# REDOX STATUS IN WOMEN WITH RHEUMATHOID ARTHRITIS

Aleksandra Vranic[1], Aleksandra Antovic[2], Nevena Draginic[3], Marijana Andjic[1], Marko Ravic[1], Vladimir Jakovljevic[3,4] and Mirjana Veselinovic[5]
[1]Department of Pharmacy, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia
[2]Karolinska Institutet, Department of Medicine, Solna, Rheumatology Unit
and Academic Specialist Center, Center for Rheumatology, Stockholm Health Services, Stockholm, Sweden
[3]Department of Physiology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia
[4]Department of Human Pathology, 1st Moscow State Medical University IM Sechenov, Moscow, Russia
[5]Department of Internal medicine, Faculty of Medical Sciences, University of Kragujevac, Serbia

# REDOKS STATUS KOD ŽENA SA REUMATOIDNIM ARTRITISOM

Aleksandra Vranic[1], Aleksandra Antovic[2], Nevena Draginic[3], Marijana Andjic[1], Marko Ravic[1], Vladimir Jakovljevic[3,4] i Mirjana Veselinovic[5]
[1]Katedra za farmaciju, Fakultet medicinskih nauka, Univerzitet u Kragujevcu, Kragujevac, Srbija
[2]Karolinska Institut, Odsek medicina, Solna, Odeljenje za reumatologiju
i Akademski specijalisticki centar, Centar za reumatologiju, Medicinski centar Stokholm, Stokholm, Svedska
[3]Katedra za fiziologiju, Fakultet medicinskih nauka, Univerzitet u Kragujevcu, Kragujevac, Srbija
[4]Odsek za humanu patologiju, Prvi Moskovski državni medicinski univerzitet I.M. Sechenov, Moskva, Rusija
[5]Katedra za internu medicine, Fakultet medicinskih nauka, Univerzitet u Kragujevcu, Kragujevac, Srbija

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## ABSTRACT

*The aim of this study was to assess oxidative status and to set baseline characteristics for female population with established rheumatoid arthritis. Total of 42 patients with rheumatoid arthritis and 48 age- and sex-matched controls were included in the study. Clinical examination was performed and assessed disease activity. Peripheral blood samples were used for all the assays. The markers of oxidative stress were assessed, including plasma levels of index of lipid peroxidation – thiobarbituric acid reactive substances, hydrogen peroxide, superoxide anion radical, nitrites and activity of superoxide dismutase, catalase and reduced glutathione levels as antioxidant parameters. In the patients group, levels of hydrogen peroxide and index of lipid peroxidation were higher than in controls. Patients with rheumatoid arthritis had decreased superoxide dismutase and catalase activity compared to healthy subjects. Interestingly, controls had higher levels of nitrites compared to patients. Patients showed a marked increase in reactive oxygen species formation and lipid peroxidation as well as decrease in the activity of antioxidant defense system leading to oxidative stress which may contribute to tissue and cartilage damage and hence to the chronicity of the disease.*

**Keywords:** *rheumatoid arthritis, oxidative stress, reactive oxygen species, women*

## SAŽETAK

*Studija ima za cilj da proceni oksidacioni status i utvrdi osnovne karakteristike ženske populacije sa dijagnozom reumato. U istraživanje je uključeno 42 pacijenta sa reumatoidnim artritisom i 48 zdrava ispitanika iste starosti i pola. Urađen je klinički pregled i procenjena je aktivnost bolesti. Uzorci periferne krvi su korišćeni za sve testove. Određivani su markeri oksidacionog stresa, uključujući plazma koncentracije indeksa lipidne peroksidacije, vodonik peroksida, superoksid anjon radikala, nitrita kao i aktivnost superoksid dismutaze, katalaze i nivo redukovanog glutationa kao antioksidacionih parametara. Kod pacijenata, nivoi vodonik peroksida i indeksa lipidne peroksidacije bili su veći nego u kontrolnoj grupi. Pacijenti sa reumatoidnim artritisom imali su smanjenu aktivnost superoksid dismutaze i katalaze u poređenju sa zdravim osobama. Interesantno, kontrole su imale veće vrednosti nitrita u poređenju sa pacijentima. Pacijenti sa reumatoidnim artritisom pokazali su značajno povećanje koncentracije reaktivnih kiseonicnih vrsta, kao i smanjenje aktivnosti antioksidacionog sistema, što dovodi do nastanka oksidacionog stresa koji može doprineti oštećenju tkiva i hrskavice, a time i hroničnom toku bolesti.*

