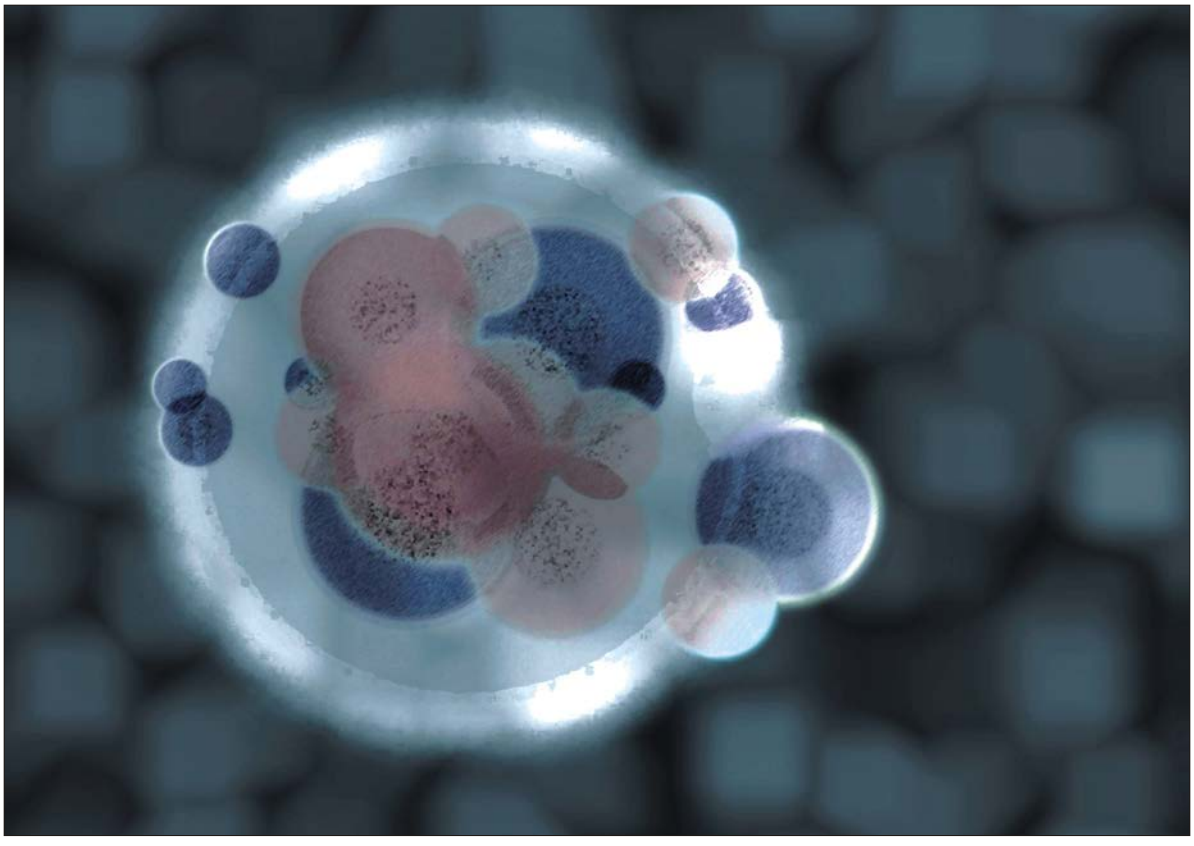

Journal of Biological Sciences

ISSN 1727-3048

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Journal of Biological Sciences 10 (1): 63-66, 2010
ISSN 1727-3048

Effects of Antioxidant Supplementations on Oxidative Stress in Rheumatoid Arthritis Patients

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Abstract: Reactive Oxygen Species (ROS) play an important role in the pathogenesis of Rheumatoid Arthritis (RA) exposing these patients to oxidative stress. The aim of the present study was to evaluate the effects of antioxidant supplementations on oxidative status and disease activity in RA patients. Forty nine RA patients (41 females, 8 males, age 48.78±12.54 years) participated in this randomized clinical trial. Patients were randomly divided into two groups to received antioxidant supplementations in combined with conventional treatment (Group I, n: 24) or conventional treatment only (group II, n: 25) for 12 weeks. Plasma concentration of malondialdehyde (MDA) and Total Antioxidant Capacity (TAC) were measured at the beginning of the study and after intervention in both groups. Disease activity was also measured before and after intervention using Rheumatoid Arthritis Disease Activity Index (RADAI). Supplementation with antioxidant yielded significantly decreased in plasma MDA concentration ($p<0.0001$) and disease activity ($p<0.0001$) and statistically increased in TAC levels ($p<0.0001$) in group I in comparison to group II after 12 weeks. This study indicates that antioxidant supplementations may play an important role in improving oxidative stress and decreasing disease activity in these patients.

