N-3 polyunsaturated fatty acids administration does not reduce the recurrence rates of atrial fibrillation and inflammation after electrical cardioversion: a prospective randomized study

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ABSTRACT

Objective: The purpose of the present prospective randomized study was to evaluate the effects of n-3 polyunsaturated fatty acids on recurrence rates of atrial fibrillation (AF) and inflammation after electrical cardioversion.

Methods: Calculation of the number of patients needed was based on the assumption of 20% and 65% chance of maintaining sinus rhythm with amiodarone and with polyunsaturated fatty acids, respectively. To observe a significant difference with an alpha level of 0.05 and a power of 0.80 it was necessary to include 22 patients in each group. A total of 47 patients were randomized to amiodarone (n=24) and amiodarone plus n-3 polyunsaturated fatty acids (n=23) groups before scheduled electrical cardioversion. The end-point was the recurrence of AF during 12-month follow-up. Effect of n-3 polyunsaturated fatty acids on inflammation was evaluated with high sensitivity C-reactive protein level measurements. Statistical analysis was performed using unpaired Student's t, Mann Whitney U and Chi-square tests. We analyzed the recurrence of AF using the Cox proportional hazards regression model to control for potentially confounding factors.

Results: Nine patients in the amiodarone group (37.5%), and 9 patients in the amiodarone plus n-3 polyunsaturated fatty acids group (39.1%) had recurrence of AF during follow-up (p=1). With the Cox proportional model, risk factors for the recurrence of AF were previous electrical cardioversion (HR 10.33, 95% CI 1.74 to 61.10, p=0.01) and high sensitivity C-reactive protein levels (HR 1.07, 95% CI 1.02 to 1.38, p=0.007). High sensitivity C-reactive protein levels at baseline, at day 15 and during AF recurrence were similar between two groups (p > 0.05 for all).

Conclusion: N-3 polyunsaturated fatty acids administration does not reduce the recurrence rates of atrial fibrillation and inflammation.

Key words: Atrial fibrillation, recurrence, polyunsaturated fatty acids, Cox proportional regression analysis

ÖZET

Amaç: Bu prosktetif randomize çalışmanın amacı, n-3 çoklu doymamış yağ asitlerinin elektriksel kardiyoversiyon sonrası atriyal fibrilasyon (AF) nüksü üzerine olan etkilerini araştırmaktır.

Yöntemler: Gereki hasta sayısı, sinüs ritim idamesinin amiyodaron grubunda %20, yağ asidi grubunda %65 olacağını tahmin edilen bulundu. Alpha değeri 0.05 ve güç %80 olacak şekilde önceden elektriksel kardiyoversiyon yapılması amacına göre 22 hastanın gerekli olduğu bulundu. Toplam 47 hasta amiyodaron (n=24) ve amiyodaron+n-3 çoklu doymamış yağ asidi (n=23) gruptarına randomize edildiler. Nüks olarak alınan 12 aylık takipte AF nüksü olan hastaların 9 hastasında (37.5%), amiyodaron+n-3 çoklu doymamış yağ asidi grubunda ise 9 hastada (39.1%) nüks gözlemlendi (p=1). Cox regresyon analizi ile potansiyel faktörlere kontrol ederek Cox oransal regresyon modeli ile değerlendirildi.

Bulgular: Amiyodaron grubundaki 9 hastada (%37.5) ve amiyodaron+n-3 çoklu doymamış yağ asidi grubundaki 9 hastada (%39.1) nüks gözlemlendi (p=1). Cox regresyon analizi ile nüks için risk faktörleri önceden elektriksel kardiyoversiyon sonrası nüks olmasının (izafı risk 10.33, %95 GA 1.74-61.10;
Introduction

Previous studies indicated that atrial fibrillation (AF) is associated with inflammation (1-3). Statins, which have anti-inflammatory actions, have been shown to be effective in preventing AF in different patient populations (4-9), including recurrence of AF after electrical cardioversion (EC) (10, 11). N-3 polyunsaturated fatty acids (PUFAs) also have anti-inflammatory actions (12).

However, conflicting results on the effects on the development of AF have been obtained with the use of these agents (13-16).

The aim of the present study was to evaluate the effects of n-3 PUFAs on recurrence of AF after EC.

