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The Effects of Antioxidants Supplementation on Asymmetric Dimethylarginine, Vascular Endothelial Growth Factor and C-reactive protein Levels in Female Patients with Rheumatoid Arthritis

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Abstract

***Background & Aims*:** Rheumatoid Arthritis (RA) is one of the common autoimmune diseases with unknown etiology. It has been suggested that antioxidant supplements may play a role in inflammation perpetuating process. This study examined the influence of combined antioxidant supplementation on serum asymmetric dimethylarginine (ADMA), vascular endothelial growth factor (VEGF) levels and high-sensitivity C-reactive protein (hs-CRP) as inflammatory markers in RA patients.

Materials & Methods: A three-month pre-post study was conducted on 40 female RA patients receiving one Selenplus capsule (Selenium 50µg, Zinc 8 mg, vitamin A 400 µg, vitamin C 125 mg and vitamin E 40 mg) daily. 5 ml venous blood samples were taken from all the participants before and after the administration period. The serum levels of ADMA, VEGF and high-sensitivity C-reactive protein (hs-CRP) were measured by standard methods.

***Results*:** 39 out of 40 patients completed the study. In comparison with the baseline, we did not find any significant differences between serum ADMA and VEGF values before and after the intervention. The 3-month use of SelenPlus supplementation resulted in decreasing hs-CRP level ($p<0.003$).

***Conclusion*:** The combined antioxidant supplements for 3 months decreased serum hs-CRP levels in RA patients that may be helpful in RA treatment. The hs-CRP reduction may due to the anti-inflammatory effects of Zinc and Selenium in SelenPlus supplement.

***Keywords*:** Rheumatoid Arthritis; Antioxidant; ADMA; VEGF; C-reactive protein

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Introduction

Rheumatoid arthritis (RA) is a chronic relapsing immune-mediated multisystem inflammatory disease

with predominant synovial proliferation and destruction of articular cartilage. It is the most common inflammatory arthritis affecting approximately 1–2% of the general population worldwide. The incidence increases with age and women being affected three times more than men (1).

Although the exact etiology of RA is not yet fully understood, oxidative stress and high reactive oxygen species (ROS) production have been implicated in its pathogenesis (2, 3). Oxidative stress in RA is due to the fact that the antioxidant systems are impaired and epidemiological studies have shown that low intake of dietary antioxidants is associated with the incidence of RA (4, 5). According to some clinical studies, antioxidant supplementation such as vitamins A, C, and E in addition to trace elements, Selenium (Se) and Zinc (Zn), may have significant roles in the management of RA (4-6).

In recent years, it has become clear that RA as a chronic inflammatory disease is associated with increased incidence of atherothrombotic cardiovascular events (7). Accumulation of Asymmetric dimethylarginine (ADMA) has been identified as independent predictors of future cardiovascular events in patients with coronary artery disease. Interestingly, it has been demonstrated that elevated ADMA levels can be detected in RA patients regardless of the presence of cardiovascular disease. In an earlier study, elevated ADMA levels were found in RA patients with subclinical carotid atherosclerosis (7).

It is apparent that autoimmune process begins in the initial stage of RA and inflammation within the joint structure can induce relative hypoxia of synovial tissue which may aggravate tissue injury by releasing hydrolytic enzymes, increasing vascular permeability and accelerating the inflammatory process. Because vascular endothelial growth factor (VEGF) which can be strongly induced by hypoxia, is a potent endothelial cell-specific angiogenic factor, the potential for VEGF production might be crucial in the initiation of RA (8). Based on recent studies, increased serum VEGF levels were correlated with disease activity in RA (8, 9). This study was designed to evaluate the effects of commercial antioxidant supplementation, "SelenPlus", on the clinical and inflammatory manifestations of RA. The assessment of supplementation-associated changes in ADMA and VEGF levels was beyond the scope of the current study.

