Effect of Omega-3 Fatty Acid on Oxidative Stress in Patients on Hemodialysis

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Introduction. Oxidative stress in patients with chronic kidney failure, particularly in hemodialysis patients, has been suggested to have a major role in the pathogenesis of atherosclerosis. We evaluated omega-3 fatty acids supplementation effects on oxidative and antioxidant factors in hemodialysis patients.
Materials and Methods. In a clinical trial, patients on hemodialysis were divided into 2 groups in order to receive either omega-3 fatty aid capsule, 1 g 3 times a day, or placebo for 2 months. The two groups were comparable in terms of sex distribution, age, medications, diabetes mellitus, hemoglobin level, serum ferritin, and serum albumin. Blood samples taken from patients before and at the end of the study period were examined for oxidative stress markers including malondialdehyde, glutathione peroxidase, superoxide dismutase, and ferric reducing antioxidant power.
Results. Seventy-five hemodialysis patients were divided into the omega-3 group (n = 37) and the control group (n = 38). Before the treatment period, the two groups were comparable in the malondialdehyde, glutathione peroxidase, superoxide dismutase, and ferric reducing antioxidant power levels. In the patients who received omega-3 fatty acids, antioxidant factors including glutathione peroxidase, superoxide dismutase, and ferric reducing antioxidant power were significantly increased after two months (P = .02, P = .02, and P = .01, respectively); however, there was no significant changes in the control group in these markers. Malondialdehyde levels were significantly reduced after the study period only in the omega-3 group (P = .007).
Conclusions. The present study revealed that the supplementation with omega-3 fatty acids may result in better antioxidation status in patients on maintenance hemodialysis.

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INTRODUCTION

Atherosclerotic disease is recognized as the most life-threatening factor in uremic patients.[1,2] Patients suffering from end-stage renal disease (ESRD), receiving renal replacement therapy, are at increased risk of developing cardiovascular disease.[3] The excess risk factors are only partially made by the traditional risk factors such as hypertension, diabetes mellitus, smoking, and dyslipidemia, while other new factors such as increased oxidation stress is presumed to substantially contribute to cardiovascular risks in uremic patients.[4] Both uremia and treatment with hemodialysis are characterized by oxidative stress, micro-inflammation, and various

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metabolic changes.[5]

Oxidative stress was originally defined as the disequilibrium between pro-oxidants and antioxidants in biological system.[6,7] Factors such as exposure of blood to dialysis membranes, high risk of acute and chronic infection, and dietary limitation in the intake of antioxidant nutrients make patients on dialysis susceptible to more oxidative stress.[8] Diets enriched with omega-3 polyunsaturated fatty acids have been associated with a lower incidence of coronary heart disease and a reduction in atherosclerotic lesions.[9] Epidemiological evidence on the benefits of fish oil and omega 3 fatty acid consumption have been accumulated over the past few decades.[10] Clinical trials with fish oil have demonstrated a significant reduction in cardiovascular events.[11,12] Omega-3 fatty acids are believed to be beneficial in prevention of atherosclerosis.[13,14] They may have antioxidant effects by inhibiting lipid peroxidation.[9] In an animal study, omega-3 fatty acid supplementation decelerated progression of oxidative stress and influenced pathways involved in oxidative stress and inflammation.[15]

In patients with chronic kidney disease, several deficiencies been demonstrated in different components of the endogenous antioxidant defense mechanisms that maintain reactive oxygen species levels at a normal range, including reduced levels of vitamin C and E (primarily due to dietary restrictions and losses during dialysis),[16] reduced superoxide dismutase (SOD) activity in erythrocytes, and deficiency in the glutathione scavenging system.[17] While several antioxidant nutrients have been shown to be beneficial in hemodialysis patients,[18] the potential effects of fish oil supplementation, which has been recommended in dialysis patients, is still unclear. In this study, we examined several parameters associated with oxidative stress in hemodialysis patients receiving omega-3 fatty acid.

MATERIALS AND METHODS

Patients included in the study were from the hemodialysis unit of Central Hospital of Tabriz Medical University. Those meeting the inclusion criteria were invited to participate and provided informed consent. The excluded patients were those with malignancy and active inflammatory disease, those who were younger than 18 years, patients who received vitamin E, B6, B complex, folic acid, iron supplement, or blood transfusion during the past 3 months, and those on dialysis therapy for less than 3 months.

