

Full Length Research Paper

Effects of omega-3 administration on concentration of serum inflammatory makers in renal transplant recipient

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Omega-3 polyunsaturated fatty acids have been shown to decrease inflammatory markers such as TNF- α , IL-1 β and IL-6 levels. It has been shown that there is association between several markers of inflammation with cardiovascular disease which is the leading cause of death in the end stage of renal disease (ESRD) patients. So it may be useful to administer this supplement in renal transplant recipients. To investigate this possibility, we studied the effect of omega-3 on the occurrence of acute rejection. In this study, we assessed the effect of 6 months of dietary supplementation with 6 g/day of omega-3 fatty acids on Lipid profile (TG, HDL-C, and LDL-C) and serum concentration of TNF- α , IL-6 and hs-CRP in renal transplant recipients. Based on these data, omega-3 fatty acids supplementation does not cause a decrease in serum level of tested inflammatory biomarkers in renal transplant recipients but decreases total cholesterol level.

Key words: Omega-3 fatty acids, renal transplantation, inflammatory biomarkers.

INTRODUCTION

Usually, in renal transplant recipients, acute rejection occurred after renal transplantation, and cytokines have an important role in immune response—in the maturation of lymphocytes and in their proliferation and function (Vandenbroecke et al., 1991). Both TNF- α and IL-1 stimulate the production of IL-6, which is the main mediator of the acute-phase response and contributes in B- and T-cell activation and maturation (Vandenbroecke et al., 1991; Yard et al., 1992; Noronha et al., 1993).

Also, there is a well-built independent link between inflammatory markers and the incidence of cardiovascular disease, and an association with IL-6 and TNF- α levels has been seen for subclinical cardiovascular disease. Significant relationships with CRP were found for congestive heart failure and coronary heart disease (Perunicic-Pekovic et al., 2007). Renal transplant recipient (RTR) has a high incidence of cardiovascular disease and rate of mortality is elevated in such patients.

The contributing causes of cardiovascular disease in these patients are complex (Bonis et al., 2005).

Omega-3 fatty acids are essential fatty acids; they are essential for human health but it cannot be produced in the body. Fish is a good source of Omega-3 fatty acids, also known as polyunsaturated fatty acids (PUFAs), omega-3 fatty acids play a central role in brain function as well as normal growth and development. They have also become popular because they may reduce the risk of heart disease. According to the literature by Joseph and Jeffrey (2006), polyunsaturated fatty acids (PUFA) are known to decrease generation of pro-inflammatory products such as interleukins-1, -2, and-6, and tumor necrosis factor alpha.

These immunomodulatory effects of fish-oil are due to induced change in the cyclooxygenase and lipoxygenase pathways that is associated with alteration in leukocyte function, and with reduction in TNF- α and IL-1 synthesis (Billiar et al., 1988). In addition, ω -3 PUFA suppresses IL-2 *ex vivo* production by mononuclear cells in healthy individuals (Endres et al., 1993). These effects could well clarify the lower occurrence of reported acute rejections and better renal function in renal transplant patients getting this compound (Vandenbroecke et al., 1991; Van der Heide et al., 1993).

The various biological effects of omega-3 fatty acids and observations in non-transplant situations afforded a basis for clinical trials in organ transplantation. The major experience has been in kidney transplantation in which laboratory, animal and early human studies showed that omega-3 fatty acid supplementation, mainly fish oil, had the potential to diminish cyclosporine (CsA) nephrotoxicity, decrease rejection, improve hyperlipidemia, and reduce hypertension. Other benefits had also been suggested such as reduction in risk factors for thrombosis, restoration of erythrocyte deformability, and blood viscosity. There is far less experience in other forms of organ transplantation, although the effects of omega-3 fatty acids have been assessed in the setting of heart, liver and bone marrow transplantation where similar benefits had been expected (Kinsella et al., 1990; Perunicic-Pekovic et al., 2007; Bonis et al., 2005). According to previous investigations and due to lack of long-term study on the potential of omega-3 fatty acids in RTRs, we performed a 6 months study investigating the effects of fish oil administrations in RTRs especially on serum inflammatory markers level.

