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Omega-3 Fatty Acid Modifies Serum HSP 27 in Patients with Cardiovascular Disease: Randomized Double-Blind Placebo-Controlled Trial

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A B S T R A C T

Background: Heat shock proteins 27(HSP-27) is involved in the onset of heart disease by inducing pro-inflammatory immune response against endothelial cells. Omega-3 fatty acids increase anti-inflammatory eicosanoids and contribute to the primary and secondary prevention of cardiovascular disease (CVD). The purpose of the current study is to assess the effect of omega-3 on serum HSP-27 in patient with atherosclerosis.

Methods: In the double-blind placebo-controlled trial study, patients were selected from Tehran Heart Hospital. Both of intervention and placebo groups were 21 males with cardiovascular disease (based on Angiography). The intervention group received omega-3 supplement and placebo group took edible paraffin. Dietary intakes, physical activity level, anthropometric parameters and body composition, were measured. Serum HSP-27 concentration was determined after two months.

Results: The difference in serum HSP-27 between two groups was statistically significant (P=0.001) after two months. The difference in change of serum HSP27 between two groups remained significant even after control for serum LDL cholesterol concentration (p=0.002).

Conclusion: The study showed that taking omega-3 fatty acids can ameliorate serum HSP-27 as inflammatory parameters. Our results suggest more investigation to assess the pathway omega-3 leads to lower incidence of CVD.

Introduction

Atherosclerotic plaques are formed in the coronary artery wall and leads to acute coronary syndrome symptoms such as angina and myocardial infarction [1]. Inflammation plays an important role in the progression of atherosclerosis. All stages of initiation and progression of atherosclerosis are associated with inflammatory responses [2].

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Stressful stimuli such as infection, biomechanical stress, oxidized Low Density Lipoprotein (ox-LDL), free radicals, heat shock and other stress induce HSP production in the walls of the arteries and other tissues[3]. Heat shock proteins classified into 7 families according to their size and molecular structure[4]: HSP-10, Small HSP (15-30 kDa), HSP 40, HSP 60, HSP 70, HSP 90, and HSP 100.

HSP-27 involves in the heart disease initiation by pro-inflammatory immune response induction against endothelial cells [5]. Jozefowicz–Okonkwo *et al* have found that serum level of HSP-27 is a possible indicator of myocardial ischemia with 2 or 3 clogged arteries [6]. HSP-27 production is measured by serum antigen-antibody concentration which is appropriate as a risk indicator for heart disease progression [5].

Omega-3 fatty acids include anti-inflammatory effects, regulate cytokine transcription and gene expressions related to the immune response and inflammation, improve endothelial function, and regression of atherosclerotic plaque [7,8]. Cardiovascular effects of omega-3 fatty acids include improved endothelial function. Anti-inflammatory effects of omega-3 fatty acids include increasing anti-inflammatory eicosanoids, reducing Tumor necrosis factor α (TNF-α) and Interleukin 1-6 (IL-1, IL-6) [9].

Several studies have demonstrated that omega-3 fatty acids, Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) involve in the primary and secondary prevention of cardiovascular disease (CVD) and sudden death [10]. The effects of omega-3 fatty acids on immune markers include decrease in inflammatory cytokines. On the other hand, the levels of anti-inflammatory cytokine concentration tend to increase [11].

With respect to anti-inflammatory effects of omega-3 fatty acids, the purpose of the current study was to investigate the effect of omega-3 on serum HSP-27 in patient with atherosclerosis. So far no study has performed on the effect of omega-3 fatty acids on serum levels of these factors in human specimens with vascular disorders.

Methods

This research is a double-blind placebo-controlled trial study. Enrolled patients were recruited from Tehran Heart Hospital. Forty four patients suffering from CVD expressed their willingness to cooperate with our investigation. Subjects were men. Forty two patients were in the study. Intervention group was 21 males with CVD proven angiographically. Inclusion criteria of study included middle-aged men (45-65 years old) (Obstruction ≥50% in at least one vessel in the last three months, approved by angiography) and body mass index <30 kg/m^2 tend to maintain normal activities and lifestyle in the period of study, willingness to cooperate, no-smoking habit and no cancer history. Exclusion criteria included liver, kidney, stomach, and thyroid diseases in addition diabetes diagnosis. Exclusion criteria were unwillingness to continue cooperation, onset of inflammatory disease that leads to long-term usage of anti-inflammatory drugs (more than 2 weeks), allergy and sensitivity to fish oil.

Patients were divided into two groups based on Randomized Permuted Block and two groups would receive either omega-3 fatty acid or placebo. All participants signed informed consent. During the investigation soft gel supplement was consumed by patients as being instructed with two main meals due to their fat solubility and enhanced absorption. The patients consumed half of prescribed daily dosage (two softgels) with each meal. The intervention group received omega-3 supplement (containing EPA: 720 mg, DHA: 480 mg per 4 one gram fish oil softgels) and the placebo group took edible paraffin (4 grams edible paraffin per day) for 2 months. The method and timing of placebo consumption were the same as instructed to omega 3 fatty acids trial arm. That amount of edible paraffin has been allowed to consume without any side effects on gastrointestinal functions or nutrients absorption. The registration code number in www.clinicaltrial.org is (IDNCT02117960).

