Effects of Coenzyme Q10 Supplementation on Inflammatory Cytokines (TNF-α, IL-6) and Oxidative Stress in Rheumatoid Arthritis Patients: A Randomized Controlled Trial

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Received for publication May 31, 2015; accepted August 25, 2015 (ARCMED-D-15-00393).

Backgrounds and Aims. Overproduction of proinflammatory cytokines is a main trait of rheumatoid arthritis. Coenzyme Q10 (CoQ10), an endogenous antioxidant, has shown anti-inflammatory effects in some diseases. In this study we aimed to assess the effects of CoQ10 supplementation on cytokines generation and oxidative stress in rheumatoid arthritis.

Methods. In this double-blind, randomized controlled clinical trial, 44 patients with rheumatoid arthritis were recruited. Twenty two patients received 100 mg/day capsules of CoQ10 and 22 patients took placebo for 2 months. At the beginning and the end of the intervention, 7 mL of fasting blood was taken from patients to measure malondialdehyde (MDA), total antioxidant capacity (TAC), interleukin (IL)-6 and tumor necrosis factor alpha (TNF-α).

Results. At the end of the study, serum MDA significantly decreased in supplemented group (mean difference = −1.47 nmol/mL; 95% confidence interval (CI), −2.52 to −0.43; p = 0.008). CoQ10 also suppressed overexpression of TNF-α (difference in median was +1.1 in placebo vs. +0.03 in CoQ10 group; p = 0.033). There was no significant difference in TAC and IL-6 levels between groups.

Conclusions. This study showed beneficial effects of CoQ10 supplementation on inflammatory cytokines and oxidative stress in rheumatoid arthritis patients. © 2015 IMSS. Published by Elsevier Inc.

Key Words: Coenzyme Q10 (CoQ10), Ubiquinol, Inflammatory cytokines, Oxidative stress, Rheumatoid arthritis.

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease in which the synovium is the first affected constitution (1). Although each articulation may be involved, symmetrical engagement of small joints in the hands and feet is more common (2,3). The main etiology of the disease is unknown; it appears that environmental factors initiate the immune cells response in genetically predisposed individuals. Stimulation of the immune system leads to secretion of a large number of proinflammatory cytokines (3). Cytokines may be produced by nearly every cell, but mostly act locally on cell receptors and have high potency (4). Their production gives rise to an inflammatory cascade. One of the most important elements in this cascade is overexpression of tumor necrosis factor-alpha (TNF-α). TNF-α causes synovitis, articular destruction and overproduction of other cytokines, especially interleukin (IL)-6 which, in turn, leads to further inflammation and joint degradation (5). Attention
to the role of TNF-α and IL-6 in RA comes from in vitro studies where they showed potential for bone and cartilage breakdown (6).

During the inflammation, immune cells (neutrophils) generate pro-oxidant substances such as reactive oxygen species (ROS) (7). In addition, ROS can be generated via mitochondria as a byproduct of electron transport chain (ETC) during adenosine triphosphate (ATP) synthesis (8). ROS, especially mitochondrial ROS (MtROS), is reciprocally implicated in secretion of proinflammatory cytokines (8,9). Regardless of the origin of ROS, it was also associated with DNA, proteins and membrane lipid damage (10). Incessant generation of ROS in involved joints leads to depletion of antioxidant systems (7). Levels of certain antioxidants are lower in both serum (11) and synovial fluid (7) of RA patients compared to healthy persons. These changes all have positive feedback on inflammation and resulting joint erosion.

New treatment strategies based on blockade of cytokine pathways in late stages of RA are progressing. In spite of their benefits, long-term utilization of these blocking agents has indicated side effects (12). Our role as nutritionists is to find nutraceuticals with similarity in function to these agents and with the least disadvantages. It has been demonstrated that antioxidant nutrients exert anti-inflammatory activity (7). Coenzyme Q₁₀ (CoQ₁₀) or ubiquinone is a lipid-soluble vitamin-like antioxidant naturally found in the diet and can also be synthesized endogenously by all cells of our body. It is one of the key components in ATP production in ETC. CoQ₁₀ protects membranes against oxidation, regenerates and reduces vitamins E and C and enzymatic antioxidant systems, and modulates prostaglandin metabolism (13–16).