**Ključne reči:** *reumatoidni artritis, oksidacioni stres, reaktivne kiseonične vrste, žene*

## ABBREVIATIONS

**Anti-CCP** – Anticyclic citrullinated polypeptide
**aPTT** – activated partial thromboplastin time
**BMI** – Body mass index
**CRP** – C-reactive protein
**DAS28** – Disease activity score
**ESR** – Erythrocyte Sedimentation Rate
**INR** – International Normalised Ratio
**LPO** – lipid peroxidation
**PT** – prothrombin time
**RA** – Rheumatoid arthritis
**RF** – Rheumatoid factor
**ROS** – Reactive oxygen species

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**Corresponding author:**
Mirjana Veselinovic,
Faculty of Medical Sciences, University of Kragujevac,
Kragujevac, SERBIA,
Email:veselinovic.m@sbb.rs

## INTRODUCTION

Rheumatoid arthritis (RA) is systemic autoimmune disease manifested as erosive polyarthritis with chronic manner, intermittent acute inflammatory episodes and extra-articular manifestation (1). It consequently leads to progressive cartilage and joints damage as well as to disability (2) and represents a significant burden both for the individual and for society (3). RA affects almost 1% of the total population whereby it is characterized by a strong systemic inflammation with a shortened lifetime and increased mortality (4). Data suggests that the risk to develop this disease in 3:1 in favor of the female (5) with a significant higher disease activity in this population (6).

Oxidative stress and decreased antioxidant status are the hallmarks in patients of RA as observed in recent years. It is a dynamic phenomenon involved in the disease pathogenesis in a complex way. Oxidative stress represents an imbalance between pro-oxidants and antioxidants in the body in favor of reactive oxygen species (ROS) (7).

As a response to inflammatory conditions in RA an ROS overproduction is usually generated from endogenous source such as mitochondrial electron transport chain by the process of oxidative phosphorylation. Generally, oxygen species in RA have different origin and it can be produced by activated macrophages in the synovial membrane, by chondrocytes, and also through activated neutrophils in the synovial cavity (8). Reaction of ROS production is followed by upregulation of different enzymes such as NADPH oxidase, nitric oxide synthase as well xanthine oxidase (9). Thereafter, NADPH oxidase complex catalyses oxygen reduction to superoxide anion radicals. Unless the concentration of this ROS is not neutralized by superoxide dismutase it consequently leads to deadly combination with NO, synthesis of toxic peroxynitrite. Components of cartilage and extracellular matrix are damaged either directly or indirectly by superoxide anion along with other oxygen and nitrogen radicals by reducing the synthesis of matrix components such as collagen and proteoglycans (10). In addition, the $H_2O_2$-dependent inhibition of proteoglycan synthesis contributes to cartilage destruction in RA through interfering with the repairing mechanisms of the proteolytic and oxidative damages (11).

Increased values od ROS are potentially harmful for different macromolecules and it may result in impairment of physiological processes and damage cell membranes, lipids, proteins and nucleic acids. This leads to fast protonation, - SH group's depletion, deamination od DNA bases and lipid peroxidation (12). Afterwards, lipid peroxidation has been implicated in pathogenesis of many diseases such as cancer, degenerative disease as well inflammatory disease. It can be measured as thiobarbituric acis reactive substance (TBARS) and it may play an important role (13). Increased lipid peroxidation has been reported in various biological samples of RA patients (14).

Body has developed an antioxidant defense system in order to prevent damaging effects of the pro-oxidants and to protect the cells from oxidative damage. Some common enzymes involved in the neutralization or elimination of ROS are superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) as well as glutathione reductase (GR), glutathione-S-transferase (GST), thioredoxin reductase (TR) and heme oxygenase (10). Catalase and reduced glutathione have affinity to $H_2O_2$. Affinity of CAT increases due to higher values of $H_2O_2$ (15) while SOD has mail effect in superoxide anion radical neutralization (10).

RA is a condition with strong influence of both genetic and environmental factors. Accordingly, data available about this disease and the biochemical aspects in our population are very minimal, limited and non-compliant. Regarding, the aim of this study was to assess oxidative status and to set baseline characteristics for female population with established rheumatoid arthritis.

