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The Effects of Fish Oil Supplementation on Hematologic Pattern of the Elderly "Kahrizak Elderly Study"

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Abstract

Background: Several studies have shown the beneficial effects of n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA) on autoimmune, allergic and inflammatory diseases. This study was aimed to determine the effects of low dose n-3 LCPUFA from fish oil supplement on some hematologic indexes which at the same time are non-specific markers of immunity and inflammation in a group of Iranian elderly subjects.

Methods: We investigated 114 elderly residents of Kahrizak Charity Foundation aged ≥ 65 years in this randomized, double-blind placebo-controlled clinical trial. During 6 months of this study, the placebo group received 1g capsule/day containing medium chain triglycerides, while the intervention group took 1g fish oil capsule/day containing 300 mg n-3 LCPUFA. Blood samples were collected before and after the intervention to determine hematologic indices, including white blood cell, platelet and red blood cell counts and hemoglobin and hematocrit values.

Results: After 6 months, there were no significant alterations in the studied variables in placebo group. In intervention group, only there was a significant elevation in hemoglobin levels compared with baseline (P=0.004). By univariate analysis after some adjustment, fish oil containing low dose n-3 LCPUFA did not show any significant overall effects on the values of red blood cells, white blood cells, platelets; also hemoglobin and hematocrit.

Conclusion: Six months low-dose supplementation with n-3 LCPUFA derived from fish oil had no effects on the hematologic markers such as leukocyte, granulocyte, lymphocyte and platelet count in this group of Iranian elderly people.

Keywords: Fish oil, n-3 fatty acids, Granulocytes, Lymphocytes, Platelet count

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Introduction

Ample evidence suggests an essential role for inflammation in the development and progression of atherosclerosis (1, 2). An association has been found between the number of leukocytes and cardiovascular disease risk in the Lyon Diet Heart Study which was a secondary prevention trial (3, 4). Lower absolute leukocyte and lymphocyte counts were seen in subjects consuming n-3 enriched foods than in controls in a study by Mukaro et al (5). Also, an obviously decreased number of leukocytes, involving monocytes, neutrophils, lymphocytes, and in the number of platelets has been shown after 4 weeks of Mediterranean-inspired diet rich in n-3 fatty acids that suggested a lower inflammatory activity than an ordinary Swedish diet (6).

Beneficial effects of supplementation with n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA) have been observed in the treatment of autoimmune, allergic and inflammatory disorders, such as psoriasis, rheumatoid arthritis, Crohn's disease, ulcerative colitis, systemic lupus erythematosus, atherosclerosis and asthma (7-13). In spite of such positive findings, the lack enough knowledge of efficacious type and dose of n-3 fatty acids (14) do not allow special recommendations for use of n-3 LCPUFA in these disorders. Differential effects of Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as the most important members of n-3 LCPUFA family on immune cells and pathways (15), may explain the different degrees of advantage achieved for various conditions in different studies. Fish oil, which is rich in EPA and DHA, has been shown to suppress several markers of immune function (16).The previous studies on immune and cardiovascular function used fish oil rich in EPA, generally an EPA: DHA ratio of 2:1 (7, 17, 18).

The objective of this study was to determine the effects of low-dose long-term n-3 LCPUFA supplementation with fish oil in a ratio of 3:2 EPA/DHA on hematological indices specially numbers of circulating leukocytes, granulocytes, lymphocytes and platelets in a group of Iranian elderly subjects.

Methods

Subjects

A total number of 120 subjects aged 65 years and older from Kahrizak Elderly Study (KES) were enrolled in this trial. KES is an ongoing prospective cohort study of health status among elderly residents of Kahrizak Charity Foundation in Tehran, Iran. Exclusion criteria were end-stage liver, cardiac and renal diseases, chronic inflammatory disease, taking any prescribed anti-inflammatory medication, consumption of more than one fish meal per week, taking fish oil supplements or vitamin supplements, allergy to fish products or fish oil, history of a coagulation disorder and treatment with warfarin for 30 days or less prior to enrollment and smoking.

During the study, six persons refused to continue taking the medication. Finally, 114 subjects were recruited. The research ethics committee of the Tehran University of Medical Sciences approved the study and written informed consent was obtained from each subject before entry into the trial.