Methods

Patient population, and study design

The population of the present prospective randomized study was chosen from a group of 67 patients with persistent AF referred to cardioversion between April 2006 and May 2009. From this group, patients with hyperthyroidism (n=3), left atrial thrombi (n=10), unsuccessful cardioversion attempts (n=5), and spontaneous conversion into the sinus rhythm (n=2) were excluded and remaining 47 patients constituted the study population. Therefore, 47 patients (27 women; mean age: 61±11 years; range: 37-81) with persistent AF were enrolled for the study with the aim of EC and were randomized in 1:1 fashion to amiodarone (n=24) and amiodarone plus PUFAs (n=23) groups.

In all the patients, the duration of AF was >48 h. Exclusion criteria included paroxysmal AF episodes; a left atrium size >5.5 cm; moderate to severe heart valve disease; coronary artery disease; unsuccessful cardioversion; New York Heart Association class 3, 4 heart failure; a history of cardiac surgery; acute reversible condition; contraindications to treatment with amiodarone; significant impairment of renal function; pregnancy; lactation; fertile female; age <18 years old; and an ejection fraction <0.30.

The local Ethics Committee approved the study protocol, and all subjects gave written informed consent.

Statistical analysis

Statistical analyses were performed using SPSS 11.0 (SPSS Inc., Chicago, IL, USA). A P-value of <0.05 (two-tailed) was considered significant. Continuous variables are expressed as mean±1 SD and categorical variables are presented as percentages. Kolmogorov-Smirnov test was used to test the distribution of numeric variables and variables with normal distribution were compared with unpaired Student t-test (age and body mass index) and those without normal distribution was compared with Mann-Whitney U test (AF duration, ejection fraction, left atrial diameter and baseline hsCRP). Categorical variables were compared with Chi-square test. Serial measurements of hsCRP in each group were compared with Friedman’s test.

We analyzed the recurrence of AF using the Cox proportional hazards regression model to control for potentially con-

Follow-up

All the patients were seen at each week in the first month, then at each month thereafter, and at any time the patient complained of any symptoms. A 24-hour Holter recording was performed at 1 and 3 months. Electrocardiography and 24-hour Holter recording were also performed at any time the patient had any symptoms. The duration of follow-up was 12 months and the end point of the study was electrocardiographically confirmed recurrence of AF lasting >10 minutes. When recurrence was documented, amiodarone and n-3 PUFAs were discontinued.

High sensitivity C-reactive protein measurement

High sensitivity C-reactive protein (hsCRP) levels were determined by Immulite 2000 (Siemens Medical Solutions Diagnostics, Los Angeles, CA, US) before the randomization, at day 15 and during recurrence. Normal reference values for hsCRP were 0-1.1 mg/L.

One capsule of this product is 500 mg (Eicosapentaenoic acid [EPA], 18%; Docosahexaenoic acid [DHA], 12%). All the patients underwent transesophageal echocardiography to rule out the possibility of intracardiac thrombi and patients with detected intracardiac thrombi were excluded from the study. After the EC, anticoagulation with warfarin to achieve an international normalized ratio of 2.0 to 3.0 for at least four weeks was done in all the patients. The dose of warfarin was arranged based on the strict international normalized ratio measurements. EC was performed in the fasting state. Propofol was administered for sedation. R wave-synchronized monophasic direct-current shocks (Zoll) were delivered in all patients with the step-up protocol of 200, 300, and 360 J with anterolateral approach. Successful cardioversion was defined as the presence of sinus rhythm lasting ≥1 minute after the shock. If unsuccessful, 2 anteroposterior shocks of 200 and 360 J were applied. Patients with unsuccessful cardioversion attempts were excluded from the study.

