ARAŞTIRMA

2007: 21 (2): 67 - 73
http://www.fusabil.org

Total Antioxidant Response and Oxidative Stress in Patients with Rheumatoid Arthritis

Ahmet IŞIK [1]
Şahabettin SELEK [2]

[1] Fırat University
Medicine Faculty
Department of
Rheumatology
Elazığ-TURKEY

[2] Harran University
Medicine Faculty
Department of Biochemistry
Şanlıurfa-TURKEY

It has been reported that various antioxidants decrease in rheumatoid arthritis (RA). Total antioxidant response (TAR), whose measurement method has been recently specified and developed, represents the total antioxidant properties of all antioxidants. This study aims to measure total antioxidant capacity in RA using TAR and evaluate its relations with oxidative stress.

The study included 47 active RA cases, and 23 age- and sex-matched healthy volunteers as the control group. TAR and total peroxide (TP) were studied using appropriate methods and oxidative stress (OSI) was calculated.

TAR level was 1,02±0,09 and 1,16±0,08 mmol Trolox eq./L in the patient group and the control group, respectively; TP level was 23,84±10,10 and 7,72±1,86 µmol H2O2/L in the patient group and the control group, respectively; and OSI value was 2,34±1,19 and 0,67±0,18 Arbitrary Units in the patient group and the control group, respectively. When compared with the control group, TAR level was significantly lower, and TP and OSI levels were significantly higher in the patient group ($p<0.001$ for each). TAR level was negatively correlated with TP and OSI levels both in the patient group ($r=-0.534$, $p<0.01$ and $r=-0.552$, $p<0.01$, respectively) and the control group ($r=-0.469$, $p<0.05$ and $r=-0.382$, $p<0.05$, respectively).

The results of our study indicate an increase in oxidative stress and lipid peroxidation products and a decrease in antioxidant capacity in RA cases.

Key Words: *Sigmoid Colon, Abnormal, Cadaver*

Romatoid Artritli Hastalarda Total Antioksidan Cevap ve Oksidatif Stres

Romatoid artrit (RA)'te çeşitli antioksidanların azaldığı bildirilmiştir. Total antioksidan kapasiteyi gösteren ve , total antioksidan cevap (TAR)'ın ölçüm metodu son zamanlarda belirlenmiş ve geliştirilmiştir olan total antioksidan cevap (TAR) tüm antioksidanların total antioksidan özelliklerini yansıtmaktadır. Bu çalışmanın amacı, RA'te total antioksidan kapasiteyi TAR ile ölçmek ve oksidatif stres ile ilişkilerini incelemektir.

Çalışmaya 47 aktif RA'li olgu ile kontrol grubu olarak yaş ve cinsiyetleri uyumlu 23 sağlıklı gönüllü alındı. Hasta ve kontrol gruplarında TAR, total peroksit (TP) uygun yöntemler ile çalışıldı ölçüldü ve oksidatif stres indeksi (OSI) hesaplandı.

TAR düzeyi hasta grubunda 1,02±0,09 mmol ve kontrol grubunda 1,16±0,08 mmol Trolox eq./L; TP düzeyi hasta grubunda 23,84±10,10 ve kontrol grubunda 7,72±1,86 µmol H2O2/L; OSI değeri ise hasta grubunda 2,34±1,19 ve kontrol grubunda 0,67±0,18 Arbitrary Unit olarak saptandı. Kontrol grubu ile karşılaştırıldığında, hasta grubundaki TAR düzeyi düşüklüğü, TP ve OSI düzeyleri yüksekliği anlamlı bulundu (herbirisi için, $p<0.001$). TAR düzeyi ile TP ve OSI düzeyleri arasında, hem hasta grubunda (sırasıyla; $r=-0.534$, $p<0.01$ ve $r=-0.552$, $p<0.01$), hem de kontrol grubunda (sırasıyla; $r=-0.469$, $p<0.05$ ve $r=-0.382$, $p<0.05$) anlamlı negatif korelasyon vardı.

Çalışma sonuçlarımız, RA'li olgularda oksidatif stres ve lipid peroksidasyon ürünlerinde artış ve antioksidan kapasitede azalma olduğunu göstermektedir.

