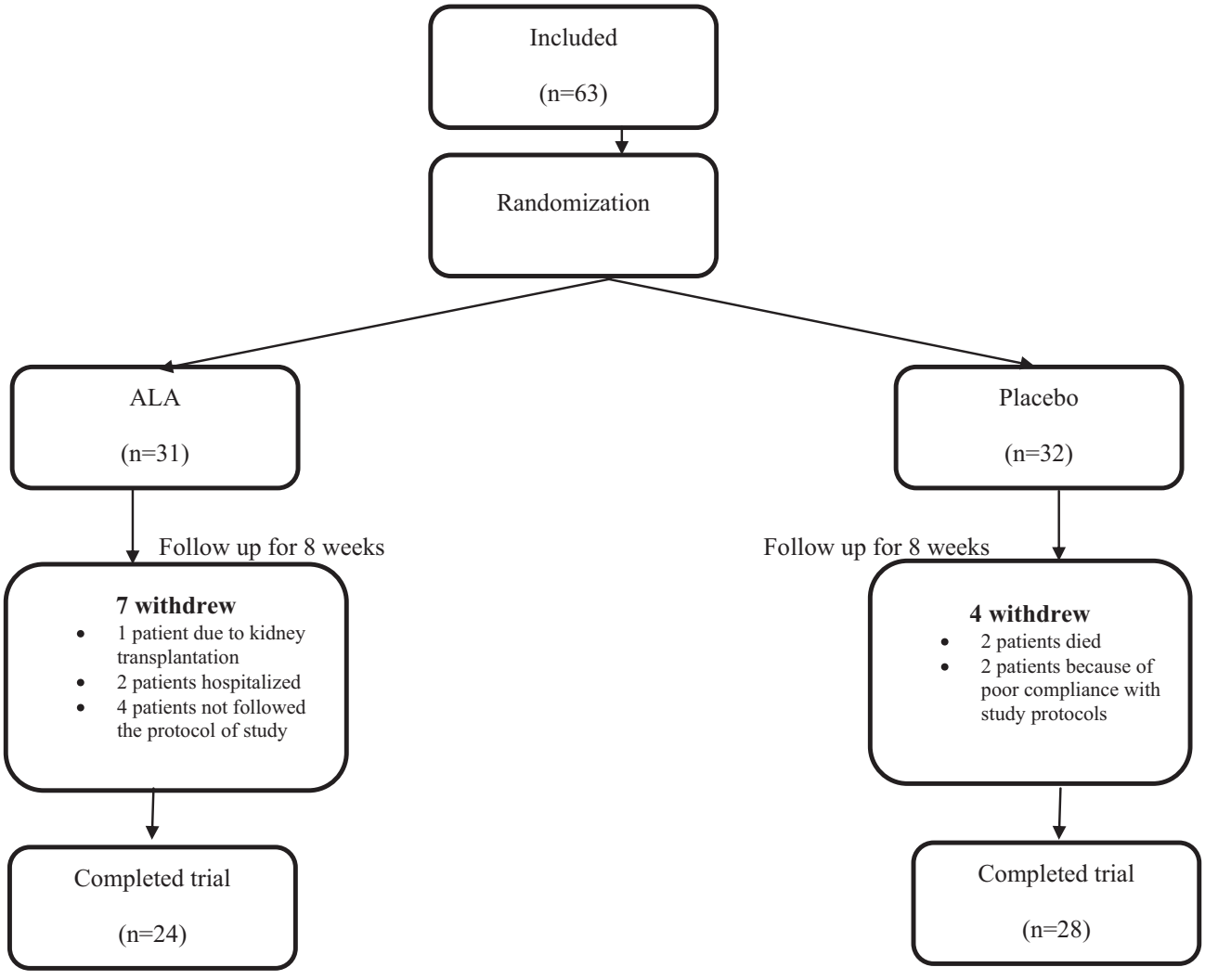

Figure 1. Flow chart for patient enrolment, randomization and retention.

