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The Effect of Antioxidant Supplement on Asymmetric Dimethyl Arginin and Vascular Endothelial Growth Factor in with Rheumatoid Arthritis Patients

Sousan Kolahi[1], Amir Ghorbanihaghjo[2*], Arezzo Ghasmebaglou[3], Mahsa Jalili[4], Amir Teimouri Dereshki[5]

1- Associate Professor of Rheumatology, Drug Applied Research Center, Collagen vascular research center, Tabriz University of Medical
2- Associate Professor of Biochemistry, Drug Applied Research Center, Tabriz University of Medical Sciences
3- Resident of Internal Medicine, Internal Medicine Department, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.
4- PhD of Nutrition, Faculty of Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran.
5. Medical student of faculty of medicine, Tabriz University of Medical Sciences
*_Corresponding author: Arezzo Ghasmebaglou (a_ghasmebaglou@yahoo.com)

Abstract: Oxygen free radicals have been implicated as mediators of tissue damage in patients with rheumatoid arthritis (RA). Purpose: This study was designed to elucidate the effect of complemented "SELEN PLUS" antioxidant supplementation on clinical manifestation and serum level of AsymEnetric Dimethyl Arginin (ADMA), high sensitive C-Reactive proteins (HS-CRP) and Vascular Endothelial Growth Factor (VEGF) in Female Patients with Rheumatoid Arthritis. Method: This is a clinical trial study, surveyed by choosing forty RA females between 40_60 ages with a moderate to severe disease. Disease activity scale (DAS- 28) and serum levels of ADMA, HS-CRP and VEGF were measured two times (before and after intervention). Results: In Patients, serum levels of Hs-CRP, ADMA, VEGF and HS-CRP were elevated in comparison with normal. (Pz0/003 and p=0/019) but VEGF and ADMA levels, number of stiffed joints and painful joints did not show significant changes after 3 months. In addition, there were a positive linear correlation between DAS-28 change score and Hs-CRP changes (with coefficient correlation of r=0/922 and p=0/001). Conclusion: Complemented "SELEN PLUS" antioxidant supplementation make significant change in Hs-CRP level but it does not make any significant changes in VEGF and ADMA levels in Female Patients with Rheumatoid Arthritis

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1. Introduction

Rheumatoid Arthritis (RA) is one of the most common chronic disorders and it is the main reason of most disabilities in elderly (Gill, 1995). RA is a systemic and chronic disorder (Monjamed and Razaviyan, 2007) which is associated with inflammatory changes in joints and immune system disorders (Poormoghim, 2007). During last 30 years, several clinical trials have studied the effects of different antioxidants on RA patients, and among them, effects of vitamin A, C, E and zinc had denoted more (Remans, 2004). Selenium as an antioxidant is a necessary element for GLOTATIUN PEROXIDASE normal function, and its antitoxic effect faces toxic oxygen radicals in body (Canter, 2007). Besides, zinc is needed for growth and immune system ordinary functions.

We know that Joint's synovial membrane grows and rises Panus, then Panus attacks joint cartilage. Because of nutritional needs of Panus, new vascular formation called Angiogenesis is the main process in Panus formation.

Prevention of Angiogenesis causes nutritional pinch for Panus, furthermore, it possibly will lead to retreating new vessels and will make clinical improvement in RA patients. It is confirmed that synovial and serum level of VEGF increases in RA and plays the most important role in angiogenes process. In addition, VEGF as a vascular penetrance enhancer increases edema and vasodilatation in RAs (Paleolog, 2002; Harada, 1998).

In spite of several vascular tissues related to RA, involved joints are hypoxic . Hypoxia and re oxygenation repeating cycles with phagocytes oxidative products causes oxidative stress in joints and produces active oxygen radicals that cooperates degeneration of tissues(Taylor and Bran, 2005).

With a quick look to the results of different surveys, the main reason of death in RA is Atherosclerosis, or other result shows that Asymmetric Diethyl Argininеis one of the most important and newly found risk factors of Atherosclerosis and cardiovascular diseases. Moreover, recent studies verified that dysfunction of Endothelial Vessels is one of the initiation factors and the reason of vascular degeneration associated with inflammatory processes and oxidative stress, ADMA and VEGF have negative effects on endothelial function.

So decreasing and changing in ADMA and VEGF in Combination with routine treatments, seem to be more effective and this is the purpose of this study.

In this survey, the effects of complemented antioxidant supplementations on RAs have been examined, besides, clinical manifestations of Atherosclerosis Rheumatoid, ADMA and VEGF level as negative endothelial increasing factors, were also considered. We predict if outcomes show adequate results, it can be helpful for RAs and other disease with inflammatory symptoms. Therefore, we were interested to know the effects of antioxidants on RA patients.

