ANTIOXIDANT ENZYMES CAPACITY IN PATIENTS WITH RHEUMATOID ARTHRITIS: THE RELATIONSHIP WITH DISEASE ACTIVITY SCORE

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SUMMARY

The aims of this study were to check the alterations in serum levels of antioxidant enzyme activities and to assess whether the disease activity scores are reflected by serum antioxidant enzyme capacity in patients with RA.

The levels of erythrocyte CuZn superoxide dismutase (CuZnSOD) and glutathione peroxidase (GSH-Px) were assessed in 20 patients with rheumatoid arthritis (RA) and 20 healthy control subjects. Disease activity score (DAS28) was calculated for all patients. Erythrocyte CuZnSOD activity levels in RA patients and control subjects were 13.8 ± 2.5 (U/mg. protein) and 8.62 ± 1.8 (U/mg. protein), respectively. There was a significant difference between the groups (p<0.001). The mean erythrocyte GSH-Px values were 27.6 ± 9.3 (µmol NADPH oxidized/min/mg.protein) and 14.5 ± 3.3 (µmolNADPH oxidized/min/mg.protein) for the RA patients and controls subjects, respectively. There was a significant difference with respect to erythrocyte GSH-Px activity values between the groups (p<0.001). The mean DAS 28 score in RA patients was 3.44 ± 1.23. There was a significant positive correlation between each of erythrocyte CuZnSOD and GSH-Px levels and DAS28 scores for the RA patients (r= 0.80, p<0.001; r= 0.81, p<0.001 respectively). Positive correlation between erythrocyte CuZnSOD and GSH-Px activity values and CRP (r=0.68, p<0.001; r=0.76, p<0.001), was not detected with ESR.

The present study indicated that increased erythrocyte CuZnSOD and GSH-Px levels that might play a role in the tissue damage and inflammation process of this disease may be a useful marker for evaluating of the disease activity in patients with RA.

Key Words: *Rheumatoid arthritis, disease activity, antioxidants, superoxide dismutase and glutathione peroxidase*

ÖZET

ROMATOİD ARTRİTLİ HASTALARDA ANTİOKSİDAN ENZİM KAPASİTESİ: HASTALIK AKTİVİTE SKORLARI İLE İLİŞKİSİ

Bu çalışmanın amacı; RA'li hastalarda antioxidan enzim aktivitelerinin serum seviyelerindeki değişiklikleri kontrol etmek ve hastalık aktivite skorlarının serum antioksidan enzim kapasitesini yansıtıp yansıtmadığını değerlendirmekti.

Eritrosit CuZn superoksit dismutaz (CuZnSOD) ve glutatyon peroksidaz (GSH-Px) seviyeleri 20 RA'li hasta ile 20 sağlıklı kontrol grubunda bakıldı. Hastalık aktivite skoru DAS28 kullanılarak tüm hastalar için hesaplandı. RA'li hastalarda ve kontrol grubunda CuZnSOD aktivite değerleri sırasıyla, 13.8 ± 2.5 (U/mg.protein) ve 8.62 ± 1.8 (U/mg.protein) idi. İki grup arasında anlamlı fark vardı (p<0.001). Ortalama eritrosit GSH-Px aktivite seviyeleri RA'li hastalar ve kontrol grubu için sırasıyla 27.6 ± 9.3 (µmol NADPH oxid/min/mg.protein) ve 14.5 ± 3.3 (µmol NADPH oxid/min/mg.protein) olarak bulundu. Eritrosit GSH-Px ak-tivite değerleri açısından gruplar arasında anlamlı fark vardı (p<0.001). RA hastalarında ortalama DAS28 skoru 3.44 ± 1.23 idi. Eritrosit CuZnSOD ve GSH-Px kapasitelerinin herbiri ile DAS28 skor-ları arasında anlamlı bir pozitif korelasyon vardı (r= 0.80, p<0.001; r= 0.81, p<0.001, sırasıyla). RA'li hasta-larda eritrosit CuZnSOD ve GSH-Px aktivite değerleri ile CRP arasında anlamlı korelasyon saptanırken, ESR ile enzim kapasiteleri arasında korelasyon yoktu.

