CLINICAL TRIAL REPORT

Effects of Alpha-Lipoic Acid Supplementation on Cardiovascular Disease Risk Factors in β-Thalassemia Major Patients: A Clinical Trial Crossover Study

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Aim: Thalassemia is one of the most common genetic diseases, and cardiovascular disease (CVD) has been considered as the leading cause of mortality in more than 50% of β-thalassemia patients. The aim of this study was to determine the effects of alpha-lipoic acid (ALA) on CVD risk factors in β-thalassemia major patients.

Methods: Twenty β-thalassemia major patients participated in this randomized crossover clinical trial study. Participants were randomly assigned to ALA (600 mg/day) or placebo groups for two 8-wk interventions that were separated by a 3-wk washout period. The CVD risk factors including serum osteoprotegerin (OPG), homocysteine, lipoprotein-associated phospholipase A_2 and trimethylamine N-oxide were measured at the beginning and the end of each intervention phase according to the standard protocol.

Results: Serum OPG reduced significantly in the ALA group in all participants (5.38 ± 2.79 to 3.27 ± 2.43 ng/mL, *P*= .003) and in the male subgroup (5.24 ± 2.56 to 3.13 ± 2.5 ng/mL, *P*= .015); this reduction was significant in comparison with the placebo group (*P*= .013). The changes in other CVD risk factors were not significant.

Conclusion: The results of this study showed that after 8-wk of ALA consumption, the serum OPG reduced significantly in β-thalassemia major patients. Therefore, controlling the serum OPG level with ALA consumption can be an important complementary therapeutic option to prevent the progression of CVD in β-thalassemia major patients.

Keywords: β-thalassemia major, alpha-lipoic acid, CVD risk factors

Introduction

Thalassemia is the most common hereditary diseases in the world, including in Iran. The frequency of disease varies from one region to another, so that the highest prevalence (10%) has been reported on the margins of the Caspian Sea and Persian Gulf, and in other parts of Iran it was 4%–8%. Currently, there are about 22,000 β-thalassemia major patients and more than 2 million carriers in Iran.[1]

This hereditary hemolytic anemia causes the body not to make the hemoglobin chains. β-Thalassemia major is the severe form of thalassemia, which begins with anemia and continues with apparent deformity, bone problems, weakness, and growth retardation.[2] Therefore, in order to compensate for the lack of hemoglobin, an inevitable treatment is the continuous infusion of blood. This causes patients to have extra complications due to increased iron load and accumulation in various organs such as the liver and heart and eventually the risk of premature death.[3] So cardiovascular disease (CVD) is one of the major complications of β-thalassemia major that could cause more

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than half of deaths in patients.[4] Despite treatment with defer-oxamine (an iron chelator), the most common cause of death in thalassemic patients (63% of all cases) is cardiac dysfunction including limiting pericarditis, heart failure, and arrhythmias.[5] CVD is a multifactorial disease with a great number of traditional and modern risk factors such as osteo-protegerin (OPG), homocysteine (HCY), lipoprotein-associated phospholipase A_2 (Lp-PLA_2), and trimethylamine N-oxide (TMAO) are considered as the new risk factors and various studies have shown that the level of these new risk factors are high in β-thalassemia major patients.[6–9]

Alpha-lipoic acid (ALA), a potent antioxidant, can be used as an adjunct therapy for many diseases such as diabetes, CVD, neurological damages, autoimmune diseases, cancers, and AIDS.[10] In addition to its antioxidant role, ALA has chelating properties for metals such as iron, copper, manganese, and zinc in both in-vitro and in-vivo conditions. Therefore, ALA can be used as an adjunct therapy to chelate iron in β-thalassemia major patients.[11]

Also, studies have shown that this antioxidant has beneficial effects in reducing serum lipids, inflammatory factors, oxidative stress, and CVD risk factors including HCY, OPG and Lp-PLA_2.[12–14]

We examined the effect of ALA supplementation on traditional CVD risk factors including cholesterol, HDL-C, LDL-C, triglyceride, etc. in another study that is currently under review.