Study design

A computer-generated random sequence was kept in a remote secure location and administered by an independent third party who was not involved with the clinical conduct of study until all study data were collected and verified. Patients and those involved in enrolling participants, administering interventions and assessing outcomes were blind to group assignments. The patients were randomly assigned into the supplemented group (n=31), receiving alpha lipoic acid oral capsule (each capsule included 600 mg ALA) once a day after breakfast [21] or a control group (n=32) receiving one placebo capsule for 8 weeks. Placebo capsules were made up of starch and were carefully matched in appearance with the active treatment. The participants were asked to keep their usual dietary intake, physical activity and medication during the study period unchanged. Patients were monitored weakly for any side-effects of ALA supplementation. Compliance was assessed by pill count. Height was measured using a non-stretchable tape to the nearest 0.1 cm with subjects standing erect bare-feet with heels touching and the eyes directed straight ahead. Weight was measured to the nearest 0.1 kg using a Seca scale, and the subjects mounted with light cloths and bare feet, after the HD session. Body-mass index (BMI) was calculated by dividing weight (in kg) by the square of height (in meters).

Clinical parameters

Fasting blood samples were collected for measurement of biochemical markers before the dialysis session at baseline and after 8 weeks of supplementation. Blood samples were divided into two parts: First part was kept in plain tubes and second part in ethylenediaminetetraacetic acid (EDTA) tubes. Samples (plain tube) were centrifuged and serum was extracted. Serum and whole blood samples were kept at −70 °C.

Hemoglobin (Hb) was measured by Cell Counter Device. Biochemical measurements including GPx and SOD were measured in whole blood by spectrophotometric kit (Ransel, Randox laboratories ltd, UK) and autoanalyzer apparatus (Abbott, model Alcyon 300, USA). Meanwhile, CAT was measured by Abei [22] method. Serum albumin (Alb) was measured using bromocresol green method [23] by Pars Azmoon kit.

Statistical analysis

Statistical analysis was performed using SPSS version 13.0 and the descriptive data were reported as means and SDs. A χ^2 test was used to compare qualitative variables between the two groups. The normal distribution of variables was tested by the Kolmogorov–Smirnov test, and also considering the mean and SD. Comparisons within groups were performed using paired samples t-test and comparison between groups was performed by independent t-test for normally distributed parameters and the Mann–Whitney test for nonparametric data. ANCOVA test was used to adjust for the effects of confounding factors (baseline values of biochemical parameters). p-value <0.05 was considered significant.

Table I. Baseline characteristics of study groups

Characteristics	ALA group (n=24) **	Placebo group (n=28) **	*P value
Age (year)	53.8 ± 13.2	54.0 ± 13.9	0.96
†Sex n (%)			0.86
Male	16 (66.7%)	18 (64.3%)	
Female	8 (33.3%)	10 (35.7%)	
†Diabetes n (%)	7 (29.2%)	9 (32.1%)	0.82
Time on dialysis (month)	59.2 ± 40.7 (7–127)	51.9 ± 40.7 (6–152)	0.58
Weight (kg)	65.0 ± 11.7	67.0 ± 13.6	0.58
Height (cm)	159 ± 7.41	159 ± 8.25	0.98
BMI (kg/m²)	25.4 ± 4.18	26.1 ± 4.75	0.58

BMI: Body mass index
*independent t-test
**Baseline data are shown just for study subjects who completed the study
†χ^2 test

Results

From a total of 63 subjects who met the inclusion criteria and entered the study, 7 patients in ALA group and 4 patients in placebo group were dropped-out from the study. As indicated in Fig. 1, data were reported for 52 patients (24 in ALA group and 28 in placebo group). All treatments were well tolerated and no serious adverse events were reported in this trial. There were no significant differences on baseline characteristics between two groups as presented in Table I. Biochemical parameters at baseline and after 8 weeks of supplementation are listed in Table II. Baseline values of these parameters were not significantly different between the two groups.

At the end of study, the mean activity of SOD increased in ALA group and decreased in placebo group when it was compared with baseline. The reduction of SOD in placebo group was statistically significant (p=0.04) (Table II). Between two groups analysis by independent t-test also showed that increased activities of SOD in ALA group was significant in comparison with placebo group (p=0.04), as shown in Table III. This finding was also significant after adjusting for baseline values of SOD (p=0.02).