Mevcut çalışma, bu hastalığın inflamatuvar proçesinde ve oluşan doku hasarında rol oynayan artmış eritrosit CuZnSOD ve GSH-Px seviyelerinin RA'li hastalarda hastalık aktivitesinin değerlendirilmesinde faydalı markerler olabileceğini gösterdi.

Anahtar Kelimeler: *Romatoid artrit, hastalık aktivitesi, superoksit dismutaz, glutatyon peroksidaz*

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INTRODUCTION

Rheumatoid arthritis (RA) is characterized by a chronic hypertrophic synovitis leading to destruction of connective tissue and functional damage of cartilage and bone structures. The causes of RA have not been completely elucidated (1). Oxidative stress can play an important role in the pathogenesis of RA. Acute and chronic oxidant stress to the vascular endothelium is a serious causative factor of vascular endothelial dysfunction and plays an important role in the pathophysiology of some diseases, including diabetes (2), panic disorder (3), and inflammatory bowel diseases (4). In recent years, increasing attention has been given to the role of reactive oxygen metabolites in the pathogenesis of inflammatory disease such as RA. Increased activity of free radicals, the unstable molecules associated with cell damage, is theorized to underlie the mucosal injury commonly seen in the various inflammatory diseases (5).

Recent data from several reports indicate that free radicals are involved in aetiopathogenesis of much human pathology including inflammatory disorders such as RA. Increased reactive oxygen species (ROS) and other free radicals which play an important role in the inflammatory process and contribute to tissue destruction can initiate lipid peroxidation and DNA damage leading to mutagenesis, carcinogenesis and cell death, if the antioxidant system is impaired (6, 7). The harmful effects of ROS are controlled in vivo by antioxidants enzymes such as glutathione peroxidase (GSH-Px) and CuZn superoxide dismutase (CuZn-SOD). It has been proposed that, inflammation of mucosa causes impairment of antioxidant defense mechanism, and makes tissue more susceptible to oxidative damage (8). Recently, antioxidant enzymes functions have been linked to anti-inflammatory properties.

The aims of this study were to check for alterations in serum levels of antioxidant enzyme activities and to assess whether the disease activity scores are reflected by serum antioxidant enzyme capacity in patients with RA.

PATIENTS AND METHODS

This study was carried out in 20 RA patients, diagnosed according to the 1987 revised criteria of the American College of Rheumatology (formerly, the American Rheumatism Association) (9). In the patient group (n=20), 16 were females and 4 were males (mean age: 37.9 7.7, range: 27-50 years). The mean disease duration was 6.45 ± 3.2 years (range 2-15 years). In the control group (n=20), 15 females and 5 males were (mean age: 38.0 ± 5.8, range: 28-48 years) healthy hospital personnel without a history of inflammatory disease.

After obtaining blood samples, low dose steroid and disease modifying anti-rheumatic drugs (DMARD) were started in RA patients at the activation period. The patients at remission period continued to their previous drugs. Thirteen cases were taking low dose steroid (7.5 mg/daily) plus methotrexate (MTX) and 7 were taking only NSAID. None of the subjects was taking alcohol and antioxidant agent (e.g. vitamins E and C) or had intestinal absorption defects and showed any clinical or laboratory signs of liver disease, diabetes mellitus (DM), thyroid disease, infectious disease, or coronary artery disease. Venous blood from patients with RA and healthy controls were collected in vacutainers without additive and in heparinized glass tubes, allowed to clot for 30 min at room temperature and centrifuged at 2000g for 5 minutes. Serum aliquots and red cells were kept in deep freeze at -80°C until they were assayed. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were determined in whole blood and serum aliquots, respectively. ESR was determined according to the Westergren method and CRP by a nephelomet-

ric method (Beckman Array Protein System, USA). Disease activity score (DAS28) was calculated using the following formula (10, 11): DAS28 = 0.56 x $\sqrt{(\text{tender28})}$ + 0.28 x $\sqrt{(\text{swollen28})}$ + 0.70 x ln (ESR) + 0.014 x GH

ESR: Erythrocyte Sedimentation Rate (mm/h), GH: Global Health measured on a Visual Analogue Scale, the number of swollen joints and tender joints were assessed using 28-joint counts (12).