Study Design and Interventions

In this randomized, double-blind, placebo-controlled clinical trial, the subjects were randomly assigned into 2 groups: intervention and placebo. During 6 months, the intervention group was supplemented with 1 g fish oil capsule per day (180 mg EPA, 120 mg DHA; total 300 mg n-3 fatty acids), and the placebo group consumed a capsule containing 1 g of medium-chain triglycerides (MCT) per day with their meals. The capsules were provided by the Zahravi Pharmacy Company, Tehran, Iran. Both participants and investigators were blinded to the intervention. Subjects were instructed to continue their usual daily diet, activity level or other lifestyle habits during the trial; also, the subjects were asked to keep their medication unchanged during the study.

Data collection

At baseline, Information on demographic characteristics, including age and sex were

collected by a trained nurse using a standard questionnaire. Weight and height were measured in light clothing without shoes by trained nurse using standard protocols and techniques at baseline. The body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Waist and hip circumferences were measured at baseline. Waist circumference at the level of the umbilicus was measured with the nearest centimeter at minimal respiration; Hip circumference was measured over the widest point of the buttocks (19). The waist-hip ratio (WHR) was calculated as the ratio of waist to hip circumference. At the beginning of the study, blood pressure measurement was performed according to guidelines of The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC VII) with the participant in a recumbent position after 5 minutes rest by trained nurse and the average of 3 measurements were recorded (20). Subjects were recommended to avoid alcohol, cigarette smoking, caffeinated beverages, and exercise for at least 30 min before their blood pressure measurement. Diabetes was defined according to American Diabetes Association criteria as diagnosis in their medical records and/or current use of hypoglycemic drugs and insulin and/or fasting serum glucose ≥126 mg/dl (21).

Before and after the intervention, blood samples were collected to determine hematologic indices, including white blood cell count, red blood cell count, hemoglobin and hematocrit levels and platelet count. White blood cells, red blood cells, hemoglobin, hematocrit and platelets were analyzed by Hycel Diagnostic cell counter. Granulocytes and lymphocytes were determined by manual differentiation of blood cells.

Statistical Analyses

Continuous variables were expressed as mean ± standard deviation (SD) and were compared using t-test. Categorical variables were expressed as percentages and were compared using the χ^2 test. Univariate analysis was used to access the comparative effects of fish oil intervention on the measured variables after adjustment for age, sex, smoking status and baseline values of each variable. A two-tailed, paired *t* test was applied for the evaluation of changes from baseline conditions to those at 6 months. Comparisons of the means between the two groups at baseline or post-intervention were performed by Student's *t* test. All data analyses were conducted using SPSS-17 software for Windows. P-values ≤ 0.05 were considered as statistically significant.

Results

The participants' mean age was 74± 10 years and 47.4% were men. The mean weight and BMI were 63.4 ± 14.5 kg and 25.4 ± 5.7 kg/m^2, respectively. At baseline, placebo and intervention groups had similar age, weight, BMI, waist circumference, waist-to-hip ratio, systolic and diastolic blood pressure measurements. There was no significant different in percentage of diabetic patients between placebo and intervention groups (18.5 *vs.* 11.9, P<0.05) (Table1).

There were no significant differences between the placebo and intervention groups for red blood cell count, hemoglobin, hematocrit, the concentration of white blood cells, granulocytes, lymphocytes and platelets at baseline. After 6 months no changes were observed in the mentioned variable levels in placebo group; while, there was a significant elevation in hemoglobin levels compared with baseline in the intervention group (P=0.004); despite no changes in other parameters (Table 2). Further, we performed univariate analysis after some adjustment to assess the comparative effects of fish oil supplementation with placebo on hematologic indices of the elderly and we found no significant overall effect of fish oil supplementation on the red blood cells, hemoglobin, hematocrit, white blood cells, granulocytes, lymphocytes and platelets.

Table 1- Baseline characteristics of the subjects *

Characteristics	All participants	Placebo group (n=54)	Intervention group (n=60)
Male (%)	47.4	48.1	46.7
Age (years)	74± 10	74± 9	74± 11
Weight (kg)	63.4 ± 14.5	62.5± 14.6	64.1± 14.6
BMI (kg/m^2)	25.4± 5.7	24.6± 5.9	26± 5.4
Waist circumference (cm)	92.5 ± 13.8	92.6± 14.4	92.4± 13.5
Waist-to-hip ratio	0.92 ± 0.07	0.92± 0.08	0.91 ± 0.06
Systolic blood pressure (mmHg)	132± 24	133 ± 25	130 ± 23
Diastolic blood pressure (mmHg)	76 ± 13	75± 12	77± 15
Diabetes (%)	15	18.5	11.9

* All values are expressed as mean ± standard deviation (SD) and/or percentage.
There was no significant difference in comparison of two groups (P > 0.05).