Anahtar Kelimeler: *Rheumatoid Arthritis, Total Antioxidant Response, Total Peroxide, Oxidative Stress Index.*

Introduction

Rheumatoid arthritis (RA) is a progressive, relapsing and chronic inflammatory disease (1). Although it affects about 1% of the adult population, its etiopathogenesis has not been fully revealed yet (1). Nonetheless, reactive oxygen species (ROS) have been reported to play important roles in RA pathogenesis (2-4). ROS, which can be produced as a result of normal aerobic metabolism and whose production is increased by active neutrophils during inflammation, have recently attracting increasing attention (5). It is known that neutrophils in the joints affected by the disease are activated in RA cases. Activated neutrophils lead to the production of ROS like hydrogen peroxide (H2O2), hydroxyl radical (OH•) and superoxide radical (O2•-), as well as, nitrogen-containing radicals like peroxynitrite (ONOO-) (2-4).

Geliş Tarihi : 28.09.2006
Kabul Tarihi : 20.11.2006

Yazışma Adresi
Correspondence

Ahmet IŞIK
Fırat Üniversitesi
Tıp Fakültesi
Romatoloji Bilim Dalı
23119
Elazığ-TÜRKİYE

drahmetisik@hotmail.com

Polymorphonuclear leukocytes increase the production of hypochlorous acid (HOCl) from H2O2 via myeloperoxidase enzyme (2). HOCl, in turn, causes tissue injury via sulphydryl oxidation and protein decarboxylation, leading to oxidative modification of macromolecules in the tissues (2).

The primary targets of ROS are lipids in the cell membrane. ROS increase lipid peroxidation (LPO), and thus, impair cell structure and functions (2-4). Increase in LPO products has been reported in RA cases (6-8). It has been demonstrated that increased LPO products and ROS inhibit antioxidant systems and impair oxidant/antioxidant balance in RA (9,10).

Blood contains many antioxidant molecules those prevent and/or inhibit harmful free radical reactions (11). Exogenous antioxidants like vitamin C and vitamin E, and endogenous antioxidants like uric acid, bilirubin, albumin and superoxide dismutase and glutathione peroxidase as the scavenger enzymes can protect the cell against the potentially detrimental effects of oxidant agents (10,11). Concentrations of these antioxidants in the plasma can be measured one by one, but this procedure is time-consuming, labour-intensive and costly and requires complicated techniques (12). On the other hand, total antioxidant response (TAR), whose measurement method has been recently specified and developed, and which can indicate the total antioxidant state of the plasma (12,13). In this method, TAR of plasma against especially potent free radical reactions which strongly lead to oxidative damage of biomolecules such as lipids, proteins and DNA, is measured.

In this study, we aimed to measure both the levels of some individual antioxidant components and the TAR values in plasma samples from active RA to evaluate their antioxidant status using a novel automated method (12,13). As a reciprocal measure, the total peroxide (TP) levels of the same plasma samples were also measured (14). The ratio of the plasma TP level to the TAR level was regarded as the oxidative stress index (OSI) (15).

Materials And Methods

The study included 47 active RA cases who applied to outpatient clinic of Rheumatology Department of Internal Medicine of Medical Center of Firat University and 23 healthy individuals who age and sex matched. RA was diagnosed according to the American Rheumatism Association (ARA) criteria (16). Approval of the local ethics committee and informed consent of the patients and healthy individuals were obtained. Individuals who had pathologies those could lead to secondary lipid disorders, cardiovascular diseases (CVD), diabetes mellitus, renal failure, chronic infection, alcohol abuse, and those who used antilipidemic and antioxidant drugs were excluded from the study.

Blood samples were obtained at 08.00-09.00 a.m. after a 8-12 hours of fasting. The samples were centrifuged at 3000 rpm for 10 minutes to obtain serums. Routine biochemical analyses were carried out in the serum samples by Olympus AU 600 Autoanalyzer using Olympus kits (Olympus Corp. Tokyo-Japan). Erythrocyte sedimentation rate (ESR) was determined by classical Westergren method immediately in whole blood with 1 mg/mL ethylene diamine tetraacetic acid. The level of C-reactive protein (CRP) was determined by immunoturbidimetric technique (Schiapparelli Biosystems, the Netherlands). The level of rheumatoid factor (RF) was studied measured by nephelometric method (BNII, Dade Behring, Germany).