Considering the high prevalence of cardiac dysfunction and premature death, the high level of new risk factors in thalassemic patients, and the lack of specific nutritional interventions regarding new CVD risk factors in these patients, we aimed to evaluate the effects of ALA supplementation on new CVD risk factors including OPG, HCY, Lp-PLA_2, and TMAO in β-thalassemia major patients.

Materials and Methods
Participants
According to available files in the Mohammad Kermanshahi Hospital, of 314 β-thalassemia major patients in this hospital, 85 individuals were assessed for eligibility, and 59 did not meet the inclusion criteria. In all, 20 eligible individuals were recruited to this randomized clinical trial (RCT). Furthermore, this number was adequate to cover potential losses during the study period, but we considered 26 individuals who had inclusion criteria (Figure 1).

Inclusion criteria include age >15 yr; no history of hepatitis B and C, CVDs (include coronary artery disease, heart failure, limiting pericarditis, arrhythmia, myocardial infarction and cardiomyopathy), liver or kidney disease, cancer, allergy and digestive problems, not being pregnant or lactating, being non-smoking, and not adhering to a specific diet. Exclusion criteria included heart failure during the study, pregnancy, non-compliance with the study protocol, and the use of certain drugs (except routine drugs of thalassemic patients) and dissatisfaction with the continuation of the study. Before the start of the study, a questionnaire asking about demographic information, medical history, and medication use was completed. After the overview of the study course was given to the participants, they were asked to provide a written informed consent; for participants under the age of 18 years, informed consent was obtained from their parents.

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Study Design
This double-blind clinical trial crossover study was conducted between August 2017 and May 2018 among β-thalassemia major patients referring to Mohammad Kermanshahi Hospital in Kermanshah, Iran. With regard to previous studies, the sufficient sample size was calculated by $(N = (Z_{1-\alpha/2} + Z_{1-\beta})^2 \times 2S^2/(\Delta)^2)$ formula[15] based on serum level of HCY,[16] in which $\alpha = 0.05$ and $\beta = 95\%$. The calculated sample size was seven individuals in each group. Due to attrition risk, 10 individuals were considered in each group.

The participants were randomly divided into two groups and randomly assigned to ALA group (600 mg/day) or placebo group (corn starch) by a third person who was not aware of the kind of capsules. Each phase of study was 8 weeks, separated by a 3-week washout period. Patients were asked to take a capsule per day for 8 weeks in addition to their routine chelating drugs. It was recommended that the ALA supplement should be consumed half an hour before dinner due to availability increment.[17] Adherence to the study protocol was assessed by counting the returned capsules.

Five milliliters of fasting blood were drawn for biochemical assessment at four steps (baseline and at the end of each intervention phase: baseline and 8th, 11th, and 19th weeks). The participants completed a 3-day dietary record at the beginning and at 4-week intervals until the end of study. Participants were instructed how to complete the dietary records. In each intervention phase, they were asked to record

their food intakes of two routine days and a weekend day. Nutritionist IV software was used for analyzing food intakes. Furthermore, participants were requested to record their daily physical activities at the beginning and by 4-week intervals until the end of the study. The recorded physical activities were multiplied by the relevant metabolic equivalents (MET) coefficients and finally converted to MET-h/d. (Figure 1).

Anthropometric Measurements

Anthropometric indices including weight, height, and body mass index (BMI) were measured baseline and at the end of each intervention phase (at baseline and 8th, 11th, and 19th weeks) by a trained expert. Using a Seca scale, weight was measured nearest to 0.1 kg. During weight measurement, participants wore light clothing and were barefoot. Height was measured with a Seca stadiometer without shoes, with 0.1 cm accuracy. BMI was calculated by dividing weight (kg) into the height square (m^2).

