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EFFECTS OF OMEGA 3 AND VITAMIN E ON ANTIOXIDANT ENZYMES AND HSCRP IN CAD PATIENTS

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Abstract -Inflammation, as a consequence of oxidative stress, is a key factor in development of cardiovascular diseases. The aim of the current study was to assess the effects of omega 3 and vitamin E supplementation on the serum level of antioxidant enzymes and hsCRP levels in CAD patients. Participants of this RC T study included 60 non-smoker male CAD patients who received 4g/day of omega 3 and vitamin E placebo (OP), omega 3 (4g/day) and 400IU/day vitamin E (OE) or omega 3 and vitamin E placebos (PP) for two months. Serum antioxidant enzymes and hsCRP levels were measured at the baseline and at the end of the study and effects of the intervention on these parameters were compared between and within the groups. Serum levels of catalase increased significantly in OE and OP groups (P=0.002 and P= 0.015, respectively). TAC increased significantly in OE group (P=0.009) but not in OP and PP groups. Serum SOD and GPX did not change significantly in any of the groups or between the groups. Omega 3 alone and combined omega 3 and vitamin E supplementations decreased hsCRP significantly in OP and OE groups (P=0.008 and P= 0.050, respectively).Co-administration of omega 3 and vitamin E in CAD patients seems to include beneficial effects on inflammation by decreasing hsCRP levels and to improve oxidative stress by influencing antioxidant enzymes.

Keywords: CAD, omega 3, vitamin E, antioxidant enzyme, hsCRP

I. INTRODUCTION

Coronary artery disease (CAD) is the most common disease in developing countries, partly due to changes in lifestyle in recent years. According to global and regional projects of mortality and burden of diseases, CAD is the leading cause of death until 2030 [1]. Approximately, 30% of all global deaths in 2008 attributed to CVD (WHO, 2011). It is estimated that someone dies from CAD in USA every minute [2].Several studies have shown that oxidative stress is the major cause of atherosclerosis[3]and free radicals enhance the expression of some chemotactic factors such as monocyte chemotactic protein (MCP) [4], matrix-metalloproteinase 9 (MMP-9) and tissue factor[5].ROS can activate the nuclear factor kappa B (NFkB) which can increase gene expressions of many atherosclerotic factors resulting in the accumulation of macrophages in arterial walls [6].Inflammation, as a consequence of oxidative stress, is a key factor in cardiovascular diseases and can result in atherosclerotic plaque formation and thrombosis [7].

It is believed that omega 3 fatty acids include beneficial effects in preventing atherosclerosis[8, 9]and contain antioxidant properties by decreasing lipid peroxidation [10]. These substances are anti-inflammatory agents and are able to reduce inflammation through inhibitory production of cytokines such as IL1, 1L2 and TNFα [11–13]. In a study, two months of supplementation with omega 3 resulted in significant increase in glutathione peroxidase (GPX) and superoxide dismutase (SOD) in hemodialysis patients [14]. Vitamin E includes an antioxidant and anti-inflammatory properties and inhibits atherogenes is progressing via affecting smooth muscle cells and endothelial cells of the arteries [15–17]. However, human studies which assessed the effects of vitamin E supplementation on CAD patients showed conflicting results. In a study by Devaraj et al., a two-year supplementation with vitamin E in CAD patients did not result in significant differences in cardiovascular diseases[18].Furthermore, results of a systemic review released in 2004 showed that vitamin E had no beneficial or adverse effects in CAD patients[19].Since vitamin E and omega 3 fatty acids include antioxidant and anti-inflammatory properties and that no studies have been carried out to assess simultaneous supplementation of these nutrients in CAD patients, the current study was designed to evaluate the effects of omega 3 fatty acids with or without vitamin E supplementation on serum antioxidant enzymes, lipid profile and hsCRP levels in CAD patients.