Key words: Rheumatoid arthritis, oxidative stress, antioxidant supplementations

INTRODUCTION

Rheumatoid Arthritis (RA) is an autoimmune disorder with unknown etiology that is associated with increased mortality risk (Sezgin *et al*., 2005). This disease affects approximately 1-2% of the general population worldwide (Shivani *et al*., 2003). The most major characteristics of RA include hyperplasia of synovial lining cells, predominant synovial proliferation, bone destruction and articular cartilage degradation (Kamanli *et al*., 2003; Gulden *et al*., 2005). Several evidences suggest that Reactive Oxygen Species (ROS) and oxidative stress are involved in pathogenesis of RA (Sezgin *et al*., 2005; Kamanli *et al*., 2003). Epidemiologic studies have shown that antioxidant system is impaired in RA and ROS can not remove effectively (Cimen *et al*., 2000; Gambhir *et al*., 1997). Because of impaired antioxidant system it seems that RA patients are exposed to lipid peroxidation which is one of the indicators of oxidative stress (Gulden *et al*., 2005; Kamanli *et al*., 2003; Ozturk *et al*., 1999).

Most studies indicate that malondialdehyde (MDA) as a product of lipid peroxidation will increase in the serum, plasma and synovial fluid in RA (Kamanli *et al*., 2003; Sang-Cheol *et al*., 2003; Gambhir *et al*., 1997). Moreover, an inverse association between serum antioxidant levels and inflammation were reported before (Paredes *et al*., 2002) and RA patients have lower levels of serum antioxidants, including vitamin E, vitamin C, β-carotene, selenium and zinc in comparison to healthy person (Sang-Cheol *et al*., 2003). Meanwhile, some studies have shown that, the use of antioxidant may improve RA clinical symptoms (Sang-Cheol *et al*., 2003). However, the results of studies are inconsistent and few studies evaluate the relationship between decreased

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oxidative stress and improvement of disease activity in RA. So, to determine the effectiveness of antioxidants in controlling oxidative stress, this study was designed to examine the effects of antioxidant supplements on oxidative stress and disease activity in rheumatoid arthritis patients.

MATERIALS AND METHODS

Study plan was approved by the ethics committee of the Health Faculty in Iran University of Medical Science. The study was done in the Rasoul-Akram Hospital (Educational hospital of Iran University of Medical Sciences) between late 2007 to early 2008. 60 inactive RA patients (age 48.78±12.54 years) fulfilled the revised American College of Rheumatology (ACR) criteria for RA. Patients with any history of chronic disorders such as renal disease, diabetes mellitus, hepatic disease, hypertension, dyslipidemia, inflammatory disease, infection, malnutrition and obesity, smoking or alcohol habits, consumption of any antioxidant supplements already or had in the previous month were excluded. Eleven patients withdrew during the study and forty-nine patients completed the study. Written informed consent was obtained from all patients.

All patients were randomly divided into two groups. Group I (n = 24, 20 females and 4 males) received conventional treatment plus antioxidant supplementations including 300 mg vitamin C, 5 mg zinc daily and 25000 IU vitamin A every other day for 12 weeks and group II (n = 25, 21 females and 4 males) received conventional treatment (including methotrexate, prednisolone, sulfasalazine and chloroquine) for the same duration only. All patients of two groups received methotrexate (MTX) and prednisolone and some patients received sulfasalazine (37.5 and 40% patients in group I and II, respectively) or chloroquine (41.7 and 40% patients in group I and II, respectively) combined with MTX and prednisolone. Anthropometric indices, including body weight, height and body mass index (BMI, kg m^{-2}) and energy and nutrients intake (specially, protein, carbohydrate, fat, fiber, vitamin A, C, E and zinc) were measured before and after intervention. All of consumed food items measured by two-day, 24 h dietary recall questionnaires (usual and vacation day) and food frequency questionnaires. Patients were asked to state the consumption of each food item according to the defined serving size. Nutrient items converted to gram and analyzed by Food Processor (FP) program III (Food processor III nutrition system. Salem, Oregan, USA, 1987). Rheumatoid Arthritis Disease Activity Index (RADAI), an established measure for the evaluation of RA activity, was determined before and after intervention in both groups. Fasting blood samples (5 mL) were taken before and after intervention. Blood samples were collected into tubes containing EDTA (1 mg mL^{-1}). Plasma MDA content was measured by the spectrophotometric method based on the reaction between thiobarbituric acid with MDA (Satoh, 1978). Plasma Total Antioxidant Capacity (TAC) levels were determined by the Ferric Reducing Ability of Plasma (FRAP) assay (Benzie and Strain, 1996).