Studies in cells (17) and animal models (18) revealed anti-inflammatory effects of CoQ₁₀. Recent studies also indicated the usefulness of this micronutrient against inflammation in coronary artery disease, neurodegenerative diseases and diabetic patients (19–21). To our knowledge, there is no clinical study reporting the effects of CoQ₁₀ on inflammation and oxidative stress in RA. For this reason, our research team was encouraged to investigate the effects of oral supplementation with this anti-inflammatory agent on serum concentration of inflammatory biomarkers (TNF-α, IL-6) and oxidative stress in RA patients.

### Materials and Methods

**Study Design and Ethics Statements**

After the ethics committee of Tabriz and Urmia Universities of Medical Sciences approved the study in accordance with the Helsinki Declaration (the ethical codes of study in Tabriz and Urmia were 92113 and umsu.rec.1392.152, respectively), this double-blind, randomized controlled clinical trial was conducted at the Imam Hospital and Sahand Clinics affiliated with Urmia University of Medical Sciences. This study was registered in the Iranian registry of clinical trials (ID: IRCT201311014105N16).

#### Sample Size and Randomization

With a standard deviation of 1.08 from a previous study (22) to detect the smallest difference in malondialdehyde (MDA) means (0.97 nmol/mL) based on 95% confidence interval and a power of 80%, sample size was determined as 20 patients for each group. In regard to dropout (35%), the sample size was considered to be 27 persons per group. Blocked randomization was run through Random Allocation Software (RAS) to assign patients into two parallel groups (1:1) with four participants in each block.

**Recruitment of Patients**

According to 1987 American College of Rheumatology (ACR) criteria, 54 RA patients (18–65 years) diagnosed at least 6 months ago with moderate and severe disease activity (DAS 28 > 3.2) were recruited from January to June 2014. Patients with a history of diabetes mellitus, renal, liver, thyroid and infectious diseases, smoking, consumption of antioxidants or omega-3 fatty acid supplements in the previous month, persons taking warfarin or oral contraceptive pills, and pregnant or lactating women were excluded. All participants signed informed written consent.

**Intervention**

Patients received daily a 100 mg capsule of CoQ₁₀ (Health Burst Inc., USA) (n = 27) or placebo (wheat starch, identical in size, color and shape to supplement) (n = 27) for 2 months in addition to their conventional medications (methotrexate, sulfasalazine, hydroxychloroquine, prednisolone). They were asked to take the capsule along with a meal and not to change their usual diet and physical activity during the study. Patients completed 3-day food records during two steps: 1 week prior to beginning and at the final week of the intervention. Dietary intakes were analyzed using modified Nutritionist IV software for Iranian foods.

At least 2 weeks supplementation with 90 mg/day is needed to reach steady state levels of CoQ₁₀ in serum (23). Considering that RA patients routinely report to the rheumatologist in the mentioned clinics every 2 months and that the long intervention period may decrease the rate of adherence (24), we designed our intervention using 100 mg/day CoQ₁₀ for 2 months. Whereas measuring CoQ₁₀ concentration in serum better reveals compliance of intervention, due to budgetary considerations we could not measure its levels in the present study. Adherence to the prescribed interventions was pursued every 14 days.
via a phone interview by questioning about side effects and time and number of capsules consumed in a day during the study, at the end of intervention it was also calculated using the following equation:

\[
\text{Compliance} = \frac{\text{consumed capsules at the end of study}}{\text{received capsules at the entry}} \times 100
\]

**Biochemical Measurements**

Seven mL of venous blood was taken from patients after fasting for 10–12 h at baseline and after 2 months of intervention in the laboratory of Imam Hospital. Two-mL blood samples were used to measure erythrocyte sedimentation rate (ESR) and the remainder was immediately centrifuged to separate serum. Serum samples were stored at −80°C until analysis. Serum IL-6 and TNF-α were assayed by relevant ELISA kit (Human IL-6 ELISA kit and Human TNF-α ELISA kit; Orgenium Inc., Finland). Serum total antioxidant capacity (TAC) was assessed using ELISA Kit (TAC ELISA kit; LDN Labor Diagnostika Nord GmbH and Co. KG, Germany). Spectrophotometric method was used to measure serum MDA by its reaction with thiobarbituric acid (25).