## PATIENTS AND METHODS

### Patients

Forty two women with diagnosed rheumatoid arthritis referred to the outpatient of Internal Clinic, Department of Rheumatology were included in the study (mean age 54.8±9.1). The mean disease duration was 12.8±8.0 years and the mean value of DAS28 was 3.8±1.1 at the moment of blood sampling. All patients were treated with the standard treatment protocol methotrexate (15-25mg per week) and prednisolone (≤10mg per day). The diagnose of rheumatoid arthritis was established according to Classification criteria for RA American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) 2010. Patients included in this study did not have history of diabetes, malignancy, inflammatory disease except rheumatoid arthritis, liver- or renal insufficiency, previous hospitalization due to cerebrovascular, cardiovascular disorders and venous thromboembolism. The patients treated with high doses of steroids (≥10 mg/day, including parenteral administration) and those treated with biologic therapy were excluded from the study. The number of swollen and tender joints of the selected patients was recorded with the help of a rheumatologist. Disease activity score (DAS28) was calculated using a universally accepted formula. Additionally, at the time of blood sampling patients were free of any medication known to influence oxidative balance.

### Healthy controls

Forty eight age- and sex-matched subjects (mean age 54.1±6.2) were included in the study as healthy controls and had a medical investigation to ensure absence of a history of diabetes, malignancy, inflammatory disease including rheumatoid arthritis, liver- or renal insufficiency. As well as in patients group, controls were free of any medication known to influence oxidative balance.

Written informed consent was obtained from all participants, and the study protocol was approved by the Ethics Committee of the Clinical Center Kragujevac prior to the onset of the study. The investigation was conducted in accordance with the principles outlined in the Declaration of Helsinki and principles of Good Clinical Practice (GCP).

**Laboratory analysis**

During blood sampling, blood was taken to perform laboratory analyzes. Biochemical and hematological analyzes (RF, anti-CCP, ESR, CRP, lipid status, leukocytes, leukocyte formula, fibrinogen, aPTT, PT, INR) for all research participants were obtained in the Central Laboratory of the Clinical Center Kragujevac.

**Biochemical assays**

Blood sampling was obtained in the same manner for all patients at the Internal Clinic, Department of Rheumatology, Clinical Center Kragujevac, Serbia. The examinations were performed in a quiet, air-conditioned, temperature-controlled room (22–24°C). Venous blood from all participants was collected between 8–10 a.m at least a 10-h fast in both patients and controls. Blood was collected in Vacutainer tubes containing 0.129 M sodium citrate (BD Vacutainer Blood Collection System) using 21-gauge polyethylene catheter for taking blood samples (BD Vacutainer needles). Blood was centrifuged to separate plasma and red blood cells (RBCs).

**Redox status**

Redox status was evaluated spectrophotometrically by measuring the levels of hydrogen peroxide, superoxide anion radical, nitrites and index of lipid peroxidation in plasma. Activities of corresponding antioxidative enzymes superoxide dismutase, catalase, and reduced glutathione were measured in erythrocytes in the same manner.

**Index of lipid peroxidation (thiobarbituric acid reactive substances)**

The degree of lipid peroxidation in plasma was estimated by measuring of thiobarbituric acid reactive substances (TBARS) using 0.4 ml 1% thiobarbituric acid (TBA) in 0.05 NaOH mixed with 0.8 ml of plasma, incubated at 100 °C for 15 min and measured at 530 nm. Distilled water was used as a blank probe. TBA extract was obtained by combining 0.8 ml plasma and 0.4 ml TCA (trichloroacetic acid). Thereafter, samples were put on ice for 10 min, and centrifuged for 15 min at 6000 rpm (16).

**Nitrite determination**

Nitric oxide (NO) decomposes rapidly to form stable metabolite nitrite/nitrate products. The method for detection of the plasma nitrite levels is based on the Griess reaction. Nitrites ($NO_2^-$) were determined as an index of NO production with Griess reagent (forms purple diazocomplex) (17). 0.1 ml 3 N PCA (perchloric acid), 0.4 ml 20 mM EDTA (ethylenediaminetetraacetic acid), and 0.2 ml plasma were put on ice for 15 min, then centrifuged 15 min at 6000 rpm. After pouring off the supernatant, 220 µl $K_2CO_3$ was added. Nitrites were measured at 550 nm. Distilled water was used as a blank probe.