The recruited patients were divided into 2 groups. They were comparable in terms of sex distribution, age, medications, diabetes mellitus, hemoglobin level, serum ferritin, and serum albumin. Patients in one group were treated with omega-3 fatty acid supplementation and were compared with the second group which was considered as the control group receiving placebo. Omega-3 fatty acid capsules (Zahravi Pharmacy, Tabriz, Iran), 1 g, were given 3 times a day to the first group. The control group received placebo of the same form and size of capsule as the omega-3 fatty acid capsules. The treatment was continued for 2 months. All of the patients were checked for oxidation and antioxidant parameters and those suspected of inflammatory or infectious conditions during the study were excluded. Five patients failed to continue the drug because of the personal reasons and were excluded. Three other patients were also excluded; 1 died and 2 underwent kidney transplantation. Finally, 75 patients completed the study.

Initially, 2 blood samples were taken from the patients, the plasma and the buffy coat were removed after centrifugation at 1000 g for 10 minutes at 4°C. The first blood sample and plasma serum were frozen and stored in -80°C until the second whole blood sample was prepared after the 2-month study period. Whole blood glutathione peroxidase (GP) was measured using a Ransel kit (Randox Laboratories, London, UK). Glutathione peroxidase catalyzes oxidation of glutathione by cumene hydroperoxide. At the presence of glutathione reductase and nicotinamide adenine dinucleotide phosphate reduced (NADPH), the oxidized glutathione is immediately converted to the reduced form along with oxidation of NADPH to NADP+. The decrease in absorbance at 340 nm is measured. Superoxide dismutase activity was measured using a Ransel kit (Randox Laboratories, London, UK), employing xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The GP and SOD activity were expressed as U/g of hemoglobin. Ferric reducing antioxidant power (FRAP) was assessed through the use of the

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ultraviolet-visible spectrophotometric technique on plasma in this method. Ferric-to-ferrous ion reduction at low pH causes a colored ferrous-tripyridyltriazine complex. The FRAP values are obtained by comparing the absorbance change at 539 nm in test reaction mixtures with those containing ferrous ions in known concentration. The level of malondialdehyde was determined in serum of the patients through use of the thiobarbituric acid method. The results were obtained in terms of nmol/mL and determined using the colorimetric method.

Statistical analyses were done using the SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, Ill, USA). Continuous variables were shown as mean ± standard deviation. Normality of the distributions was checked for each variable using the Kolmogorov-Smirnov test. The paired *t* test was used to compare values before and after treatment in each group. Independent *t* test was used to compare variables between the two groups. A *P* value less than .05 was considered significant.

RESULTS

Seventy-five patients, including 26 women and 49 men with a mean age of 49.3 years, completed the study. They were 37 patients in the omega-3 group and 38 in the control group. Demographical characteristics of the patients in the omega-3 and control groups are shown in Table 1. There were no significant differences between the two groups in age, gender, diabetes mellitus, mean duration of dialysis, serum albumin level, hemoglobin, and serum ferritin level.

Table 1. Characteristics of Hemodialysis Patients in Omega-3 and Control Groups*

Variables	Patients on Hemodialysis		*P*
	Omega-3	Control	
Number of patients	37	38	
Mean age, y	48.8 ± 2.7	49.3 ± 1.8	.50
Gender			
Male	25 (67.6)	24 (63.2)	
Female	12 (32.4)	14 (36.8)	.80
Diabetes mellitus	8 (21.6)	13 (34.2)	.50
Mean dialysis duration, mo	43.1 ± 7.4	38.0 ± 5.7	.70
Mean serum albumin, mg/dL	3.90 ± 0.10	4.01 ± 0.12	.10
Mean hemoglobin, g/dL	10.95 ± 0.29	10.89 ± 0.35	.10
Mean serum ferritin, µg/L	632.0 ± 81.2	647.5 ± 73.1	.50

*Values in parentheses are percents.

Laboratory findings at baseline and after 2 months in the omega-3 and control groups are shown in Table 2. After 2-month administration of omega-3 fatty acid, SOD, GP, and FRAP levels significantly increased, while no significant changes were seen in these antioxidant indicators in the control group. Also, a significant decrease was seen in malondialdehyde level in the omega-3 group, but not in the controls.

Comparing the two groups before starting treatment showed no differences in the oxidative and antioxidant factors. Whereas, GP and SOD values were significantly higher in the omega-3 group after the treatment than those in the control group ($P = .007$ and $P = .003$, respectively). Also, the same significant difference was observed the FRAP level between the two groups after 2 months ($P = .02$). Malondialdehyde plasma level was significantly lower in the omega-3 group after the treatment period than that in the control group ($P = .009$).

DISCUSSION

The results of this placebo-controlled study demonstrated that omega-3 fatty acid supplementation decreased malondialdehyde and could increase levels of the antioxidant markers including GP, SOD, and FRAP. To our knowledge, this is the first study evaluating the impact of supplementation with omega-3 fatty acid on oxidative stress in hemodialysis patients.