Materials and Methods

Study design and patients selection:

400 RA patients recorded in the center of Sheikh al-Raeis and Sina Clinics of Tabriz University of Medical Sciences, and 40 women aged between 40 and 60 with RA based on proven inclusion criteria were included in this study.

Recognition of RA in the patients was confirmed by a rheumatologist according to the American College of Rheumatology (ACR) criteria which are as the following: (1) morning stiffness for at least an hour, (2) stiffness of more than three joints, (3) arthritis of hand joints, (4) symmetrical arthritis, (5) rheumatoid nodules, (6) positive rheumatoid factor, (7) typical clinical radiographic changes in wrists and hands; and that the patients have not changed their treatment protocol for at least 2 months.

The exclusion criteria include having diabetes mellitus, hypertension, thyroid disorders, renal failure, liver dysfunction, Cushing's syndrome, smoking as well as being exposed to smoke at home daily. Also, any changes in treatment protocol and drug therapy for any reason, led to omission from the study. The study was a pre-post design and the intervention period was three months. The patients were asked to take a daily dose of "SelenPlus" capsule (Euro vital Pharmaceutical Company, Germany) that contain Se (50 μg), Zn (8 mg), vitamin A (400 μg), vitamin C (125 mg) and vitamin E (40 mg). The containers of capsules did not have any commercial logo.

Before entering the study, a detailed clinical examination was performed by a rheumatologist and the following forms were filled in: personal data,

medications and food intake for 3 days (2 working days and a regular holiday). A trained nutritionist also filled in a food frequency questionnaire by analyzing the energy intake, macronutrients and antioxidant micronutrients by Nutritionist III software (MAM research soft co, USA).

Anthropometric indices including weight and height were also measured and Body Mass Index (BMI) was calculated. Weight was measured by a calibrated digital scale for each individual with minimal clothing and without shoes, and height was determined by a stadiometer according to the contact of 4 points of the body (back of the heel, hip, shoulder and head) with the wall by controlling the stadiometer precision for each measurement.

Five ml fasting venous blood samples (8-12 h after fasting) were taken from all the participants. The samples, then, stayed in the room temperature for half an hour in order to separate serum samples. All the samples were centrifuged (Hettich Universal, D-7200; Germany) at 3000 g for 15 min and the sera were then stored at -70°C (Snider's, Germany) until conducting biochemical measurements. Following the patents' taking the supplements, ongoing calls were taken every 2 weeks. After 3 months of intervention, the process before biochemical measurements was repeated.

Biochemical measurements:

The serum levels of VEGF were measured by using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Immuno-Biological Laboratory Co. Japan). The serum levels of ADMA were also measured by an ELISA kit (Wuhan EIAab Science Co. Ltd. China). High-sensitivity C-reactive protein (hs-CRP) was determined by immunoturbidometry method using the Pars-Azemoon kit (Pars Azmoon Co. Tehran, Iran).

Statistical analysis:

SPSS statistical software version 13 (SPSS for windows, Chicago, IL, USA) was used for statistical analysis. In order to evaluate normal distribution of the data, Q-Q plot and Kolmogrov-Smirnov test were used. In order to analyze the mean differences of before-after data, the paired t-test was used for parametric data and wilcoxon-rank test was used for non-parametric data. Linear regression model was used to adjust for confounding factors. All the tests were done two-sided and the p-value of less than 0.05 ($p<0.05$) was considered significant. Qualitative data were expressed as median (minimum, maximum).

Ethical issues:

After calling and explaining the method of performing the intervention for each patient, written informed consent forms were collected from all the participants. The registration code of the local ethics committee of Tabriz University of Medical Sciences was 8912 and the registration number in the registration center for clinical trials in Iran was IRCT201203116934N2. In case of any side effects, patients could leave the study and the prescribed dose of each antioxidant nutrient was kept at the Recommended Dietary Allowances (RDA) level.