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II. MATERIALS AND METHODS

The participants of this randomized double-blind placebo-controlled clinical trial included 65non-smoker male patients with CAD who had at least 50% stenos is in one coronary artery in the past three months as proven by angiogram. These volunteers were selected from the Heart Medical Center of Tehran University of Medical Sciences, Tehran, Iran. An informed consent was obtained from all participants. The study plan was approved by the Ethical Committee of Tehran University of Medical Sciences (ID: 23605) and registered in www.clinicaltrial.org under the registry number of NCT02011906.The inclusion criteria included BMI ≤30 and no history of allergies, diabetes, thyroid malfunction and kidney and liver disorders. Participants of this RCT study were divided into three groups of omega 3 and vitamin E (OE), omega 3 and vitamin E placebo (OP) and omega 3 and vitamin E placebos (PP) using random permuted blocks. The OE group received four1g of omega-3 fatty acids softgels and 400 IU of vitamin E daily, OP group received four1g of omega-3 fatty acid softgels and vitamin E placebo daily and PP group received both omega-3 fatty acid and vitamin E placebo softgels. Each 1 g of omega 3 softgels contained 120 mg of docosahexaenoic acid (DHA) and 180 mg of eicosapentaenoic acid (EPA). All supplements and paraffin placebos were provided by Minoo Pharmaceutical, Cosmetic and Hygienic Company, Tehran, Iran.

Height, hip and waist circumferences were measured before and after the intervention with maximum accuracy, minimal clothing and no shoes. BMI was calculated as the weight(kg) divided by the square of height (m)and waist to hip ratio (WHR) was calculated by dividing the waist circumference to the hip circumference. At the baseline and after two months of the intervention, a two-day food recall was filled and 10 ml of blood samples were collected after 12–14 h of fasting. Serums were separated by centrifuging and stored at -80 °C until use. Serum hsCRP was assessed using a commercially available ELISA kit (LDN, Germany).Serum levels of total antioxidant capacity (TAC) were assessed using 2,2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid (ABTS)[20]. Catalase activity was assessed according to Aebi's method [21] and serum GPX and SOD were measured spectrophotometrically using Palgia et al. and Sun et al. methods, respectively[22, 23].Nutritionist IV Software was used for the analysis of dietary data and statistical analysis was performed using SPSS Software V.18. Data were expressed as mean ±SE (standard error) of the parameter. The Kolmogorov Smirnoff test was used for determining normality of the parameters distribution. One-way analysis-of-variance (ANOVA) test was used to compare the mean of the variables between the groups and paired t-test was used to compare parameters within the groups before and after the intervention. *P*values < 0.05 were considered as significant.

III. RESULTS

The current study was begun with 65 male CAD patients, but three of them were hospitalized for heart surgery during the intervention and two other patients discontinued the supplements consumption due to personal reasons. Therefore, the study was completed with 60 CAD patients at the end of the intervention, including 21, 20 and 19 patients in OE, OP and PP groups, respectively. These three groups were not statistically different from each other for the mean patient ages and the disease duration at the beginning of the intervention (*P*=0.079 and *P*= 0.299, respectively). Anthropometric parameters of the three groups at the baseline are described in Table 1. No significant differences were seen between these parameters in the study groups at the baseline. Dietary intakes of the three groups based on the recall analysis are described in Table 2. As shown in the table, energy and macronutrient intakes were not statistically different in the study groups at the baseline and at the end of the intervention. Furthermore, no difference was seen in dietary intakes of vitamin E and fatty acids and it seems that diets were not changed during the intervention.

Table 3 illustrates fasting serum biochemical values and serum hsCRP in the three groups at the baseline and at the end of two months of intervention. As it described, fasting serum triglycerides (TG) decreased significantly in OE and OP groups (*P*=0.020 and *P* = 0.001, respectively). Omega 3 supplementation increased FBS significantly in OP group (*P*= 0.004). No difference was seen between the mean FBS of the three groups at the end of the intervention. Omega 3alone and combined omega 3 and vitamin E supplementation decreased hsCRP significantly in OP and OE groups (*P*=0.008 and *P* = 0.050, respectively). Serum low-density lipoprotein (LDL), high-density lipoprotein (HDL) and total cholesterol (TC) did not change significantly in any of the groups or between the groups. Adjustment of the BMI and duration of the disease did not change the significance of the differences. Table 4 shows the antioxidant enzymes of the study groups at the baseline and the end of two months of intervention. As shown in the table, serum level of catalase increased significantly in OE and OP groups (*P*=0.002 and *P* = 0.015, respectively). Omega 3 and vitamin E supplementation increased serum level of GPX, but it was not statistically significant (*P*=0.086). TAC increased significantly in OE group (*P*=0.009), but not in OP and PP groups. Serum SOD did not change significantly in any of the groups or between the groups.