PATIENTS AND METHODS

This study was established by the Ethics Commission of MUMS (Mashhad University of Medical Sciences). All patients signed an agreement form proceeding to entry into the study. 40 hemodialysis (HD) patients (all candidates for future transplantation) who fulfilled inclusion and exclusion criteria were selected. All patients were experienced kidney transplantation; the patients were in the 19 to 62 years old age range, received the kidney from live subjects and treated by immunosuppressant drugs. People excluded from the study were patients with heart failure, thyroid disease, liver dysfunction,

diabetes, acute infection and acute rejection and who were treated by statins or their immunosuppressive regimen was changed. After transplantation, all 40 HD patients were divided into two groups randomly. One group (A) was administered omega-3 supplements (6 g/day: 720 mg of DHA and 1080 mg of EPA daily (oral)) for 6 month, whereas the control group (B) received placebo treatment; placebo pills contained starch. The other Group (B) received placebo in the same condition which constituted 20 patients with placebo treatment. Since the maximum risk of incidence acute rejection is during the first 3 months after transplantation, fish oil was administered during this time and later.

Blood samples were obtained from patients just before and 6 months after transplantation after a 12 h fast. Demographic data and para-clinical test results including traditional cardiovascular risk factors and ESRD etiology were recorded.

The serum concentration of inflammatory markers (TNF- α , IL-6 and hs-CRP) was determined by enzyme-linked immunosorbant assay (ELISA) kit (Axies-shield). Two independent sample t test and paired student t test was used for data analyzing. P values < 0.05 were considered significant.

RESULTS

Characteristics of the study population

The median age was 36.5 ± 14.34 years (range from 19 to 62). We evaluated other risk factors, such as age, hypertension, hypercholesterolemia, smoking, diabetes, and previous CV disease (Table 1).

Comparison of inflammatory markers concentrations between two groups

After 6 month of treatment of group A and B, the changes in TNF- α , IL-6 and hs-CRP levels were not significant (data not shown).

Comparison of total cholesterol level between two groups

After 6 month of treatment of group A and B, a significant decrease in total cholesterol level was observed in group A compared with group B (Figure 1).

DISCUSSION

Our study showed that 6 months of fish oil supplementation does not affect evaluated end points including the concentration of inflammatory marker levels after renal transplantation in RTRs except on lipid profile. There was no significant difference in inflammatory markers serum concentration between control and treatment groups.

There are several studies indicating anti-inflammatory and immune-modulating effects of PUFA, though sometimes the results have been controversial (Kinsella et al., 1990; Meydani, 1990). However this effect is in agreement with the effects of fish oil on the cyclooxygenase

Table 1. Demographic data, laboratory tests and traditional cardiovascular risk factors of patients. There is no significant ($p>0.05$) difference between two groups after statistical analysis (Chi square/ Student t test).

Demographic data	
Age (year)	36.5 ± 14.34
Female/Male ratio	0.57
Laboratory tests	
Calcium (mg/dl)	9.5 ± 0.50
Phosphorus (mg/dl)	6.8 ± 6.9
Parathyroid hormone (pg/ml)	238.0 ± 228.23
HDL cholesterol (mg/dl)	38.2 ± 5.64
LDL cholesterol (mg/dl)	108.9 ± 27.37
Total cholesterol (mg/dl)	168.4 ± 35.93
Triglycerides (mg/dl)	174.3 ± 112.79
Mean CCIMT (mm)	0.62 ± 0.11
Traditional cardiovascular risk factors	
Hypertension (%)	54.84
Dyslipidemia (%)	12.9
Positive family history (%)	25.8
Diabetes (%)	0
Current smoking (%)	9.68