Study protocol

Transthoracic echocardiography and baseline laboratory analysis were performed and electrocardiography was taken before the randomization. Patients were randomized to amiodarone (n=24) and amiodarone plus n-3 PUFAs (n=23) groups. N-3 polyunsaturated fatty acids treatment and amiodarone were started after the cardioversion and both were given during 12-month follow-up or until the recurrence. Amiodarone was given intravenously 1 g/d for the first day, followed by 800 mg/d for the first week, 600 mg/d for the second week, 400 mg/d for the third week and 200 mg/d thereafter. The dose of commercially available n-3 PUFAs was 2 g/d (Marincap, Koçak, Turkey).
founding factors. The strength of the association between N-3 PUFAs treatment and the occurrence of AF was represented by hazard ratios (HRs) and accompanying 95% confidence interval (CI). We employed an epidemiological approach and factors that have been shown to be multivariate predictors of AF recurrence in the previous studies (10, 17-19) have been accepted as potential risk factors for the recurrence of AF. Therefore, age, left atrial diameter, treatment with beta-blockers, treatment with statins, body mass index, AF duration, previous EC and hsCRP have been included in the Cox proportional hazards regression model. A p value of <0.05 was considered significant.

Sample size calculation
Calculation of the sample size was performed using InStat (GraphPad Software, Inc. La Jolla, CA, USA) software.
Calculation of the number of patients needed was based on the assumption of 20% and 65% chance of maintaining sinus rhythm at 12 months with amiodarone and with amiodarone plus n-3 PUFAs, respectively. To observe a significant difference with an alpha level of 0.05 and a power of 0.80 it was necessary to include 22 patients in each group.

Results
Baseline characteristics
A total of 47 patients were randomized to amiodarone and amiodarone plus n-3 PUFAs groups before scheduled EC. The baseline characteristics (Table 1) were similar between two groups (p>0.05 for all).

Laboratory findings
HsCRP levels at baseline, at day 15 and during recurrence (Table 2) all were similar in the both groups (p>0.05 for all).

Follow-up findings
Nine patients in the amiodarone group (37.5%), and 9 patients in the amiodarone plus PUFAs group (39.1%) had recurrence during follow-up (p=1). The duration of follow-up was 12 months. One patient in each group lost to follow-up. Three patients in the amiodarone group (12.5%) and 5 patients in the amiodarone plus n-3 PUFAs group (21.7%) developed hyperthyroidism requiring cessation of amiodarone (p=0.46). One patient in the combination group had liver enzyme elevation and amiodarone was also stopped in this patient. One patient in the amiodarone group (4.2%) had transient ischemic attack and one patient in the combination group (4.3%) had ischemic stroke during follow-up (p=1). Hospitalization rates were also similar in the both groups (p=0.7; Table 3).

With the Cox proportional model, risk factors for the recurrence of AF were previous electrical cardioversion (HR 10.33, 95% CI 1.74 to 61.10, p=0.01) and hsCRP levels (HR 1.07, 95% CI 1.02 to 1.38, p=0.007; Table 4).

Discussion
The main finding of the current study is that n-3 PUFAs administration does not reduce the recurrence rates of AF and inflammation after EC.

Conflicting results have been obtained about the effects of fish oil on AF. Data from experimental, epidemiological and randomized studies are controversial.

Hearts from rabbits fed a PUFA-rich diet have demonstrated an increased resistance to stretch-mediated changes in atrial electrophysiological properties. AF episode duration was shorter in rabbits with a high PUFA content in the atrial myocyte membranes (20).

There are two positive epidemiological studies showing antiarrhythmic effects of PUFAs on AF. Mozaffarian et al. (14) conducted a prospective study in 4,815 adults aged ≥65 years. They reported that there was a negative correlation between the consumption of fish oil and the risk of AF; AF risk at hospital discharge records and annual ECGs at 12-year follow-up was 31% lower when fish was consumed ≥5 times weekly. A lower AF incidence was associated with consumption of broiled or baked fish but not fried fish. They noted that frying can increase the content of n-6 and trans fatty acids, and oxidation species. Another study supported the findings of Mozaffarian et al. (14); n-3 PUFA reduced the risk of the hospitalization for AF.