Table II. Comparison of biochemical parameters before and after supplementation

Biochemical parameters	ALA group (n=24)			Placebo group (n=28)		
	Baseline	Week 8	*P	Baseline	Week 8	*P
SOD (U/gHb)	1032 ± 366	1149 ± 502	.30	1021 ± 384	862 ± 414	†.04
GPx (U/gHb)	18.9 ± 5.09	19.1 ± 7.19	.84	17.6 ± 3.51	17.4 ± 2.58	.79
CAT (U/gHb)	191 ± 82.7	208 ± 86.6	.36	171 ± 41.2	180 ± 54.8	.43
Hb (g/L)	11.6 ± 2.55	11.7 ± 2.83	.84	12.3 ± 2.02	12.3 ± 1.97	.99
BMI(kg/m²)	25.4 ± 4.18	25.4 ± 4.21	.64	26.1 ± 4.75	26.4 ± 4.49	.27
Weight (kg)	65.0±11.7	64.9±12.0	.76	67.0±13.5	67.7±12.9	.28
Alb(g/dl)	4.05 ± 0.30	4.07 ± 0.34	.82	4.16 ± 0.27	4.06 ± 0.31	.91

SOD: Superoxide dismutase; GPx: glutathione peroxidase; CAT: catalase; Hb: hemoglobin; BMI: Body mass index; alb: albumin
*p values are based on the paired t-test.
†statistically significant (P<.05)

Table III. Comparison of changes in biochemical parameters between two groups

Biochemical parameters	ALA group (n=24)	Placebo group (n=28)	*p
SOD (U/gHb)	117 ± 537	−158 ± 392	†.04
GPx (U/gHb)	0.26 ± 6.26	−0.22 ± 4.35	.75
CAT (U/gHb)	17.4 ± 91.0	9.30 ± 61.2	.70
Hb (g/L)	.10±2.40	0.00 ± 2.87	.90
BMI (kg/m²)	−0.05 ± 0.46	0.28 ± 1.35	.26
Weight (kg)	0.07 ± 1.08	0.73 ± 3.50	.26
Alb (g/dl)	0.02 ± 0.36	−0.09 ± 0.28	.42

SOD: Superoxide dismutase; GPx: glutathione peroxidase; CAT: catalase; Hb: hemoglobin; BMI: Body mass index; alb: albumin
(−) means: decrease
*p values are based on the independent sample t test for normally distributed parameters and Mann− Whitney test for nonparametric variables.
†statistically significant (P<.05)

At the end of intervention, the mean GPx concentrations increased in ALA group and decreased in placebo group, but the changes of GPx were not statistically significant in both groups (Table II). Furthermore, between the groups analysis by independent t-test showed that the changes in GPx were not also statistically significant between two groups (Table III).

As illustrated in Table II, although the activities of CAT increased in the study groups, these changes were not statistically significant.

The changes in weight, BMI, Alb and Hb concentration are summarized in Table II. No significant alterations were also observed in these parameters within each group during the study.

Discussion

Oxidative stress is an important risk factor for CVD in HD patients. The first lines of defense against ROS in erythrocytes are enzymes, such as SOD, catalase, or GPx, by which the ROS are turned into less reactive species [4].

SOD neutralizes $O_2^{\bullet -}$ by transforming it into hydrogen peroxide (H_2O_2), thereby preventing the formation of aggressive compounds such as peroxynitrite ($ONOO^-$) and hydroxyl radical ($HO^\bullet$) [24].

As documented, HD is a case of excessively depleted antioxidant status [8]. Decrease in SOD activity was demonstrated in patients on HD, a change that seems to contribute to the occurrence of oxidative disorders [3, 4]. Mekki et al. also reported that plasma SOD activity was lower in HD compared to chronic renal failure (CRF) and peritoneal dialysis patients [5]. Decreased SOD levels in HD patients are related to some possible factors such as inflammation [24]. Inflammation is more prevalent in HD patients [25]. The reduced SOD activity in HD patients may be an adaptation to oxidative stress and this situation exists probably because of the prevention to H_2O_2 formation which could generate the toxic $HO^\bullet$ [4]. Moreover, low plasma activity of SOD could be the consequences of food restriction. It has been reported that HD patients usually have a reduced intake of fruits and vegetables which are rich sources of antioxidants such as vitamins (A, C, E), trace – elements and polyphenols [5].