Statistical analysis was carried out using statistical package for social sciences (SPSS 12.0 for Windows, SPSS Inc. ® Headquarters, Chicago, USA). Values were expressed as Mean±SD. Independent-sampled t-test was used for comparisons between the two groups and paired-sampled t-test was used to compare the differences between different time points in each group. P-value less than 0.05 was regarded as significant differences.

RESULTS

Table 1 shows the demographic and anthropometric characteristics of RA patients in the two groups. At the beginning of study, we have not found any statistical differences in regard of mean age, sex, mean disease duration, type of drug consumption, body weight, Height and BMI between two groups. Meanwhile, after intervention, no differences were seen between body weight, height and BMI of two groups.

Analysis of the daily nutrients intake, shown in Table 2, reveals that there were no significant differences in regard of mean of energy, protein, carbohydrate, fat, fiber, vitamin A, C, E and zinc intake between two groups, before and after intervention.

Table 3 shows the TAC and MDA levels and disease activity in two groups. No significant differences were observed in regard to MDA, TAC and disease activity between the groups at the beginning of study. But there was a statistically significant decrease in the concentration of MDA in group I and II after intervention ($p<0.0001$). Meanwhile, MDA reduction was significantly higher in group I ($p<0.0001$). Plasma TAC levels were statistically increased in Group I and II ($p<0.011$, $p=0.041$, respectively) at the end of 12 weeks. However, the

Table 1: Demographic and anthropometric characteristic of RA patients in two groups

Variable	Group I (n = 24)	Group II (n = 25)	p-value
Age (year)	48.79±12.61	48.76±12.72	0.993
Sex (M/F)	4/20	4/21	1*
Disease duration (month)	59.33±32.40	65.04±31.43	0.535
Height (cm)	159.25±7.58	160.08±8.48	0.720
Weight (kg)			
Before	66.5±7.44	65.72±8.55	0.735
After	66.2±7.28	65.48±8.59	0.751
BMI (kg m^{-2})			
Before	26.30±3.14	25.72±3.37	0.536
After	26.15±3.18	25.62±3.4	0.575

Values are given as Mean±SD; *Fisher test

Table 2: Daily intake of nutrients of RA patients in two groups

Nutrients	Group I (n = 24)	Group II (n = 25)	p-value
Energy (Kcal)			
Before	1526.90±558.10	1335.46±430.21	0.184
After	1409.31±493.58	1349.22±495.24	0.673
Protein (g)			
Before	61.29±19.90	57.77±17.87	0.518
After	58.76±22.25	55.17±17.69	0.535
Carbohydrate (g)			
Before	236.76±108.25	207.22±72.88	0.27
After	228.20±88.49	217.76±96.36	0.695
Fat (g)			
Before	33.32±13.83	34.61±17.3	0.774
After	34.68±17.24	34.38±17.34	0.952
Fiber (g)			
Before	15.83±8.96	12.94±5.97	0.19
After	13.80±8.27	12.47±7.18	0.551
Vitamin A (µg)			
Before	604.75±524.26	614.27±530.81	0.95
After	685.6±594.09	517.335±398.52	0.248
Vitamin C (mg)			
Before	87.58±60.35	73.55±65.73	0.981
After	62.63±39.95	63.19±57.15	0.965
Vitamin E (µg)			
Before	2.39±0.78	4.28±4.72	0.06
After	2.38±1.00	2.90±2.43	0.335
Zinc (µg)			
Before	6.16±2.22	6.17±1.82	0.445
After	6.32±5.43	5.43±1.95	0.16

Values are given Mean±SD. Difference between groups is compared with independent t-test., Difference in each group before and after intervention are compared with a paired t-test

Table 3: Plasma MDA, TAC and disease activity (RADAI) of RA patients in two groups

Nutrients	Group I (n = 24)	Group II (n = 25)	p-value
MDA (nmol mL^{-1})			
Before	3.15±0.602	3.27±0.75	0.537
After	1.92±0.65	2.75±0.681	<0.0001[1]
Change	-1.23±0.46	-0.525±0.28	<0.0001[2]
TAC (µmol L^{-1})			
Before	1458.55±600±64	1459.49±532.81	0.995
After	1942.86±721.23	1577.95±470.23	0.011, 0.043[1]
Change	484.31±232.22	118.45±214.05	<0.00012
RADIA			
Before	5.06±1.32	4.96±1.23	0.829
After	2.59±0.95	3.52±1.22	0.005[1]
Change	-2.46±0.66	-1.44±0.75	<0.0001[2]

Value are given as Mean±SD. Difference between groups is compared with independent t-test. Before and after values within the groups are compared with paired t-test. [1]Significantly difference, before and after intervention in each group. [2]Significantly difference, between changes in two groups

increase was significantly higher in group I ($p<0.0001$). Also, the RADAI score was significantly decreased in group I and II ($p<0.005$) at the end of 12 weeks, but the decrease of RADAI score was significantly higher in group I ($p<0.0001$).