CuZn-SOD assay: An aliquot of the erythrocyte hemolysate was mixed with 0.25 times the volume of ethanol and 0.15 times the volume of chloroform to remove MnSOD (13). After centrifugation, the supernatant was assayed according to the method of Misra and Fridovich (14), using the photochemical o-dianisidine-riboflavin assay. Briefly, 0.2 ml supernatant and 2.9 ml reaction mixture (containing 2.7 ml of 50 mM potassium phosphate, 0.1 mM ethylenediamino-tetraacetate (EDTA, pH 7.8). 0.1 ml of 6 mM o-dianisidine dihyrochloride (Sigma) in water, and 0.1 ml of 0.39 mM riboflavin (Sigma) in 10 mM potassium phosphate (pH 7.8) were placed into a quartz cuvette and after 4 min of incubation at room temperature the absorbance was determined at 460 nm. Enzyme activity was expressed as units per milligram of protein and 1 unit of CuZn-SOD activity was defined as the amount of enzyme required to decompose 1µmol of H_2O_2 in 1 min.

GSH-Px assay: GSH-Px was assayed by the method of Levander et al (15) as modified by Paglia and Valentine (16). The protein content in the samples was determined by the method of Lowry et al (17). Briefly, 200 L of Buffer K (0.25 M potassium phosphate buffer containing prepared by combining 0.25 M K_2HPO_4 with 25 mM Na_2EDTA pH= 7.4), containing 5 units of glutathione reductase per milliter (Sigma), 50 L of 40 mM GSH (Boehringer- Mannheim), 500 L supernatant and 200 L of deionized water were added in a quartz cuvetta and mixture was incubated for 10 minutes at 370 C. After blanking spectrophotometer at 340 nm, 10 L of 20 mM NADPH (Sigma) dissolved in 0.1 % $NaHCO_3$ was added. The mixture was mixed and then incubated for 2 minutes at 37°C. The reaction was initiation by adding 20 L of 15 mM t-butyl hydro-peroxide (Sigma). The mixture was stirred and absorbance was read immediately and at 1 minute intervals for 4 minutes. A standard curve was prepared using bovine erythrocyte GSH-Px. The absorbance change during the 2 to 4 minute interval was used to calculate enzyme activity. Enzyme activity was expressed as 1 mol NADPH oxidized per milligram of protein per minute.

Statistical analysis: Data were processed using the Statistical Package for the Social Science (SPSS) program. Laboratory results were given as mean ± standard deviation (SD). Differences between groups were performed using the Mann-Whitney U test. The correlations between variables were assessed using Spearman's rank correlation coefficient. P values of < 0.05 were regarded as significant.

RESULTS

The clinical features and laboratory findings of the patients and control subjects are shown in Table I. There were no significant differences according to age or the female-male ratio between the patients and controls ($p>0.05$). Compared with the control subjects, the serum levels of ESR and CRP in the patients with RA were significantly elevated ($p<0.001$).

Erythrocyte CuZnSOD and GSH-Px activities were determined in the RA patients and the healthy subjects. In a comparison of the 2 groups, it was found that the activities of these 2 antioxidant enzymes were significantly increased in patients with RA. Erythrocyte CuZnSOD activity levels in RA patients and con-

Table I. Baseline characteristics of erythrocyte CuZnSOD and GSH-Px levels in rheumatoid arthritis patients and healthy controls.

Patients	Healthy control	p	
N (F/M)	20 (16/4)	20 (15/5)	ns
Duration of disease (years)	6.45 ± 3.23	-----	
Age (years)	38.0 ± 7.6	39.5 ± 5.8	ns
Erythrocyte sedimentation rate (mm/h)	29.90 ± 12.77	16.23 ± 8.88	<0.001
C-reactive protein (mg/L)	1.44 ± 1.07	0.34 ± 0.14	<0.001
CuZnSOD (U/mg protein)	13.8 ± 2.56	8.64 ± 1.85	<0.001
GSH-Px (µmol NADPH oxidized/min/mg.protein)	27.6 ± 9.3	14.5 ± 3.3	<0.001
DAS28 scores	3.44 ± 1.23	-----	

ns: not significant

trol subjects were 13.8 ± 2.5 (U/mg.protein) and 8.6 ± 1.8 (U/mg.protein) respectively. There were statistically significant differences between the groups (p<0.001). The mean erythrocyte GSH-Px levels were 27.6 ± 9.3 (µmol NADPH oxidized/min/mg.protein) and 14.5 ± 3.3 (µmol NADPH oxidized/min/mg.protein) for the RA patients and control subjects, respectively. There was a statistically significant difference with respect to erythrocyte GSH-Px activity values between the groups (p<0.001).

