Beneficial Role of Antioxidants on Clinical Outcomes and Erythrocyte Antioxidant Parameters in Rheumatoid Arthritis Patients

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ABSTRACT

Background: This study aims to investigate the effect of antioxidants supplement on clinical outcomes and antioxidant parameters in rheumatoid arthritis (RA).

Methods: The pre-post study was conducted on 40 female patients with RA in 12 weeks that taken daily one "Selenplus" capsule contained 50 μg selenium, 8 mg zinc, 400 μg vitamin A, 125 mg vitamin C, and 40 mg vitamin E. About 5 mL venous blood sample was taken from all participants and disease activity score (DAS) was determined by DAS-28 formula and high-sensitive C-reactive protein (hs-CRP). Glutathione peroxidase (GPX) and superoxide dismutase (SOD) were measured by spectrophotometric kit and catalase (CAT) was measured by Abei method. Total antioxidant capacity (TAC) was determined by spectrophotometric kit. Distribution of the variables was assessed using histogram with normal curve as well as Kolmogorov-Smirnov test and data were analyzed with paired *t*-test for differences between pre-post data using SPSS software version 13.5.

Results: Out of 40 patients, 39 completed the study. DAS-28 score and hs-CRP have changed ($P < 0.01$ for both), while the number of swollen and painful joints did not reduce significantly. TAC, GPX, SOD, and CAT increased significantly ($P < 0.01$ for all variables).

Conclusions: Our findings showed that antioxidants may improve disease activity significantly, but it did not affect the number of painful and swollen joints and increased erythrocyte antioxidant levels. Antioxidants may be useful for controlling of clinical outcomes and oxidative stress in RA.

Keywords: Antioxidants, dietary supplements, nutrition, oxidative stress, rheumatoid arthritis

Original Article

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease that about 0.5%-1% of world population are affected by its complaints.[1] The prevalence of RA in females is 3 times

higher than males. The RA affects blood vessels, heart, lungs, muscles, and joints, resulting in bone deformity and osteoporosis.[2]

Several studies have reported that oxidative stress and production of oxygen-free radicals have important role in RA development[3,4] and epidemiologic studies have revealed a reverse relationship between dietary intake of antioxidants and RA incidence[5,6] and due to reduction of intake and absorption of dietary antioxidants in RA patients, the levels of blood antioxidants are decreased too. The antioxidants supplements such as vitamin E,[7,8] vitamin C,[4,9] and selenium[10,11] may control the disturbance of lipid peroxidation and loss of antioxidants markers in patients with RA. Vitamin E can interact with nitric oxide and may trigger the gene expression of catalase (CAT), glutathione peroxidase (GPX), and superoxide dismutase (SOD) enzymes,[12] vitamin C may demolish the peroxides of macrophage activities, zinc may strengthen the immune system,[13] and selenium has an important role as a cofactor of GPX enzyme in reduction of oxidative stress.[14]

In past 30 years, controlled trials have conducted to compare the effect of dietary antioxidants and antioxidant-rich diets in controlling of RA clinical outcomes;[15,16] however, they could not find a clear statement about antioxidants in RA prevention and treatment due to difference in study period, dose, and different types of antioxidants.[12] Regarding to integrity of antioxidant defense system and few clinical studies on combined antioxidant supplements in RA, the aim of this study is to evaluate the effect of combined antioxidant supplements as daily oral capsule on clinical outcomes and antioxidant parameters in female patients with RA for 3 months.

METHODS

Study design and patients

A pre-post clinical trial was conducted on female patients with RA for 12 weeks. The study group was selected from 400 registered RA patients in Sheikh-al-Rais and Sina clinics of Tabriz University of medical sciences, Iran according to inclusion criteria. The inclusion criteria were RA diagnosis by rheumatologist according to American College of Rheumatology guidelines-1987, 40-60 years old, no change in treatment approach in past 2 months. The exclusion criteria were diabetes mellitus, hypertension, thyroid disorders, liver and kidney failure, Cushing syndrome, severe infection, gastric illnesses, smoking, and exposure to daily smoking at home. We followed-up the intake of daily supplement use and type and dose of medications by regular phone calls, so change in type and dose of drugs and antioxidant supplement resulted in omission from study.

We asked from selected patients to take daily a "selenplus" capsule (Eurovital pharmaceutical company, Germany) that contained 50 μg selenium, 8 mg zinc, 400 μg vitamin A, 125 mg vitamin C, and 40 mg vitamin E. The supplement has been given to patients without trading label. After explanation of the study risks and benefits, written consent form was taken from all subjects. Registration code of the local ethics committee of Tabriz University of Medical Sciences is 8912 and registration number in the registration center for clinical trials in Iran is IRCT138901183655N1. In the case of any side effects, patients could leave the study and dose of each antioxidant nutrient was at the Recommended Dietary Allowances amount.