**Superoxide anion determination**

The level of superoxide anion radical ($O_2^-$) was measured using Nitro Blue Tetrazolium (NBT) reaction in TRIS-buffer with plasma and read at 550 nm. Distilled water was used as a blank probe (18).

**Hydrogen peroxide determination**

Determination of hydrogen peroxide ($H_2O_2$) concentration is based on oxidation of phenol red using hydrogen peroxide, in reaction catalyzed by enzyme peroxidase from horse radish (POD) (19). 200 µl sample with 800 µl PRS (phenol red solution) and 10 µl POD were combined (1:20) and measured at 610 nm.

**Determination of catalase, superoxide dismutase, and reduced glutathion**

Isolated RBCs were washed three times with 3 volumes ice-cold 0.9 mmol/l NaCl and hemolysates containing about 50 g Hb/l, prepared according to McCord and Fridovich (20), were used for the determination of catalase (CAT) activity. Determination of CAT activity was determined according to Beutler (21). Lysates were diluted with distilled water (1:7 v/v) and treated with chloroform–ethanol (0.6:1 v/v) to remove hemoglobin. Then 50 µl CAT buffer, 100 µl sample, and 1 ml 10 mM $H_2O_2$ were added to the samples. Detection was performed at 360 nm. Distilled water was used as a blank probe. Determination of superoxide dismutase (SOD) activity is based on epinephrine method of Misra and Fridovich (22). A 100 µl lysate and 1 ml carbonate buffer were mixed, and then epinephrine in a volume of 100 µl was added. Detection was performed at 470 nm. This method belongs to'negative' type group of methods, since it monitors decrease of autoxidation speed in alkaline medium, which is dependent of $O_2^{-\cdot}$. The level of reduced glutathione (GSH) concentration was determined based on GSH oxidation with 5.5-dithiobis-6.2-nitrobenzoic acid, using Beutler method (23). Measurement of the absorbance is carried out at a wavelength of maximum absorption of 420 nm.

**Statistical analysis**

The statistical analysis was performed using the statistical package SPSS 20.0 for Windows. The results are expressed as means ± standard deviation of the mean (SD). The differences between parameters in different time measurements were assessed by analysis of variance test with repeated measures and independent samples t-test as post hoc. The alpha level for significance was set to $p < 0.05$.

**Table 1.** Baseline characteristics of healthy subjects (controls) and patients with rheumatoid arthritis

| Features of the participants | Controls | RA patients |
|---|---|---|
| Number of patients | 48 | 42 |
| Age (years) | 54.15 (6.29) | 54.81 (9.05) |
| Weight (kg) | 71.75 (12.04) | 72.02 (13.37) |
| Height (cm) | 166 (6) | 166 (6) |
| BMI (kg/m$^2$) | 26.13 (3.90) | 26.16 (4.87) |
| Disease duration (years) | / | 12,81 (7.99) |
| Number of tender joints | / | 5 (5) |
| Number of swollen joints | / | 2 (2) |
| DAS28 - ESR | / | 3.75 (1.09) |
| RF (IU/ml) | / | 91.50 (71.43) |
| RF, n (%) | / | 36 (85.71) |
| Anti-CCP antibodies (U/ml) | / | 173.05 (128.68) |
| Anti-CCP antibodies, n (%) | / | 40 (95.24) |
| Menopause (Yes/No) | 34/14 | 31/11 |
| Smokers, n (%)<br>Current smokers<br>Non-smokers<br>Past smokers | <br>20 (41.7)<br>20 (41.7)<br>8 (16.6) | <br>17 (40.5)<br>19 (45.24)<br>6 (14.26) |

The values are expressed as means ± SD. **BMI** – Body Mass Index; **DAS28** – Disease Activity Score 28; **ESR** – Erythrocyte Sedimentation Rate; **RF** – Rheumatoid factor; **anti-CCP** – Anticyclic citrullinated polypeptide.