Levels of serum total protein, albumin, bilirubin and uric acid were measured by using commercial kits (Olympus AU Autoanalyzer). TAR and TP, the specific parameters, were measured in the plasma samples obtained by centrifugation of blood put in heparinized tubes, using appropriate methods. Plasma samples were stored at -80 20 oC until the day of analysis.

Measurement of the total antioxidant status of plasma: The total antioxidant status of the plasma was measured using a novel automated colorimetric measurement method for the TAR developed by Erel (12,13). In this method, the hydroxyl radical, the most potent biological radical, is produced by the Fenton reaction, and reacts with the colourless substrate O-dianisidine to produce the dianisyl radical, which is bright yellowish-brown in colour. Upon the addition of a plasma sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction mix are suppressed by the antioxidant components of the plasma, preventing the colour change and thereby providing an effective measure of the total antioxidant capacity of the plasma. The assay results are expressed as mmol Trolox eq./L, and the precision of this assay is excellent, being lower than 3% (17).

Measurement of total plasma peroxide (TP) concentration: The total plasma peroxide concentrations were determined using the FOX2 method (14) with minor modifications (15). The FOX2 test system is based on the oxidation of ferrous iron to ferric iron by the various types of peroxides contained in the plasma samples, in the presence of xylenol orange which produces a coloured ferric-xylenol orange complex whose absorbance can be measured. The FOX2 reagent was prepared by dissolving ammonium ferrous sulphate (9.8 mg) in 250 mM H2SO4 (10 ml) to give a final concentration of 250 μM ferrous iron in acid. This solution was then added to 90 ml HPLC-grade methanol containing 79.2 mg of butylated hydroxytoluene (BHT). Finally, 7.6 mg of xylenol orange was added, with stirring, to make the working reagent (250 μM ammoniumferrous sulphate, 100 μM xylenol orange, 25 mM H2SO4, and 4 nM BHT, in 90% (v/v) methanol in a final volume of 100 ml). The blank reagent contained all the components of the solution except ferrous sulphate.

Aliquots (200 μL) of plasma were mixed with 1.8 ml FOX2 reagent. After incubation at room temperature for 30 min, the vials were centrifuged at 12,000 g for 10 min. The absorbance of the supernatant was then determined at 560 nm. The TP content of the plasma samples was determined as a function of the difference in absorbance between the test and blank samples using a solution of

H2O2 as standard. The coefficient of variation for individual plasma samples was less than 5%.

Oxidative stress index (OSI): The ratio of the TP to the total antioxidant potential gave the OSI, an indicator of the degree of oxidative stress (15).

Statistics: All results were expressed as means□SD. Student's t-test and Spearman's correlation analysis were performed using SPSS for Windows Release 11.0 (SPSS Inc. Chicago, Illinois, USA). The P value less than 0.05 was considered to be significant.

Results

The patient group included 40 females and 7 males, whose mean age was 50,4±12,5 years and mean duration of the disease was 4,4±6,2 (0-16) years. The patients were diagnosed as RA according to ARA criteria (16). All RA patients were active according to the criteria for complete clinical remission of RA, as suggested by Pinals et al. (18). The control group consisted of 17 females and 6 males healthy volunteers whose mean age was 49,7± 4,2 years. Of the patients in the RA group, 11 had been recently diagnosed and had not used corticosteroid and any disease-modifying antirheumatic drugs (DMARDs) in their history. Of the 36 cases who were diagnosed previously, 22 were receiving methotrexate, salazopyrin sulfasalazine, chlorochine and corticosteroid combination; 14 were using corticosteroid in addition to one of the DMARDs. RF was positive in 38 cases and negative in 9 cases. Nine cases in the patient group and 5 cases in the control group were diagnosed as having hypertension. Demographical characteristics and laboratory findings of the patient group and the control one are presented in Table 1.