Dietary supplementation with ALA has been successfully employed in a variety of in vitro and in vivo models of diseases associated with oxidative stress [8, 26]. Gastrointestinal absorption of ALA appears to be quite variable. Most commercially available ALA supplements are a mixture of both R and S enantiomers that have different bioavailability. The efficiency of ALA uptake was also lowered by its administration in food, suggesting uptake competition with other nutrients for the carrier protein(s) involved [21].

In this study, ALA administration significantly increased the activity of SOD by 11.3% (117 U/g Hb) after 8 weeks when compared with the result of placebo group (P=0.04). No research was found in the available published

data about the effect of ALA supplementation on blood activities of SOD in HD patients to be compared with the results of this study. However, in Wang and colleagues study on diabetic rats, the decreased activities of SOD in the serum and renal cortex, were significantly improved by ALA treatment [27]. Stankovic et al. also observed similar results in NAFLD male mice treated with ALA [28]. Xu et al. evaluated the effects of flaxseed oil and ALA combination therapy in patients with nonalcoholic fatty liver disease (NAFLD) and observed a remarkable increase in the activities of SOD [29]. The exact mechanism for this action of ALA is unknown; however, it may be attributed to the indirect antioxidant properties of ALA. ALA and its reduced form dihydrolipoic acid (DHLA) create a potent redox couple that has been called "universal antioxidant", this redox couple appears to be able to regenerate other antioxidants [26]. ALA can increase the SOD levels through the activation of sirtuin 1 (SIRT1) and sirtuin 3 (SIRT3) [30].

GPx is an antioxidant enzyme that scavenges H_2O_2 and lipid peroxides, and may thus protect the cells from the deleterious effects of peroxides [5]. The activities of GPx in HD patients were investigated in many studies and low levels were reported in most of them [5, 7]. Roehrs and colleagues reported that the increase of lipid peroxidation was negatively correlated with GPx activity deficiency demonstrating that ROS generation can contribute to decreased GPx activity. This decrease in GPx activity may represent an early consequence of active nephron mass reduction, reinforcing the suggestion that the renal tubule is the predominant site of synthesis of GPx. Thus, in HD patients when renal impairment is increased, GPx activity is lower [2]. Furthermore, low levels of GPx in HD patients may be due to unbalanced diet in this population [5].

In our study, after 8 weeks of supplementation, the activities of GPx increased in ALA group and decreased in placebo group, but the changes were not statistically significant. Similarly Asci et al. reported that GPx activities increased insignificantly with ALA treatment in rats with amikacin-induced nephrotoxicity [31]. Xu et al. reported that flaxseed oil and ALA combination therapy in NAFLD patients significantly increased the activities of GPx [29].

CAT is an antioxidant enzyme that catalyses the dismutation of hydrogen peroxide to oxygen and water [32]. It also oxidizes different toxins, such as formaldehyde, formic acid, phenols and alcohols [33]. Changes of CAT levels in HD patients were reported in many studies; however, the results of different studies are sometimes contradictory. Several studies reported that activities of CAT are low in HD patients [5, 7] which may be due to oxidative stress or food restrictions. Some of studies reported an elevated levels of CAT [2, 4] justifying that CAT increases in HD patients to compensate for the oxidative stress that results from the HD process [2].