Laboratory Analysis

12–14 h fasting blood samples were taken at the beginning of study (week 1) and at the 8th, 11th, and 19th weeks. Blood samples were centrifuged at 3000 rpm for 10 min and then frozen at 70 °C until performing analysis. ELISA kits were used to measure the serum level of HCY, Lp-PLA$_2$, OPG, and TMAO (SHANGHAICRYSTAL DAY BIOTECH CO., CHINA).

Statistical Analyses

Normal distributions of all variables were assessed by the Kolmogorov–Smirnov test. Except dietary intakes, all variables had normal distributions. Therefore, paired *t*-test and Wilcoxon were used to compare dietary intakes. Paired *t*-test was used to compare baseline values of variables between the two groups, baseline and end values of variables within each group, and changes of variables between the two groups. ANCOVA was used to compare the end point values. In this analysis, end values were adjusted for baseline values of variables and monounsaturated fatty acids (MUFA), Vit E, and Vit B$_6$. To determine the Pgroup, Ptime and Pgroup*time, repeated measure analysis of variance was applied. SPSS (SPSS Inc, Chicago, IL, USA, version 16) was used for all statistical analyses. $P < .05$ was considered as the significant level.

Results

The participants included 13 (65%) men and seven (35%) women. Baseline characteristics of the study population are presented in Table 1.

Dietary intakes of participants are presented in Table 2. Participants in the ALA group had higher intake of MUFA ($P= .017$) and lower intake of vitamin E ($P= .037$) and vitamin B$_6$ ($P= .017$) compared with the placebo group.

There was no statistical difference in the body weight and BMI between the two phases of intervention ($P> .05$). Also, there was no statistical difference in the amount of physical activity between the two phases of the study (31.65 ± 1.82 MET-h/d in ALA group versus 31.8 ± 2.15 MET-h/d in the placebo group; $P= .726$).

The effects of ALA on CVD risk factors are shown in Table 3. As shown, there was no significant difference according to baseline values of variables between ALA and placebo groups. In the ALA group the OPG levels significantly reduced during 8-wk intervention in all participants ($P= .003$) and in the male subgroup ($P= .015$); also in the ALA group, a significant difference was seen in regard to the endpoints of OPG values in comparison with the placebo group in all participants ($P= .013$) and in the male subgroup ($P= .032$). However, there was no significant difference in regard to the serum level of HCY, Lp-PLA$_2$, and TMAO during the 8-wk intervention in the ALA group or between the two groups.

The changes of CVD risk factors after intervention are presented in Table 4. Comparison of intragroup changes of variables between the ALA and placebo groups showed that only changes in serum OPG were significant ($P= .006$). Additionally, intragroup changes of serum OPG between the ALA and placebo groups in the male subgroup had a trend toward being significant ($P= .059$). Also, the interaction of group, time, and time×group for all CVD risk factors were not significantly different. For all variables, carry-over effect and period effect were not significant ($P> .05$).

