

Circulating lipid peroxidation, plasma and erythrocyte antioxidant status in patients with rheumatoid arthritis

Palanisamy Pasupathi¹, Mathiyalagan Deepa², P. Rani³ and R. Ramesh Sankar⁴

¹Institute of Laboratory Medicine, K.G. Hospital and Postgraduate Medical Institute, Coimbatore 641 018, Tamil Nadu; ²Department of Biochemistry & ³Gynecology, Raajam Hospital, Karruppur, Salem 636 012, Tamil Nadu; ⁴Sai Bhrenthavan Homeo Clinic, Omalur, Salem 636 455, Tamil Nadu, India.
e-mail: drppasupathi@gmail.com

Abstract

The aim of the study was to investigate the levels of lipid peroxidation, and both plasma and erythrocyte antioxidant states in patients with rheumatoid arthritis. The population consisted of 60 subjects divided into two groups, 30 subjects had evidence of rheumatoid arthritis and age and sex matched healthy subjects were studied as controls. The level of plasma and erythrocyte thiobarbituric acid reactive substances (TBARS) was markedly increased in both the rheumatoid arthritis patients when compared to control subjects. The activities of plasma and erythrocyte antioxidants were significantly decreased in rheumatoid arthritis. C reactive protein, rheumatoid factor and anti-streptolysin-O were significantly higher in rheumatoid arthritis patients than in healthy subjects. In conclusion, on the basis of enhanced lipid peroxidation in rheumatoid arthritis patients with concomitant failure of both the plasma, and erythrocyte antioxidants defense mechanism. These results are consistent with the underlying hypothesis that there is an imbalance between reactive oxygen species production and the antioxidant defense system in inflammatory rheumatoid arthritis disease.

Introduction

Rheumatoid arthritis is a chronic progressive autoimmune disorder characterized by symmetric erosive synovitis and sometimes shows multi-system involvement. The long-term outcome of this disease is characterized by significant morbidity, loss of functional capacity, and increased mortality. This disease affects about 1% of the general population worldwide¹.

Reactive oxygen species (ROS) are clearly involved in the pathogenesis of various diseases, including rheumatoid arthritis. ROS have been implicated as mediators of tissue damage in patients with rheumatoid arthritis. The synovial fluid of the inflamed rheumatoid joint swarms with activated neutrophils, which produce large amounts of ROS². ROS are formed in oxidative processes that normally occur at relatively low levels in all cells and tissues. Under normal conditions, a variety of antioxidant mechanisms serve to control this ROS production. In contrast, high doses and/or inadequate removal ROS result in oxidative stress, which may cause severe metabolic malfunctions

and damage to biological macromolecules. Further, decomposition of peroxidized lipid yields a wide variety of end products, including malondialdehyde. Elevated levels of malondialdehyde have been observed in the serum (or plasma) and synovial fluid of rheumatoid arthritis patients³. In recent years, increasing attention has been given to the role of reactive oxygen metabolites in the pathogenesis of inflammatory disease such as rheumatoid arthritis. 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 diseases⁴.

Several antioxidant systems have been reported which have different activities. Circulating human erythrocytes possess the ability to scavenge O₂⁻ and H₂O₂ generated extracellularly by activated neutrophils, superoxide dismutase, catalase, and glutathione peroxidase-dependent mechanisms. Superoxide dismutase, the first line of defense against ROS, catalyzes the dismutation of the superoxide anion into hydrogen peroxide. Catalase

can then transform hydrogen peroxide into H₂O and O₂. Glutathione peroxidase is a selenoprotein, which reduces lipidic or non-lipidic hydroperoxides as well as H₂O₂ while oxidizing glutathione^{5,6}. Therefore, the present study was aimed at comparing lipid peroxidation indices [as thiobarbituric acid reactive substances (TBARS)] and plasma concentrations of various antioxidants in rheumatoid arthritis patients as well as inflammatory and biochemical parameters in patients with rheumatoid arthritis and healthy controls matched by age and sex.