Antioxidant nutrients were proven to be effective in hemodialysis patients.[18] Several oxidations

Table 2. Antioxidant Enzymatic Activities in Serum of Hemodialysis Patients in Omega-3 and Control Groups Before and After Treatment With Omega-3 Fatty Acid and Placebo*

Parameter	Omega-3			Control		
	Before	After	*P*	Before	After	*P*
MDA, nmol/mL	2.2 ± 0.9	1.7± 0.7	.007	1.9 ± 0.8	2.3 ± 1.1	.10
SOD, U/g	1019.9 ± 201.1	1112.4 ± 244.1	.02	1019.8 ± 196.2	962.1 ± 181.1	.10
GP, U/g	32.18 ± 7.82	34.54 ± 7.64	.02	31.85 ± 5.38	30.20 ± 5.75	.05
FRAP, mmol/L	1.24 ± 0.26	2.06 ± 1.80	.01	1.32 ± 0.25	1.29 ± 0.43	.70

*MDA indicates malondialdehyde; SOD, superoxide dismutase; GP, glutathione peroxidase; and FRAP, ferric reducing antioxidant power.

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markers are produced during hemodialysis treatment.[19] Malondialdehyde and a lipid peroxidation production was noted to be significantly increased in hemodialysis patients.[20] Allard and associates[21] demonstrated that supplementing the diet with n-3 fatty acids resulted in an increase in lipid peroxidation, as measured by plasma malondialdehyde release and lipid peroxide products in healthy humans. Similarly, supplementation with omega-3 fatty acid in our study showed significant increase in malondialdehyde plasma level in hemodialysis patients.

Fisher and colleagues[22] reported that modest dietary omega-3 fatty acid supplementation could reduce stimulated human monocyte-free-radical production and might impair the capability of macrophages derived from monocytes to promote oxidation of low-density lipoprotein cholesterol and associated cellular toxicity, so that it seemed have a beneficial effect on prevention of oxidation stress in human. In our study, SOD was significantly increased in hemodialysis patients treated with omega-3 fatty acids, which potentially improved oxidation state.

Plasma ferric reducing antioxidant power levels can be dramatically affected by both uremia and dialysis.[23] According to our results, omega-3 fatty acid supplementation increased the FRAP in hemodialysis patients. Glutathione plays a key role in cellular resistance against oxidative damage,[24] and plasma GP is an important antioxidant enzyme that is mainly produced in the kidneys.[25] Ahmadpoor and coworkers[8] showed that glutathione levels and GP activity were markedly lower in the hemodialysis patients than in the healthy individuals, and the total antioxidant capacity of plasma was decreased in patients on dialysis. Depletion of glutathione as a key antioxidant component and disturbances in its related enzymes are associated with oxidative stress. Iraz and colleagues showed that in animal models, 30 days of dietary supplementation with fish oil containing of omega-3 acid (0.4 g/kg/d) helped to prevent lipid peroxidation and prevent tissue from oxidative injury.[26] Dietary supplementation with omega-3 fatty acids might possibly protect tissues from oxygen-free radical injury in various diseases in which the oxidant/antioxidant defense mechanisms are disturbed.[26] In another animal experiment, supplementation with omega-3 fatty acids was shown to have a protective effect against the toxicity of formaldehyde on the kidney.[27] Rats were administered omega-3 fatty acid while exposed to formaldehyde and showed increased SOD and GP enzyme activities and decreased levels of malondialdehyde. Romieu and colleagues[28] showed in the elderly exposed to environmental particulate matter that supplementation with omega-3 unsaturated fatty acids appeared to modulate the adverse effects of particulate matter on these biomarkers, particularly in the fish oil group. Supplementation with omega-3 unsaturated fatty acids could modulate oxidative response to particulate matter exposure. In another animal study by Kenar and colleagues,[29] SOD and GP antioxidative enzyme activities in the colorectal tissue were increased in omega-3 groups in comparison with rats of the control group, and malondialdehyde levels significantly decreased. Their results suggested that the dietary supplementation of omega-3 fatty acids be useful and had a healthy effects by prevention of oxidative stress formation. Similarly, our present results showed that the activity of SOD in the hemodialysis patients was markedly increased in the omega-3 group when compared with the control group. Also, omega-3 fatty acids induced a positive effect on the activity of GP.

CONCLUSIONS

We conclude that the use of omega-3 fatty acid, 3 g/d, is well tolerated and have a positive effect at least on the improvement of oxidation markers, which have the main roles in pathogenesis of atherosclerosis in dialysis patients. Further studies with more patients on dialysis and longer administration of unsaturated fatty acids are recommended.

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CONFLICT OF INTEREST

None declared.

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