IV. DISCUSSION

To the best of the authors' knowledge, this is the first study to assess the effects of omega 3 and vitamin E supplementation on antioxidant enzymes and serum hsCRP in CAD patients. Results of this study have shown that omega 3 alone and a combination of omega 3 and vitamin E can increase serum catalase level, but includes no significant effects on GPX and SOD levels. Elevation in ROS levels is a common feature in cardiovascular diseases and can result in endothelial dysfunction [24, 25]. Although omega 3 fatty acids can decrease lipid peroxidation and have antioxidant properties [10], some studies have suggested that increased consumption of omega 3 fatty acids may exacerbate oxidative stress [26, 27].Vitamin E is a potential antioxidant agent and its supplementation possibly affects oxidative stress. As the current study showed, only a combination of omega 3 and vitamin E positively influenced serum TAC levels. In a study on romatoid arthritis patients, Kolahi et al. reported that omega 3 supplements with or without vitamin E had no effects on SOD and GPX levels. However, unlike the current study, serum TAC did not change significantly in their report [28]. Another study on diabetic patients showed that serum TAC increased significantly in groups receiving a

combination of EPA and vitamin E and vitamin E alone, but catalase increased only in EPA and vitamin E group [29].

Several studies have shown that vitamin E is an anti-inflammatory agents and its supplementation can decrease the release of IL-1β from monocytes. IL-1β is a cytokine which can elevate serum IL-6 level [15, 17, 30]. Results of a meta-analysis showed that supplementation with vitamin E could reduce serum CRP levels significantly [31].Omega 3 fatty acids can decrease production of inflammatory cytokines such as IL1, 1L2 and TNFα[11–13]. Furthermore, these fatty acids can reduce serum CRP levels[32, 33].In the current study, omega 3 supplementation alone or in combination with vitamin E decreased serum hsCRP Levels significantly. However, the omega 3 and vitamin E group experienced more decline in serum CRP than omega 3 group. Vitamin E and omega 3 fatty acids seem to act synergistically to reduce inflammation processes via decreasing CRP levels. Results of another study on CAD patients showed that omega 3 alone and omega 3 and vitamin E supplements could decrease hsCRP levels significantly [34]. Saboori et al. in one Randomized Clinical Trial study showed that co-administration of omega 3 fatty acids with vitamin E can improve the outcomes of CAD patients by increasing the gene expressions of SIRT1 and PGC1(1).

In the present study, serum TG levels decreased significantly in OE and OP groups. Several studies have shown that omega 3 fatty acids include a dose-dependent effect on serum triglycerides and can reduce TG concentration up to 30% [35, 36]. Omega 3 fatty acids seem to be able to inhibit hepatic production of TG and apo-lipoprotein B [37, 38] and to increase fatty acid oxidation [39, 40].Results of the current study have shown that intake of omega 3 supplements alone can increase serum glucose levels significantly. However, serum glucose was still in normal range. The effect of omega 3 fatty acids on glucose homeostasis is conflicting. While it was previously suggested that using omega 3 supplements could adversely affect glucose control [41, 42], studies have concluded that omega 3 fatty acids can improve glucose uptake and insulin sensitivity [43–45].In the current study, although it is not clear that why this increase in FBS did not occur in OE group, it might be linked to concurrent using of vitamin E. Some studies have revealed that vitamin E can improve glucose homeostasis because it can influence insulin sensitivity [46–48].One limitation of the current study includes the relatively short duration of the intervention. If supplementation was continued for more than two months especially with higher doses of omega 3, possibly more antioxidant enzymes would be influenced. In conclusion; results of this study showed that omega 3 fatty acids alone or in combination with vitamin E could positively affect antioxidant status of CAD patients and also improve inflammation condition via decreasing hsCRP levels.

Conflicts of Interest

The authors declare no conflict of interest.