Materials and Methods

Study population: The population consisted of 60 subjects divided into two groups, 30 subjects had evidence of rheumatoid arthritis patients (15 females, 15 males; mean age 40.4 ± 8.3 years). The other 30 subjects age and sex matched healthy subjects were studied as controls (15 females, 15 males; mean age 42.2 ± 7.6 years). The patients with rheumatoid arthritis were diagnosed by the diagnostic criteria of the American Rheumatism Association and those who had been receiving corticosteroid agents, gold preparations, and/or d-penicillamine for at least 3 months before the consultation were excluded from this study. However, patients who had been receiving ordinary dosages of non-steroidal anti-inflammatory drugs (NSAID) were not excluded as almost all the patients with rheumatoid arthritis had been undergoing NSAID treatment. None of the patients or controls was a smoker or consumed alcohol or had any other chronic disease. The Ethics Committee of the Medical Faculty approved the study plan, and all subjects volunteered for the trial.

Preparation of blood samples: Blood samples from the antecubital vein, using a monovette system of blood collection, were taken before breakfast for determining the blood parameters. One ml of anticoagulated blood containing sodium EDTA was used for hematological analysis. The remaining anticoagulated blood was separated into plasma by centrifugation for 10 min at 1,000x g at +4°C. Two ml of serum was used for the detection of C reactive protein, rheumatoid factor and anti-streptolysin-O. Plasma and serum samples were stored at -30°C. Plasma was stored for <3 months pending measurement of enzymic activity. The remaining plasma was used for immediate lipid peroxidation assays. Blood hematological and serum inflammatory markers were measured within 6 hours of blood collection.

Blood collection and erythrocyte lysate preparation: Blood samples were collected by

venous puncture in heparinized tubes and the plasma was separated by centrifugation at 1,000x g for 15 min. After centrifugation, the buffy coat was removed and the packed cells were washed three times with physiological saline. A known volume of the erythrocytes was lysed with hypotonic phosphate buffer (pH 7.4). The hemolysate was separated by centrifugation at 2,500x g for 15 min at 2°C.

Biochemical investigation: Fasting blood glucose and serum uric acid were determined using by fully automated clinical chemistry analyzer (Hitachi 912, Boehringer Mannheim, Germany). Red blood cell count, total white blood cell count, platelet count, total hemoglobin and ESR were determined using fully automated hematology analyzer (ABX Pentra XL 80, USA). C reactive protein, rheumatoid factor and ASO level was estimated using fully automated clinical chemistry analyzer (Architect C 8000, Abbott Diagnostics, Japan)

Estimation of lipid peroxidation: Lipid peroxides were estimated by measurement of TBARS in plasma by the method of Yagi⁷ and in erythrocytes by the method of Donnan⁸. The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation was estimated. Results were expressed as nmol/mL for plasma and pmol/mg Hb for erythrocytes.

Assay of superoxide dismutase and catalase: Superoxide dismutase was assayed utilizing the technique of Kakkar et al.⁹ based on inhibition of the formation of nicotinamide adenine dinucleotide, phenazine methosulfate and amino blue tetrazolium formazan. A single unit of enzyme was expressed as 50% inhibition of nitroblue tetrazolium reduction/min/mg Hb. Catalase was assayed colorimetrically at 620 nm and expressed as μmol of H₂O₂ consumed/min/mg Hb as described by Sinha¹⁰. The reaction mixture (1.5 mL) contained 1.0 mL of 0.01 M pH 7.0-phosphate buffer, 0.1 mL of hemolysate and 0.4 mL of 2 M H₂O₂. The reaction was stopped by the addition of 2.0 mL of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio).

Estimation of reduced glutathione: Reduced glutathione content was determined by the method of Ellman¹¹. 1.0 mL of plasma was treated with 0.5 mL of Ellmans reagent (19.8 mg of 5,5'-dithiobisnitro benzoic acid (DTNB) in 100 mL of 0.1% sodium nitrate) and 3.0 mL of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm. Erythrocyte reduced glutathione content was determined with dithionitrobenzoic acid using the method described by Beutler and Kelley¹².

Reduced glutathione content was expressed as mg/dL.

