Evaluation of Omega-3 Effect as Adjuvant Therapy to Methotrexate on Alkaline Phosphatase Level in Iraqi Patients with Active Rheumatoid Arthritis

Shaimaa Saleh Khudhur
Department of Clinical Pharmacy, College of Pharmacy, University of Tikrit, Tikrit, Iraq

Abstract
Rheumatoid arthritis (RA) is a common inflammatory disease associated with many extra-articular features. **The aim:** of the current study is to assess the influence of omega-3 fatty acids (EPA, DHA) on serum alkaline phosphatase in patients with active RA. A single blinded placebo controlled clinical trial with 12 weeks follow up period at which 50 patients with active RA using methotrexate were randomized into 2 groups to receive either omega-3 (1000 mg) capsule three times daily or capsules prefilled with glucose as placebo and were evaluated at zero time (baseline) and after 12 weeks for alkaline phosphatase level in serum. In addition to twenty five healthy subjects as control group. The age of RA patients in omega-3 group ranged from 25-80 years (50.36±2.46), whereas the age of RA patients in placebo group ranged from 35-68 years (50.08±1.84), while the age of apparently healthy subjects in control group where ranged from 40-60 years (49.20±1.12). The RA disease activity was measured using disease activity score of 28 joints(DAS28-ESR) and clinical disease activity index **(**CDAI). After 12 weeks of starting adjuvant treatment with either omega-3 or placebo, the results showed that there were no significant difference between the effect of omega- 3 and placebo on alkaline phosphatase level. In Conclusion, omega-3 significantly decreased alkaline phosphatase level in patient with active rheumatoid arthritis.

Keywords: Active rheumatoid arthritis, Methotrexate, omega-3, alkaline phosphatase.

تقييم تأثير أوميغا 3- كعلاج مساعد للميثوتريكسايت على مستوى الفوسفاتيز القلوية في المرضى العراقيين المصابين بالتهاب المفاصل الرثوي الفعال

شيماء صالح خضر

الخلاصة
التهاب المفاصل الروماتويدي (RA) هو مرض التهابي مزمن وشائع غير معروف السبب والذي يستهدف في المقام الأول الأنسجة الزليلية والذي يؤثر على أنسجة وأجهزة مفصلية وغير المفصلية. **الهدف**: من هذه الدراسة هو تقييم تأثير دواء اللأوميغا-3 كعلاج مساعد للميثوتركسايت على مستوى الفوسفاتيز القلوية في المرضى العراقيين المصابين بالتهاب المفاصل الرثوي الفعال. خمسون مريضا مصابا بألتهاب المفاصل الرثوي الفعال يتعاطون10ملغ من الميثوتريكسايت اسبوعيا لمدة ثلاثة اشهرمتتابعة على الاقل قد شاركوا في هذه الدراسة، بالإضافة إلى خمسة وعشرين شخصا اصحاء ظاهريا متوسط اعمارهم (49.20±1.12) اخذوا كعينة ضبط. تم تقسيم هؤلاء المرضى الى مجموعتين،أعطيت المجموعة الاولى(اوميغا-3) 1000ملغم ثلاث مرات يوميا ،وقد كان متوسط اعمارهم (50.36±2.46)، بينما أعطيت المجموعة الثانية دواء وهمي وقد كان متوسط اعمارهم (50.08±1.84). تم تقييم جميع المرضى سريريا بواسطة قياس نتائج فعالية المرض اعتمادا على معيار

ال28 مفصل(DAS28) ومعيار نشاط المرض السريري(CDAI). سحبت عينات الدم من المرضى وكذلك الاشخاص الاصحاء لغرض تقييم مستوى الفوسفاتيز القلوية عند بدء الدراسة وبعد 12 اسبوع من بدء العلاج. **يستنتج**: من الدراسة ان دواء الأوميغا -3 له القابلية على تقليل مستوى الفوسفاتيز القلوية بشكل ملحوظ في المرضى المصابين بالتهاب المفاصل الرثوي الفعال.

