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Original Article

Beta-Carotene, Vitamin E, MDA, Glutathione Reductase and Arylesterase Activity Levels in Patients with Active Rheumatoid Arthritis

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Abstract

Background: Many studies have investigated the possible role of reactive oxygen species in the etiology and pathogenesis of Rheumatoid Arthritis (RA). The aim of this study was to investigate the activities of some antioxidants in RA patients.

Methods: In this case-control study, 59 RA patients and 60 healthy sex and age-matched controls were selected. Vitamin E and Beta-carotene were determined using HPLC. Erythrocytes glutathione reductase (GR) activity was measured spectrophotometrically, and malondialdehyde (MDA) was determined by colorimetric method. Arylesterase activity (AEA) was measured by Phenylacetate. The clinical data were determined by a rheumatologist, medical history and filling the questionnaire by interview. Statistical analyses were carried out using the SPSS software.

Results: In patients with RA, serum MDA level was significantly higher and plasma concentration of vitamin E, Beta-carotene and GR activity, were significantly lower than healthy control ($P< 0.001$). AEA activity differences between two groups were non-significant.

Conclusions: Oxidative stress may play an important role in the inflammation and pathogenesis of RA.

Keywords: *Beta-carotene, Vitamin E, MDA, Glutathione reductase, Arylesterase activity, rheumatoid arthritis*

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease with damage of articular cartilage and synovial proliferation. It affects about 1% of the world population and is the most common inflammatory disease worldwide (1, 2). Incidence increases with age, and women are affected three times more than men (1). The exact etiology of RA remains unknown. Recently, many of studies have investigated the possible role of reactive oxygen species (ROS) in the aetiology and pathogenesis of RA(2-5). ROS have been implicated as mediators of tissue damage in patients with RA (5). Free radicals from oxygen metabolism destroy antioxidant systems (6). Researchers such as Heliövaara have suggested that enzymatic and/or nonenzymatic antioxidant systems are impaired in RA (3). RA patients are therefore exposed to oxidant stress (6). The synovial fluid of the inflammed rheumatoid joint swarms with activated neutrophils which produce large amounts of ROS (4). ROS are formed in oxidative processes which occured normally at low levels in all cells and tissues. Under normal conditions, a variety of antioxidant mechanisms act to control ROS production (3). Moreover, high levels and/or inadequate removal of ROS result in oxidative stress. Oxidative stress may in turn cause severe metabolic dysfunctions and destruction of biological molecules (3). Antioxidants may convert ROS into harmless

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products (6) and so they can break the destructive chain reaction initiated by ROS.
Trace elements like copper, zinc and selenium may act as antioxidants since they are implicated in specific antioxidant enzymes activity, specially SOD, catalase and glutathione peroxidase (GSH-Px) activities (7, 8). Reduced glutathione (GSH) plays an important role in metabolic processes, transport and cellular protection in nearly all cells of the body by its thiol groups (5, 6) and GR converts oxidised glutathione to reduced glutathione these enzymatic and non- enzymatic antioxidants are also essential for inhibition of inflammation related to the function of neutrophils (9).
Many studies exist on the relation of some antioxidant vitamins and enzymes with inflammatory conditions (10-14), but limited data are available on the antioxidant, lipid peroxidation status and inflammatory parameters in patients with RA and so to our knowledge; this is the first study, which investigated on arylesterase activity (AEA) in RA patients.

Materials and Methods

Patients and controls

This was a case-control study performed on 59 patients with RA aged between 18 and 69 yr fulfilling ACR (American College of Rheumatology) criteria, the version of ACR was 1987 revised criteria (15) with a disease duration of more than 2 yr. The patients had not received any systemic therapy, which might affect cellular immunity, before the blood sample was collected. The control group consisted of 60 healthy volunteers matched for sex and age in 5 yr groups. The study plan was approved by the Ethics Committee of the Faculty of Medicine and all subjects volunteered for the trial and a written informed consent was obtained from all participants.