Assay of glutathione peroxidase and glutathione reductase: Glutathione peroxidase activity was measured by the method described by Rotruck et al.¹³ with modifications. Briefly, reaction mixture contained 0.2 mL of 0.4 M Tris-HCl buffer pH 7.0, 0.1 mL of 10 mM sodium azide, 0.2 mL of hemolysate, 0.2 mL glutathione and 0.1 mL of 0.2 mM hydrogen peroxide. The contents were incubated at 37°C for 10 min. The reaction was arrested by 0.4 mL of 10% TCA, and centrifuged. The supernatant was assayed for glutathione content by using Ellmans reagent. Glutathione peroxidase activity was expressed as μ moles of reduced glutathione consumed/min/g Hb. Glutathione reductase activity was assayed using oxidized glutathione as a substrate according to the method described by Carlberg and Mannervic¹⁴. The method was based on the absorbance change at 340 nm due to oxidation/reduction of NADPH/NADP⁺. Glutathione reductase activity was expressed as μ mol of NADPH oxidized/hour/mL.

Assay of glutathione-S-transferase: Glutathione-S-transferase activity was determined spectrophotometrically by the method of Habig et al.¹⁵. The reaction mixture (3 mL) contained 1.0 mL of 0.3 mM phosphate buffer (pH 6.5), 0.1 mL of 30 mM 1-chloro-2, 4-dinitrobenzene (CDNB) and 1.7 mL of double distilled water. After preincubating the reaction mixture at 37°C for 5 min, the reaction was started by the addition of 0.1 mL of hemolysate and 0.1 mL of glutathione as substrate. The absorbance was followed for 5 min at 340 nm. Glutathione-S-transferase activity was expressed as μ mol of CDNB-GSH conjugate formed/min/mg Hb.

Statistical analysis: All data were expressed as mean \pm SD. The statistical significance was evaluated by the Student 't' test using Statistical Package for the Social Sciences (SPSS Cary, NC, USA) version 10.0.

Results and Discussion

The mean age limit was 40.4 ± 8.3 years in rheumatoid arthritis patients and 42.2 ± 7.6 years in healthy control subjects (Table I). The decreased body mass index (BMI) in rheumatoid arthritis patients (23 ± 2.2 kg/m²) when compared to control subjects (26 ± 2.1 kg/m²) was statistically significant. Systolic blood pressure was significantly high in patients groups as compared with controls.

The levels of C reactive protein, rheumatoid factor, ASO, total WBC count, hemoglobin, platelet count and ESR were significantly increased in rheumatoid arthritis patients (Table II). However, the levels of blood glucose fasting, uric acid and total RBC counts in rheumatoid arthritis patients compare with healthy subjects did not differ significantly.

Table I: Demographic characteristic of healthy subjects and rheumatoid arthritis patients

Parameter	Healthy subjects (n=30)	RA patients (n=30)
Age (years)	42.2 \pm 7.6	40.4 \pm 8.3*
Sex- (M/F)	15/15	15/15
Body mass index (kg/ m ²)	23 \pm 2.2	26 \pm 2.1**
Smokers	9	18
Hypertension	2	12

Values are given as mean \pm S.D; RA patients compare with normal healthy subjects (*p<0.05, **p<0.001)

Table II: Levels of parameters related to biochemical, inflammation and hematological parameters in normal healthy subjects and rheumatoid arthritis patients

Parameter	Healthy subjects (n=30)	RA patients (n=30)
Fasting glucose (mg/dL)	101 \pm 9	99 \pm 17 ^{NS}
Uric acid (mg/dL)	4.44 \pm 0.96	4.72 \pm 1.01 ^{NS}
C reactive protein (mg/L)	3.13 \pm 1.41	16.67 \pm 1.92**
Rheumatoid factor (IU/mL)	5.92 \pm 1.03	11.3 \pm 3.10**
ASO (IU/mL)	0.04 \pm 0.00	102 \pm 59**
Hemoglobin (g/dL)	14.8 \pm 1.6	10.0 \pm 1.3**
RBC count (Cells/10 ⁶ μ L)	5.5 \pm 0.95	5.6 \pm 0.31 ^{NS}
WBC count (Cells/10 ⁶ μ L)	7.8 \pm 2.0	11.3 \pm 2.6**
ESR (mm/hour)	7 \pm 2	52 \pm 12**
Platelet count (Cells/10 ³ μ L)	270 \pm 67	350 \pm 80*

Values are given as mean \pm S.D; RA patients compare with normal healthy subjects (*p<0.05, **p<0.001, NS- Not significant)

Lipid peroxidation indicated by TBARS level was significantly higher in both plasma and erythrocytes levels in patients with rheumatoid arthritis patients compared to normal healthy subjects (Table III). The significantly decreased in the levels of plasma and erythrocyte glutathione, glutathione reductase and C reactive protein in rheumatoid arthritis patients compare with normal healthy subjects was statistically significant. A significant decrease in the activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase in erythrocyte lysate was seen in rheumatoid arthritis patients as compared to normal healthy subjects (Table IV).