In our study, ALA supplementation in HD patient for 8 weeks did not exert a significant effect on CAT. No research was found in the available published data about the effect of ALA on blood activities of CAT to be compared with the results in this study. However, Asci et al. showed that ALA significantly increased the plasma levels of CAT in rats with amikacin-induced nephrotoxicity [31]. In Xu et al. study, flaxseed oil and ALA combination therapy in patients with NAFLD, also resulted in a remarkable increase in the activities of CAT as compared with placebo group [29]. Insignificant alteration of GPx and CAT levels in our patients may be due to inadequate dose of ALA or short period of study. Moreover, the relatively small sample size of the study may hinder beneficial effects in this population [8]. It also may be attributed to the fact that ALA acts synergistically with other endogenous antioxidants; so antioxidant disturbance in HD patients limiting ALA's ability to perform its complete antioxidative role [8]. Previous published results of this study showed that the mean concentrations of MDA (malondialdehyde) were 2.65 mmol/L (supplemented group: 2.81 ± 1.24; placebo group: 2.49 ± 1.06 mmol/L), which indicated a condition of oxidative stress in these patients. After 8 weeks, the serum concentration of MDA increased in both groups; however, the increased level in ALA group was approximately 3 times lower than the placebo group (0.09 vs. 0.24 mmol/L). This may be attributed to the anti-inflammatory and antioxidative actions of ALA. Therefore, it seems that ALA consumption may inhibit further increase in MDA levels by ameliorating the inflammation and oxidative stress. Inhibition of NF-kB activation may be a possible mechanism for the anti-inflammatory actions of ALA. NF-kB is a transcription factor that is activated by oxidative stress and inflammation and induces expression of many genes involved in inflammation. The serum concentration of TAS (total antioxidant status) did not change significantly in both groups that may be because of the consumption of ALA as an antioxidant [19].

We found that the weight, BMI, Hb and albumin of the patients remained stable during the study. BMI is a simple and useful marker in the assessment of body size. Although an increased BMI is associated with higher mortality in general population, many epidemiologic studies have shown that overweight and obesity may play a protective role in HD patients [34, 35]. The albumin level is an important predictor of mortality in HD patients, and the presence of hypoalbuminemia may increase lipid peroxidation and contribute to oxidative stress in these patients [34]. Decreased serum albumin levels are a sign of malnutrition which is more prevalent in this category of patients [34, 36]. Noori et al. indicated that combined administration of ALA and pyridoxine improves albuminuria in diabetic nephropathy [37].

Although, based on the results of the present study, supplementation with ALA may be beneficial for HD patients by increasing SOD; further studies with more sample size, long period of study and/or higher doses of ALA are needed to achieve precise results.

