Original Article

Effect of Fish Oil Supplements on Serum Paraoxonase Activity in Female Patients with Rheumatoid Arthritis: A Double-blind Randomized Controlled Trial

Amir Ghorbanihaghjo PhD[1], Sousan Kolahi MD[1,4], Soroush Seifirad MD•[1,2], Nadereh Rashtchizadeh PhD[3], Hassan Argani MD[3], Mehrzad Hajialilo MD[4], Alireza Khabazi MD[4], Samira Alizadeh PhD[1], Elham Bahreini PhD[1]

Abstract

Background: This study was conducted to determine the effect of fish oil (FO) supplements on high density lipoprotein cholesterol (HDL-C), apolipoprotein-AI (Apo-AI), malondialdehyde (MDA), arylesterase (Aryl), and paraoxonase-1 (PON1) activity in female patients with rheumatoid arthritis (RA).

Methods: A total of 90 RA patients were randomly allocated into two groups that were treated with one FO pearl (1 gr) daily or placebo for three months in addition to conventional treatment. HDL-C, Apo-AI, and MDA levels as well as PON1 and Aryl activities were measured before and after treatment. Independent t-test was used to match basal parameters of case and control groups. Paired t-test was used to assess significance of the differences. Correlation was evaluated by Pearson's test and the statistical significance was set at $P < 0.05$.

Results: No significant differences were noted between FO and placebo patients with regards to age, disease duration, post-menopausal status, conventional therapy, body mass index (BMI), and numbers of swollen and tender joints at the beginning of the study. There were 83 patients who completed the three-month follow up. Serum levels of HDL-C ($P = 0.018$), Apo-AI ($P = 0.165$), Aryl ($P = 0.026$), and PON1 ($P = 0.049$) activity increased, whereas MDA levels decreased significantly with FO supplementation ($P = 0.077$). Significant correlations between increased PON1 activity and both HDL-C ($P = 0.007$, r = 0.419) and Apo-AI ($P < 0.001$, r = 0.742) concentrations as well as between HDL-C and Apo AI levels ($P = 0.01$, r = 0.403) were found.

Conclusion: According to the results of this study, FO could increase serum HDL-C and PON1 levels and Aryl activity in female patients with RA.

Keywords: Apolipoprotein-AI, arthritis, cholesterol, HDL, paraoxonase, rheumatoid

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Introduction

Rheumatoid arthritis (RA) patients have higher cardiovascular mortality and morbidity compared to the healthy population.[1] Proinflammatory cytokines and acute phase reactant protein plasma levels are increased in RA patients.[2] Increased free radical production and lipid peroxidation, in addition to decreased antioxidant levels are also reported.[3]

Low density lipoprotein cholesterol (LDL-C) oxidation in the arterial walls is accepted as an important mechanism for atherosclerosis, and many studies have focused on preventing LDL-C oxidation mechanisms. Paraoxonase-1 (PON1) activity is one of the main mechanisms of LDL-C oxidation. Because of the key role of LDL-C oxidation in atherosclerosis, reduction of serum activity of PON1 may explain one of the essential mechanisms for increased risk of atherosclerosis and cardiovascular disease in RA patients.[4]

It has also been shown that serum levels of high density lipoprotein cholesterol (HDL-C) and apolipoprotein-AI (Apo-AI) are decreased in RA patients compared to the healthy population. Decreased HDL-C levels also play a key role in increased risk of cardiovascular disease in RA patients.[5] Interventions which increase paraoxonase activity and HDL-C levels may decrease atherosclerosis progression.

Fish oil (FO) and omega-3 fatty acid supplements have been used for more than two decades in the treatment of RA patients because of their probable anti-inflammatory effects.[6,7] It has been shown in several studies that omega-3 fatty acids could reduce the production of inflammatory cytokines such as tumor necrosis factor-α (TNFα) and interlucin-1 (IL-1) in RA patients.[3,8,9]

This study determines the effect of FO supplements on HDL-C, Apo-AI, malondialdehyde (MDA), arylesterase (Aryl), and PON1 activity in female RA patients. To the best of our knowledge this is the first study which investigates the effect of FO supplements on paraoxonase activity in RA patients. The results of this study may help to reduce cardiovascular morbidity and mortality in RA patients.