**Statistical Analysis**

Statistical analysis was performed using the Statistical Package for Social Sciences v. 16 (SPSS Inc.). Normality of variables was evaluated by Kolmogorov-Smirnov test. Paired sample \(t\)-test and Wilcoxon were used to examine differences in each group; independent sample \(t\)-test and Mann-Whitney tested differences between groups. Analysis of covariance (ANCOVA) assessed differences between groups at the end of the study after adjustment for changes of disease duration, medication, and total energy intake. Data were expressed as mean ± standard deviation (SD) for normally distributed variables and median (interquartile range [IQR]) for non-normally distributed variables; \(p < 0.05\) was considered significant.

**Results**

As shown in Figure 1, 45 patients (39 females and six males) completed the study. Because of the low amount

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**Figure 1.** CONSORT flow diagram of supplementation with 100 mg/day coenzyme Q10 for 2 months in rheumatoid arthritis patients. (A color figure can be found in the online version of this article.)
of serum specimen, one patient in the placebo group was excluded from analysis. Age and disease duration of participants as mean ± SD were 49.59 ± 11.33 and 6.92 ± 6.12 years, respectively. Some characteristics of patients at the beginning of the study are shown in Table 1 and were not significantly different between placebo and CoQ10 groups. Dietary intake of antioxidant nutrients such as β-carotene, vitamins A, C, E, α-tocopherol, zinc, and selenium were assessed and showed no differences between groups at baseline and did not change (except vitamin C) at the end of 2 months (Table 2).

Table 3 shows serum levels of TAC and MDA during the two steps of measurement and their changes throughout the study. MDA concentration in the CoQ10 group significantly decreased at the end of the intervention (mean difference = −1.47 nmol/mL; 95% confidence interval (CI), −2.52 to −0.43; p < 0.05). CoQ10 led to a significant decrease in MDA compared with placebo (p < 0.05). IL-6 and TNF-α values as median (IQR) are shown in Table 4. Serum level of TNF-α regarding its baseline value increased significantly in the placebo group (p < 0.05). Comparison of the TNF-α changes in patients who received CoQ10 supplement and placebo showed a significant difference (median difference was +0.03 vs. +1.1, respectively; p < 0.05). In this interventional study, CoQ10 supplementation did not reveal any significant effect on serum TAC and IL-6 concentration. Adverse effects of CoQ10 were not seen during the intervention.

### Discussion

The purpose of the current study was to evaluate the effects of oral CoQ10 supplementation on serum concentration of MDA, TAC, IL-6 and TNF-α in RA patients. This study indicated that CoQ10 supplementation for 2 months in RA patients led to a significant decrease in MDA compared with placebo. Also, a nonsignificant increase was observed in TAC in both groups. Although the augmentation was larger in the supplemented group than in placebo group (+0.19 vs. +0.14) the difference between groups was not significant.

MDA level as an indicator of free radical-induced injuries or oxidative stress (26) is much higher and antioxidant levels are much lower in RA patients than healthy persons (27). Numerous investigators evaluated effects of CoQ10 on oxidative stress and inflammation in other diseases. Lee et al. observed a significant increase in plasma Q10, catalase (CAT), superoxide dismutase (SOD) and a significant decline in serum MDA level after 12 weeks administration of 150 mg/day CoQ10 in patients with coronary artery disease (CAD); however, they could not see the same effect using 60 mg/day of CoQ10 (26). Also, in their investigations, 300 mg/day CoQ10 raised plasma vitamin E and glutathione peroxidase (GPx) levels in addition to reported effects via 150 mg/day of supplement (28). In
Another study, Sanoobar et al., following supplementation with 500 mg/day CoQ10 for 12 weeks, found a meaningful decrease in serum MDA and a rise in SOD of multiple sclerosis (MS) patients; however, no effect on TAC was seen. These authors declared that availability of various methods of TAC assessment may be responsible for dissimilarity of their finding from other authors (29). Singh et al. reported that supplementation of 120 mg/day CoQ10 in heart disease patients after 4 weeks (30) and 8 weeks (31) led to a significant increase in TAC concentration may be attributed to its low dose in our study. It must be kept in mind that even though improvement of TAC with supplements is necessary, the extent of safety is not clear and a high increase may induce detrimental results in some conditions (39).