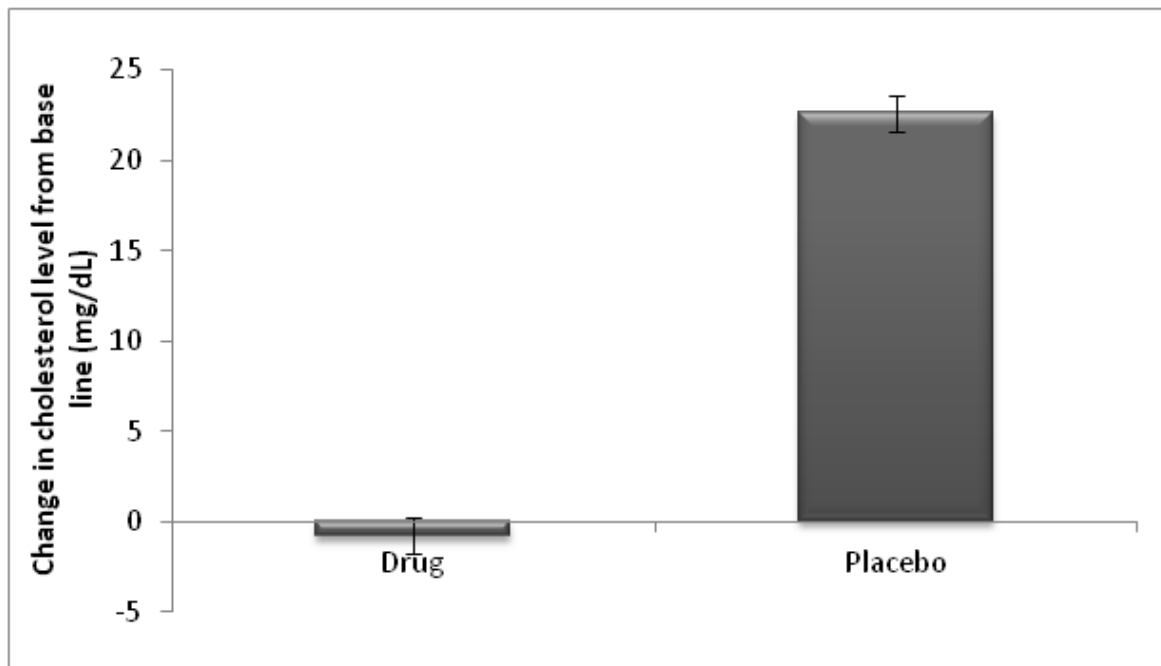


Figure 1. Comparison of change in total cholesterol level between two groups ± SE (mg/dl).

and lipoxygenase pathways, and the final changes in the production of eicosanoids; all main compounds in the progression of renal allograft rejection. Changes induced in the leukotriene profile have been related to a

significant decrease in the chemotactic properties of leukocytes and to a diminished production of TNF- α and IL-1 β (Endres et al., 1989; Anne et al., 1995). Also, ω -3 PUFA n-fatty acids restrain interleukine-2 production and

proliferation of mononuclear cells in healthy people (Perunicic-Pekovic et al., 2007). These effects have also resulted in a reduction in PGE2 and LTB4 formation, although there might be an increase in trienoid prostaglandins and LB5 (Billiar et al., 1988; Endres et al., 1993). In addition, reduced production of thromboxane A2 may be another effective factor in the rejection process as reported previously (Coffman et al., 1989). Several studies in healthy volunteers have shown that n-3 fatty acid supplementation reduces the potential of mononuclear cells to produce TNF, IL-1 and IL-2 (Billiar et al., 1988; Endres et al., 1989; Endres et al., 1993). These substances may suppress the immunological responses in animal models [30]. In a study Perunicic-Pekovic showed that 2 month administration of 2.4 g EPA in hemodialysis patients caused a significant decrease in TNF- α and IL-6. Also PUFA administration could delay the renal autoimmune diseases because of anti oxidant activity, reduction in NF-KB activity and decreased TNF- α , IL-1 and IL-6 gene expression in renal tissue. On the other hand in diabetic patients (type II), there was no change in TNF- α , CRP and IL-6 serum levels after EPA+DHA administration for 3 month. In a clinical trial in young Japanese women, PUFA could not alter the CRP concentration. In the recent study, we did not find that fish oil treatment had any significant effect on the concentration of inflammatory marker levels during the first 6 month post-transplant period, thus confirming other reports (Kooijmans-Coutinho et al., 1996).

It is probable that the beneficial effect of ω -3 PUFA may be masked in the setting of intragraft immune events. The dosage and period of fish oil administration in this research was similar to other studies with either positive (Van der Heide et al., 1993) or negative (Kooijmans-Coutinho et al., 1996) results. Since the effect of ω -3 PUFA on plasma phospholipids and cell membranes is in a dose-dependent manner (Van der Heide et al., 1993; Calder, 1997) and because of immunosuppressive drugs received by such patients, maybe higher doses are required in order to get a clinical advantage. However, significant reduction in triglyceride levels showed that at least a biologically effective dose had been used.