2. Material and Methods

Methods of study and patient selection:

By an experimental human study from all female RA patients who referred to SHEYKH-OL-RAYIS and SINA clinics in Tabriz, 40 females between 40-60 years old were chosen by a Rheumatologist based on ACR indexes. Including factors: ACR indexes (at least 4 indexes for 6 weeks from these: 1-Morning stiffness for at least 1 hour in 3 Joints or more. 2-Arthritis in 3 joints or more. 3- Hands Joint arthritis. 4- Symmetric arthritis. 5- Rheumatoid nodules. 6-positive Rheumatoid factor 7- typical Radiographic changes in hand Joints) in RA females, and with a permanent treatment at least during last 2 months. Excluding factors: 1- new DM, HTN, thyroid dysfunctions, renal failure, Liver dysfunctions, Cushing syndrome.2- smoking (active or passive) 3-taking any other antioxidant complements 4- any changes in treatment protocol during the study.

Executing Method:

Patient selection:

All patients with including factors were examined and explained by a Rheumatologist about the purpose of the study and its methods and the Rheumatologist assured them about safeness of complements.

Disease activity scale (DAS) forms and general information, such as: age, occupation, education, physical activities, past medical history and drug history were studied. Then, some measurements were done. including: 1- weight (with digital balance by 0/1 kg of precisions without shoes and with minimum dressing), 2- height (with a wall fixed meter. It was in contact with back of heel bottom, scapula and back of head and with 0/1 cm of precision) 3- BMI. Then, 5ml fasting venous blood (after 8-12 hours of fasting) was taken and after 10 minutes of spinning in 3000 RPM, samples were transferred to a freezer with -70 degrees of centigrade.

"SELEN PLUS" capsule (selenium 50 µ gram, zinc 8 mg, Vitamin A 400 µ gram, vitamin C 125 mg, vitamin E 40 mg) prescribed daily for 3 months, patients was asked to give back the empty boxes of Capsules to be confident of taking them and they were followed up daily by phone calls. After intervention, we repeated clinical examinations and all the measurements again.

Sample size

With attention to standard deviation of examined indexes in past studies, sample size calculated by this formula:

$$N= [2(SD) \dots (Z \alpha/2+Z\beta)\dots]/ \dots$$

It was calculated with assurance level of %95 and power of %90 and with Mean discrepancy of 2.2 in Inflamed joints and also with highest standard deviation of 2.7, sample size predicted 23.6. Because of long period of intervention and high probability of "lost to follow up", thus it was necessary to choose more than 24 patients, so we selected 40 patients to prevent Bias effects.

Biochemical tests:

Serum level of ADMA-VEGF and HS-CRP measured two times (before and after intervention)

Medical ethics:

Purpose, methods and safeness of antioxidants were explained to patients before starting the study. Patients were able to leave the study whenever they want.

Statistic analysis:

Data analysis was done by SPSS (ver. 13.5). Quantity data were calculated in (mean ISD) and the quality data were calculated in frequency.

We used Shapiro-wilk test and normal distribution curve Histogram to test the normal being of data distribution.

To compare the mean of quantity data before and after intervention, we used Paired t test (if it was normal) and non-parametric wilcoxon rank (if it was abnormal). Significant statistic level was defined as $P<0.05$.

3. Results

This study wants to show the effects of complemented antioxidants on clinical manifestations, VEGF, ADMA and HS-CRP levels in female RA patients in a period of 3 months. One patient out of 40 patients was removed due to non-medical reasons. Patients profiles are documented in table 1 (based on BMI). All Patients were over weighted at the beginning of study (2818±4.1).

2- Clinical manifestations based on DAS-28

All clinical changes after study are shown in table 2. There is a linear and positive correlation (r=0.922 and P=0.001) between DAS-28 and HS-CRP (Figure -1).

Table 1- basic features of female RAs at the beginning of study

Study group	
Number	40
Sex	Female
*Age (year)	52.65±5.26
Period of RA (month)	72 (18, 420)
Education Under diploma	87.5%
Diploma	12.5%
Prednizolon use	35(87.5)
Use	34(85)
Use	6(15)
Use	17(42.5)
NSAID use	4(10)
Use	1(2.5)
Use	8(25)

3- ADMA and VEGF serum level

ADMA and VEGF levels were higher than normal at the beginning of study. Table 3 (P<0.05)

In all patients, ADMA and VEGF levels measured two times, before and after study. Mean levels of both ADMA and VEGF did not show any significant statistical changes (P<0.492 and P<0.675). There was no statically significant correlation between ADMA changes, VEGF changes and DAS-28. However, there was a considerable correlation between ADMA and HS-CRP changes (P=0.003).And also there was no correlation between VEGF and Hs-CRP changes.

Table 2-changes in clinical indexes in RA females before and after 3 months.

	Before intervention	After intervention	P
*DAS-28	2.71±1.19	2.65±1.17	0.019
Number of stiffed joints	1(0,17)	1(0,14)	0.839
Number of painful joints	0(0,15)	0(0,14)	0.736
*Hs-CRP(mg/L)	5.5±0.51(mg/L)	4.20±0.51(mg/L)	0.003

4. Discussions and conclusion

This was a clinical trial study, and we desired to discover the effects of antioxidants on clinical features of RA patients**.** Antioxidants of macro and micronutrients did not show any significant effects on indexes of study, so they were not considered as confounding factors in linear regression model (Regimen factors were known as controlled factors).