Results

One of the 40 patients was removed from the study due to unrelated medical problems. The mean age and BMI of the patients were 52.6 ± 5.3 (years) and 28.85 ± 4.11 (kg/m^2), respectively (Table 1). As shown in Table 2, three months after the SelenPlus supplementation, there were significant ($p<0.05$) changes in disease activity score-28 (DAS-28) and hs-CRP levels but demographic characteristics, ADMA and VEGF levels did not alter significantly ($p>0.05$).

Table 1. Demographic characteristics of female RA patients in the baseline of the study

Variables	Study group
Gender (number)	Female (40)
Age (years)*	52.6±5.3

Variables	Study group
Weight (kg)*	71.1±11.6
Height (cm)*	156.9±7.2
BMI (kg/m2)*	28.85 ± 4.1
Disease duration (months) ‡	72 (18, 420)
Prednisolone use†	35 (87.5)
Methotrexate use†	34 (85)
Sulfasalazine use†	6 (15)
Cloroquine use†	17 (42.5)
Cyclosporine use†	1 (2.5)
NSAIDs use†	4 (10)
Imuran use†	1(2.5)
Other drugs use †	8 (25)

BMI, body mass index; NSAID, nonsteroidal anti-inflammatory drugs; RA, rheumatoid arthritis

* mean±SD; ‡ median (min, max); † number (percent)

Table 2. Clinical and laboratory variables in female RA patients before and after the antioxidant intervention

Variables	Before Intervention	After Intervention	p-value
DAS-28	2.71 ± 1.19	2.65 ± 1.17	0.019*
hs-CRP (mg/L)	5.50 ± 0.51	4.20 ± 0.51	0.003*
ADMA (μmol/L)	0.72 ± 0.09	0.71 ± 0.14	0.675
VEGF (pg/mg)	376.51 ± 207.97	390.49 ± 187.29	0.492

ADMA, asymmetric dimethylarginine; DAS-28, disease activity score-28; hs-CRP, high-sensitivity C-reactive protein; RA, rheumatoid arthritis; VEGF, vascular endothelial growth factor

* Significant ($p<0.05$)

Discussion

Previous studies have estimated the global prevalence of RA was about 0.24% with no discernible change from 1990 to 2010 and it has been shown that antioxidant supplements may play a role in inflammation perpetuating process (10).

Antioxidant supplementation is one of the effective solutions in the treatment of RA (1, 11). The exact etiopathogenesis of RA is not yet understood but recent researches implicate ROS as mediators of the disease (12, 13). Many different pathways can lead to oxidative stress and increase ROS production in inflamed joints of RA patients. Increased ROS can gradually lead to tissue damage and have important role in inflammation perpetuating process in rheumatoid synovium (1, 14, 15). Several defense mechanisms have evolved to protect tissues from oxidative damage including intracellular enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase and other peroxidases (3). The failure of these endogenous

defense mechanisms in keeping pace with oxidant generation may either be due to decrease in antioxidant defense or increased generation of ROS as is the case in RA (1, 16). In addition to the endogenous antioxidant defense mechanisms, exogenous antioxidants can also protect cellular systems from the damaging effects of ROS (6, 11, 17). The main antioxidant micronutrients as exogenous protectors are vitamin A (retinol and metabolites), vitamin C (ascorbic acid), vitamin E (α-tocopherol), Se and Zn (3, 18).

Vitamin A, a water-soluble vitamin, is a free-radical scavenger that controls the propagation of reactive species and influences lipoxygenase activity. Vitamin C, one of the first lines of defense from oxidative stress, can prevent lipid peroxidation by trapping water-soluble peroxyl radicals before their diffusion into lipid membranes; it also reacts with superoxide, peroxy, and hydroxyl radicals, and is important in recycling other antioxidants such as vitamin E. Vitamin E has lipid soluble properties that allow it to act as a chain-breaking reagent in lipid peroxidation (3).