Table 1. Clinical characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>Amiodarone (n=24)</th>
<th>Amiodarone plus PUFAs (n=23)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>61±11</td>
<td>62±12</td>
<td>0.8</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>9 (37.5)</td>
<td>11 (47.8)</td>
<td>0.56</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>5 (20.8)</td>
<td>3 (13)</td>
<td>0.7</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>12 (50)</td>
<td>13 (56.5)</td>
<td>0.77</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>60±8 (40-70)</td>
<td>61±7 (40-70)</td>
<td>0.89</td>
</tr>
<tr>
<td>Left atrial diameter, mm</td>
<td>44±4 (36-51)</td>
<td>44±4 (34-54)</td>
<td>0.75</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28±4</td>
<td>28.8±5.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Duration of atrial fibrillation, day</td>
<td>58±97 (2-365)</td>
<td>103±179 (2-730)</td>
<td>0.86</td>
</tr>
<tr>
<td>Previous electrical cardioversion, n (%)</td>
<td>4 (16.7)</td>
<td>8 (34.8)</td>
<td>0.19</td>
</tr>
<tr>
<td>Medications use, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta- blockers</td>
<td>6 (25)</td>
<td>6 (26.1)</td>
<td>1</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>3 (12.5)</td>
<td>3 (13)</td>
<td>1</td>
</tr>
<tr>
<td>Statins</td>
<td>5 (20.8)</td>
<td>7 (30.4)</td>
<td>0.51</td>
</tr>
<tr>
<td>ACEIs/ARBs</td>
<td>11 (45.8)</td>
<td>17 (73.9)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Table 2. Laboratory findings

<table>
<thead>
<tr>
<th>Variables</th>
<th>CRP- baseline</th>
<th>CRP-15th day</th>
<th>CRP recurrence</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiodarone (n=24)</td>
<td>6.7±11</td>
<td>5.1±7.4</td>
<td>6.5±10.9</td>
<td>0.16</td>
</tr>
<tr>
<td>Amiodarone plus PUFAs (n=23)</td>
<td>9.9±16</td>
<td>7.9±22</td>
<td>14±16</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Values are presented as mean±SD (range) or number (percentage)
*p *Student t- test, Mann-Whitney U test and Chi-square test
ACEIs - angiotensin converting enzyme inhibitors, ARBs - angiotensin receptor blockers, PUFAs - N-3 polysaturated fatty acids
Table 3. Follow-up findings

<table>
<thead>
<tr>
<th>Variables</th>
<th>Amiodarone (n=24)</th>
<th>Amiodarone plus PUFA (n=23)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AF recurrence</td>
<td>9 (37.5%)</td>
<td>9 (39.1%)</td>
<td>1</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td>3 (12.5%)</td>
<td>5 (21.7%)</td>
<td>0.46</td>
</tr>
<tr>
<td>Liver enzyme elevation</td>
<td>-</td>
<td>1 (4.3%)</td>
<td>0.48</td>
</tr>
<tr>
<td>Thromboembolism</td>
<td>1 (4.2%)</td>
<td>1 (4.3%)</td>
<td>1</td>
</tr>
<tr>
<td>Hospitalization</td>
<td>4 (16.7%)</td>
<td>5 (21.7%)</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Values are presented as mean±SD (range) or number (percentage)

*Chi-square test

AF - atrial fibrillation, PUFA - N-3 polyunsaturated fatty acids

Table 4. Multivariable predictors of recurrence with Cox proportional regression analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard ratio</th>
<th>95% Confidence intervals</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous electrical cardioversion</td>
<td>10.33</td>
<td>1.74 to 61.10</td>
<td>0.01</td>
</tr>
<tr>
<td>HsCRP levels</td>
<td>1.07</td>
<td>1.02 to 1.38</td>
<td>0.007</td>
</tr>
</tbody>
</table>

HsCRP - high sensitivity C-reactive protein

Danish Diet, Health, and Cancer Study (15), Physicians’ Health Study (21) Rotterdam study (22), Women’s Health Initiative Study (23) and small randomized study (24) were unable to indicate any beneficial effects of fish consumption or PUFA treatment on AF. Danish Study (15) was a prospective cohort study performed in 47,949 adults aged 50 to 64 years who were free of coronary artery disease at baseline. At a mean follow-up of 5.7 years, there was no benefit on AF with the consumption of fish. Interestingly, the Danish Study (15) and the Physicians’ Health Study (21) actually reported a trend toward increased risk of developing AF in those with greater fish intake.

There is only one study that evaluated the effects of PUFA on AF recurrence after EC. In a placebo-controlled study that enrolled 180 patients with persistent AF, Erdoğan et al. (24) have shown that PUFA treatment had no effect on the recurrence rate of AF, which is in agreement with the result of the current study.