Table 1. Demographical characteristics and laboratory findings in the patient group and the control group.

	RA (n:47)	Controls (n:23)	*P*
Age (years)	50,4±12,5	49,7±4,2	0.333
Sex (M/F)	7/40	6/17	0.456
Disease Duration (years)	4,4±6,2	-	-
BMI (kg/m^2)	26,1±4,2	25,1±3,1	0.084
ESR (mm/h)	42,4±27,8	10,2±8,6	0.002
CRP (mg/L)	35,7±39,4	3,1±2,2	0.001
RF (U/L)	201±214	-	-
Hemoglobin (gr/dL)	12,4±1,5	14,2±1,4	0.033
WBC ($10^3/\mu L$)	9,1±2,5	6,2±1,5	0.020
Fasting blood glucose (mg/dL)	95,3±14,6	91,5±9,3	0.333
Total cholesterol (mg/dL)	179,7±40,4	170,4±31,4	0.602
LDL (mg/dL)	113,6±33,2	109,7±22,2	0.095
HDL (mg/dL)	49,8±12,5	46,6±8,6	0.197
Triglyceride (mg/dL)	141,4±72,4	145,5±77,8	0.823
Total protein (gr/dL)	7,28±0,8	6,95±0,6	0.081
Albumin (gr/dL)	4,01±0,79	4,37±0,37	0.035
Total bilirubin (mg/dL)	0,82±0,34	0,86±0,31	0.651
Direct bilirubin (mg/dL)	0,11±0,07	0,09±0,03	0.202
Uric acid (mg/dL)	4,44±0,96	4,72±1,01	0.657

M: male, F: female, BMI: body mass index, ESR; erythrocyte sedimentation rate, CRP; C-reactive protein, RF; rheumatoid factor, WBC; white blood cell count.

Table 2. TAR, TP and OSI findings in the patient group and the control group.

	RA (n:47)	Controls (n:23)	P
TAR (mmol Trolox eq./L)	1,02±0,09	1,16±0,08	0.000
TP (μmol H_2O_2/L)	23,84±10,1	7,72±1,86	0.000
OSI (Arbitrary Unit)	2,34±1,19	0,67±0,18	0.000

TAR; total antioxidant response, TP; total peroxide and OSI; oxidative stress index.

The comparison between the patient group and the control group revealed that white blood cell count (WBC), ESR and CRP values were significantly higher ($p<0.05$, $p<0.01$, $p<0.001$, respectively) and hemoglobin level was significantly lower ($p<0.05$) in the former. There was not any significant difference between the groups in terms of fasting blood glucose, total cholesterol, triglyceride, HDL and LDL levels ($p>0.05$, for each).

The level of albumin, an individual antioxidant, was significantly lower in the patient group ($p<0.0501$), but

there was not any significant difference between uric acid and bilirubin levels of the groups (p>0.05, for each).

TAR level was 1,02±0,09 and 1,16±0,08 mmol Trolox eq./L in the patient group and the control group, respectively; TP level was 23,84±10,1 and 7,72±1,86 µmol H2O2/L in the patient group and the control group, respectively; and OSI value was 2,34±1,19 and 0,67±0,18 Arbitrary Units in the patient group and the control group, respectively. When compared with the control group, TAR level was significantly lower, and levels of TP and OSI were significantly higher in the patient group (p<0.001, for each, Table 2, Figure 1 and 2). TAR level was negatively correlated with TP (Figure 3) and OSI levels both in the patient group (r=-0.534, p<0.01 and r=-0.552, p<0.01, respectively) and the control one (r=-0.469, p<0.05 and r=-0.382, p<0.05, respectively).

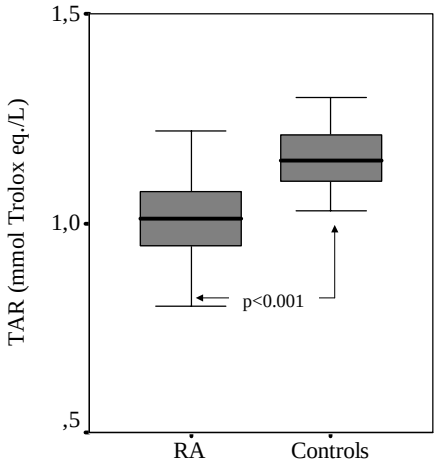

Figure 1. The levels of TAR in the patient group and the control group.