**Table 2.** Comparison of values of biochemical, hematological and hemostatic analysis between healthy subjects (controls) and patients with rheumatoid arthritis

| Parameter | Controls | RA patients |
|---|---|---|
| ESR (mm/h) | 13,33 (8,83)** | 22,95 (13,50) |
| CRP (mg/L) | 2,54 (2,40)** | 11,55 (26,42) |
| Cholesterol (mmol/L) | 6.50 (1.23) | 6.06 (1.01) |
| Triglycerides (mmol/L) | 1.5 (0.65) | 1.49 (0.62) |
| HDL (mmol/L) | 1.60 (0.30) | 1.49 (0.29) |
| LDL (mmol/L) | 4.21 (1.12) | 3.81 (0.94) |
| Leukocytes (10$^9$/L) | 6,51 (1,98) | 7,58 (3,02) |
| Neutrophils (10$^9$/L) | 3.77 (1.62)* | 4.76 (2.69) |
| Neutrophils (%) | 56,95 (6,40)** | 61,92 (9,28) |
| Lymphocytes (%) | 32,71 (5,68)** | 27,87 (7,79) |
| Fibrinogen | 3.26 (0.52)* | 3.55 (0.69) |
| aPTT | 28.16 (3.17) | 28.35 (3.12) |
| PT | 11.15 (0.67)** | 11.89 (1.95) |
| INR | 1.01 (0.06)** | 1.08 (0.2) |

The values are expressed as means ± SD. Statistical significance between control and experimental group presented as* ($p < 0.05$); **($p < 0.01$). **ESR** – Erythrocyte Sedimentation Rate; **CRP** – C-reactive protein; **HDL** – High-Density Lipoprotein; **LDL** – Low-density lipoprotein; **aPTT** – activated partial thromboplastin time; **PT** – prothrombin time; **INR** – International Normalised Ratio.

## RESULTS

### Subjects characteristics

Demographic and clinical characteristics of the study population as well as results regarding the presence of traditional CV-risk factors are presented in Table 1. Thirty six out of 42 patients were RF seropositive and 40 of 42 had anti-CCP antibodies. There wasn't any statistical difference between healthy controls and patients regarding age, BMI and smoking.

### Parameters of laboratory analysis

Patients with RA had higher ESR, CRP, fibrinogen levels as well as values of PT and INR. Furthermore, leukocyte formula showed higher percent of neutrophils and lower percent of lymphocytes in RA patients compared to healthy controls. Also, higher value of neutrophils was noticed in patients. These results can indicate increased inflammation and changes in hemostatic balance in RA patients. Results for biochemical, hematological and hemostatic analysis are presents in Table 2.

### Redox status parameters

The dynamics of oxidative stress parameters ($H_2O_2$, $O_2^-$, $NO_2^-$ and TBARS) in healthy control and patients with rheumatoid arthritis are shown in Figure 1, while activities of the antioxidative enzymes (SOD, CAT, GSH) are shown in Figure 2.

### Oxidative parameters

Values of hydrogen peroxyde were lower in healthy subjects compared to patients suferring of RA (0.82 ± 0.33 vs. 1.05 ± 0.41 nmol/min/g wt, respectively; $p<0.01$) (Fig 1a). When it comes to $O_2^-$ values, there was no statistically significant difference between controls compared to patients (26.92 ± 5.68 vs. 23.05 ± 5.64 nmol/min/g wt, respectively) (Fig 1b). Values of nitrites were higher in control group relative to patients with RA (4.98 ± 0.9 vs. 3.83 ± 0.52 nmol/min/g wt, respectively; $p<0.01$) (Fig 1c). Futhermore, the same trend as for the $H_2O_2$ was noticed for TBARS. The values were lower in controls relative to RA patients (1.04 ± 0.11 vs. 0.94 ± 0.13 μmol/min/g wt, respectively; $p<0.01$) (Fig 1d).

### Activity of antioxidant parameters

Activity of CAT was statistically increased in control group compared to patients (3.39 ± 0.58 vs. 3.10 ± 0.51 U/gHbx10$^3$, respectively; $p<0.05$) (Fig 2a). The same trend was noticed in SOD activity with statistically higher values in controls relative to RA group (23.57 ± 5.27 vs. 13.95 ± 5.80 U/gHbx10$^3$, respectively; $p<0.05$) (Fig 2b). Values of GSH were not statistically different between controls and patients (75071.02 ± 8616.41 vs. 69814.68 ± 8503.38 nmol/ml RBCs, respectively) (Fig 2c).

### Correlation

There was no correlation between the measurements of the serum oxidative stress markers and antioxidant capacity and diseases duration, DAS28, CRP, ESR, fibrinogen, the values of RF, anti- CCP antibodies.