There is only one positive randomized study indicating beneficial effects of PUFA on AF: Calo et al. (13) randomized 160 patients undergoing coronary artery bypass surgery to receive PUFA (fish oil capsules containing 850-882 mg EPA and DHA in a ratio of 1:2, with a dose: 2 g/day) or placebo control, starting 5 days before surgery and continuing until hospital discharge. They reported that the incidence of AF was reduced by PUFA (p=0.013).

Although previous studies have shown that PUFA have anti-inflammatory actions (12, 25), however, current study indicated that hsCRP levels, an indicator of inflammation, were similar between PUFA groups and control group.

Mechanisms of action of n-3 PUFA

Eicosapentaenoic acid and DHA are found in varying proportions in PUFA extracted from fish oil. N-3 polyunsaturated fatty acids inhibit Na and Ca current and Na-H+ exchanger and at higher concentrations, K currents. Thus, may prevent electrical remodeling. They have also anti-inflammatory and anti-oxidant effects and thus may prevent structural remodeling (25). Li et al. (26) have shown that omega-3 fatty acids inhibit l(ito), l(Kur) and l(Na) in human atrial patch clamp preparation.

Possible mechanism of negative results obtained in the present study

We used fish oil with a dose of 2 g/d in the present study. One capsule of the commercially available fish oil product used the present study is 500 mg and EPA and DHA ratios are 18% and 12%, respectively. However, fish oil capsules containing 850-882 mg EPA and DHA in a ratio of 1:2, with a dose: 2 g/d were used in the study of Calo et al. (13) the difference in this ratio might be a reason of the negative result. Calo et al. (13) started the fish oil 5 days before the operation and we started it after the EC. We used amiodarone in the both group. These differences might also affect the results. Dietary intake of fish oil might have been higher in the amiodarone group, causing us not to obtain beneficial effect in the combination group. As shown in a previous study (14) more patients in the amiodarone group might have eaten broiled or baked fish and/or more patients in the combination group might have eaten fried fish. Baseline left atrial diameters were higher in the both group (about 45 mm), this might be one of the reasons of the negative results of PUFA. Follow-up duration is relatively short in the current study. Larger randomized studies with longer duration of follow-up could show positive effect of PUFA on AF. The results of ongoing prospective randomized studies would be very helpful in this issue (27, 28).

Although statistically insignificant compared with amiodarone group, follow-up period was shorter in combination group. Last possible mechanism is that, epidemiological studies that demonstrated no benefit of PUFA on AF generally enrolled younger patients than studies showing benefit (15, 21); the mean age in negative studies was 56 and 60 years, however, the mean age in CHS study, which showed beneficial effects of PUFA was 73 years (14). The mean age in the present study is 61 therefore this might also be another mechanism of the negative result in the present study.

Study limitations

The sample size was small. We did not evaluate plasma phospholipid EPA and DHA concentrations. Therefore, the dose of fish oil in the combination group might not be high enough to show a positive effect on AF and inflammation. Atrial fibrillation recurrence represent periodic and widely spaced snapshots of rhythm status, so the information that comes from these periodic assessments addresses prevalence of AF in the population at these time points, not the time when AF may have occurred. This is a continuous variable; it has far more statistical power as a continuous longitudinal repeated measure than a binary variable. However, to overcome this problem, instead of the employed multivariable regression analysis, we employed a Cox analysis. The duration of n-3 PUFA treatment was too short to have an effect on structural remodeling. It remains controversial as to whether elevated CRP in itself results in electrical remodeling or is a marker of ongoing electrical disturbance.
may not be relevant to be dependent on CRP alone. The duration of follow-up was short. It is not a placebo-controlled double-blind trial. We might have missed short-lived asymptomatic paroxysmal AF recurrences between scheduled follow-up visits. However, we did not include patients with paroxysmal and self-terminating AF; the follow-up visits were relatively frequent and we used Holter ECG recording to detect recurrence. The two groups were not perfectly balanced concerning pre-procedural ACEIs/ARBs. However, this variable was inserted in the regression analysis. The study was underpowered because of different patients had to be discontinued amiodarone/PUFA therapy during follow-up. Another potential limitation is that the n-3 PUFAs administration started after the cardioversion. Finally, we did not evaluate possible mechanisms other than inflammation.

Conclusion

N-3 PUFAs administration does not reduce the recurrence rates of AF and inflammation after EC.

Conflict of interest: None declared.

References