Table 2- Hematologic pattern at baseline and after the intervention*

Characteristics	Placebo group (n=54)		Intervention group (n=60)	
	Before	After 6 months	Before	After 6 months
Red blood cells (×10^{12} /L)	5± 0.6	5± 0.6	4.8 ± 0.7	4.9 ± 0.5
Hemoglobin (g/dL)	14.6± 2.2	14.8± 2	14.2± 1.8	14.7± 1.7†
Hematocrit (%)	43.3± 6.2	43.4± 5.7	41.9± 5.8	42.9± 5.1
White blood cells (×10^9 /L)	7± 1.9	6.9 ± 1.8	7.1± 1.8	7.3± 2.1
Granulocytes (×10^9 /L)	4.6± 1.4	4.6± 1.5	4.7± 1.2	4.8± 1.3
Lymphocytes (×10^9 /L)	2.2 ± 0.9	2.7± 4.3	2.4 ± 0.8	2.4± 1.5
Platelets (×10^9 /L)	199.8± 60.5	200.8± 46.3	191.9± 49.9	203.7± 57.5

* Data are mean ± standard deviation (SD).
No significant difference between the two groups at baseline and post intervention by Student's *t* test (P > 0.05).
†P<0.05 vs. before determined by two-tailed, paired *t* test.
‡ P-values of univariate analysis were adjusted for age, sex, smoking status and baseline value of each variable.

Discussion

In present study, fish oil containing 180 mg EPA and 120 mg DHA did not show any overall effect on white blood cells, granulocytes, lymphocytes and platelets. Our findings confirms the results of the previously performed study that revealed 20 g of encapsulated seal oil supplement containing 1.3 g EPA and 1.7 g DHA did not change white blood cell count in healthy male (22). Conversely, Ambring *et al.* showed a significant reduction in total number of leukocytes and platelets after Mediterranean-inspired diet rich in n-3 LCPUFA compared with ordinary Swedish diet (6). Also, in a study by Mukaro *et al*. absolute leukocyte and lymphocyte numbers were lower in subjects consuming 1 gram n-3 enriched foods than in controls although there were no changes in the number of neutrophils and monocytes (5).

In addition, we found no effect of low-dose fish oil on red blood cells, hemoglobin and hematocrit in intervention group compared with placebo group which are in agreement with the results of previous such studies (6, 22). Mediterranean-inspired diet rich in n-3 LCPUFA for 4 weeks had no effect on red blood cell count compared with ordinary Swedish diet in normocholesterolemic healthy individuals (6). In addition, there were no changes in hematocrit after supplementation with 20 g of encapsulated seal oil containing 4.3 g n-3 LCPUFA (22). Conversely, Miller *et al*. showed that fish oil concentrate intake for eight weeks reduced whole blood viscosity in both non-diabetics and in diabetics (23).

Our study had several limitations. We did not measure serum fatty acid profile in subjects which could help to clarify the effects of dietary fatty acids on serum fatty acid profile. Therefore, without measurement of concentrations of n-6 and n-3 fatty acids, we could not determine a serum ratio of n-6 to n-3 fatty acids which could partly be an

indicator of fatty acid content of body tissues. This ratio could help us to define an optimal dietary n-6/n-3 ratio.

The role of platelets in regulation of homeostasis and thrombosis and consequently major cardiovascular complications (24, 25), has promoted researchers to study the effects of n-3 LCPUFA on platelet function. We did not focus on this subject which was another shortcoming of our study. However, most studies have shown that supplementation with n-3 LCPUFA may cause inhibition of platelet function (26); which is pathogenically associated with prevention of cardiovascular events. Inhibition of platelet aggregation and also thromboxane A2 production which is a potent aggregator and vasoconstrictor has been demonstrated after supplementation with fish oil in various studies (27–35). Various beneficial effects of EPA and DHA in different doses on platelet reactivity have also been demonstrated by various authors (36-42).

Our study showed that fish oil supplement containing a total of 300 mg n-3 LCPUFA per day for 6 months had no effects on the hematologic markers such as white blood cells, granulocytes, lymphocytes which on the other hand are nonspecific markers of immunity and inflammation as well as other indices including platelets, red blood cells, hemoglobin, hematocrit in this group of elderly people. However, more studies are warranted to determine proper doses and types of n-3 LCPUFA in order to protection against platelet-related cardiovascular events.

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