There was no significant correlation among biochemical findings, TAR, TP and OSI values of the cases in the patient group in regard with gender, duration of disease, RF positivity (p>0.05, for each).

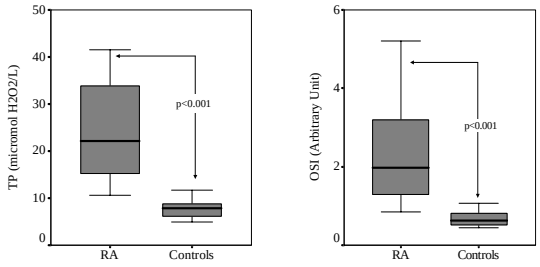

Figure 2. The levels of TP and OSI values in the patient group and the control group.

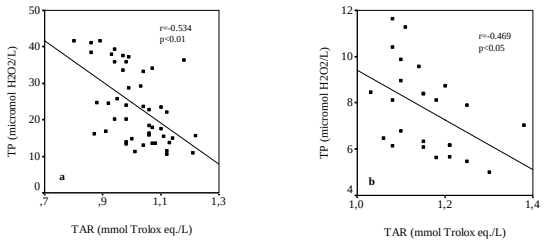

Figure 3. Correlation between TAR and TP in the patient group (a) and the control group (b).

Discussion

ROS and other oxidants have been shown to be formed even in the normal physiological process (19,20). It is known that activated inflammatory cells lead to ROS production in RA a systemic autoimmune disease (1,2). Increased ROS, in turn, increase LPO products and cause tissue injury (1-5).

Hydrogen peroxide and other derivatives of peroxides, produced physiologically and which increase in some conditions, diffuse into plasma. When TP is measured, it means that the sum of many peroxides like protein peroxide, lipid peroxide and H2O2 are measured (19). Although it is known that H2O2 and lipid peroxides increase in RA (1-3,7,8), oxidative stress has not been evaluated via measurement of TP. However, it has been reported that TP level increases in passive smokers (21), preeclampsia cases (22) and skin leishmaniasis (23). It was shown in our study that TP level also increased in RA cases. Possible causes of this increase in TP might be the inevitable increase in lipid peroxides and ROS including H2O2 in RA.

Many antioxidant molecules found in blood prevent or inhibit the harmful effects of free radicals (11). Whenever there is a decrease in antioxidants and/or an increase in oxidants, oxidant/antioxidant balance is impaired in favor of oxidants and this is known as oxidative stress (19,20). It is known that oxidative stress is responsible for tissue injury in many diseases and contributes to the development of atherosclerosis (19,24,25). Antioxidant activity indicates the antioxidant characteristics of only one antioxidant, whereas total antioxidant capacity (TAC) represents the total antioxidant characteristics of all antioxidants found in the plasma. TAR and total antioxidant status (TAS) are used synonymously with TAC (20). It is doubtlessly more advantageous to evaluate TAR, instead of individual antioxidant activities. Many methods have been developed recently for this aim. Total radical trapping antioxidant parameter (TRAP), oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) are colorimetric methods previously developed to assess TAC (12,13,20). It has been reported that TAR, a new measurement method developed by Erel (12,13) has correlated with the data obtained by other measurement methods and has had some additional advantages (12,13).