Authors' Affiliations: [1]Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, [2]Endocrinology and Metabolism Research Center (EMRC), Tehran University of Medical Sciences, Shariati Hospital, Tehran, Iran , [3]Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, [4]Rheumatology Division, Tabriz University of Medical Sciences, Tabriz, Iran.
•Corresponding author and reprints: Soroush Seifirad MD, Endocrinology and Metabolism Research Center (EMRC), Tehran University of Medical Sciences, Shariati Hospital, North Kargar St., Tehran, Iran, P. O. Box: 1411413137. Tel: +98 9355799979,+98 2188220038, Fax: 00982188220052,
E-mail: sseifirad@gmail.com
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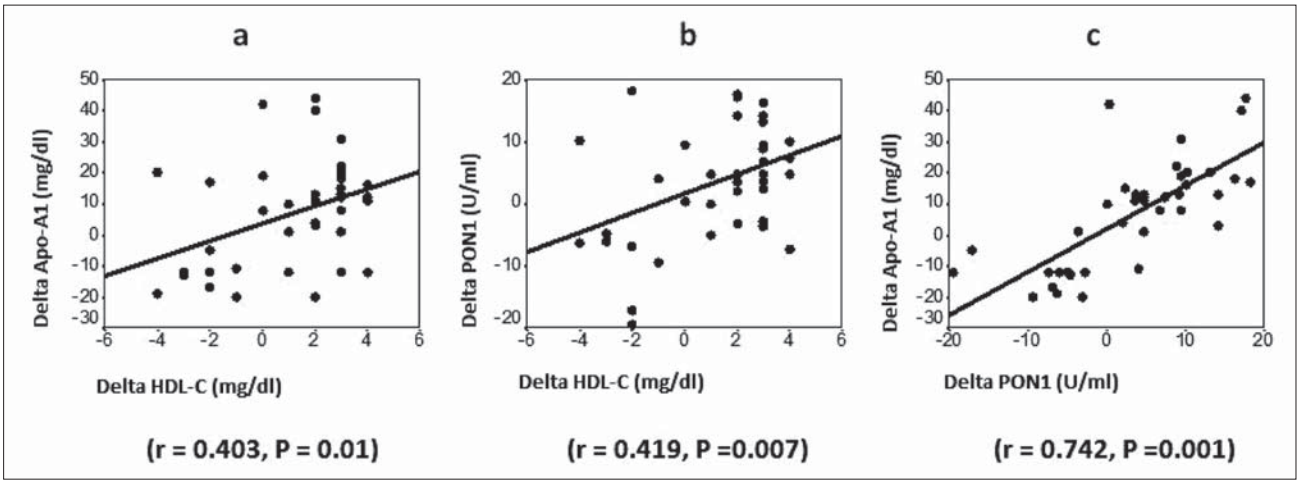

Figure 1. Correlations between the changes in (A) APO-A1 and HDL-C, (B) PON1 and HDLC, and (C) Apo-A1 and PON1

Materials and Methods

A total of 90 RA patients were enrolled in this prospective, double-blind, placebo-controlled, randomized clinical trial. Patients were randomly allocated to two groups, on a 1:1 fashion via the balanced block randomization method. The experimental group received FO (1 g/day) versus placebo in addition to RA conventional treatment for the control group. FO and placebo (containing paraffin) pearls were matched in appearance and packaging. Each FO (1 g) pearl (Zahravi Co., Iran) was composed of 180 mg eicosapentaenoic acid (EPA, 20:5 Omega-3) plus 120 mg docosahexaenoic acid (DHA, 22:6 Omega-3). FO pearls were orally administered at a dose of 1 g daily in the fasting state.

The study protocol was reviewed and approved by the Ethics Committee of Tabriz University of Medical Sciences. Written informed consent was obtained from all patients.

Patients

A total of 90 female patients diagnosed with RA, as confirmed by the American College of Rheumatology (ACR) criteria were included in the study. Inclusion criteria were female patients with fixed therapeutic schedules at least two months before study entry. Exclusion criteria were as follows: patients with Body Mass Index (BMI) > 30 kg/m^2, cardiovascular or liver diseases, uncontrolled hypertension (systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg), diabetes mellitus, thyroid disorders or other metabolic diseases, patients treated with micronutrient supplements, lipid lowering drugs and those on hormone replacement therapy. Alcoholics, smokers and patients who needed to change their conventional therapeutic protocol were also excluded from the study.

Clinical assessments

A rheumatologist performed the clinical examinations. Disease Activity Score 28 (DAS28) was calculated for all patients using high-sensitivity C-reactive protein (hs-CRP), the numbers of swollen and tender joints, and patient-reported general health using the Visual Analog Scale (VAS).[10]

Sample collection and analysis

Fasting venous blood samples were collected before the start of FO supplementation and following three months of supplementation, at the end of the study. Serum levels of HDL-C were determined using commercial reagents with an automated chemical analyzer (Abbott Analyzer, USA). Serum levels of Apo-AI and hs-CRP were assayed by immunoturbidimetric kits (Pars Azemoon, Iran) using the same analyzer. Serum PON1 and Aryl activity was determined spectrophotometrically using paraoxon (O, O-diethyl-O-P-nitrophenylphosphate) and phenylacetate as substrates, respectively.[11] MDA level as an index of lipid peroxidation was measured by the thiobarbituric acid (TBA) method.[12]

Statistical analysis

Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) version 13.0 was used to perform statistical analysis. Results were expressed as mean ± SD. Chi square and independent t-tests were used to match basal parameters of the case and control groups. Kolmogorov–Smirnov test was used to evaluate data distribution. The paired t-test was used to assess significance of differences between baseline and three months after supplementation. Correlation was evaluated by Pearson's test and the statistical significance was set at $P < 0.05$.