In a stage of 3 months, DAS-28 Score and Hs-CRP show significant changes. Cerhan et al showed that, higher complemented C and E vitamins are in reverse relation with improving clinical manifestations in RA patients. In addition, in a prospective study of 55-70 years old RA females (1986-1997) it was shown that, Zinc is in reverse relation with improving of clinical manifestations (Cerhan, 2003).

Table 3- Comparison of serum levels of VEGF and ADMA in patients with normal serum

	Before intervention	Normal	P
VEGF(pg/mg)	208±376.50	173±107	0.001
ADMA(μ mol/L)	0.715±0.090	0.46±0.045	0.001

Some studies find broadly similar results to ours (taking antioxidant micronutrients can prevent progression of RA). Furthermore, the role of non-genetically factors like: Smoking, regimen and psychological stresses are more effective in elderly than juveniles are, so controlling these factors is recommended (Hasstedt, 1994; Nourmohammadi, 2010).

In an other study with similar outcomes, taking complemented antioxidant complex (vitaminC 300 mg. vitamin A 2500 IU) daily, for 12 weeks had significantly decreased RA activity (P<0.0001) and RADAI index got better (Nourmohammadi, 2010).

Based on similar results of another study, taking complemented antioxidant complex (400 Mg Alpha tocopherol,10 Mg Lycopene,10mg Carotenoid of palm oil, Lutein 10mg,Vitamin C200 mg) daily, for 10 weeks decreased number of painful joints and also DAS-28 Score improved (Van Vugt, 2008).

In another study, taking complemented antioxidant (Vitamin E 37.5mg. Vitamin C 150mg) with 1.4gr.Eicosapentaenoic ***acid***, 0.2 gr. Docosahexaenoic ***acid*** , 0.5 gr. ***gamma-linolenic acid*** showed no significant effects on clinical manifestations, number of inflamed Joints and number of painful Joints (Remans, 2004) In another study, complemented antioxidants (***Quercetin*** 166 mg, Vitamin C 133 mg, Alpha Lipoic ***Acid*** 300 mg) for 4 weeks did not show significant changes in clinical manifestations (this could be because of short intervention period and low dosage). (Sang-Cheol, 2009).

In a study by Pretez et al , selenium improved Ritchie index, number of painful and inflamed joints, morning stiffness and CRP, without statistically changes, But there was a significant improve in general health (P<0.01). In other studies, single antioxidant complements were used in different dosage, for example, Trap et al used 256 mg selenium for 6 months, serum selenium levels increased, but there was no significant anti rheumatic effects. This study also used other indexes like Rithchie index, which, number of joints with limited range of motions, inflamed joints, morning stiffness period and number of Painful joints before and after study, did not show any significant changes (Yoshikawa, 2000).

In Senthilmohan study, complemented antioxidant complexes (PinusRadiata 240mg with 120mg vitamin C per day) for 12 weeks showed positive significant effects on clinical manifestations but in a 6-month period of intervention, could not made any significant changes. These outcomes showed that besides the adequate doses of antioxidant complements, period of intervention also has great effect on clinical improvement of RA patients. Another study reported that Zinc sulphate complemented (10mg) for 6 weeks could not create significant changes in RA activity. Maybe it is because of non-adequate dosage and short period of intervention (Mattingly and Mowat, 1982).

We could not find any studies about effects of antioxidant complements on serum level of VEGF and ADMA, so here are some studies about ADMA and VEGF serum level.

Lee SS study showed that, hypoxia and inflammatory cytokaines stimulate Platlets and neutrophils, to secrete VEGF .So VEGF level is significantly high in RA patients. VEGF level in RA patients is in relation with disease activity, and VEGF serum level concentration in early stage of Rheumatoid Arthritis, is a prognostic factor for progressive disease in future (Lee, 2001).

Cluff study showed that, 15mg Vitamin E (for 4 weeks), decreases VEGF level (Cluff, 2009).

In Rajiv Saran study, ADMA level in chronic kidney disease was higher than normal and 8 weeks of antioxidant treatment with vitamin E, considerably decreased ADMA serum level (Rajiv Saran, 2003).

Sundeept Ballara showed that serum levels of VEGF decreases with improving in clinical manifestations. Patients with early stages of RA and good Response to DMARDS (Disease Modifying anti rheumatic drags) have low levels of VEGF rather than those with poor response (Ballara, 2001).

Charalambos Antoniades showed that serum levels of ADMA in inflammatory settings like RA and atherosclerosis is raised and they are simultaneous with raise in IL-6 levels (Charalambos, 2007).

Beatakwaśny- krochin showed that serum levels of ADMA are considerably higher than its levels in normal population.

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Disclosure of potential conflict of interest

The authors have declared no potential conflicts of interest.

Corresponding Author:

Dr. Arezzo Ghasmebaglou

Emam Reza Hospital, Internal medicine Department, Faculty of Medicine, Tabriz University of Medical Sciences, Iran.

Email: a_ghasmebaglou@yahoo.com

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