References

1. Putri, A.Y., & Thaha, M. (2014) Role of oxidative stress on chronic kidney disease progression. Acta Med Indones. 46, 244–252.
2. Roehrs, M., Valentini, J., Paniz, C., Moro, A., Charão, M., Bulcão, R., Freitas, F., Brucker, N., Duarte, M., Leal, M., Burg, G., Grune, T., & Garcia, S.C. (2011) The relationships between exogenous and endogenous antioxidants with the lipid profile and oxidative damage in hemodialysis patients. BMC Nephrology. 12, 1–9.
3. Lima de Castro, L., De Carvalho, E.M.M.D., Mendes Garcez, A., Rocha Pacheco, J.F., Macedo Cunha, F.V., Moita Neto, J.M., Carine Lima de Freitas, M., & De Melo Cunha, L.A. (2014) Hypoalbuminemia and oxidative stress in patients on renal hemodialysis program. Nutr Hosp. 30, 952–959.
4. Knap, B., Prezelj, M., Buturovic-Ponikvar, J., Ponikvar, R., & Bren, A.F. (2009) Antioxidant enzymes show adaptation to oxidative stress in athletes and increased stress in hemodialysis patients. Ther Apher Dial. 13, 300–305.
5. Mekki, K., Taleb, W., Bouzidi, N., Kaddous, A., & Bouchenak, M. (2010) Effect of hemodialysis and peritoneal dialysis on redox status in chronic renal failure patients: a comparative study. Lipids Health Dis. 9, 93.
6. Kayabasi, H., Sit, D., Atay, A.E., Yilmaz, Z., Kadiroglu, A.K., & Yilmaz, M.E. (2010) Parameters of oxidative stress and echocardiographic indexes in patients on dialysis therapy. Ren Fail. 32, 328–334.
7. Guo, C.-H., Wang, C.-L., Chen, P.-C., & Yang, T.-C. (2011) Linkage of some trace elements, peripheral blood lymphocytes, inflammation, and oxidative stress in patients undergoing either hemodialysis or peritoneal dialysis. Perit Dial Int. 31, 583–591.
8. El-Nakib, G.A., Mostafa, T.M., Abbas, T.M., El-Shishtawy, M. M., Mabrouk, M.M., & Sobh, M.A. (2013) Role of alpha-lipoic acid in the management of anemia in patients with chronic renal failure undergoing hemodialysis. Int J Nephrol Renovasc Dis. 6, 161–168.
9. Gomes, M.B., & Negrato, C.A. (2014) Alpha-lipoic acid as a pleiotropic compound with potential therapeutic use in diabetes and other chronic diseases. Diabetol Metab Syndr. 6, 80.
10. Bulmus, F.G., Gursu, M.F., Muz, M.H., Yaman, I., Bulmus, O., & Sakin, F. (2013) Protective effects of alpha-lipoic Acid on oleic Acid-induced acute lung injury in rats. Balkan Med J. 30, 309–314.
11. Shay, K.P., Moreau, R.F., Smith, E.J., Smith, A.R., & Hagen, T. M. (2009) Alpha-lipoic acid as a dietary supplement: molecular mechanisms and therapeutic potential. Biochim Biophys Acta. 1790, 1149–1160.
12. Papanas, N., & Ziegler, D. (2014) Efficacy of alpha-lipoic acid in diabetic neuropathy. Expert Opin Pharmacother. 15, 2721–2731.
13. Konrad, D., Somwar, R., Sweeney, G., Yaworsky, K., Hayashi, M., Ramlal, T., & Klip, A. (2001) The antihyperglycemic drug alpha-lipoic acid stimulates glucose uptake via both GLUT4 translocation and GLUT4 activation: potential role of p38 mitogen-activated protein kinase in GLUT4 activation. Diabetes. 50, 1464–1471.
14. Mijnhout, G.S., Kollen, B.J., Alkhalaf, A., Kleefstra, N., & Bilo, H.J. (2012) Alpha lipoic Acid for symptomatic peripheral neuropathy in patients with diabetes: a meta-analysis of randomized controlled trials. Int J Endocrinol. 2012, 1–8.
15. Mendes, P.R., Félix Ddos, S., Silva, P.C., Pereira, G.H., & Simões, M.O. (2015) Effect of alpha lipoic acid on the blood cell count and iron kinetics in hypertensive patients. Nutr Hosp. 31, 883–889.
16. Shinto, L., Quinn, J., Montine, T., Dodge, H.H., Woodward, W., Baldauf-Wagner, S., Waichunas, D., Bumgarner, L., Bourdette, D., Silbert, L., & Kaye, J. (2014) A randomized placebo-controlled pilot trial of omega-3 fatty acids and alpha lipoic acid in Alzheimer's disease. J Alzheimers Dis. 38, 111–120.
17. Gualandri, W., Gualandri, L., Demartini, G., Esposti, R., Marthyn, P., Volontè, S., Stangoni, L., Borgonovo, M., & Fraschini, F. (2003) Redox balance in patients with Down's syndrome before and after dietary supplementation with alpha-lipoic acid and L-cysteine. Int J Clin Pharmacol Res. 23, 23–30.
18. Omran, O.M., & Omer, O.H. (2015) The effects of alpha-lipoic acid on breast of female albino rats exposed to malathion: Histopathological and immunohistochemical study. Pathol Res Pract. 211, 462–469.
19. Khabbazi, T., Mahdavi, R., Safa, J., & Pour-Abdollahi, P. (2012) Effects of alpha-lipoic acid supplementation on inflammation, oxidative stress, and serum lipid profile levels in patients with end-stage renal disease on hemodialysis. J Ren Nutr. 22, 244–250.
20. Himmelfarb, J., Ikizler, T.A., Ellis, C., Wu, P., Shintani, A., Dalal, S., Kaplan, M., Chonchol, M., & Hakim, R.M. (2014) Provision of antioxidant therapy in hemodialysis (PATH): a randomized clinical trial. J Am Soc Nephrol. 25, 623–633.
21. Teichert, J., Tuemmers, T., Achenbach, H., Preiss, C., Hermann, R., Ruus, P., & Preiss, R. (2005) Pharmacokinetics of alpha-lipoic acid in subjects with severe kidney damage and end-stage renal disease. J Clin Pharmacol. 45, 313–328.
22. Aebi, H. (1984) Catalase in vitro. Methods Enzymol. 105, 121–126.
23. Doumas, B.T., Watson, W.A., & Biggs, H.G. (1971) Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta. 31, 87–96.
24. Afonso, Vr., Champy, R., Mitrovic, D., Collin, P., & Lomri, A. (2007) (Review) Reactive oxygen species and superoxide dismutases: Role in joint diseases. Joint Bone Spine. 74, 324–329.
25. Tsirpanlis, G. (2005) The pattern of inflammation and a potential new clinical meaning and usefulness of C-reactive protein in end-stage renal failure patients. Kidney Blood Press Res. 28, 55–61.
26. Rochette, L., Ghibu, S., Richard, C., Zeller, M., Cottin, Y., & Vergely, C. (2013) Direct and indirect antioxidant properties of alpha-lipoic acid and therapeutic potential. Mol Nutr Food Res. 57, 114–125.
27. Wang, L., Wu, C.G., Fang, C.Q., Gao, J., Liu, Y.Z., Chen, Y., Chen, Y.N., & Xu, Z.G. (2013) The protective effect of alpha-Lipoic acid on mitochondria in the kidney of diabetic rats. Int J Clin Exp Med. 6, 90–97.
28. Stankovic, M.N., Mladenovic, D., Ninkovic, M., Ethuricic, I., Sobajic, S., Jorgacevic, B., de Luka, S., Vukicevic, R.J., & Radosavljevic, T.S. (2014) The effects of alpha-lipoic acid on liver oxidative stress and free fatty acid composition in methionine-choline deficient diet-induced NAFLD. J Med Food. 17, 254–261.