Exclusion criteria

Exclusion criteria included usage of supplemental vitamins (vitamin A and its precursor, vitamin E and C for at least 6 mo according to their statements before the study), smoking, and presence of diabetes mellitus, coronary artery disease, malignancy, hypertension, hyperlipidemia, acute-chronic liver diseases, and renal dysfunction.
The method for evaluation of disease activity was GPA (Global Physician Assessment) determined by rheumatologist, pain in joints, morning stiffness, inflammation and tenderness and by regarding in acute phase reactant such as CRP, ESR, PLT and Hb. Pain was assessed by VAS (Visual Assessment Scale) with a 0-100 mm scale.

Blood sample collection

Blood samples collected into 4 tubes Serum, plasma, Hb and ESR. Blood was washed three times with 9 g/l NaCl solution. Cell membranes were removed by centrifugation at 1200Xg for 5 min at 4 °C and the hemolysates were used for determining antioxidant enzyme activity. Catalase activity of blood samples was measured within 1 wk after sampling. Blood hematological and serum inflammatory markers were measured within 6 h after blood collection. Red blood cells, plasma and serum were stored at −70 °C for a time no longer than 1 wk and used for analyzing of the GR activities (RBC), Beta-carotene and vitamin E (plasma), AEA, CRP and RF (Serum).
AEA was measured by Phenylacetate that was used as a substrate to measure the AEA. Enzymatic activity was calculated from the molar absorptive coefficient of the produced phenol, one unit of AEA was defined as 1 mmol phenol generated per min under the above conditions and expressed as units/l of serum (16). GR Erythrocytes activity was measured spectrophotometrically by Ransod kit (Manual) at 37 ºC and 340 nm according reduction of glutathione (GSSG) in the presence of NADPH, which is oxidised to $NADP^+$ due to GR. Beta-carotene and α-tocopherol were determined by high performance liquid chromatography (17) CRP and RF values were determined with Immunoturbidometric assay which in method CRP gives a complex with polyclonal antibody and creates turbidity that has positive relation with CRP in sample. RF gives a complex with Human IgG and creates turbidity that its amount has positive related with RF in samples. Haematological analyses counts by an automated

blood counter (Sysmex K21, Japan). ESR was measured using an ESR apparatus (Greiner Labor Technic GmbH, Germany).

Statistical analyses

Descriptive statistics were applied for calculating the distribution of various characteristics. All results were expressed as means±standard deviation. Statistical analyses were carried out using the SPSS. Students t-test was used to compare continuous variable between two groups and if its distribution was not normal, Mann-Withney test was used for comparison of quality and rational variable Q-Square test were used. *P* values of less than 0.05 were regarded as statistically significant.

Results

Study was performed in 59 RA patients (The control group consisted of 59 healthy volunteers matched for sex and age and BMI). Pain, morning stiffness, number of joints with inflammation, tenderness, and GPA (Table 1) in patients with active RA is shown. The CRP and RF levels were significantly $P=0.011$, $P<0.001$) respectively higher in RA patient groups than in controls (Table 2). Erythrocytes GR activities, the mean concentrations of vitamin E and Beta-carotene, were significantly lower in the patient group than in the control group ($P< 0.001$) and AEA was non-significantly ($P>0.05$) lower in RA patients compared to control groups, but MDA was significantly higher in patients group ($P = 0.003$) (Table 3). Level of Hb was nonsignificantly lower in RA patient groups than in controls ($P= 0.13$). ESR was significantly higher in RA patient groups than in controls ($P< 0.001$).

Table1: Demographic, anthropometric data and some clinical variables in patients with active RA and control subjects (mean±SD)

Variables	Case (n=59)	Control (n=59)	*P –Value*
Male/Female*	21/38	21/38	*P>0.05*
Age (years)	41.95±11.20	39.16±8.72	*P>0.05*
BMI	26.37±4.71	25.94±3.65	*P>0.05*
Duration of RA (years)	8.27±7.62	-	-
Morning Pain(mm)	46.65±29.13	-	-
Night Pain (mm)	48.68±31.09	-	-
After activity Pain(mm)	55.72±35.31	-	-
Morning stiffness(hour)	1.20±0.92	-	-
NO of swollen Joints	8.21± 10.02	-	-
NO Joints with tenderness	6.33±7.15	-	-
GPA (percent)		-	-
Non active	0(0)	-	-
Mild	21(35.6)	-	-
Moderate	28(47.5)	-	-
Severe	10(26.9)	-	-