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Table 1.Anthropometric parameters of the study groups before the intervention

	OE (*n* = 21)	**OP (*n* = 20)**	**PP (*n* = 19)**	***P*. value***
Height (cm)	170.32 ±1.19	169.04 ±1.36	170.92±1.58	0.623
Weight (kg)	78.54 ±2.17	79.95 ±2.68	78.35 ±1.87	0.864
BMI (kg/cm^2)	27.08 ±0.70	27.95 ±0.83	26.85 ±0.61	0.530
Waist circumference (cm)	95.76 ±1.58	98.72 ±2.11	96.18 ±1.88	0.479
Hip circumference (cm)	100.33 ±1.15	101.12 ±1.59	99.63 ±0.80	0.701
WHR	0.95 ±0.01	0.97 ±0.01	0.96 ±0.01	0.463

OE, omega-3 fatty acid and vitamin E; OP, omega-3 fatty acid and placebo; PP, placebo and placebo; BMI, body mass index; WHR; waist hip ratio; *ANOVA analysis

Table 2. Dietary intakes of the study groups before and after the intervention

Treatment group		**OE (*n* = 21)**	**OP (*n* = 20)**	**PP (*n* = 19)**	***P*. value***
Energy (Kcal)	Baseline	1469.10 ±93.33	1450.74±114.37	1528.49 ±111.25	0.867
	Post-intervention	1649.48 ±122.47	1684.55 ±131.39	1508.20 ±130.59	0.596
	difference	180.38 ±162.53	233.81 ±169.99	32.36 ±173.82	0.688
	P. value#	0.281	0.185	0.854	
Carbohydrate (g)	Baseline	228.44 ±16.93	231.47 ±22.93	246.01 ±18.52	0.800
	Post-intervention	259.51 ±21.96	271.56 ±25.45	238.00 ±21.37	0588
	difference	31.06 ±25.68	40.09 ±26.02	-8.01 ±30.04	0.426
	P. value#	0.241	0.140	0.793	
Protein (g)	Baseline	69.64 ±8.76	62.25 ±7.75	62.27 ±7.71	0.770
	Post-intervention	66.16 ±6.98	60.44 ±5.74	59.30 ±6.49	0.722
	difference	-3.48 ±11.49	-1.81 ±10.58	3.42 ±10.64	0.992
	P. value#	0.765	0.866	0.751	
Fat (g)	Baseline	33.45 ±2.96	33.05 ±2.79	35.08 ±3.82	0.895
	Post-intervention	41.80 ±4.10	42.88 ±3.75	37.01 ±3.86	0.538
	difference	8.34 ±5.28	9.82 ±4.97	1.93 ±5.53	0.538
	P. value#	0.131	0.063		
Vitamin E (mg)	Baseline	2.72 ±0.74	2.70 ±0.55	2.29 ±0.65	0.427
	Post-intervention	4.04 ±0.97	4.19 ±1.04	2.77 ±0.45	0.311
	difference	1.33 ±1.35	1.48 ±1.29	0.48 ±0.78	0.971
	P. value#	0.338	0.265	0.456	
Omega-3 fatty acids (g)	Baseline	0.13 ±0.04	0.12 ±0.03	0.11 ±0.04	0.963
	Post-intervention	0.11±0.05	0.21±0.10	0.10 ±0.03	0.464
	difference	-0.01±0.06	0.09 ±0.11	-0.01 ±0.05	0.570
	P. value#	0.821	0.428	0.781	
Omega 6 fatty acids (g)	Baseline	10.80 ±1.17	11.47 ±0.93	10.89 ±1.76	0.926
	Post-intervention	13.58 ±2.15	13.76 ±1.95	12.87 ±2.19	0.148
	difference	2.78 ±2.59	2.29 ±2.21	1.98 ±2.91	0.534
	P. value#	0.380	0.273	0.506	
Saturated fatty acids (g)	Baseline	9.73 ±1.05	8.17 ±6.28	10.33 ±1.19	0.333
	Post-intervention	8.98 ±0.77	9.16 ±0.80	10.11 ±1.13	0.649
	difference	-0.75 ±1.28	0.99 ±1.07	-0.22 ±1.65	0.643
	P. value#	0.566	0.367	0.897	

OE, omega-3 fatty acid and vitamin E; OP, omega-3 fatty acid and placebo; PP, placebo and placebo; *ANOVA analysis; #paired T test

Table 3. Serum biochemical characteristics and hsCRP levels in the study groups before and after the intervention