Blood has an important function in the oxidant/antioxidant balance, as it carries and distributes antioxidants through the body (20). Plasma has various antioxidant molecules. Albumin, uric acid, bilirubin and ascorbic acid are the major antioxidant components of plasma (12,13,20). TAR represents practically all of them (12,13). Albumin consists of about half of the TAC of the plasma (12,13). Albumin has several biological functions, particular as a ligand binder (26). Plasma thiol contents originate from albumin. Thiol groups, on the surface of albumin, bind oxidants (26). Low level of albumin can cause oxidative stress via leading to increase oxidants like homocysteine (26). In chronic inflammation, albumin is reduced. We attribute the low albumin level in our patient group to this protein's being a negative acute phase reactant, but this decrease is also expected to negatively influence antioxidant capacity. Bilirubin, a powerful endogenous antioxidant, is one of the catabolits of heme oxygenases (27). However, Harma et al. (22) have reported that bilirubin did not correlated with TAR, in their clinical study. Uric acid is another well-known low molecular weight water-soluble plasma antioxidant (12,13). Uric acid has a strong antioxidant activity and its concentration in the plasma is about 10 fold than antioxidants like vitamin C and vitamin E (20). In the present study, although a significant decrease was found in the level of albumin, an individual antioxidant, in the patient group, uric acid and bilirubin levels were not significantly different from those in the control group. A positive correlation was reported amongst uric acid, bilirubin and total protein levels, and TAC level (20). However, uric acid concentration changes depending on gender, diet, heavy exercise, renal failure, and in some metabolic diseases (20,28). Methotrexate treatment might alter uric acid level has been also reported in RA cases (29). We suggest that uric acid might not appropriately reflect the TAC. However, it has been also reported that uric acid was not a strong antioxidant and might not protect against free radicals (30).

Orem et al. (31) have reported a decrease in TAR level in Behcet's disease. It was established in our study that TAR level decreased, while TP and OSI levels increased and these increases negatively correlated with TAR level in the patient group. We think that the decrease in TAR level may have resulted from increased oxidative stress. As uric acid and bilirubin levels have not been significantly different in the patient and control groups, it has seemed reasonable to assume that the decrease in TAR level is a result of the decrease in other antioxidants. It has been reported that PON1 activity (32,33) and SH level (34), both of which are antioxidants, have decreased in RA. Ece et al. (35) have noted that PON1 is positively correlated with TAR and negatively correlated with TP and OSI in cases with nephrotic syndrome. The decrease in TAR level in RA may be associated with the decrease in PON1 and other antioxidants.

Plasma TAR level has been reported to be lower in those with CVD, compared with without CVD, in smokers, compared with non-smokers, in diabetic cases, compared with non-diabetic cases, and in hyperlipidemic cases, compared with those who have a normal lipid profile (36). Additionally, children who are exposed to passive cigarette smoking have found to have a decrease in TAR level, and an increase in TP and OSI levels (21). As TAR is a fairly good representative of antioxidant capacity, while TP and OSI are indicators of oxidant capacity, decreased TAR and/or increased TP levels indicate oxidative stress (12,13,28).

It has been reported in the literature that atherosclerotic diseases increase in RA cases, but the causes of this accelerated atherosclerosis cannot always be explained by classical risk factors (5). Oxidative stress is blamed to be responsible in the pathogenesis of atherosclerosis (24,25). Atherosclerosis must also be considered when evaluating RA cases, as it has been shown that oxidative stress relates with atherosclerosis and TAR levels decrease in atherosclerosis (36,37).

It is possible that the administration of DMARDs and steroids affect the levels of oxidants and antioxidants either negatively or positively. Previous experimental studies had demonstrated that using steroid impaired antioxidant systems and leading to overproduction of ROS (38,39). In RA patients, it has been reported that MTX alone (40) or combined with sulfasalazine (41) lead to increased plasma homocysteine level. In our study, we did not observe any difference between the cases with use or without use of these medications with regard to oxidant and antioxidant levels. Not observing this difference might be resulted from that the cases enrolled to the study were in their active phases.

The limitation of our study might be not to evaluate relationships amongst drugs used in our cases, level of cytokines like IL-1, Il-6 and TNF-α, rheumatological data reflecting the activity, severity and function of RA patients (pain, DAS-28, disability, articular index, radiological severity etc.).

In conclusion, oxidant/antioxidant balance is impaired in RA and TAR, a new measurement method, effectively shows this balance.

Kaynaklar

1. Fox DA. Etiology and pathogenesis of rheumatoid arthritis. In: Koopman WJ, Moreland LW, eds. Arthritis and allied conditions a textbook of rheumatology. Fifteenth edition. Philadelphia: Lippincot Williams and Wilkins. 2005:1089-1115.