Increasing amount of inflammatory cytokines is the best defined characteristic of RA patients. Numerous approaches (medications and dietary interventions) have been introduced to treat RA patients and ameliorate the situation by reducing the executioner cytokines. We applied supplementation with CoQ10 to examine its anti-inflammatory effects claimed in previous reports (18, 28). CoQ10 suppressed overproduction of TNF-α in CoQ10-treated group and led to a significant change in comparison to placebo group. Although in our study the concentration of IL-6 decreased in the CoQ10 group, the reduction was not significant.

The effects of CoQ10 on inflammatory cytokines have previously been assessed. In one study in patients with MS, 500 mg/day CoQ10 supplementation for 12 weeks decreased serum levels of TNF-α and IL-6; however, there was no significant effect on anti-inflammatory cytokines such as transforming growth factor-beta (TGF-β) and IL-4 (40). In another study, 12-week consumption of carnitine Q-gel (270 mg/day CoQ10 and 2250 mg/day L-carnitine) in patients with heart failure reduced both TNF-α and IL-6 but did not alter IL-10 level (32).

Table 3. Serum levels of oxidative stress-related markers in RA patients before and after 2 months supplementation with CoQ10

<table>
<thead>
<tr>
<th>Variables</th>
<th>Placebo group</th>
<th>CoQ10 group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 22)</td>
<td>(n = 22)</td>
<td></td>
</tr>
<tr>
<td>TAC</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(nmol/L)</td>
<td>Baseline</td>
<td>0.81 ± 0.47</td>
<td>0.77 ± 0.55</td>
</tr>
<tr>
<td></td>
<td>End point</td>
<td>0.96 ± 0.47</td>
<td>0.96 ± 0.58</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.275</td>
<td>0.312</td>
</tr>
<tr>
<td>Mean changes</td>
<td>0.15 ± 0.62</td>
<td>0.19 ± 0.87</td>
<td>0.847</td>
</tr>
<tr>
<td>MDA</td>
<td>(nmol/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.07 ± 1.71</td>
<td>3.61 ± 1.51</td>
<td>0.274</td>
</tr>
<tr>
<td>End point</td>
<td>3.16 ± 1.71</td>
<td>2.13 ± 1.44</td>
<td>0.006***</td>
</tr>
<tr>
<td>p</td>
<td>0.755</td>
<td>0.008*</td>
<td></td>
</tr>
<tr>
<td>Mean changes</td>
<td>0.09 ± 1.39</td>
<td>−1.47 ± 2.36</td>
<td>0.009**</td>
</tr>
</tbody>
</table>

RA, rheumatoid arthritis; TAC, total antioxidant capacity; MDA, malondialdehyde.

Table 4. Serum levels of inflammatory markers in RA patients before and after 2 months supplementation with CoQ10

<table>
<thead>
<tr>
<th>Variables</th>
<th>Placebo group</th>
<th>CoQ10 group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 22)</td>
<td>(n = 22)</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(pg/mL)</td>
<td>Baseline</td>
<td>0.09 (0.05–5.72)</td>
<td>0.45 (0.04–18.15)</td>
</tr>
<tr>
<td></td>
<td>End point</td>
<td>0.09 (0.06–47.10)</td>
<td>0.07 (0.04–9.32)</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.485</td>
<td>0.570</td>
</tr>
<tr>
<td>TNF-α</td>
<td>(pg/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.36 (2.21–16.86)</td>
<td>3.36 (1.89–26.79)</td>
<td>0.916</td>
</tr>
<tr>
<td>End point</td>
<td>4.45 (3.41–37.72)</td>
<td>3.39 (1.91–24.25)</td>
<td>0.033**</td>
</tr>
<tr>
<td>p</td>
<td>0.008*</td>
<td>0.858</td>
<td></td>
</tr>
</tbody>
</table>

RA, rheumatoid arthritis; IL-6, interleukin 6; TNF-α, tumor necrosis factor-α.