## DISCUSSION

In addition, it is well known that a pro-inflammatory mediator such as cytokines and prostaglandins plays an important role in the RA pathogenesis. However there is strong evidence that oxidative stress has additional impact through the overproduction of ROS at inflammation sites. Oxidative stress occurs either due to the overproduction

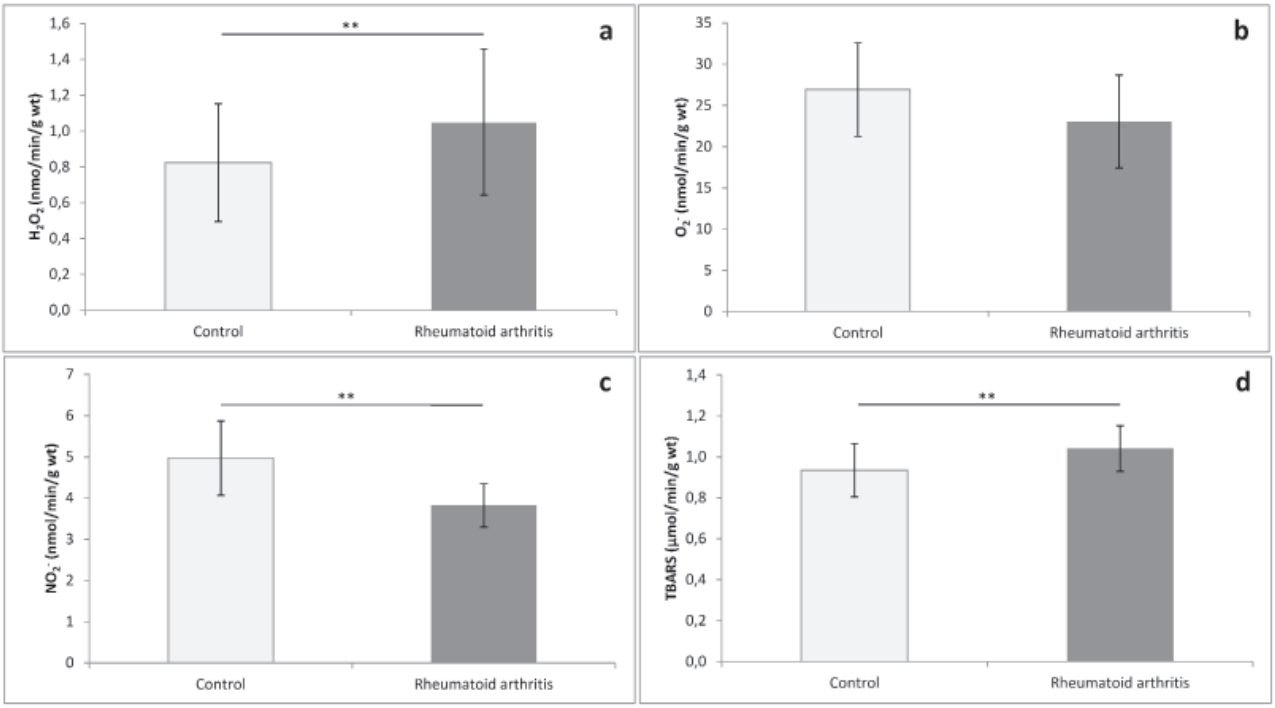



**Figure 1.** Oxidative stress parameters in control group compared to patients with rheumatoid arthritis. Data are presented as following: **a** – Level of hydrogen peroxide ($H_2O_2$); **b** – Level of superoxide anion radical ($O_2^-$); **c** – Level of nitrites ($NO_2^-$); **d** – Level of index of lipid peroxidation measured as thiobarbituric acid reactive substances (TBARS). Data are expressed as mean ± SD (*p<0.05; **p<0.01).