DISCUSSION

Many studies show that ROS play an important role in pathogenesis of RA (Sezgin *et al*., 2005; Kamanli *et al*., 2003). Macrophages, neutrophils and lymphocytes are present in synovial fluid in high levels which produce ROS (Gulden *et al*., 2005). The increased ROS is due to oxidative stress and lipid peroxidation. In this study, we used MDA concentration as an indicator of oxidative stress and our results showed that in comparison with MDA concentration in normal control group of similar studies, plasma MDA levels were higher in patients of our study (Sezgin *et al*., 2005; Gambhir *et al*., 1997; Karatas *et al*., 2003; Taysi *et al*., 2002; Cimen *et al*., 2000; Ozgunes *et al*., 1995). One of possible reason that MDA concentration in our patients was higher than normal persons in comparison to previous studies is: In reviewing the literature, Hakimi *et al*. (2006) detected 17-43% of population has zinc deficiency in different regions of Iran. Meanwhile, we have marginal vitamin A deficiency at the national level and sub clinical vitamin A deficiency in many part of country in different age and sex groups (WHO, 2009) and one of recent national dietary survey shows that 30.9, 40 and 42% of population in the country had less than 70% of their daily needs of vitamin C, Vitamin B_2 and Vitamin A intake, respectively. Also, other studies show that RA patients have lower serum levels of these nutrients (Sang-Cheol *et al*., 2003) and there is an inverse association between serum antioxidant levels and inflammation in these patients (Paredes *et al*., 2002). Therefore, because of roles of these nutrients as antioxidant and probability of their deficiency, it seems that there is increased lipid peroxidation in these patients and thus supports the need for studies evaluating the role of antioxidant as free radical scavengers in RA patients. In this study we found that the concentration of plasma MDA was decreased in both groups after intervention, although, MDA reduction was significantly higher in group I that received antioxidant supplementations combined with conventional drugs. Similar results were also reported by other study (Shivani *et al*., 2003).

Also, we measured TAC as a marker of plasma antioxidant status. Present results showed that TAC levels were statistically increased in the two groups after 12 weeks, but supplementation with antioxidants resulted in a much higher increase in TAC levels in group I in comparison to group II. These results were similar to other study (Shivani *et al*., 2003), but in Shivam's study, instead of measuring of TAC levels, other markers of antioxidant status including vitamin C, glutathione and thiol concentrations were measured and there was a statistically significant increase in the post treatment concentrations of these antioxidant markers. However, the increase was significantly higher in patients on antioxidant therapy. Because dietary antioxidants may affect antioxidant status, so in contrast to previous studies, we assessed dietary intake of antioxidant nutrients (especially, vitamin A, C and zinc) in patients of two groups before and after intervention. No significant differences, were observed, demonstrating that

antioxidant intake of patients in two groups are almost similar, thus significant increased TAC levels in group I mainly resulted from supplementation therapy.

Finally, we evaluated the effects of antioxidant supplementation on disease activity. Present results showed that RADAI score was significantly decreased in both groups after intervention, although, decrease of RADAI score was significantly higher in group I that received antioxidant supplementation plus conventional treatment. Similar results have been observed by other studies (Shivani *et al*., 2003). Thus, it is obvious that oxidative stress can affect disease activity in RA patients. Increased TAC levels associated with decreased MDA concentration and disease activity score were found also in group II patients, suggesting that the drug treatment reduces the oxidative stress in RA patients, although, use of antioxidants as supplements with conventional drugs bring about better results.

In conclusion, our results confirm the role of oxidative stress in RA patients and indicates that antioxidant supplementation play an important role in controlling oxidative stress and decreasing disease activity in these patients. However, these findings suggest the need for designing more randomized controlled clinical trials to evaluate the impact of antioxidant therapy in different stage of disease activity for the treatment of RA.

ACKNOWLEDGMENTS

The study was supported by a grant from the office of the Vice Chancellor for Research, Iran University of Medical Sciences. We are grateful to the Cellular-Molecular Research Center at the Iran University of Medical Sciences and the patients from the Rasol Akram Hospital for making this study possible. Also, the authors thank Dr. Mohammad A. Hafezi for assistance with the study design.

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