Discussion

The aim of the present study was to determine the effects of ALA supplementation on CVD risk factors in β-thalassemia major patients. ALA significantly reduced OPG levels during the 8-wk of intervention in comparison with placebo group in all participants and in the male subgroup. However, it had no significant effect on serum level of HCY, Lp-PLA$_2$, and TMAO.
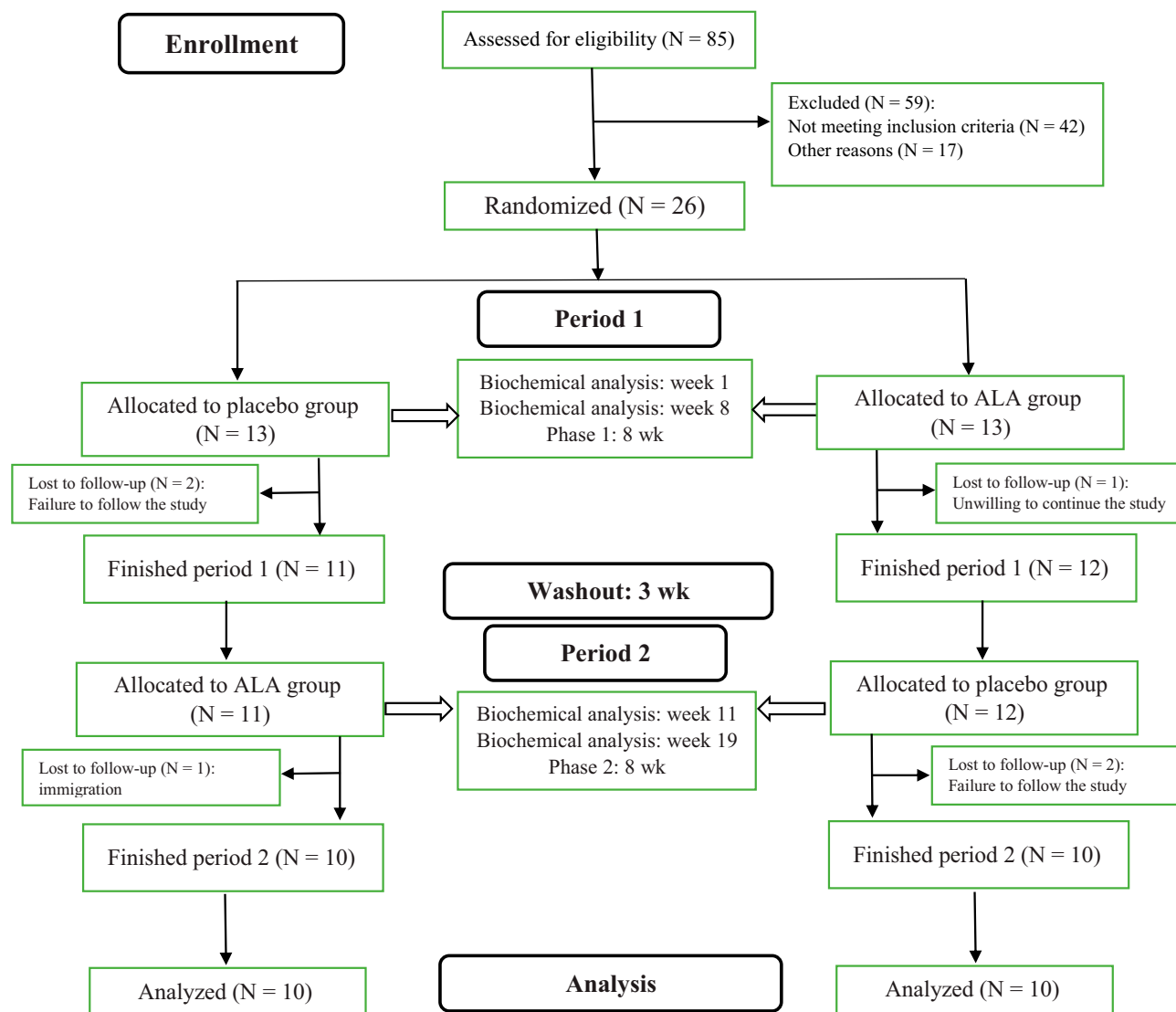
Figure 1 Flowchart of study progress.