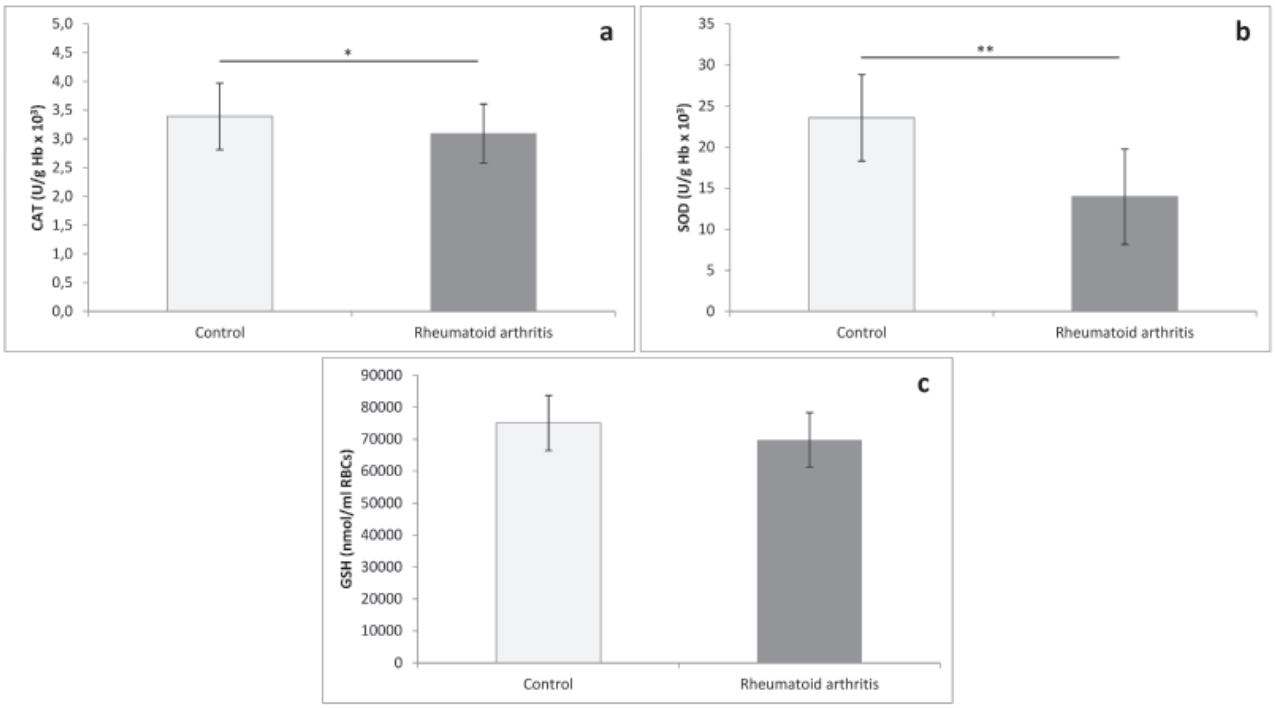



**Figure 2.** Activity of antioxidant parameters in control group compared to patients with rheumatoid arthritis. Data are presented as following: **a** – Catalase activity (CAT); **b** – Superoxide dismutase activity (SOD); **c** –Level of reduced glutathione (GSH). Data are expressed as mean ± SD (*p<0.05; **p<0.01).

of oxidants or their elimination weakness by antioxidant defense system. In this study, balance of pro-oxidative and oxidative parameters was assayed to gain insight into the oxidative stress status in RA.

Previously, it has been shown that NO values were statistically higher in patients with RA compared with healthy controls (1) (8) NO is a reactive nitrogen species produced mainly by macrophages and it is an important component of the oxidative burst during inflammation and autoimmunity. Interestingly, in our study, statistically higher values of nitrites were noticed in healthy control compared to patients suffering of RA (Fig 1c). Some of possible explanation for these results is in the fact that combined NO and superoxide anion radical can induce deadly peroxynitrite formation. Nitric oxide ($NO^{\cdot}$) is produced by the deamination of L-arginine from nitric oxide synthase. This oxide reacts with superoxide radical in favor of peroxynitrite production (24). In our study we noticed lower values of $O_2^-$ in patients compared to control group without statistical significance (Fig 1b). This can be result of the previously mentioned peroxynitrite synthesis due to primary higher values of NO and $O_2^-$ in RA patients that induced reaction between themselves and reduce $O_2^-$ and NO levels. $O_2^-$ has a half-life of $10^{-6}$ of a second, and NO molecules last for few seconds, both of which support this possibility (25). Furthermore, it is quite possible that the highest values of NO is in inflammation is *in situ* on the original place of inflammation origin, in joints. It has been found that one more important source of NO were articular chondrocytes and synovial fibroblasts. During chronic inflammation in affected joints, different type of cells such as neutrophils, lymphocytes, mast cells and macrophages could result with increased production of NO (1). Kundu et al showed higher concentration of NO in different biological fluids. The levels of intracellular NO were 1.56 times higher in isolated monocytes from the blood of RA patients relative to healthy as well as in monocytes of the synovial infiltrate (12). Increased levels of the marker molecule for peroxynitrite, nitrotyrosine, were found in synovial fluid samples from rheumatoid patients (24). During long-term of disease duration and high disease activity measured as DAS28 it is possible that in the place of ROS origin in inflammated joint there is strong and fast reaction between NO and $O_2^-$ and production of peroxynitrite. But, that remains unknown considering that in our study we didn't measure values of this radical.