Table III: Levels of plasma and erythrocytes TBARS, reduced glutathione (GSH) and glutathione reductase (GSHR) levels in normal healthy subjects and rheumatoid arthritis (RA) patients

Parameter	Healthy subjects (n=30)	RA patients (n=30)
Plasma TBARS (nmol /mL)	2.31 ± 0.21	5.12 ± 0.25*
Erythrocyte TBARS (pmole mg/Hb)	3.02 ± 0.41	6.16 ± 0.73*
Plasma GSH (mg/dL)	30.13 ± 1.37	23.03 ± 2.14*
Erythrocytes GSH (µmol g/Hb)	4.71 ± 1.78	2.56 ± 1.12*
Plasma GSHR (mg/dL)	52.21 ± 6.42	40.43 ± 5.11*
Erythrocytes GSHR (Umin/g/Hb)	11.20 ± 3.71	5.15 ± 2.37*

Values are given as mean ± S.D; RA patients compare with normal healthy subjects (*p<0.001)

Table IV: Activities of erythrocyte superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-Transferase (GST) in control and rheumatoid arthritis (RA) patients

Parameter	Healthy subjects (n=30)	RA patients (n=30)
SOD (Unit ^A /mg Hb)	3.71 ± 0.41	2.32 ± 0.21*
CAT (Unit ^B /mg Hb)	60.5 ± 6.71	47.27 ± 5.51*
GPx (Unit ^C mg /Hb)	7.35 ± 1.39	5.71 ± 0.72*
GST (Unit ^D mg /Hb)	2.50 ± 0.45	1.10 ± 0.37*

Values are given as mean ± S.D; RA patients compared with normal healthy subjects (*p<0.001); A – One unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in one min; B – µmole of H₂O₂ consumed/min; C – µg of GSH consumed/min; D – µmoles of CDNB – GSH conjugate formed/min

The results of the study indicate that the TBARS level in plasma and erythrocyte was higher in the patient group than in the control group. ROS and other oxidants have been shown to be formed even in the normal physiological process^{16,17}. It is known that activated inflammatory cells lead to ROS production in rheumatoid arthritis a systemic autoimmune disease¹⁸. Increased ROS, in turn, increase lipid peroxidation products and cause tissue injury¹⁹. Oxidant stress constitutes a serious pathophysiological factor for a wide variety of connective tissue disorders such as rheumatoid arthritis. Pathogenic mechanism of chronic inflammation is associated with increased production of ROS and free radicals (superoxide anion and hydrogen peroxide). Elevated free radical generations in inflamed joints and impaired antioxidant system have been implicated in rheumatoid arthritis. In this study, rheumatoid arthritis is a chronic degenerative disease; oxidative stress (e.g. TBARS) was higher in patients with rheumatoid arthritis patients than in healthy subjects. Furthermore, there was a close relationship between oxidative stress and

inflammation in patients with rheumatoid arthritis. In addition, activities of some plasma and erythrocyte antioxidant enzymes in the rheumatoid arthritis group were lower than in the healthy subjects²⁰.

Human synovial fluid contains little catalase, glutathione peroxidase, reduced glutathione and superoxide dismutase. Thus, the superoxide radicals and hydrogen peroxide generated by phagocytes in the inflamed rheumatoid joint would not be efficiently scavenged. Therefore, synovial fluid from the knee joints of human rheumatoid patients contains increased levels of TBARS, suggesting increased lipid peroxidation in vivo. The TBARS is transported from the synovial fluid to the blood by the blood circulation system. TBARS levels were found to be significantly elevated in the patients with rheumatoid arthritis compared to controls. Increased plasma and erythrocyte TBARS levels in patients with rheumatoid arthritis observed in this report and previously published reports and are indirect indicators of increased ROS production²¹.