In the present study, ALA significantly reduced serum OPG. Tumor necrosis factor-α (TNF-α) is one of the key intermediates of inflammatory processes which increases the secretion of OPG by stimulating the OPG-Von-Willebrand factor.[10] Studies have shown that TNF-α decreases following nutritional interventions with ALA consumption. In a systematic review and meta-analysis of RCTs, findings showed that ALA supplementation decreased TNF-α, C-reactive protein (CRP), and interleukin-6 (IL-6) among patients with metabolic syndrome.[12] In a study on obese people, a significant reduction in CRP and TNF-α concentrations was observed following ALA supplementation.[18] In another study, ALA supplementation decreased TNF-α and IL-6 and increased adiponectin levels in obese subjects with impaired glucose tolerance.[15]

ALA intake may reduce inflammatory markers through scavenging free radicals, downregulating pro-inflammatory redox-sensitive signal transduction processes including nuclear factor kappa B translocation, leading to lower release of other free radicals and cyto-toxic cytokines.[19] Moreover, ALA improves cellular anti-oxidant capacity and phase 2 enzymes such as catalase, reduced glutathione, glutathione reductase, and glu-tathione-S-transferase.[20]

It can be said that ALA decreases serum OPG and inhibits vascular calcification by reducing inflammatory mediators such as TNF-α. Therefore, controlling the serum OPG level can be an important complementary therapeutic option to prevent the progression of CVD in β-thalassemia major patients.

Table 1 Baseline Characteristics of All Study Participants at Entry (*N*= 20)

Variable	Mean ± SD
Age (y)	23.5 ± 5.47
Height (cm)	161.52 ± 13.02
Weight (kg)	55.55 ± 12.22
BMI (kg/m^2)	21.04 ± 2.4
OPG (ng/mL)	4.87 ± 3.04
HCY (μmol/L)	12.12 ± 5.42
Lp-PLA$_2$ (ng/mL)	23.14 ± 8.96
TMAO (pg/mL)	30.42 ± 14.57

Abbreviations: OPG, osteoprotegerin; HCY, homocysteine; Lp-PLA$_2$, lipoprotein-associated phospholipase A$_2$; TMAO, trimethylamine *N*-oxide.

Table 2 Dietary Intakes of Individuals in ALA and Placebo Groups

Variable	ALA Group (N=20)	Placebo Group (N= 20)	P-value
Energy (kcal)	1715.5 ± 296.0	1703.4 ± 271.47	0.724
Protein (g)	69.6 ± 15.8	68.19 ± 22.83	0.774
Carbohydrate (g)	229.5 ± 43.6	240.8 ± 33.6	0.284
Fat (g)	60.2 ± 18.9	54.04 ± 20.91	0.08
Saturated fat (g)	19.4 ± 8.3	17.0 ± 7.77	0.097
MUFA (g)	18.5 ± 7.4	15.9 ± 7.4	0.017*
PUFA (g)	14.6 ± 7.9	14.64 ± 9.06	0.973
Cholesterol (mg)	219.7 ± 30.1	235.42 ± 30.29	0.691
DHA (g)	0.2 ± 0.1	0.11 ± 0.03	0.15
EPA (g)*	0.1 ± 0.1	0.1 ± 0.0	0.092
Calcium (mg)	625.6 ± 52.4	668.9 ± 52.5	0.477
Vitamin E (mg)	2.6 ± 0.5	4.7 ± 1.0	0.037*
Vitamin B$_6$ (mg)	1.2 ± 0.4	1.4 ± 0.5	0.017*
Folate (μg)	236.8 ± 21.3	223.3 ± 25.4	0.645

Notes: Data are presented as Mean ± SD. *P*-values are obtained from paired *t*-test or Wilcoxon signed-rank test*.
Abbreviations: MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

Given that OPG is a new risk factor, there is still no reliable source to report the normal range of OPG. However, comparing postintervention OPG level in thalassemic patients with OPG level in healthy subjects in the studies, it was found that the OPG level in thalassemic patients after intervention in this study is similar to the OPG level in healthy subjects and it seems that this level of serum OPG would reduce CVD risk and thus can be considered beneficial to thalassemic patients' health.[21,22]

Lp-PLA$_2$ is a macrophage-derived enzyme that after oxidation of LDL can decompose oxidized phosphatidylcholine into lysophosphatidylcholine (LysoPC) and oxidized nonesterified free fatty acids (oxNEFA).[23] These compounds significantly contribute to inflammation associated with atherosclerosis and can stimulate monocytes and macrophages

to secrete cytokines, which in turn cause more liberation of Lp-PLA$_2$ from macrophages.[24] In the present study, ALA consumption had no significant effect on Lp-PLA$_2$.