The primary targets of ROS are double bonds in polyunsaturated fatty acids in the cell membrane, which increase lipid peroxidation (LPO) and result in more oxidative damage. Deleterious by-product is generated by oxidative damage mediated by ROS as well as a thiobarbituric acid reactive substance (TBARS), malondialdehyde (MDA) which leads to synthesis of various adducts with protein inducing pathogenic antibodies and tissue damage in patients with RA. MDA has been reported as a primary biomarker of free radical induced lipid damage and oxidative stress (26). Increased LPO has been shown in previous studies confirming the presence of oxidative stress in rheumatoid arthritis. Vasanthi et al showed statistically higher values of MDA in patients compared to control group (1). Desai and coworkers pointed out that mean level of blood malondialdehyde (in nmol/ml) in controls was lower compared to cases (27). Large number of previous studies indicated increased levels of MDA in RA patients relative to healthy subjects in different biological fluids (14, 28, 29). Pasupathi et al showed that level of plasma and erythrocyte thiobarbituric acid reactive substances (TBARS) was markedly increased in the rheumatoid arthritis patients when compared to control subjects (30) as well as Veselinovic et al (31) who correlate with our study.

In our study values of $H_2O_2$ were statistically increased in patients compared to controls (Fig 1a). That follows decreased CAT activity in RA patients due to detoxified concentration of $H_2O_2$ by CAT. Several studies in patients with RA documented evidence of increased values of $H_2O_2$ levels compared to healthy control. Veselinovic and coworkers noticed higher serum concentrations in RA patients compared to healthy subjects that correlate with our results (31). The higher values of $H_2O_2$ were noticed in previous study by Khojah et al (8).

In our study, statistically higher SOD and CAT activity as antioxidant defense system enzymes was noticed in controls as well as higher values of GSH without statistic significance in the same group (Fig 2a-c). These enzymes have an important function in antioxidant defense system. Catalase has a different role in physiological and pathological conditions. Under physiological condition, catalase has low affinity for $H_2O_2$ (compared to glutathione peroxidase) but CAT affinity increases due to increased concentration of $H_2O_2$ (15). Glutathione peroxidase uses reduced glutathione as hydrogen donor for metabolizing $H_2O_2$ to water. In addition, previous findings promoted glutathione (GSH), the main thiol antioxidant, as an important anti-inflammatory mediator (32). Two enzymes of antioxidative protection, gluthathione peroxidase and catalase are very important in metabolism regulation of hydrogen peroxide which in excess can cause damage to DNA, RNA and lipids. The role of SOD is in superoxide anion neutralization one of the mail ROS involved in inflammation (10).

Futhermore, SOD and CAT activity was higher in healthy subjects relative to patients (Fig 2 a-b). The same trend was noticed in Feijóo and coworkers research. In control group there were higher SOD and CAT activity compared to RA patients (7). Likewise, higher activity of SOD and CAT followed by decreased level of pro-oxidative parameters in healthy control compared to RA patients were noticed in other studies (14, 26, 27) that correlate to our results. In previous studies values of GSH were higher in control group compared to RA patients (7, 14, 26, 27). But, our results showed correlation with Cimen and coworkers study. They showed unchanged values of GSH in controls compared to RA patients (33).

Our results are in agreement with other recent studies that indicate that oxidative stress generated within an inflamed joint can produce connective tissue destruction leading to joint and periarticular deformities in RA.

## CONCLUSION

This study confirmed that redox status in RA patients is changed and that antioxidant defense system is compromised in these patients. There is a shift in the oxidant/antioxidant balance in favor of lipid peroxidation, A marked increase in ROS formation and lipid peroxidation as well as decrease in the activity of antioxidant defense system can lead to oxidative stress which may contribute to the tissue and cartilage damage observed in RA and hence to the chronicity of the disease. These results may suggest a potential benefit from exogenous antioxidant supplements in women with RA.

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