2. Jasin HE. Mechanisms of tissue damage in rheumatoid arthritis. In: Koopman WJ, Moreland LW, eds. Arthritis and allied conditions a textbook of rheumatology. Fifteenth edition. Philadelphia: Lippincot Williams and Wilkins. 2005:1141-1164.

3. Griffiths HR, Lunec J. The C1q binding activity of IgG is modified in vitro by reactive oxygen species: implications for rheumatoid arthritis. FEBS Lett 1996;388:161-164.

4. Nurcombe HL, Bucknall RC, Edwards SW. Activation of the neutrophil myeloperoxidase-H2O2 system by synovial fluid isolated from patients with rheumatoid arthritis. Ann Rheum Dis 1991;50:237-242.

5. Sattar N, McCarey DW, Capell H, McInnes IB. Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. Circulation 2003;108:2957-2963.

6. Miesel R, Murphy MP, Kroger H. Enhanced mitochondrial radical production in patients which rheumatoid arthritis correlates with elevated levels of tumor necrosis factor alpha in plasma. Free Radic Res 1996;25:161-169.

7. Jaswal S, Mehta HC, Sood AK, Kaur J. Antioxidant status in rheumatoid arthritis and role of antioxidant therapy. Clin Chim Acta 2003;338:123-129.

8. Jira W, Spiteller G, Richter A. Increased levels of lipid oxidation products in low density lipoproteins of patients suffering from rheumatoid arthritis. Chem Phys Lipids 1997;87:81-89.

9. Gambhir JK, Lali P, Jain AK. Correlation between blood antioxidant levels and lipid peroxidation in rheumatoid arthritis. Clin Biochem 1997;30:351-355.

10. Ozturk HS, Cimen MY, Cimen OB, Kacmaz M, Durak I. Oxidant/antioxidant status of plasma samples from patients with rheumatoid arthritis. Rheumatol Int 1999;19:35-37.

11. Young IS, Woodside JV. Antioxidants in health and disease. J Clin Pathol 2001;54:176-186.

12. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004;37:277-285.

13. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem 2004;37:112-119.

14. Miyazawa T, Saeki R, Inaba H. Detection of chemiluminescence in lipid peroxidation of biological systems and its application to HPLC. J Biolumin Chemilumin 1989;4:475-478.

15. Harma M, Harma M, Erel O. Increased oxidative stress in patients with hydatidiform mole. Swiss Med Wkly 2003;133:563-566.

16. Arnet FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-324.

17. Cao G, Prior RL. Comparison of different analytical methods for assessing total antioxidant capacity of human serum. Clin Chem 1998;44:1309-1315.

18. Pinals RS, Masi AT, Larsen RA. Preliminary criteria for clinical remission in rheumatoid arthritis. Arthritis Rheum 1981;24:1308-1315.

19. Abuja PM, Albertini R. Methods for monitoring oxidative stress, lipid peroxidation and oxidation resistance of lipoproteins. Clin Chim Acta 2001;306:1-17.

20. 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;29:1106-1114.

21. Kosecik M, Erel O, Sevinc E, Selek S. Increased oxidative stress in children exposed to passive smoking. Int J Cardiol 2005;100:61-64.

22. Harma M, Harma M, Erel O. Measurement of the total antioxidant response in preeclampsia with a novel automated method. Eur J Obstet Gynecol Reprod Biol 2005;118:47-51.

23. Kocyigit A, Keles H, Selek S, Guzel S, Celik H, Erel O. Increased DNA damage and oxidative stress in patients with cutaneous leishmaniasis. Mutat Res 2005;585:71-78.

24. Madamanchi NR, Hakim ZS, Runge MS. Oxidative stress in atherogenesis and arterial thrombosis: the disconnect between cellular studies and clinical outcomes. J Thromb Haemost 2005;3:254-267.

25. Violi F, Cangemi R. Antioxidants and cardiovascular disease. Curr Opin Investig Drugs 2005;6:895-900.

26. Sengupta S, Wehbe C, Majors AK, Ketterer ME, DiBello PM, Jacobsen DW. Relative roles of albumin and ceruloplasmin in the formation of homocystine, homocysteine-cysteine-mixed disulfide, and cystine in circulation. J Biol Chem 2001;276:46896-46904.