According to previous studies and our results, it seems that omega-3 administration in post-transplantation does not have significant effect on inflammatory marker level but it decreased total cholesterol level. To decrease other cardiovascular risk factors other than LDL and TG we suggest the simultaneous administration of other supplements with omega-3. We postulate that the mechanism through which omega-3 decreases inflammatory marker level in other patients is altered in renal transplant patients and thus omega-3 could not decrease these factors in such patients. In summary, 6 months of treatment with fish oil after renal transplantation does not affect serum inflammatory markers (TNF- α , IL-6 and hs-CRP) in such patients properly.

REFERENCES

- Billiar TR, Bankey PE, Svingen BA, Curran RD, West MA, Holman RT, (1988). Fatty acid intake and Kupffer cell function: fish oil alters eicosanoid and monokine production to endotoxin stimulation. *Surg.* 104(2):343-349.
- Bonis P, Chung M, Tatsioni A, Sun Y, Kupelnick B, Lichtenstein A (2005). Effects of Omega-3 Fatty Acids on OrganTransplantation. Evidence Report/Technology Assessment, AHRQ: 1-11.
- Calder PC (1997). n-3 polyunsaturated fatty acids and cytokine production in health and disease. *Ann. Nutr. Metab.* 41(4):203-234.
- Coffman TM, Ruiz P, Sanfilippo F, Klotman PE (1989). Chronic thromboxane inhibition preserves function of rejecting rat renal allografts. *Kidney Int.* 35(1):24-30.
- Endres S, Ghorbani R, Kelley VE, Georgilis K, Lonnemann G, van der Meer JWM (1989). The Effect of Dietary Supplementation with n ω -3 Polyunsaturated Fatty Acids on the Synthesis of Interleukin-1 and Tumor Necrosis Factor by Mononuclear Cells. *New Engl. J. Med.* 320(5):265-271.
- Endres S, Meydani SN, Ghorbani R, Schindler R, Dinarello CA (1993). Dietary supplementation with n-3 fatty acids suppresses interleukin-2 production and mononuclear cell proliferation. *J. Leukoc. Biol.* 54(6):599-603.
- Joseph CM, Jeffrey WB (2006). ω -3 Fatty acids (fish oil) as an anti-inflammatory: an alternative to nonsteroidal anti-inflammatory drugs for discogenic pain. *Surg. Neurol.* 65(4):326-331.
- Kinsella JE, Lokesh B, Broughton S, Whelan J (1990). Dietary polyunsaturated fatty acids and eicosanoids: potential effects on the modulation of inflammatory and immune cells: an overview. *Nutr.* 6(1):24-44; Discussion 59-62.
- Kooijmans-Coutinho MF, Rischen-Vos J, Hermans J, Arndt JW, van der Woude FJ (1996). Dietary fish oil in renal transplant recipients treated with cyclosporin-A: no beneficial effects shown. *J. Am. Soc. Nephrol.* 7(3):513-518.
- Meydani SN (1990). Dietary Modulation of Cytokine Production and Biologic Functions. *Nutr. Rev.* 48(10):361-369.
- Noronha IL, Kruger C, Andrassy K, Ritz E, Waldherr R (1993). In situ production of TNF-alpha, IL-1 beta and IL-2R in ANCA-positive glomerulonephritis. *Kidney Int.* 43(3):682-692.
- Perunicic-Pekovic GB, Rasic ZR, Pljesa SI, Sobajic SS, Djuricic I, Maletic R (2007). Effect of n-3 fatty acids on nutritional status and inflammatory markers in haemodialysis patients. *Nephrol. (Carlton).* 12(4):331-336.
- Van der Heide J, Bilo H, Donker JM, Wilmink JM, Tegzess AM (1993). Effect of Dietary Fish Oil on Renal Function and Rejection in Cyclosporine-Treated Recipients of Renal Transplants. *New Engl. J. Med.* 329(11):769-773.
- Vandenbroecke C, Caillat-Zucman S, Legendre C, Noel LH, Kreis H, Woodrow D (1991). Differential in situ expression of cytokines in renal allograft rejection. *Transplant.* 51(3):602-609.
- Yard BA, Daha MR, Kooijmans-Couthino M, Bruijn JA, Paape ME, Schrama E (1992). IL-1 alpha stimulated TNF alpha production by cultured human proximal tubular epithelial cells. *Kidney Int.* 42(2):383-389.