In Gajos et al's study, omega-3 reduced levels of Lp-PLA$_2$ and ox-LDL in CAD patients. One of the potential mechanisms is that omega-3 protect LDL from oxidation and thereby reduce the substrate for Lp-PLA$_2$.[25] However, in the studies of Nelson et al and Pedersen et al, omega-3 did not have a significant effect on Lp-PLA$_2$ in healthy subjects with normal lipid profiles.[26,27] In the former study, participants were patients with atherosclerosis that had high level of low-dnsity lipoprotein (LDL); while in the latter study, the levels of lipid profiles were normal and baseline Lp-PLA$_2$ level was lower than the borderline risk. In the present study, similar to Pedersen and Nelson groups' studies, baseline Lp-PLA$_2$ level was lower than the borderline risk.

Chang et al did not find any significant difference in triglyceride or oxidized-LDL levels in diabetic patients who received 600 mg/day ALA compared to the control group.[28] In the study of Skulas-Ray et al, a low dose of omega-3 did not affect Lp-PLA$_2$ in hypertriglyceridemia individuals, while its high dose reduced Lp-PLA$_2$ level.[29] Also, in Jameel et al's study, ALA inhibited PLA$_2$ in a dose–response manner in the laboratory environment.[30] It sounds like Lp-PLA$_2$ responds to the intervention as dose-dependent and in high prescriptive doses of ALA, while the applied dose in this study was the usual dose.

TMAO is produced from the TMA in the liver by the flavin-containing monooxygenase-3 (FMO3) enzyme. Various factors such as some foods, intestinal bacteria, and liver activity on FMO3 enzyme have an effect on TMAO concentration.[31] Because TMA is produced from choline, betaine, and carnitine by intestinal microbial metabolism, the consumption of animal products such as egg, liver and meat, or L-carnitine supplementation are considered to be the main source of TMAO in humans.[32]

In Obeid et al's study, vitamin D alone or with vitamin B supplementation reduced TMAO.[33] Vitamin D supplementation has been shown to reduce choline.[34] Vitamin D can also alter the intestinal environment and stimulate bacteria to produce dimethylglycine as an alternative to TMA, or use betaine and thereby reduce TMAO.[33] In an animal model, through affecting choline, a probiotic reduced the level of TMAO. Probiotics can reduce the production of TMAO by removing choline out of reach TMA-producing bacterial species.[35] In Chen et al's study, resveratrol supplement reconstructed the gut microbial environment in mice, which resulted in decreased TMAO production.[36]

Table 3 Baseline and End Values of CVD Risk Factors After 8-Wk of ALA Consumption