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of unknown aetiology characterized by articular and extra-articular involvement (1). Extra-articular features and non-articular complications of RA are not only common but are generally related to worse disease so they need to be recognized early and managed promptly (2). RA is associated with increased morbidity and mortality (3, 4).Therefore RA should be treated aggressively and rapidly (5). Rheumatoid arthritis can occur at any age (6); however, its onset typically occurs between the third and fourth decades of life (7). In RA, the synovium is the site of the pathologic process and synovial joints as well as tendon sheaths are involved. In the course of the disease, adjacent structures such as the bone, tendons, capsule, and ligaments typically are involved (8). Under normal circumstances, the body can distinguish between self (i.e., proteins found within the body) and non-self-substances (i.e., foreign substances such as bacteria and viruses); in RA this system no longer can differentiate self from non-self-tissues and attacks the synovial tissue and other connective tissues (9). Methotrexate is the most important disease modifying antirheumatic drugs (DMARDs) for the treatment of RA, and currently considered as the central drug for the standard care and the management of RA and it is internationally accepted as first choice drug (10). Omega-3 FA, found primarily in fatty fish with high oil content, consists of both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (11). Both fatty acids and EPA in particular, have close homology mode with arachidonic acid (AA), with EPA and AA differing only in the presence or absence of the omega-3 (n-3) double bond respectively (12). Research has shown increasing evidence for anti-inflammatory, antithrombotic, antiarrhythmic and antiatherogenic effects of fish oil. Fish oil is the most significant source of dietary omega-3 FA (11). The anti-inflammatory activity of EPA and DHA are due to inhibition the oxidation of arachidonic acid (AA) by the cyclooxygenase (COX) and lipoxygenase enzymes that are pivotal in the production of the C20 oxylipids, known as eicosanoids (eicosa means twenty in Greek). These mediators are important in regulating various homeostatic functions, including the gastric mucosal integrity, vascular patency, homeostasis and inflammation (13). The concentration of serum alkaline phosphatase may be increased in patients with rheumatoid disease, Raised serum alkaline phosphatase (ALP) activity in rheumatoid arthritis (RA) has been reported, although its aetiology is not clear. (14,15). The aim of the current study is to assess the influence of omega-3 fatty acids (EPA, DHA) on serum alkaline phosphatase in patients with active rheumatoid arthritis.

Methods

Study design

Seventy two patients with active RA using MTX were participated in this study; only fifty patients completed the follow up. This study was carried out at Tikrit teaching hospital from October 2011 till June 2012. Active RA patients who participated in this

study were diagnosed by a specialized physician depending on: patient medical history, physical examination and laboratory data. Patients consent inform and ethical approval were performed for each patient. Patients were allocated to take either omega-3, 1000 mg capsule (300 mg EPA, 200 mg DHA) three times daily or a capsule prefilled with glucose as placebo. In addition to twenty five apparently healthy subjects participated in this study. Omega-3 was supplied by AdrienGagnon, Canada. Whereas glucose was supplied by SDI, Samarra, Iraq, and Diagnostic kit is alkaline phosphatase supplied from Biomerieux, france. Patients were evaluated at baseline and at week12.

Patients groups

Patients were recruited in to 2 groups according to the given treatment:-

Omega-3 group:- Includes 25 patients with a mean age of (50.36±2.46), have active RA and treated with MTX in dose (10 mg /week) plus omega-3 1000 mg three time daily for three months.

Placebo group:- Includes 25 patients with a mean age of (50.08±1.84), have active RA and treated with MTX in dose (10 mg /week) plus placebo for three months.

In addition to that, 25 subjects with a mean age of (49.20±1.12), who were apparently healthy selected for the purpose of comparison.

Sample selection

Patients with RA as defined by the American college of rheumatology(ACR 1987) revised criteria (16) and proved to have active RA by calculating either DAS28 or CDAI; all selected patients were on methotrexate treatment. The exclusion criteria included Patients with juvenile RA, patients with coexistence other connective tissue diseases, patients already on omega-3, presence of contraindication to omega-3 (patients with chronic anticoagulant treatment and hemorrhagic disorder), known allergy to or intolerance of omega-3, severe liver disease, pregnancy, breast feeding, patients using high dose of steroid ≥ (7.5 mg of steroid), diabetic patients, and patients with inactive RA.