In our study, reduced glutathione, glutathione reductase levels, glutathione peroxidase and catalase activities in plasma were lower in the patient group than in control. The TBARS levels in plasma samples were higher in the patient group than in the control group. Plasma and erythrocytes may be important in regulating oxidant reactions in the surrounding medium, thereby preventing free-radical-mediated cytotoxicity. However, the relationship between erythrocyte superoxide dismutase and rheumatoid arthritis is not clear. Our result that the activity of erythrocyte superoxide dismutase is unaltered is in agreement with most studies. Erythrocyte and serum glutathione peroxidase activities in patients with rheumatoid arthritis are controversial; as some reports show decreased^{22,23} while others show unaltered²⁴ activity levels. Our results of erythrocyte glutathione peroxidase and catalase activities, similar to Kamanli et al.²⁵ showed significantly lower levels in patients with rheumatoid arthritis than in the other two groups. The statistically significant decreases in plasma glutathione peroxidase and catalase activities between healthy subjects and rheumatoid arthritis patients may be due to the inactivation of the enzymes by H₂O₂ and suggest that these enzymes may play an important role in the rheumatic process and increased oxidative stress

ASO, C reactive protein and rheumatoid factor are active components related to the phagocytic system of polymorphonuclear leucocytes. 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 ASO, C reactive protein and rheumatoid factor) are liberated from the granule into the cytoplasm and play an important part in destroying phagocytosed materials. Also, these cells are able to produce superoxide radicals and other oxidant species when activated by diverse stimuli. The values of lipid peroxidation, mediated by neutrophils, depend on the severity of the inflammatory status. It was found that C reactive protein and rheumatoid factor in patients with rheumatoid arthritis might be sensitive inflammation markers for reflecting the presence and activity of the disease. The present study showed that malondialdehyde and parameters related with inflammation such as ASO, C reactive protein and ESR showed an increase in the patient group compared to the control group. Circulating human red blood cells possess the ability to scavenge ROS generated extracellularly by activated neutrophils. Hence, the RBC with decreased antioxidant levels is easily destroyed. The significantly decreased values of RBC and hemoglobin in the blood of rheumatoid arthritis patients observed in our study are supported by other workers who reported that increased ROS production is indicative of RBC destruction in patients with rheumatoid arthritis²⁵.

This study indicates that the antioxidant defense system is compromised in patients with rheumatoid arthritis, as evidenced by increased TBARS concentrations (the most potent marker of oxidative stress) and decreased levels of both plasma and erythrocyte antioxidant enzymes. We have also demonstrated that ESR, a serologic marker of inflammation, shows a significant correlation to malondialdehyde levels in patients with rheumatoid arthritis. These significant correlations between ESR, antioxidant states and ESR vs. TBARS in rheumatoid arthritis patients may be useful in predicting disease activity. Parallel to our findings, Yildirim et al.²⁶ recently reported that serum C reactive protein, among the various acute phase reactants, was the most useful biochemical marker for evaluating the disease activity of patients with rheumatoid arthritis.

In conclusion, on the basis of enhanced lipid peroxidation in rheumatoid arthritis patients with concomitant failure of both the plasma, and erythrocyte antioxidant defense mechanism. These results are consistent with the underlying hypothesis that there is an imbalance between ROS production and the antioxidant defense system in inflammatory rheumatoid arthritis disease. The potential role of increased lipid, protein and DNA

oxidation and impaired antioxidant capacity as a result of inflammatory character of rheumatoid arthritis seems to be related to disease severity.