The mean DAS 28 score in RA patients was 3.44 ± 1.23. There was a significant positive correlation between each of erythrocyte CuZnSOD and GSH-Px levels and DAS28 scores for the RA patients (r= 0.80, p<0.001; r= 0.81, p<0.001 respectively).

Positive correlation between erythrocyte CuZnSOD and GSH-Px activity values and CRP values was clearly detected in RA patients (r=0.68, p<0.001; r=0.76, p<0.001), but not detected between erythrocyte CuZnSOD and GSH-Px values and ESR.

DISCUSSION

Oxidant stress constitutes a serious pathophysiological factor for a wide variety of connective tissue disorders such as RA. Pathogenic mechanism of chronic inflammation is associated with increased production of ROS and free radicals (superoxide anion and hydrogen peroxide) (18). Elevated free radical generations in inflamed joints and impaired antioxidant system have been implicated in rheumatoid arthritis (RA). In the neutralization process of those anions, erythrocyte CuZnSOD and GSH-Px are key enzymes. Overexpression of the antioxidant enzymes, erythrocyte CuZnSOD and GSH-Px may block ROS and free radicals-induced events, reducing the inflammatory response and tissue destruction in joints (19).

Other important result of increased free radical and ROS is lipid peroxidation (20). These reactive oxygen species, if not scavenged, lead to lipid peroxidation. The activity of CuZnSOD and GSH-Px impaired because of excessive production of ROS and free radicals. Higher concentrations of CuZnSOD and GSH-Px are needed to protect against these radicals. CuZnSOD catalyses the dismutation reaction of the toxic superoxide radical to molecular oxygen and hydrogen peroxide and thus forms a crucial part of the cellular antioxidant defence mechanism (21). Glutathione peroxidase catalyzes the reduction of various organic hydroperoxides, as well as hydrogen peroxide, with glutathione as hydrogen donor. This enzyme functions has been suggested in more times as a mechanism of protecting the cellular membrane system against peroxidative damage (22). In this study,

we focused our attention on two enzymes, CuZn SOD and GSH-Px, which serve key functions in the elimination of reactive oxygen species (23). In recent years, activities of some antioxidant enzymes have been reported previously in the various diseases (24, 25).

In the present study we have found that the values of erythrocyte CuZnSOD and GSH-Px are elevated in the serum of patients with RA. However, we also detected a positive correlation between erythrocyte CuZnSOD and GSH-Px values and disease activity scores (DAS28). Several studies have also shown that the values of erythrocyte CuZnSOD and GSH-Px significantly increased in RA patients compared with normal controls (26, 27). Increased enzymes levels could be in response to the excessive production of free radicals which need erythrocyte CuZnSOD and GSH-Px for elimination. In our study, increased erythrocyte CuZnSOD and GSH-Px values in inflammatory conditions also confirm this. However, there are also publications contradicted our results (28-30). There was a statistically significant positive correlation between erythrocyte CuZnSOD and GSH-Px values and CRP levels. However, the presence of increased ESR levels was not correlated with elevated antioxidant enzymes capacity. The ESR may be affected by many factors. CRP is a more direct measure of inflammation than ESR, and it is more sensitive to short-term changes (31). Different values of antioxidant enzymes activities have been reported by several researches (32-34). As seen, studies do not agree on the activity of erythrocyte CuZnSOD and GSH-Px among RA patients.

The present study indicates that erythrocyte CuZnSOD and GSH-Px levels that might play a role in the tissue damage and inflammation process of this disease may be a useful marker for evaluating of the disease activity in patients with RA.

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