Variable	All (N= 20)		P-value	Men (N= 13)		P-value	Women (N= 7)		P-value
	ALA Group	Placebo Group		ALA Group	Placebo Group		ALA Group	Placebo Group	
OPG									
Baseline	5.38 ± 2.79	4.09 ± 2.64	0.096ᵃ	5.24 ± 2.56	5.06 ± 2.54	0.783	5.65 ± 3.39	2.28 ± 1.86	0.069
End point	3.27 ±2.43	4.54 ± 2.89	0.013ᵇ	3.13 ± 2.5	5.03 ± 3.1	0.032	3.54 ± 2.45	3.64 ± 2.41	0.547
P-valueᵃ	0.003	0.411		0.015	0.957		0.122	0.287	
HCY									
Baseline	12.29 ± 5.55	13.02 ± 5.86	0.303	13.06 ± 4.86	13.37 ± 5.04	0.651	10.85 ± 6.83	12.38 ± 7.56	0.373
End point	11.72 ± 6.92	10.52 ± 7.19	0.329	11.85 ± 6.71	10.47 ± 6.79	0.526	11.47 ± 7.83	10.6 ± 8.44	0.376
P-value	0.665	0.061		0.511	0.126		0.739	0.314	
Lp-PLA₂									
Baseline	24.93 ± 7.61	21.23 ± 9.3	0.076	24.8 ± 5.75	23.73 ± 5.92	0.619	25.18 ± 10.82	16.58 ± 12.84	0.053
End point	22.42 ± 9.94	22.72 ± 9.11	0.311	23.85 ± 8.2	25.37 ± 7.21	0.402	19.76 ± 12.88	17.81 ± 10.76	0.647
P-value	0.186	0453		0.649	0.363		0.18	0.807	
TMAO									
Baseline	31.66 ± 13.89	27.72 ± 14.29	0.085	31.74 ± 13.4	30.26 ± 10.51	0.526	31.5 ± 15.87	23 ± 19.65	0.093
End point	29.52 ± 12.64	29.54 ± 14.51	0.602	29.37 ± 11.84	30.85 ± 13.17	0.783	29.78 ± 15.02	27.1 ±1 7.59	0.734
P-value	0.418	0.551		0.514	0.855		0.667	0.554	

Notes: Data are presented as Mean ± SD. Data were analyzed using ᵃpaired t-test and ᵇanalysis of covariance.
Abbreviations: OPG, osteoprotegerin; HCY, homocysteine; Lp-PLA₂, lipoprotein-associated phospholipase A₂; TMAO, trimethylamine N-oxide.

Table 4 Changes of CVD Risk Factors After 8-Wk of ALA Consumption

Variable	ALA Group (N= 20)	Placebo Group (N= 20)	P-valueᵃ	P-valueᵇ		
				Group	Time	Time×Group
OPG	−2.11 ± 2.76	0.45 ± 2.42	0.006	0.984	0.058	0.987
Men (N= 13)	−2.11 ± 2.7	−0.03 ± 1.95	0.059	0.263	0.36	0.059
Women (N= 7)	−2.11 ± 3.11	1.35 ± 3.07	0.06	0.093	0.688	0.06
HCY	−0.56 ± 5.79	−2.5 ± 5.63	0.332	0.784	0.078	0.332
Men (N= 13)	−1.2 ±6 .4	−2.9 ± 6.37	0.541	0.669	0.11	0.541
Women (N= 7)	0.61 ± 4.64	−1.77 ± 4.28	0.413	0.715	0.582	0.413
Lp-PLA₂	−2.51 ± 8.19	1.49 ± 8.73	0.145	0.252	0.712	0.145
Men (N= 13)	−0.95 ± 7.35	1.63 ± 6.24	0.272	0.903	0.825	0.272
Women (N= 7)	−5.41 ± 9.44	1.2 3 ±12.79	0.347	0.081	0.472	0.347
TMAO	−2.14 ± 11.56	1.82 ± 13.43	0.335	0.387	0.936	0.335
Men (N= 13)	−2.36 ± 12.72	0.59 ± 11.45	0.583	0.999	0.678	0.583
Women (N= 7)	−1.71 ± 10.04	4.1 ± 17.32	0.402	0.349	0.789	0.402

Notes: Data were analyzed using ᵃpaired t-test and ᵇrepeated measures analysis of variance.
Abbreviations: OPG, osteoprotegerin; HCY, homocysteine; Lp-PLA₂, lipoprotein-associated phospholipase A₂; TMAO, trimethylamine N-oxide.

In the study of Boutagy et al, consumption of probiotic supplementation in healthy men for 4 weeks did not affect TMAO or L-carnitine, choline and betaine levels.[37] In the study of Baugh et al, daily intake of 10 g of inulin for 6 weeks did not affect TMAO in individuals at risk for diabetes.[38] In the present study, TMAO decreased in the ALA group and increased in the placebo group, but similar to Boutagy and Baugh's studies the observed changes were not significant.