Zn is an anti-oxidant with anti-inflammatory properties. It is a cofactor of SOD. The role of Zn in SOD is generally thought to be that of a stabilizing component. The other antioxidative function of Zn is the binding to and stabilization of protein thiols. These thiols are stabilized by Zn, and the enzyme is rendered less prone to inactivation by oxygen (19). Also, Zn plays a critical role in the immune system where it helps to regulate the production and activity of T-lymphocytes and natural killer cells. Se is required in very small amounts and it is a powerful antioxidant. It is part of the GPx that works closely with the vitamins C and E to neutralize ROS (21). It also attenuates inflammatory responses and is required for immune system function. Zn and Se appear to have positive synergistic effects. Supplementation of these trace elements may decrease oxidative stress and inflammatory status (21). In the present study we determined the effect of combined antioxidant supplementation of "SelenPlus" (containing Se, Zn, Vitamins A, C and E) on the disease activity and the serum levels of VEGF and ADMA in RA female patients. The disease activity was evaluated via DAS-28. After 3 months of "SelenPlus" intervention, a significant reduction in DAS-28 was observed. In the study of van Vugt et al. (22), a significant reduction in DAS-28 was observed following antioxidants intervention of 10 weeks [a mix of a-tocopherol (400 mg), lycopene (10 mg), palm oil carotenoids (5 mg; mainly α-carotene), lutein (10 mg) and vitamin C (200 mg)]. This was accompanied by significant increases in blood levels of the antioxidants administered. In a study by Peretz et al. (23), there was no significant difference in the measured clinical outcomes between the Se and placebo groups. Our study also revealed that hs-CRP level as a sensitive inflammatory marker decreased significantly after 3 months of intervention with antioxidant supplements. This result may be due to the anti-inflammatory effects of Zn and Se in SelenPlus after 3 months of supplementation (21). Contrary to our study, Bae et al. (24) showed that dietary supplementation of antioxidants for 4 weeks did not change the disease severity and the inflammatory markers [tumor necrosis factor-alpha (TNF-alpha), interleukin-1 beta (IL-1 beta), interleukin-6 (IL-6), and CRP] in the RA patients under conventional medical treatments.

The most important part of this study was the evaluation of antioxidant supplementation-related change in ADMA and VEGF levels. Kwaśny-Krochin B et al. (25) in their study demonstrated the associations between the markers of inflammation, oxidative stress and ADMA in RA. We observed that the serum concentrations of ADMA and VEGF were not significantly altered after three months of SelenPlus intervention. This may be due to the inadequate period of the antioxidant intervention (three months) to be effective on VEGF and ADMA levels in this study. But other reasons could also be considered. Antioxidant supplementation can lead to increase in nitric oxide (NO) bioavailability (25). NO has pivotal role in VEGF production (26). Recently, some evidences such as

Dulak et al. (27), Kuwabara et al. (28) and Abe et al. (29) showed that NO could induce VEGF synthesis. So, antioxidant supplementation may lead to increase in VEGF level indirectly by enhancing NO bioavailability. On the other hand, it has been demonstrated that ADMA is a potent inhibitor of NO synthase (NOS) and, therefore, decreases NO bioavailability (30). Therefore, this probable process may cause no significant changes in ADMA and VEGF serum levels of RA patients after antioxidant supplementation in the present study.

Some weaknesses of our study were: no measurement of stress oxidative markers such as total antioxidant capacity and the possible effects of consumed drugs in the RA patients on ADMA, VEGF and hs-CRP as well as DAS-28 levels.

Conclusion

In conclusion, according to the results of this study, antioxidant supplementation may lead to the relief of RA clinical symptoms by reducing inflammation and disease activity. The ADMA and VEGF levels did not change after three months of antioxidant intervention. This may be because of insufficient period of the intervention and/or due to the other related factors such as NO. These data are promising but further studies with a more period of antioxidant intervention and evaluation of additional and prominent related factors are needed to determine the exact effects of antioxidant supplementation on RA disease.

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Conflict of interest

The authors have declared no potential conflicts of interest.

The authors have no proprietary interest in any aspect of this study.

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