27. Dohi K, Satoh K, Ohtaki H, Shioda S, Miyake Y, Shindo M, et al. Elevated plasma levels of bilirubin in patients with neurotrauma reflect its pathophysiological role in free radical scavenging. In Vivo 2005;19:855-860.

28. Puig JG, Mateos FA, Ramos TH, Capitan CF, Michan AA, Mantilla JM. Sex differences in uric acid metabolism in adults: evidence for a lack of influence of estradiol-17 beta (E2). Adv Exp Med Biol 1986;195:317-323.

29. Smolenska Z, Kaznowska Z, Zarowny D, Simmonds HA, Smolenski RT. Effect of methotrexate on blood purine and pyrimidine levels in patients with rheumatoid arthritis. Rheumatology (Oxford) 1999;38:997-1002.

30. Hawkins CL, Davies MJ. Hypochlorite-induced damage to proteins: formation of nitrogen-centred radicals from lysine residues and their role in protein fragmentation. Biochem J 1998;332:617-625.

31. Orem A, Yandi YE, Vanizor B, Cimsit G, Uydu HA, Malkoc M. The evaluation of autoantibodies against oxidatively modified low-density lipoprotein (LDL), susceptibility of LDL to oxidation, serum lipids and lipid hydroperoxide levels, total antioxidant status, antioxidant enzyme activities, and endothelial dysfunction in patients with Behcet's disease. Clin Biochem 2002;35:217-224.

32. Baskol G, Demir H, Baskol M, Kilic E, Ates F, Kocer D, et al. Assessment of paraoxonase 1 activity and malondialdehyde levels in patients with rheumatoid arthritis. Clin Biochem 2005;38:951-955.

33. Tanimoto N, Kumon Y, Suehiro T, Ohkubo S, Ikeda Y, Nishiya K, et al. Serum paraoxonase activity decreases in rheumatoid arthritis. Life Sci. 2003;72:2877-2885.

34. Hall ND, Blake DR, Bacon PA. Serum sulphydryl levels in early synovitis. J Rheumatol 1982;9:593-596.

35. Ece A, Atamer Y, Gurkan F, Davutoglu M, Kocyigit Y, Tutanc M. Paraoxonase, total antioxidant response, and peroxide levels in children with steroid-sensitive nephrotic syndrome. Pediatr Nephrol 2005;20:1279-1284.

36. Demirbag R, Yilmaz R, Kocyigit A. Relationship between DNA damage, total antioxidant capacity and coronary artery disease. Mutat Res 2005;570:197-203.

37. Zawadzka-Bartczak E. Activities of red blood cell anti-oxidative enzymes (SOD, GPx) and total anti-oxidative capacity of serum (TAS) in men with coronary atherosclerosis and in healthy pilots. Med Sci Monit 2005;11:CR440-4.

38. Iuchi T, Akaike M, Mitsui T, Ohshima Y, Shintani Y, Azuma H, Matsumoto T. Glucocorticoid excess induces superoxide production in vascular endothelial cells and elicits vascular endothelial dysfunction. Circ Res 2003;92(1):81-7.

39. Orzechowski A, Ostaszewski P, Wilczak J, Jank M, Balasinska B, Wareski P, Fuller J Jr. Rats with a glucocorticoid-induced catabolic state show symptoms of oxidative stress and spleen atrophy: the effects of age and recovery. J Vet Med A Physiol Pathol Clin Med 2002;49(5):256-63.

40. van Ede AE, Laan RF, Blom HJ, Boers GH, Haagsma CJ, Thomas CM et al. Homocysteine and folate status in methotrexate-treated patients with rheumatoid arthritis. Rheumatology (Oxford) 2002;41(6):658-65.

41. Haagsma CJ, Blom HJ, van Riel PL, van't Hof MA, Giesendorf BA, van Oppenraaij-Emmerzaal D, van de Putte LB. Influence of sulphasalazine, methotrexate, and the combination of both on plasma homocysteine concentrations in patients with rheumatoid arthritis. Ann Rheum Dis 1999;58(2):79-84.