Treatment group		OE (*n* = 21)	OP (*n* = 20)	PP (*n* = 19)	*P.* value*
FBS (mg/dl)	Baseline	92.19 ±3.27	87.75±3.25	89.32±4.78	0.697
	Post-intervention	89.38±2.32	99.13±3.78	94.5±3.53	0.108
	difference	2.81 ±3.79	11.37±3.46	4.74±4.13	0.034
	P.value#	0.264	0.004	0.267	
TG (mg/dl)	Baseline	185.48±17.39	180.38±18.02	174.53±19.41	0.914
	Post-intervention	145.38±9.44	131.28 ±14.53	154.58±19.87	0.458
	difference	-40.10±15.92	-49.10±12.25	-19.95±12.25	0.325
	P.value#	0.020	0.001	0.121	
TC(mg/dl)	Baseline	186.57±8.71	157.95±7.79	170.47±15.85	0.190
	Post-intervention	165.00±12.35	154.55±6.16	169.76±10.87	0.568
	difference	-21.57±16.04	-3.40 ±9.09	-0.71 ±13.69	0.484
	P.value#	0.194	0.713	0.959	
LDL (mg/dl)	Baseline	109.90±4.38	100.10±3.77	105.21±5.26	0.302
	Post-intervention	107.14±6.60	95.40±4.05	109.21±5.93	0.189
	difference	-2.76 ±7.43	-4.70 ±3.94	4.00 ±3.836	0.512
	P.value#	0.311	0.248	0.714	
HDL (mg/dl)	Baseline	34.35 ±1.92	31.30 ±1.66	34.42 ±1.89	0.394
	Post-intervention	37.14 ±2.38	35.55 ±1.62	37.95 ±1.95	0.702
	difference	2.73 ±2.03	4.25 ±2.21	3.52 ±0.97	0.854
	P.value#	0.120	0.065	0.182	
hsCRP (mg/l)	Baseline	2.76 ±0.48	3.12 ±0.55	3.34 ±0.45	0.720
	Post-intervention	1.80 ±0.18	1.60 ±0.23	3.57 ±0.64	0.001
	difference	-0.96 ±0.46	-1.21 ±0.48	0.23 ±0.64	0.132
	P.value#	0.050	0.008	0.716	

OE, omega-3 fatty acid and vitamin E; OP, omega-3 fatty acid and placebo; PP, placebo and placebo; hsCRP, high-sensitivity C-reactive protein; *ANOVA analysis; #paired T test

Table 4. Serum antioxidant enzyme and TAC values of the study groups before and after the intervention

Treatment group		OP (*n* = 20)	OE (*n* = 21)	PP (*n* = 19)	*P.*value*
Catalase (U/l)	Baseline	71.35±2.22	64.43 ±2.16	65.00 ±2.67	0.073
	Post-intervention	76.00 ±2.13	71.19 ±2.62	66.58 ±2.83	0.042
	difference	4.65 ±1.74	6.76 ±1.94	2.44 ±2.16	0.303
	P.value#	0.015	0.002	0.273	
SOD (U/ml)	Baseline	175.05 ±25.97	139.47 ±7.46	194.85 ±26.63	0.183
	Post-intervention	167.48 ±18.31	134.68 ±6.40	183.34 ±25.35	0.164
	difference	-7.55 ±13.11	-4.16 ±6.04	-8.94 ±7.55	0.936
	P.value#	0.571	0.499	0.252	
GPX (U/ml)	Baseline	1.74 ±0.65	1.70 ±0.045	1.85 ±0.056	0.163
	Post-intervention	1.81 ±0.068	1.81 ±0.045	1.79 ±0.057	0.959
	difference	0.068 ±0.072	0.12 ±0.065	-0.052 ±0.078	0.243
	P.value#	0.353	0.086	0.514	
TAC (μmol/l)	Baseline	112.26 ±23.04	113.91 ±21.15	79.40 ±3.13	0.342
	Post-intervention	116.30 ±22.81	119.32 ±21.07	81.88 ±3.50	0.316
	difference	4.04 ±2.61	7.58 ±2.62	2.45 ±3.15	0.418
	P.value#	0.138	0.009	0.446	

OE, omega-3 fatty acid and vitamin E; OP, omega-3 fatty acid and placebo; PP, placebo and placebo; *ANOVA analysis; #paired T test