Results

At the end of the study, 83 patients completed the three-month follow up; of these, 40 patients were treated with FO and 43 patients were treated with placebo. A total of seven patients did not complete the study protocol.

The demographic data and baseline characteristics of the two groups are shown in Table 1. No significant differences were noted between FO versus placebo-treated patients with regards to age, disease duration, postmenopausal status, type of conventional therapy (except chloroquine), BMI, swollen and tender joint counts, and DAS28 at the beginning of the study. The comparison between basal serum levels of HDL-C, Apo-A1, MDA, Aryl and serum PON1 activity of case and control groups before intervention and the changes of these parameters following three months of supplementation are shown in Table 2.

There was a significant correlation between delta PON1 and delta HDL-C ($P = 0.007$, r = 0.419) and delta PON1 and delta Apo-AI ($P = 0.001$, r = 0.742). A significant, positive correlation between

Table 1. Demographic data and baseline characteristics in fish oil (FO) vs. placebo groups.

Variable	Fish oil (FO) group (n=40)	Placebo group (n=43)
Age (years)[a]	50 (18–74)	50 (19–74)[f]
Post-menopause [n (%)]	22 (55.0%)	25 (58.1%)[f,d]
Pre-menopause [n (%)]	18 (45.0%)[c,d]	18 (41.9%)[c,d]
Conventional therapy [n (%)]		
Prednisone	38 (95.0%)	41 (95.3%)[f,d]
Methotrexate	35 (87.5%)	40 (93.0%)[f,d]
Sulfasalazine	9 (22.5%)	10 (23.3%)[f,d]
Chloroquine	23 (57.5%)	35 (81.4%)[g,d]
Cyclosporine	1 (2.50%)	4 (9.30%)[f,d]
Azathioprine	4 (10.0%)	5 (11.6%)[f,d]
NSAIDs	1 (2.50%)	2 (4.70%)[f,d]
Disease duration (months)[a]	57 (12–360)	54 (9–360)[f]
BMI (kg/m^2)[b]	25.3±3.8	25.5±4.4[f]
Swollen joint count [n (%)]		
0–5	(95.0%)	(94.9%)[f,d]
5–10	(2.50%)	(3.40%)[f,d]
>10	(2.50%)	(1.70%)[f,d]
Tender joint count [n (%)]		
0–5	(90.0%)	(87.9%)[f,d]
5–10	(7.50%)	(3.40%)[f,d]
>10	(2.5%)	(3.4%)[f,d]
DAS28[a]	2.42 (1.11–5.25)	2.98 (0.99–5.70)[f]
Baseline	2.22 (0.99–6.05)	2.80 (0.99–6.46)
3 months	(P=0.76)[e]	(P=0.54)[e]

BMI: Body Mass Index; DAS28: Disease Activity Score 28; Data are expressed as [a] median (minimum–maximum) and [b] mean±SD. [c] $P>0.9$ vs. Post-menopausal group. [d] Performed by chi-square test. [e] Before treatment vs. after treatment with fish oil (FO) or placebo. [f] $P>0.05$ and [g] $P<0.05$ vs. baseline FO group.

Table 2. Laboratory findings at baseline and after 3 months treatment with fish oil (FO) vs. placebo.

Variable	Fish oil (FO) group (n=40) Mean±SD	Placebo group (n=43) Mean±SD	P
HDL-C (mg/dl)			
Baseline	40.8±7.1	38.6±6.8	(P=0.83)[a]
3 months	41.9±7.4	38.2±5.9	(P=0.018)[b]
Apo-AI (mg/dl)			
Baseline	148.3±26.0	150.9±18.6	(P=0.61)[a]
3 months	154.8±22.5	149.2±16.0	(P=0.165)[b]
PON1 (U/ml)			
Baseline	61.5±13.6	59.8±11.4	(P=0.51)[a]
3 months	64.7±12.8	59.5±9.4	(P=0.049)[b]
Aryl(U/l)			
Baseline	73.1±10.1	73.3±10.8	(P=0.62)[a]
3 months	76.5±7.7	72.5±10.1	(P=0.026)[b]
MDA (nmol/ml)			
Baseline	3.6±0.6	3.4±0.5	(P=0.15)[a]
3 months	3.3±0.5	3.5±0.6	(P=0.077)[b]

HDL-C: High density lipoprotein cholesterol; Apo-AI: Apolipoprotein-AI; PON1: Paraoxonase; Aryl: Arylesterase; MDA: Malondialdehyde. [a] Baseline fish oil (FO) group vs. baseline placebo group. [b] Final comparison after treatment with FO or placebo.

delta Apo-AI and delta HDL-C was also noted (P = 0.01, r = 0.403; Figure 1).