Height and weight were measured before and after intervention. All anthropometric measurements were performed according to the method proposed by the World Health Organization (WHO) [12]. Seca digital scale was used for weighing (Accuracy of 0.01g). Weight was measured with light clothing and without shoes. Height was measured using a Seca stadiometer without shoes (sensitivity of 0.1 cm). Height of the patient was specified in an upright position without shoes.

Body mass index was calculated by BMI equation (the ratio of weight in kilograms to the square of height in meters). Body composition

was determined by bioelectrical impedance analysis (BIA) (BC-418, Japan). BIA output presents data about the amount and percentage of fat and lean body mass in kilograms (kg). Daily intakes of fatty acid (Linoleic and Linolenic fat) were estimated using 24-hr food recall (a usual day and a holiday) at the beginning and end of the study. Nutritionist IV software (version 4.1; First Databank Division, the Hearst Corporation) was used to analyse dietary intake. It was based on USDA database with minor modification for the special national foods like breads. International Physical Activity Questionnaires was used to consider the average of physical activity.

Serum HSP-27 was measured by Enzyme-Linked Immunosorbent Assay (ELISA) kits (Cat no. E1786Hu Bioassay Technology Laboratory, china). Serum levels of glucose, Triglyceride, Total cholesterol, Low-density lipoprotein (LDL), High-density lipoprotein (HDL) was measured by commercial kits (Pars azmoon, Iran) and auto-analyzer system (Selectra E,Vitalab, Netherland).

The dietary intake data were analyzed by Nutritionist 4 software. The gathered data was controlled and transferred to SPSS software version 21 (Chicago, Illinois, USA) for statistical analysis. The mean and standard error were used to describe data. Normal distribution of data was evaluated by Kolmogorov-Smirnov test. Comparisons between before and after intervention in each group were analyzed using paired t-test. Independent sample t test was applied to compare two groups. Confounding effect was removed by ANCOVA test. If P value had been ≤ 0.05, it was considered as statistically significant.

Results

Comparison of the anthropometric characteristics and dietary fat intake

According to Table 1 there was no significant difference between the two groups in terms of age, anthropometric Indices, lean body mass and fat mass, physical activity, dietary intake of Linoleic fat and Linolenic fat before and after the intervention.

Comparison of Biochemical markers

As Table 2 shows, the serum levels of cholesterol, triglyceride, LDL cholesterol, HDL cholesterol, FBS shows no significant difference between two groups before and after the intervention except LDL. There was an significant difference in serum LDL concentration in comparison with baseline level(P=0.04).

Serum HSP 27 concentrations

The mean serum concentration of HSP-27 of the group receiving omega-3 reduced after two months omega-3 fatty acid supplementation (P=0.001). The difference was remained significant even after control for serum LDL cholesterol (P=0.002).

Discussion

That is the first investigation of assessing the effect of omega-3 fatty acid on serum HSP 27 in patients with CVD. Omega-3 fatty acids decreased serum HSP27 concentration. Even though serum total cholesterol and triglyceride have been decreased in placebo group during

Table 1. Anthropometric characteristics and fatty acid intake in intervention and placebo groups

		Omega3 (n=21)	Placebo (n=21)	P value*
Age(y)	before	56.19±1.35	57.86±1.45	0.40
Height(cm)	before	169.11±1.20	167.36±1.47	0.36
Weight(kg)	before	80.61±1.81	74.70±2.45	0.06
	after	80.42±1.74	75.40±2.41	0.10
BMI (kg/m^2)	before	28.22±0.64	26.68±0.86	0.16
	after	28.15±0.60	26.91±0.83	0.23
Physical activity score (met- minutes/week)	before	1.57±0.13	1.48±0.13	0.61
	after	1.62± 0.14	1.43± 0.13	0.33
Fat mass(kg)	before	19.19±1.37	16.80±1.46	0.12
	after	19.72±1.21	17.51±1.26	0.21
Fat free mass(kg)	before	60.01±1.32	57.86±1.45	0.28
	after	60.55±1.23	58.00±1.48	0.19
Linoleic fat intake (g/day)	before	11.91±1.30	8.61±1.69	0.27
	after	15.08±1.74	12.76±1.68	0.81
Linolenic fat intake (g/day)	before	0.15±0.06	0.07±0.01	0.27
	after	0.08±0.03	0.07±0.017	0.81

*Independent sample t test (Between two groups). BMI = Body mass index

Table 2. Serum biomarkers before and after omega-3 supplementation in male patients with cardiovascular disease