29. Xu, J., Gao, H., Song, L., Yang, W., Chen, C., Deng, Q., Huang, Q., Yang, J., & Huang, F. (2013) Flaxseed oil and alpha-lipoic acid combination ameliorates hepatic oxidative stress and lipid accumulation in comparison to lard. Lipids Health Dis. 12, 12–58.
30. Valdecantos, M.P., Pérez-Matute, P., González-Muniesa, P., Prieto-Hontoria, P.L., Moreno-Aliaga, M.J., & Martínez, J.A. (2012) Lipoic acid improves mitochondrial function in nonalcoholic steatosis through the stimulation of sirtuin 1 and sirtuin 3. Obesity (Silver Spring). 20, 1974–1983.
31. Asci, H., Saygin, M., Cankara, F.N., Bayram, D., Yesilot, S., Candan, I.A., & Ilhan, I. (2015) The impact of alpha-lipoic acid on amikacin-induced nephrotoxicity. Ren Fail. 37, 117–121.
32. Nicholls, P. (2012) Classical catalase: ancient and modern. Arch Biochem Biophys. 525, 95–101.
33. Dalvi, S.M., Patil, V.W., Ramraje, N.N., & Phadtare, J.M. (2012) Lipid peroxidation, Superoxide dismutase and catalase co-relation in pulmonary and extra pulmonary tuberculosis. Free Radicals and Antioxidants. 2, 1–5.
34. Caetano, C., Valente, A., Oliveira, T., & Garagarza, C. (2016) Body composition and mortality predictors in hemodialysis patients. J Ren Nutr. 26, 81–86.
35. Sakao, Y., Ojima, T., Yasuda, H., Hashimoto, S., Hasegawa, T., Iseki, K., Tsubakihara, Y., & Kato, A. (2016) Serum creatinine modifies associations between body mass index and mortality and morbidity in prevalent hemodialysis patients. PLoS One. 11, e0150003.
36. Rusu, C., Kacso, I.M., Potra, A., Patiu, I.M., & Gherman-Caprioara, M. (2016) Mineral and bone disorders, morbidity and mortality in end-stage renal failure patients on chronic dialysis. Clujul Med. 89, 94–103.
37. Noori, N., Tabibi, H., Hosseinpanah, F., Hedayati, M., & Nafar, M. (2013) Effects of combined lipoic acid and pyridoxine on albuminuria, advanced glycation end-products, and blood pressure in diabetic nephropathy. Int J Vitam Nutr Res. 83, 77–85.

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Declaration of interest
The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Tannaz Khabbazi
Nutritional Research Center
Tabriz University of Medical Sciences
Tabriz
Iran
Tel. +98 4113341113

t.khabbazi@gmail.com