There were no significant differences between groups by Qi-Square*

Table 2: Hemoglobin Level, ESR, platelet count, RF and CRP in rheumatoid arthritis patients and control subjects (mean±SD)

Variable	Group	no	Mean	SD	Results
Hb (g/dl)	Case	58	13.05	1.50	*P*= 0.13
	Control	57	13.43	1.23	
ESR(mm/h)	Case	59	31.25	20.78	*P*< 0.001
	Control	59	12.02	11.40	
Platelet count 1000/UL)	Case	58	291.22	85.14	*P*< 0.001
	Control	59	222.57	59.13	
RF µg/ml	Case	59	68.15	79.18	*P*< 0.001
	Control	59	7.82	15.95	
CRP mg/dl	Case	59	5.85	8.40	*P*= 0.011*
	Control	59	2.94	1.54	

*Group t-test

Table 3: Plasma levels of Aryl Esterase activity (AEA),Vitamin E, Malondialdehyde (MDA), Glutathione (GR) and Betacarotene, in Rheumatoid Arthritis patients and control subjects (mean±SD)

Variable	Case (n=59) mean±SD	Control (n=59) mean±SD	Results
AEA(IU/ml)	146.03±38.78	147.32±34.74	*P*>0.05
MDA(ng/ml)	2.22±1.12	1.54±1.33	*P*=0.001*
GR(U/gHb)	3.68±1.16	5.46±2.09	*P*<0.001*
Betacarotene(µg/ml)	17.71±8.82	31.98±18.98	*P*<0.001*
Vitamin E (µg/ml)	12.88±3.62	14.92±3.09	*P*<0.001*

*Group *t*-test

Discussion

The results of the study indicate that the antioxidant vitamins and enzymes in the plasma of the patient group were lower than in the control group. It was shown in the previous studies that low intake of the vitamin E and vitamin A can be regarded as a risk factor for RA (18-22). Heliovaara et al. reported elevated risks of RA at low levels of α-tocopherol and Beta-carotene (3). Helmy et al. reported that high dose vitamin E treatment decreased disease activity in patients with RA (18). Cerhan et al. hypothesized that consumption of Vitamin E and Beta-carotene was inversely associated with the risk of developing RA in the elderly (23). In Kamanli et .al study low level of vitamin E, vitamin A, Beta-carotene, GSH-Px, GSH, catalase and increase in MDA, CRP, ASO have been shown in RA patients. In our study, GR, vitamin E, Beta-caroten was lower and MDA was higher in the patient group than in controls (10). Similarly, Cimen et al. reported that patients with RA had higher MDA and GR levels and a lower activity (24). Unlike to our data Bazzichi et al. reported that patients with RA had higher GR levels activity than in patients with osteoarthritis (OA) (25). Their results confirmed a high activity of collagenase and elastase in the SF of patients with RA, which is about 30 times higher than that found in the SF of patients with OA. These data underline the synergic action of these enzymes in the pathogenesis of joint damage. RA patients also exhibit higher levels of GR, which is important for the detoxification pathway of oxygen free radicals. However, compared with findings for collagenase and elastase, the increase in GR is only three times higher than level found

in the SF of OA patients. A small limited increase in glutathione reductase activity during the inflammatory process might lead to an insufficient protective effect at the joint level in RA, but Hassan et al. have shown that RA was associated with significant depletion (50%) in GSH levels compared with normal control subjects. Serum levels of the detoxifying enzymes GR like of our data decreased (32.4% reduction) (26) Kerimova et al. examined the activities of antioxidant enzymes GSH-Px, GR and catalase in the blood of RA patients and healthy controls. Similar to our data activity of catalase was decreased significantly, while activities of GSH-Px and GR remained unchanged (27).

Mulherin et al. studied on 91 patients with RA, and 220 healthy controls. Similar to our study basal GR activity in the red blood cells and polymorphonuclear leucocytes of patients with RA was low (28). Braven et al. found a 30% increase in erythrocyte GSH-Px activity was found in patients with RA compared with healthy controls whereas the increase in GR was statistically insignificant (29). Ozkan et al. studied in 22 patients with active RA and 18 age- and gender-matched control subjects. While serum MDA levels were significantly increased in patients with RA compared with the control group ($P< 0.03$), the total oxidative status levels were decreased in patients with RA compared with the control group ($P< 0.008$) (30).