Discussion

Oxidative stress plays a key role in accelerated atherosclerosis and is involved in increased risk of cardiovascular disease in RA patients.

It has been suggested that medication and supplements that contain lipid lowering properties can alter the products of lipid peroxidation, such as MDA in RA patients. Such therapies could reduce atherosclerosis in these patients, therefore possibly decreasing morbidity and mortality. It has been shown that serum levels of HDL-C and Apo-AI are decreased in RA patients compared to the healthy population. Decreased HDL-C levels may play a role in increased risk of cardiovascular disease in RA patients.[13,14] Metabolism of lipid peroxides produce a variety of metabolites such as MDA. It also has been shown that MDA serum levels are increased in RA patients, and there is a negative correlation between serum levels of MDA and serum activity of PON1.[4]

Evaluating the effect of three-month omega-3 fatty acid supplementation in our patients demonstrated borderline significant decreases in MDA levels (P = 0.077). A comparison of MDA levels before and after FO supplementation revealed a significant difference in the FO group (P = 0.007).

HDL-C protects against atherosclerosis by returning excess cholesterol from peripheral tissues back to the liver for reuse or excretion into the bile.[15] Reports suggest an antioxidative function for HDL-C, which may contribute to its anti-atherogenic activity.[15,16]

Human PON1 is a calcium-dependent esterase closely related with HDL-C-containing Apo-AI that has been shown to confer antioxidant properties to HDL-C by decreasing accumulation of lipid peroxidation products.[15,17]

The results of this study indicated that treatment with FO supplements significantly increased HDL-C levels and PON1 activity. We found a significant correlation between increased PON1 with

both increased Apo-AI and HDL-C levels. With respect to the pre-treatment values of the FO group, Apo A1 levels significantly increased after supplementation ($P = 0.022$). Although final ApoA1 levels of the FO group were higher than the placebo group, they were not statistically significant ($P = 0.165$). It seemed that FO has increased PON1 activity by increasing HDL-C and Apo-AI and/or a cooperative increase of both factor concentrations in RA patients; but the results of published studies are controversial. A significant correlation between serum PON1 and both HDL-C and Apo-AI plasma levels after omega-3 polyunsaturated fatty acid supplementation has been observed in a study by Calabresi et al.[18] Bays, however, declared that omacor (an omega-3 polyunsaturated, DHA, EPA, fatty acid concentrate) increased plasma PON1 levels without increasing Apo-AI levels.[19]

Many studies have explained the mechanism of anti-inflammatory effects of omega-3 fatty acids as a competition between eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) with arachidonic acid (an omega-6 fatty acid) in the production of inflammatory cytokines. Arachidonic acid metabolizes to prostaglandins such as prostaglandin E2 (PGE2) via the cyclooxygenase pathway and leukoterines such as leukoterine B4 (LTB4) via the lipoxygenase pathway, but omega-3 fatty acids metabolize to prostaglandins such as prostaglandin E3 (PGE3) and leukoterines such as leukoterine B5 (LTB5) with notable lower inflammatory effects.[20] It has been shown that EPA and DHA metabolize to anti-inflammatory lipids such as resolin and protectin. The anti-inflammatory effects of resolin and protectin have been demonstrated in a transgenetic rat model in a later study.[21] The results of our study suggest another mechanism for anti-inflammatory/atherosclerotic properties of FO in RA patients. With respect to our results, FO supplements could increase paraoxonase and Aryl activity, and HDL-C serum levels; with these mechanisms, it could decrease the process of atherosclerosis by decreasing inflammation and lipid peroxidation in RA patients who are at increased risk of atherosclerosis because of increased ROS formation, lipid peroxidation, and inflammation.

To the best of our knowledge, this is the first study which investigates the effect of FO supplements on paraoxonase and Aryl activity of RA patients; however there are limitations in the design and results of this study. Although a significant increase in HDL and Aryl concentrations have been observed, the respective changes were actually quite small. Future studies to evaluate the effect of higher doses are recommended. Only adult female patients have been included in this study, further more comprehensive studies could increase the impact of our findings and hypotheses. Extended larger studies are needed to evaluate the impact of FO supplementation on cardiovascular mortality and morbidity in RA patients. Conclusively FO could increase serum level of HDL-C and PON1 activity in female RA patients.

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Authars Contribution

Amir Ghorbani haghjo and Sousan Kolahi Contributed equally to this work.

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