		Omega-3 (n=21)	Placebo (n=21)	P value*
FBS(mg/dl)	before	87.62±2.90	93.93±4.17	0.22
	after	95.98±3.06	97.67±3.22	0.70
T.Cholesterol (mg/dl)	before	162.12±7.59	173.26±12.18	0.44
	after	153.76±6.71	163.26±8.35	0.38
Triglyceride (mg/dl)	before	148.24±10.35	190.07±19.55	0.06
	after	126.07±19.40	167.29±20.15	0.14
HDL (mg/dl)	before	33.24±1.79	32.19±1.43	0.64
	after	36.48±1.53	36.90±1.75	0.85
LDL (mg/dl)	before	102.48±4.39	104.57±4.67	0.74
	after	96.67±5.35	112.43±5.58	0.04*

*Independent sample t test (Between omega 3 and placebo group).
DBP= Diastolic Blood pressure, FBS=*Fasting blood sugar,* HDL=High-density lipoprotein, LDL=Low-density lipoprotein, T. Cholesterol=total cholesterol.

Table 3. HSP-27 serum levels before and after supplementation

		Omega3(n=21)	placebo(n=21)	P value [a]	P value[c]
HSP-27(ng/ml)	before	157.52±21.97	115.28±16.05	0.12	
	after	88.04±3.08	121.52±15.31	0.04 [a]	
	difference	-69.47±20.09	6.23±5.53	0.001 [a]	0.002[c]
	P-value [b]	0.002 [b]	0.273		

[a] Independent sample t test
[b] Paired t-test
[c] ANCOVA
HSP-27 = Heat shock protein-27

intervention, this decrease has not been statistically significant. Therefore it seems it would not be due to paraffin eaten by placebo members.

HSP are considered as intracellular proteins [3]. There is insignificant numbers of HSP in normal condition but stressful conditions cause increasing amount of them [13]. However, with certain conditions, they are released in the extracellular environment [14] where they act as auto antigenic agent [15]. Autoimmune response against HSP leads to atherosclerosis pathogenesis[16]. HSPs are released into the blood stream and converted to soluble heat shock proteins HSPs (HSPs). Binding of the soluble HSPs (sHSPs) to the complex of Cluster of Differentiation 14 (CD14) / Toll like receptor-4 (TLR-4) leads to proinflammatory response and autoimmune reaction to the intervention and has an important role in atherosclerosis [3]. Activation of receptors like TLR4 and CD14 leads to nuclear factor **κ**B activation. This results in various pro inflammatory cytokine expressions such as TNF, interleukin 1α and interleukin 1β [17].

The antibodies to HSPs can enter injured cells. It links to the intracellular HSP in macrophages and foam cells and causes cell lysis. This involve in the creation of the necrotic core that exists in more atherosclerosic plaques [14, 18].

Patients with acute coronary syndromes have shown augmented serum levels of HSP27 [19]. Heidari-Bakavoli *et al* have indicated that patients with acute coronary syndrome (ACS) have meaningfully higher HSP 27 concentration than control group [20].

In a study, normal vessel part and different areas plaque were incubated in vitro with protein-free medium. It was indicated that the release of HSP-27 was reduced sharply in atherosclerotic plaques. HSP-27 levels in blood flow in patients with stenosis carotid was significantly decreased compared to healthy subjects [21]. The reason is explicable according to Wick project. Decreased expression of HSP-27 in the center of the plaque can be due to proteolytic activity in this area. The expression of HSP27 in the normal area adjacent to atherosclerotic plaque is because of the highest amount of inflammation in that area [22].

Possible mechanisms of HSP-27 confronting oxidative stress included up-regulation of glucose 6-phosphate dehydrogenase, glutathione peroxidase and reduced intracellular iron[23, 24]. When cells are exposed to oxidative stress, they over-express HSP-27 [25]. Also the other HSP27 preservation mechanisms is effect on necrotic cell death, but it has no effect on apoptotic cell death[26].

Quaternary structure and chaperone role of HSP27 is altered following stress condition by phosphorylation in two or three serine residues. The phosphorylation and changed structure are important for protection [27]. Stress condition causes phosphorylation and oligomerization of Hsp27. Phosphorylation of HSP27 results in that the actin cytoskeleton within endothelial and/or smooth muscle cells become stable. In this way it preserve against vascular disease [28].

Ebrahimi *et al* have shown that patients with metabolic syndrome who had taken fish oil supplements (EPA: 180 mg, DHA: 120 mg) for 6 months had a significant reduction in HSP antibody level and no significant changes was observed in the control group [29]. Current study is in consistent with their study, revealed the effect of omega-3 on serum anti-HSP-27 level.

It is recommended to consider omega 3 fatty acids effect on HSP27 gene expression in future. In addition further intervention period and number is suggested.

Conclusion

Serum HSP-27 decreased after omega 3 fatty acids consumption in patients with atherosclerosis. To confirm these results more investigations are recommended with well-designed randomized clinical trials and larger samples.

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Conflict of interest

The current clinical trial study has not previously published in any other journal. The author declares no actual or potential conflicts of interest that affect the judgments of any author.

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