Clinical and laboratory evaluation

For all patients enrolled in this study, direct interview was performed to evaluate disease manifestations, symptoms, medical history, and laboratory findings. Clinical evaluation of patients for tender and swelling joints was done by specialized rheumatologist at zero time (baseline) and after 12 weeks. The RA disease activity was measured using DAS28-ESR (17) and CDAI (18). DAS28 and CDAI can be calculated according to the following formula:

DAS28 = 0.56 (TJC) 0.5+0.28(SJC TJC) 0.5+0.70ln (ESR) +0.014(VAS)

CDAI=TJC +SJC +PrGA + PtGA

TJC -Tender joint count **SJC- Swollen joint count**

ESR- Erythrocyte sedimentation **VAS- Visual analogue scale**

PrGA- Provider global assessment **PtGA- Patients global assessment**

Blood specimen collection and laboratory analysis (at baseline and after 12 weeks) of alkaline phosphatase was done by specialized laboratory researchers who did not participate in this study.

Determination of Serum Alkaline Phosphatase (ALP):

Colorimetric determination of alkaline phophatase activity according to the following reaction:

$$\textit{Phenylphosphate} \xrightarrow[\text{pH10}]{\text{ALP}} \textit{phenol + phosphate}$$

The liberated phenol is measured in the presence of 4-aminoantipyrine and potassium ferricyanide. The presence of sodium arsenate in the reagent stops the enzymatic reaction (19, 20). The intensity of color produced is directly proportional to ALP concentration in the sample. Absorbance was measured spectrophotometrically at wave length 510 nm, and the results were expressed as U/L by using a standard of ALP

Statistical analysis

Statistical software (SPSS version 19, Chicago, IL, USA) was used for data input and analysis. The results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to examine the degree of difference among studied groups. Chi-square test was used to test the significance of association between variables. Paired T test was used to test the significance of difference in means of pre and post treatment. Unpaired T test was used to test the significance of difference in the mean of two independent samples. Value less than 0.05 were considered significant.

Results

Out of 72 patients who were randomized in this study, only 50 patients completed the 12 weeks of treatment (25 from the omega-3 group and 25 from the placebo). In addition to twenty five apparently healthy subjects participated in this study as a control group. The three groups did not differ significantly in baseline characteristics ($p>0.05$, table1) and also for baseline clinical and laboratory values between patients in omega-3 and placebo group (Table 2). Table 3 and Figure 1 showed a serum ALP level in RA patients of both omega-3 and placebo group was highly significantly ($P = 0.02$) higher than that in the control group (a). Additionally there was a non-significant ($P = 0.265$) difference in ALP at the baseline level between omega-3 and placebo group, but there was a significant ($P = 0.025$) decrease in serum ALP (-27.58%) for patients in omega-3 group (b) and there was a highly significant ($P = 0.001$) increase in serum ALP (38.88%) for those in placebo group (c) at the end of the study. Moreover, there was a very highly significant difference ($P = 0.000$) between the effect of omega-3 and placebo on serum ALP (d).

Table (1):- Baseline demographic data

parameter	Omega-3 n=25	Placebo n=25	Control n=25	p-value
Age(yr)	50.36±2.46	50.08±1.84	49.20±1.12	p>0.05
Gender	female 22(88%)	female 23(92%)	female 21(84%)	p>0.05

	male 3(12%)	male 2(8%)	male 4(16%)	p>0.05
Smoking [n (%)]	0 (0%)	3(12%)	5(20%)	p>0.05

Table(2):- Baseline disease, clinical and lab.data

Parameter	**Omega-3 n=25**	**Placebo n=25**	**Control n=25**	**p-value**
Disease duration (yr)	4.92±1.21	7.16±1.42	-	p>0.05
Family history of RA [n (%)]	6(24%)	10(40%)	-	p>0.05
Disease activity score of 28 joints	5.97±0.12	6.02±0.16	-	p>0.05
Positive RF [n (%)]	18(72%)	20(80%)	-	p>0.05
Subcutaneous nodules [n (%)]	5(20%)	%)20(5	-	p>0.05

Table (3):- Effect of Omega-3 on serum ALP

Group	**serum ALP**			**%change**
	baseline	**After 3months**	**Change**	
Control	68.80±8.08	-	-	-
Omega-3	114.68±57.64a	83.04±54.81 b	-31.64±66.14d	-27.58%d
Placebo	97.52±49.75a	135.44±45.65c	37.92±48.36	38.88%

Values presented as Mean±SD.