Considering that the intestinal flora plays a decisive role in the formation of TMAO from dietary choline,[39] probiotics or other treatments that can alter the intestinal bacteria may affect the concentration of TMAO in clinical populations with high TMAO level compared to healthy individuals. In Boutagy and

Baugh's studies, the samples had low TMAO concentrations that are associated with a relatively low risk of CVD.

The present study did not investigate the changes in the composition of the intestinal bacteria that may be created during the interventional period; therefore, considering the role of intestinal flora in the production of TMAO,[39] examination of intestinal flora and effective factors on its alteration is essential. Another factor that may affect any intervention is duration. Although it is known that TMAO is a biomarker that can be rapidly altered by food interventions,[40] ALA may require more time to affect TMAO.

In the present study, the level of HCY in ALA and placebo group decreased, but the observed changes were not significant. Various studies have been done to evaluate the effects of supplements on serum HCY levels in different populations with different doses and different duration. In the study of Tayebi Khosroshahi et al, omega-3 significantly decreased the level of HCY in hemodialysis patients.[16] In Pakfetrat et al's study, zinc supplementation significantly reduced the level of HCY in renal patients.[41] In Hu et al's study, ALA inhibited HCY-induced cellular apoptosis in human aortic endothelial cells that play a key role in CVD, and suppressed HCY stimulation.[14]

One reason for the inconsistency of our findings with those of Tayebi Khosroshahi, Pakfetrat and Hu is that in these studies the level of HCY in the samples was higher than the normal level (5–15 μmol/L).[9] However, the level of HCY in our study was normal at the onset of the study, which could be due to the use of folic acid, an essential supplement in the routine treatment program for thalassemic patients. Another reason for the normal level of HCY in the present study could be the relatively low age of thalassemic patients (23.5±5.47 y), as it has been shown that with increasing age the level of HCY increases.[42]

The limitations of the present study were low dose of supplementation, short-term intervention time and a relatively small sample size (20 subjects). Another limitation was the lack of measurement of serum ALA due to limited financial resources. Also, considering that there has not been a similar study in the field of ALA supplementation in thalassemia, inevitably we compared our results with other studies that evaluated the effects of ALA or other compounds in different diseases.

Conclusion

The findings of this study showed that 600 mg of ALA supplementation for 8-wk in β-thalassemia major patients resulted in a significant decrease in serum OPG. However, it had no significant effect on serum HCY, Lp-PLA$_2$, and TMAO. To the best of our knowledge, this is the first study that has investigated the effects of ALA on CVD risk factors in β-thalassemia major patients; therefore, further studies are needed.

Abbreviations

ALA, alpha-lipoic acid; CVD, cardiovascular disease; OPG, osteoprotegerin; HCY, homocysteine; Lp-PLA$_2$, lipoprotein-associated phospholipase A$_2$; TMAO, trimethylamine *N*-oxide; BMI, body mass index; ELISA, enzyme-linked immunosorbent assay; ANCOVA, analysis of covariance; MUFA, monounsaturated fatty acids; TNF-α, tumor necrosis factor-α; RCT, randomized clinical trials; CRP, C-reactive protein; IL-6, interleukin-6; LDL, low-density lipoprotein; LysoPC, lysophosphatidylcholine; OxNEFA, oxidized nonesterified free fatty acids; Ox-LDL, oxidized-LDL; CAD, coronary artery disease; FMO3, flavin-containing monooxygenase-3.

Registration Number

This study was approved by the research deputy of Kermanshah University of Medical Sciences (Code No: IR. KUMS.REC.1397.192) and registered in the Iranian Clinical Trials Registry (registration number IRCT20170617142 37N2).

Data Sharing Statement

The data sets used during the current study are available from the corresponding author on reasonable request.

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Disclosure

The authors declare that they have no conflict of interest.

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