Procedure

At the beginning of the study, accurate clinical examination such as counting the swollen and painful joints was done by a rheumatologist and the validated DAS-28 form was filled to calculate disease activity index[17] according to following formula.[18]:

$$DAS-28-CRP(3) = [0.56 * Sqrt(TYC28) + 0.28 * Sqrt(SYC28) + 0.36 * Ln(CRP+1)] * 1.10 + 1.15$$

TYC28: Number of painful joints, SYC28: Number of swollen joints, Sqrt: Square, Ln: $\log_e$

Also, dietary intake questionnaire including food frequency questionnaire (FFQ) and 24 h recall questionnaire for 3 days (2 working days and 1 weekend) were completed by an expert nutritionist. The FFQ was composed of 168 food items that assessed the frequency of the intake in day, month, season, and year semiquantitavely. The recall questionnaire was a dietary detailed form in 6 parts: Breakfast, lunch, and dinner with three snacks that filled by face to face interview about the type and amount of the food items. Dietary intake of energy,

macronutrients, and antioxidant micronutrients analyzed by nutritionist III software (MAM soft research Co, USA 1993). The weight of patients was measured by digital scale (after calibration and without shoes), the height was measured by stadiometer (after attachment of 4 points of body to the wall) and the body mass index calculated by Quetelet formula. The patients were followed-up every 2 weeks by phone calls and the measurements were repeated after 3 months.

Five milliliter fasting venous blood samples (8-12 h after fasting) were taken from all participants and were kept in -70°C freezer (Snider's, Germany) until conducting biochemical measurements. Biochemical measurements including GPX and SOD were measured by spectrophotometric kit (Ransel, Randox laboratories ltd, UK) and autoanalyzer apparatus (Abbott, model Alcyon 300, USA) and CAT was measured by Abei method.[19] TAC was determined by spectrophotometric kit (Randox TAC kit, Randox laboratories Ltd, UK). Serum high-sensitive C-reactive protein (hs-CRP) was quantified by photometric kit (Pars Azmoon Company Ltd, Iran).

Statistical analysis

SPSS software version 13 (SPSS for windows, Chicago, IL, USA) was used for statistical analysis. Distribution of data was tested by Q-Q plot and Kolmogorov-Smirnov test. For parametric data, paired *t*-test and in case of nonparametric data, Wilcoxon signed rank test was used. The linear regression model was used for adjusting confounding factors such as dietary intake of some selected nutrients. $P < 0.05$ was defined significant.

RESULTS

A total of 39 patients sustained in the study after 12 weeks. The baseline characteristics and dietary intake have been reported in reference no. 20.[20] One was left in reason of unrelated medical problem. Table 1 indicates basic characteristics of the subjects at the start point of trial and the median of the duration of the disease was 72 months [Table 1]. The pharmacotherapy regimen did not change during the period of the study in the selected patients and any changes in the dose and type of the drugs resulted in the omission of the study. Dietary intake of energy and selected nutrients during 12 weeks of intervention did not differ significantly [Table 2] and in the linear regression findings, no significant linear relationship between dietary antioxidants values with biochemical indices was observed.

In our study, DAS-28 score and serum hs-CRP have changed during 12 weeks of intervention ($P < 0.01$), while the number of swollen and painful joints did not change significantly [Table 3]. The antioxidant markers of patients including TAC, GPX, SOD, and CAT increased significantly after 12 weeks supplementation ($P < 0.01$) [Table 4].

DISCUSSION

In our study, antioxidants supplement for 12 weeks reduced significantly serum hs-CRP and DAS-28 score. The literature review indicates that zinc and selenium supplementation have been used in RA remission and prevention for several years[21] and the similar results of these studies were resulted from multicomponent antioxidants and nutrients as Koracevic *et al.*,[22] showed concurrent supplementation with 37.5 mg vitamin E, 150 mg vitamin C, 1.4 g eicosapentaenoic acid, 0.2 g docosaenoic acid, and 0.5 g gamma linolenic acid could not significantly reduce the number of swollen and painful joints.[22] As the results of alike studies revealed intake of simultaneous antioxidant micronutrients can have a helpful effect against RA progress.[22] In another similar study, 300 mg vitamin C, 5 mg zinc, 25000 International Unit vitamin A for 12 weeks reduced the disease activity ($P < 0.0001$).[23] Also, Pretez *et al.*,[10] study showed that 12 weeks selenium supplementation decreased the number of swollen and painful joints; however, the results were not statistically significant. Some studies have used higher doses of one antioxidant although they did not observe significant improvement in clinical outcomes.[24] It seems that the reason of these findings is due to no increase in antioxidants levels in polymorphonuclear leukocytes and antioxidant defense system in blood cells.[25] As Onal *et al.*,[26] study indicated the pharmacotherapy in patients with RA results in lower levels of zinc and selenium and higher levels of copper in red blood cells, so intake of oral drugs such as corticosteroids and chloroquine elevates the required amount of the antioxidants to suppress inflammatory-like substances.