Baseline: Before start adjuvant therapy with either omega-3 or placebo in RA patient already on MTX; After 3 months: After 3 month of starting the adjuvant therapy.

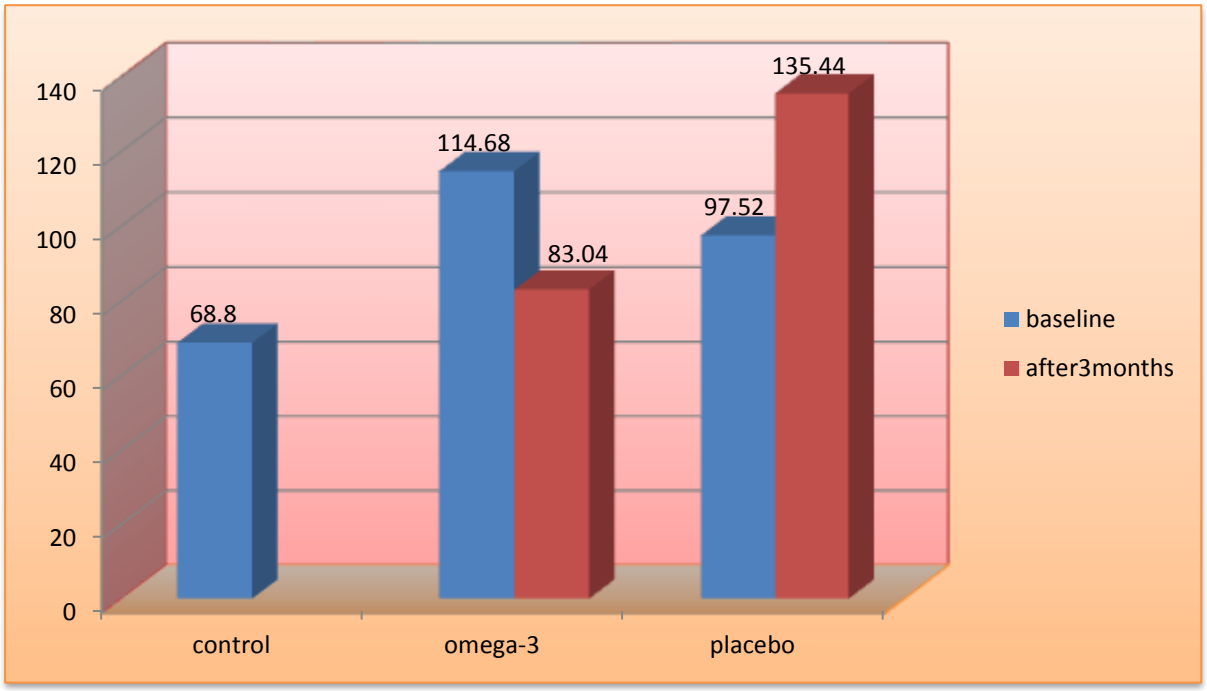

Figure (1):- Effect of Omega-3 on serum ALP

Discussion

The effect of treatment with omega-3 as adjuvant to MTX on serum level of alkaline phosphate (ALP) was investigated in this study. The result of this study showed a significant difference in serum alkaline phosphatase level between RA patients and control subjects, but there was a non-significant difference in ALP at baseline level between omega-3 and placebo group. This result was consistent to 8-week randomized double-blind clinical trial performed by Naini *et al* (21) who evaluate the effect of omega-3 on ALP level in continuous ambulatory peritoneal dialysis patients. The result of current study showed that omega-3 has the ability to reduce the level of serum ALP to a significant value, whereas the ALP serum level has been increase and reach the statistical significant effect in the placebo group. Moreover, there was a very highly significant difference between the effect of omega-3 and placebo on serum ALP. This result was disagree to 16-week randomized double blind placebo-controlled study performed by Park *et al* (22), who investigated the effect of omega-3 fatty acids supplementation on clinical symptoms, inflammatory markers, levels of bone turnover markers and liver function in patients with RA, and they found no significant difference in ALP level when both groups (omega-3 treated patients and placebo) were compared.

Conclusion

Omega-3 is significantly reducing ALP serum level in patient with active rheumatoid arthritis.

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