Karatas et al. studied on 22 patients with RA and 20 healthy volunteers and found that MDA levels in patients was significantly ($P< 0.005$) higher than controls whereas levels of vitamins A, E, C was lower. Bae et al. studied on RA patients (n= 97) and their age, gender-matched controls (n= 97) participated in this cross-sectional case-control study. Nutrient intake was estimated using a semi-quantitative food frequency questionnaire. Twenty subjects from each group provided blood samples, and plasma concentrations of alpha-tocopherol and malondiadehyde (MDA) were measured. Also, plasma activities of SOD and GSH-Px were measured. The mean calorie intake of RA patients was lower than that of the healthy controls. Energy-adjusted intake of fat, vitamin A and Beta-carotene were significantly lower in patients than those of the control subjects. RA patients as in our study had a decreased mean plasma α-tocopherol level comparing to control subjects ($P< 0.05$) (31).

Although the exact causes of RA are unknown, involvement of ROS is suspected. Several studies suggested a beneficial effect of antioxidants such as vitamin E in RA (32-34). In inflammation, activation of T cells and macrophages leads to a large increase in oxygen consumption, whose corollary is increased release of ROS (4).

Peroxynitrite production is associated with diminished of type 2 collagen and aggrecan and with a decrease chondrocyte response to the growth factor IGF-1, in vitro. In addition, peroxynitrite increases the expression of MMP-3 (Matrix Metallo Proteinase -3) and MMP-13 and decreases the production and activity of the tissue inhibitors of MMPs (TIMPs). These changes lead to increased matrix breakdown. TNF-α overproduction is thought to be the main contributor to increased ROS release in patients with RA (4-6).

The significantly decreased values of hemoglobin in the blood of RA patients observed in our study are supported by other workers who reported that increased ROS production is indicative of RBC destruction in patients with RA (35), and there is a negative relationship between the activity of disease and haemoglobin in RA patients (2) that our study indicate it. In this study RF and acute phase reactants, including ESR and CRP were higher in the patient group as was accepted (Table 2). There was a negative relationship between CRP and RF with GSH-Px, catalase, vitamin A and vitamin E. CRP and RF are active components related to the phagocytic system of polymorphonuclear leukocyte (3-5). In the event of neutrophils and macrophages being stimulated by pathogens, cytokines or other inflammation mediators, complements (C3 and C4) together with other mediators such as CRP and RF are liberated from the granule into the cytoplasm and play an important part in destroying phagocytosed materials (36). These cells are able to produce superoxide radicals and other oxidant species when activated by diverse stimuli (33-34). It was found that CRP and RF in patients with RA may be sensitive inflammation markers

for reflecting the presence and activity of the disease (3, 9) and several studies show that these parameters increase in the patient group compared to the healthy control group (35-37). In our study, non-significant reduction in serum AEA of lipoprotein-associated enzymes, PON1 in patients was shown. In RA disease, chronic systemic inflammation may contribute to a higher incidence of CVD (38). The presence of PON1 on HDL particles is considered to be a major source of protection from lipoprotein oxidation. Therefore, reduction of PON1 activity may be considered to be prooxidant and proatherogenic (39-40), PON1 activity is related to genetic, environmental, pharmacological, life-style and dietary factors, as well as age and certain disease conditions. Various in vitro and in vivo studies in animals and humans have provided initial evidence that antioxidants can increase PON1 activity (41).

In conclusion, due to increased lipid peroxidation and decreased levels of antioxidant vitamins and enzymes in plasma, RA patients are subject for oxidative stresses. These results are consistent with the underlying hypothesis that there is an imbalance situation between ROS production and the antioxidant defence system in inflammatory diseases such as RA. Antioxidant systems, lipid metabolism and inflammatory reactants are impaired in RA. There was an increased oxidative stress and a low antioxidant status in patients with RA. These changes are probably due to efforts for reducing lipid peroxidation and hence to lower tissue damage. The change in relative levels of antioxidants and free radical formation could be used as indicators for effective and earlier diagnosis of RA.

Ethical Considerations

Ethical issues including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc. have been completely observed by the authors.

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