Table 1: The basic characteristics of subjects in the study

Variables	Study group
Gender (number)	Female (40)
Age (years)*	52.6±5.3
Weight (kg)*	71.1±11.6
Height (cm)*	156.9±7.2
Body mass index (kg/m^2)*	28.8±4.1
Disease duration (months)‡	72 (18, 420)
Oral prednisolone use†	35 (87.5)
Oral methotrexate use†	34 (85)
Oral sulfasalazine use†	6 (15)
Oral chloroquine use†	17 (42.5)
Oral cyclosporine use†	1 (0.025)
Local or oral NSAIDs use†	4 (10)
Oral Imuran use†	1 (2.5)

*Mean±standard deviation, †Number (%), ‡Median (min, max), NSAIDs: Nonsteroidal antiinflammatory drugs

Table 2: Dietary intake of selected nutrients before and after 12 weeks intervention

Variables*	Before the study (*n*=40)	After the study (*n*=39)	*P* value†
Energy (kcal)	1534.5 (101, 5253)	1603.0 (797, 3741)	0.59
Protein (g)	48.3 (15, 123)	43.3 (16.8, 122)	0.45
Carbohydrate (g)	195.0 (84.9, 535)	183.0 (76.6, 563)	0.95
Fat (g)	75.8 (21.7, 149)	73.5 (17.8, 363)	0.83
Zinc (mg)	5.2 (1.7, 13)	5.1 (0.3, 14.5)	0.76
Vitamin A (IU)	4699.5 (133, 353 12)	3998.0 (239, 33375)	0.49
Vitamin E (mg)	32.1 (2.9, 83.7)	32.5 (2.8, 317.0)	0.44
Vitamin C (mg)	102 (15.1, 446)	94.0 (18.4, 361.0)	0.79

*Median (min, max), †Wilcoxon rank test
IU: International unit

Table 3: The changes of clinical outcomes in subjects of study before and after 12 weeks intervention

Variable	Before the study (*n*=40)	After the study (*n*=39)	*P* value
Disease activity score-28*	2.71±1.19	2.65±1.17	0.019†
Number of painful joints‡	1 (0, 17)	1 (0, 14)	0.839
Number of swollen joints‡	0 (0, 15)	0 (0, 14)	0.736
High-sensitive C-reactive protein (mg/L)§	5.50±0.5	4.20±0.51	0.003†

Mean±standard deviation, paired *t* test,†Significant differences in *P*<0.01, ‡Median (min, max), Wilcoxon signed rank test, §Mean±standard deviation, paired *t* test

Table 4: The changes of erythrocyte antioxidant parameters in subjects of study before and after 12 weeks intervention

Variable	Before the study (*n*=40)*	After the study (*n*=39)*	*P* value
Glutathione peroxidase (U/L)	284.42±11.76	288.27±12.13	0.011†
Superoxide dismutase (U/L)	2.94±0.31	3.00±0.30	0.009†
Catalase (U/L)	21.74±3.96	23.90±6.46	0.008†
Total antioxidant capacity (mmol/L)	1.02±0.23	1.13±0.27	<0.001†

*Mean±standard deviation, paired t-test, †Significant differences in *P*<0.01

In our study, erythrocyte antioxidant markers including TAC, GPX, SOD, and CAT increased significantly during 12 weeks supplementation due to probable direct effect of oral antioxidants on antioxidants levels. Similarly, Shinde *et al.*,[27] have shown that 400 mg vitamin E and 500 mg vitamin C could increase the reduced form of erythrocyte glutathione ($P < 0.001$), probably because vitamin E is the most important fat-soluble antioxidant and protects the cell membranes against oxidative stress just as vitamin C preserves cytosol and membranes of free radicals activity.[28] Furthermore, the results of VanVugt *et al.*,[29] study indicated that 400 mg alpha-tocopherol, 10 mg lycopene, 5 mg alpha carotene, 10 mg lutein, and 200 mg vitamin C for 12 weeks increased plasma levels of vitamin E, lycopene, lutein, alpha-carotene, and vitamin C and reduced F2-isoprstanes as the oxidative stress marker. Shah *et al.*,[30] illustrated that there is a strong association among the disease activity with antioxidant enzymes markers and they have showed that production of reactive oxygen substances can disturb the immune defense system and modulate inflammation processes to reduce the antioxidant molecules in blood cells. It seems that mixture of antioxidants help to reduce required dose of pain killer drugs and diminish the complaints of disease.

Since autoimmune diseases such as RA are accompanying with reduction of cellular immune

level that results in high coincidence of other chronic diseases, healthy antioxidant-rich diet can improve immune system and compensate the inadequate intake of micronutrients, especially antioxidant-rich supplies of RA patients in northwest of Iran.[27,28] Also, consumption of antioxidant micronutrients in the form of dietary items or supplements may be helpful in enhancement of enzymatic and nonenzymatic antioxidants due to strengthen the antioxidant defense system of the body.[29,30] Lack of the control group is the major limitation in this pre-post clinical trial due to limited financial support. One of the strengths is the high response rate of participants (97.5%) and low loss to follow-up during the intervention. Also, mild to moderate severity of RA was considered as a criterion of the study and the dietary intake of antioxidants was supposed as confounding factors.

CONCLUSIONS

The combined antioxidant supplement may improve DAS-28 score significantly, but it did not change the number of painful and swollen joints statistically significant during 12 weeks, while it could increase TAC, GPX, SOD, and CAT levels. It seems that supplementation with antioxidants may be useful as a complementary treatment in control of clinical outcomes and oxidative stress in patients with RA.

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