References

1. Sarban S, Kocyigit A, Yazar M, Isikan UE. Plasma total antioxidant capacity, lipid peroxidation, and erythrocyte antioxidant enzyme activities in patients with rheumatoid arthritis and osteoarthritis. *Clin Biochem.* 2005; 38: 981-86.
2. Kuloglu M, Atmaca M, Tezcan E, Ustundag B, Bulut S. Antioxidant enzyme and malondialdehyde levels in patients with panic disorder. *Neuropsychobiology* 2002; 46: 186-89.
3. Tuzun A, Erdil A, Inal V, Aydin A, Bagci S, Yesilova Z, Sayal A, Karaeren N, Dagalp K. Oxidative stress and antioxidant capacity in patients with inflammatory bowel disease. *Clin Biochem.* 2002; 35: 569-72.
4. Yyldyrym K, Karatay S, Gureser G, Kyzyltunc A, Uour M, Penel K. Antioxidant enzymes capacity in patients with rheumatoid arthritis: The relationship with disease activity score. *Romatizma Cilt.* 2004; 19: 81-86.
5. Michiels C, Raes M, Toussaint O, Remacle J. Importance of Se-glutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress. *Free Radic Biol Med.* 1994; 17: 235-48.
6. Cimen MYB, Cimen OB, Kacmaz B, Ozturk HS, Yorgancioglu R, Durak I. Oxidant/antioxidant status of the erythrocytes from patients with rheumatoid arthritis. *Clin Rheumatol.* 2000; 19: 275-77.
7. Yagi K. Lipid peroxides and human diseases. *Chem Phys Lipids* 1978; 45: 337-51.
8. Donnan SK. The thiobarbituric acid test applied to tissues from rats treated in various ways. *J Biol Chem.* 1950; 182: 415-19.
9. Kakkar PS, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Indian J Biochem Biophys.* 1984; 21: 130-32.
10. Sinha KA. Colorimetric assay of catalase. *Anal Biochem.* 1972; 47: 389-94.
11. Ellman GL. Tissue sulfhydryl groups. *Arch. Biochem Biophys.* 1959; 82: 70-77.
12. Beutler E, Kelley BM. The effect of sodium nitrate on red cell glutathione. *Experientia* 1963; 19: 96-97.
13. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical roles as a component of glutathione peroxidase. *Science* 1973; 179: 588-90.
14. Carlberg I, Mannervik B. Glutathione reductase, In: *Methods in enzymology.* Meister A (ed). New York, Academic Press, 1985, pp 484-90.
15. Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferases, the first enzymatic step in mercapturic acid formation. *J Biol Chem.* 1974; 249: 7130-39.
16. Abuja PM, Albertini R. Methods for monitoring oxidative stress, lipid peroxidation and oxidation

- resistance of lipoproteins. *Clin Chim Acta*. 2001; 306: 1-17.
17. Ghiselli A, Serafini M, Natella F, Scaccini C. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. *Free Radic Biol Med*. 2000; 29: 1106-14.
 18. Fox DA. Etiology and pathogenesis of rheumatoid arthritis. In: *Arthritis and allied conditions a textbook of rheumatology*. Koopman WJ, Moreland LW (eds). 15th ed. Philadelphia, Lippincot Williams and Wilkins, 2005, pp 1089-115.
 19. Jasin HE. Mechanisms of tissue damage in rheumatoid arthritis. In: *Arthritis and allied conditions a textbook of rheumatology*. Koopman WJ, Moreland LW (eds). 15th ed. Philadelphia, Lippincot Williams and Wilkins, 2005, pp 1141-64.
 20. Ahmet I, Şahabettin S. Total antioxidant response and oxidative stress in patients with rheumatoid arthritis. *Arastirma* 2007; 21: 67-73.
 21. Gambhir JK, Lali P, Jain AK. Correlation between blood antioxidant levels and lipid peroxidation in rheumatoid arthritis. *Clin Biochem*. 1997; 30: 351-55.
 22. Taysi S, Polat F, Gul M, Sari RA, Bakan E. Lipid peroxidation, some extracellular antioxidants, and antioxidant enzymes in serum of patients with rheumatoid arthritis. *Rheumatol Int*. 2002; 21: 200-04.
 23. Nivsarkar M. Improvement in circulating superoxide dismutase levels: Role of non-steroidal anti-inflammatory drugs in rheumatoid arthritis. *Biochem Biophys Res Commun*. 2000; 270: 714-16.
 24. Kerimova AA, Atalay M, Yusifov EY, Kuprin SP, Kerimov TM. Antioxidant enzymes; possible mechanism of gold compound treatment in rheumatoid arthritis. *Pathophysiology* 2000; 7: 209-13.
 25. Kamanli A, Naziroglu M, Aydilek N, Hacievliyagil C. Plasma lipid peroxidation and antioxidant levels in patients with rheumatoid arthritis. *Cell Biochem Funct*. 2004; 22: 53-57.
 26. Yildirim K, Karatay S, Melikoglu MA, Gureser G, Ugur M, Senel K. Associations between acute phase reactant levels and disease activity score (DAS28) in patients with rheumatoid arthritis. *Ann Clin Lab Sci*. 2004; 34: 423-26.
-