Data are presented as median (interquartile range). Data were analyzed by *Wilcoxon Signed Ranks Test and **Mann-Whitney U test on parameter changes (after-before); p value < 0.05 was considered significant.
Anti-inflammatory abilities of CoQ10 have been studied under other conditions. In agreement with the findings of the present study, Díaz-Castro et al. (35) found that in athletes who demonstrate overproduction of inflammatory cytokines during strenuous exercise, administration of CoQ10 reduced overexpression of TNF-α and had no effect on IL-6 values. Supplementation of 100 mg/day CoQ10 for 8 weeks in the study by Gökböel and colleagues (42) did not affect TNF-α and IL-6 concentration in healthy males. Lack of effect may be due to the reality that healthy subjects have normal levels of these cytokines. Diminishing effects of CoQ10 on TNF-α levels have been reported in animal and in in vitro studies by Fouad et al. (43), Schmelzer et al. (44), and Tawfik (45); the last researcher also noted that CoQ10 had a significant effect on IL-6 levels. Bauerova et al. (46) indicated that CoQ10 in a rat model of rheumatoid arthritis through decrease of lipid and protein oxidation (MDA-adducts, 4-hydroxy-2-nonenal [HNE]-adducts, and carbonyl) and of IL-1α could potentiate antioxidant and anti-inflammatory effects of methotrexate. In a study by Bessler et al. (17), production of IL-6 was not affected by incubation of peripheral blood mononuclear cells with CoQ10, whereas TNF-α secretion significantly declined.

In our study the effect of CoQ10 in suppression of TNF-α overexpression without further decrease in IL-6 may be due to the fact that inflammatory cytokine production as previously mentioned is increased in rheumatoid arthritis or due in part to the low dose of supplement or short duration of the intervention. A presumptive mechanism by which CoQ10 exerts inhibitory effects on TNF-α secretion can be attributed to its capability in inhibition of nuclear factor-kappa B (NF-kB) signaling pathways (35,47). NF-kB is a family of transcriptional factors with beneficial effects in normal physiology. Dysregulation of NF-kB leads to inflammatory diseases, in particular rheumatoid arthritis and osteoarthritis; therefore, inhibition of NF-kB seems to be a logical objective in the treatment of these diseases (48).

There are at least two limitations to our study. First, we did not assay antioxidant enzymes. Because total antioxidant capacity does not contain all present antioxidants in the defense system and encompasses only free radical scavengers (49), measurement of enzymatic antioxidants will be helpful and can better describe the suitable influences of CoQ10 on the defense system. Second, we did not evaluate CoQ10 intake in patients because of inadequate information about the amount of this micronutrient in nutritional databanks.

In conclusion, our findings demonstrated that supplementation with 100 mg/day CoQ10 for 2 months has an important role in attenuating MDA and TNF-α levels. It appears that oral CoQ10 supplement can be applied as an adjunct treatment in rheumatoid arthritis patients. Evaluation of other parameters related to oxidative stress and inflammation including enzymatic and nonenzymatic antioxidants, different metabolites of oxidative stress, anti-inflammatory and other proinflammatory cytokine levels using longer clinical trials with different doses of CoQ10 in RA patients may be necessary to confirm results of the present study.

Acknowledgments
This research formed part of the Ph.D. thesis of Hadi Abdollahzad (No. D/38) and was supported by a grant from research vice chancellor of Tabriz University of Medical Sciences (No. S97/3196). We thank the research vice chancellor of Urmia University of Medical Sciences and Dr. Seyed Mostafa Seyed Mardani for collaboration in patient recruitment. We express our appreciation to all patients who participated in the study.

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