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ORIGINAL ARTICLE

Evaluation used disease-modifying antirheumatic drugs oxidative stress parameters in rheumatoid arthritis patients

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ABSTRACT

BACKGROUND: The aim of our study is to evaluate the stress markers at the patients with rheumatoid arthritis that used Disease-modifying antirheumatic drugs (DMARD) medication.

METHODS: Blood taken from the patients diagnosed with romatoid artrit according to American Rheumatism Association ACR 2010 criteria, employed the medication of DMARD in order to examine the stress parameters of total oxidant status, total antioxidant status, paraoxonase, paraoxonase NaCl, arylesterase, myeloperoxidase, catalase, thiol concentration. Besides, routine assessment of patient index data 3 (RAPID3) activities of patients were monitored.

Thirty seven patients with early RA and 61 healthy controls were included in the study, and clinical examination and investigations were performed and disease activity was assessed. Peripheral blood samples were used for all the assays.

RESULTS: The average age of the patients were 46.52±12.37, and their average BMI was 28.93±4.81. The value of catalase was significantly higher at the experimental group comparing to the control group; the values of thiol, arylesterase, myeloperoksidase, total oxidant status at experimental group were significanly lower than the control group ($p<0.05$). The patients rapid 3 values and other oksidatif stres markers were not statistically significantly different. ($p>0.050$).

CONCLUSİON: Stronger response in patients with early RA suggests that oxidative stress markers may be useful in evaluating the progression of RA as well as in elucidating the mechanisms of disease pathogenesis.

KEYWORDS: Rheumatoid arthritis, oxidative stress parameters, DMARD.

INTRODUCTION

Rheumatoid Arthritis (RA) is a chronic, systemic disease, in which various joints in the body are inflamed, leading to swelling, pain, stiffness, and the possible loss of function. It is an autoimmune disease in which the body's immune system attacks itself. Rheumatoid Arthritis affects approximately 1-2% of the total world's population (1).

Oxidative stress due to damage brought about by free radicals is also known to influence the response of these patients to therapy. Moreover the body's defense mechanisms would play an important role in the form of antioxidants and try to minimize the damage, adapting itself to the above stressful situation. Antioxidants are compounds that dispose, scavenge, and suppress the formation of free radicals, or

oppose their actions and two main categories of antioxidants are those whose role is to prevent the generation of free radicals and those that intercept any free radicals that are generated (2).

The formation and scavenging activity of free radicals in biological systems have been linked closely to a number of pathological conditions. In healthy individuals, ROS and associated oxidative stresses are kept in check by a combination of antioxidant activities (3). Human cells have developed a formidable antioxidant defense against oxidant reactions. In particular, they possess enzymatic and nonenzymatic antioxidant molecules, including thiols [mainly glutathione], for defense. One key chemical barrier against stress-induced damage is the redox equilibrium of sulfhydryl/disulfides, by which low molecular weight thiols can be oxidized reversibly to disulfides and/or protein mixed disulfides in response to an oxidative stress (4). There is increasing evidence that ROS and the resulting prooxidant/antioxidant imbalance play a major role in RA, as well as in other disease states (5). It has been shown that lymphocytes, which are highly sensitive to thiol modification, are impaired in many of their immune functions when exposed to oxidative stress or sulfhydryl modifiers such as those found in the cellular microenvironment (6).

Several studies have reported that oxidative stress and production of oxygen-free radicals have important role in RA development and epidemiologic studies have revealed a reverse relationship between dietary intake of antioxidants and RA incidence and due to reduction of intake and absorption of dietary antioxidants in RA patients, the levels of blood antioxidants are decreased too (7). The antioxidants supplements such as vitamin E, vitamin C, and selenium may control the disturbance of lipid peroxidation and loss of antioxidants markers in patients with RA (8,9). Vitamin E can interact with nitric oxide and may trigger the gene expression of catalase, glutathione peroxidase, and superoxide dismutase enzymes, vitamin C may demolish the peroxides of macrophage activities, zinc may strengthen the immune system, and selenium has an important role as a cofactor of glutathione peroxidase enzyme in reduction of oxidative stres (10).

Enzymatic protection against ROS and the breakdown products of peroxidized lipids and oxidized protein and DNA are provided by many enzyme systems such as superoxide dismutase, catalase, glutathione peroxidase. Apart from these important enzymatic antioxidants, paraoxonase-1 appears to have antioxidative properties as well (11). Paraoxonase-1 is enzyme with three activities which are paraoxonase, arylesterase and dyazoxonase. Paraoxonase-1 hydrolyses organophosphates, such as paraoxon, aromatic esters, for instance, phenyl acetate, and also lipid peroxidation products, and reduces the accumulation of them. Thus, paraoxonase-1 prevents the acceleration of atherosclerosis and assumes an antiatherogenic property (12). Recent articles indicated that paraoxonase-1 reduce oxidative stress in serum and tissues, thus protecting against cardiovascular disease (13).

Our purpose in this study is find the relevance oxidation and anti oxidative effects in rheumatoid arthritis with the evaluate the values of total oxidant status, total anti oxidant status, paraoxonase, paraoxonase NaCl, arylesterase, myeloperoxidase, catalase, thiol concentration levels.

METHODS

This randomized, prospective, controlled, single blind study was conducted in Physical medicine and Rehabilitation department of Bezm-i Alem Vakif University, faculty of Medicine. In this context Bezmialem Foundation University physical medicine and rehabilitation department depends on the rheumatology outpatient clinic refer to the ACR-2010 (14) rheumatoid arthritis criteria based on the diagnosed 37 diagnose the patient disease activity related forms creating a face to face interview method were filled. Blood taken from the patients diagnosed with romatoid artrit according to American Rheumatism Association ACR 2010 criteria, employed the medication of DMARD (Disease-modifying antirheumatic drugs) in order to examine the stress parameters of total oxidan status, total antioxidan status, paraoxonaz, paraoxonaz

NaCl, arylesterase, myeloperoxidaz, katalaz, thiol concentration. DMARDs include conventional compounds, such as methotrexate (MTX), sulfasalazine, leflunomide, and hydroxychloroquine. Besides, RAPID3 activities of patients were monitored. In addition to their demographic characteristics (age, gender, weight, height, body mass index [BMI]), the patients were also questioned for occupation, main symptoms, time of diagnosis. The patients were between the ages of 20 and 65 included in this study. Patients with arthritis due to other disease, such as gout, ankylosing spondylitis, Reiter's syndrome, psoriasis, inflammatory bowel disease, systemic lupus erythematosus, Behçet's disease, and adult onset Still disease, neoplastic disease, established deficiency of vitamin B12 or folate and having received any drugs were also excluded.

Control group was consisted of 61 healthy individuals. Controls had no joint complaints, any rheumatological disease, substance abuse, smoking, alcohol use, excessive exercise, with comorbid disease and infectious diseases with metabolic. Age and sex distributions in the group of control subjects were similar to those of RA patients. Informed consent was obtained from each control.

RAPID3 is a pooled index of the 3 patient-reported American College of Rheumatology rheumatoid arthritis (RA) Core Data Set measures: function, pain, and patient global estimate of status. Each of the 3 individual measures is scored 0 to 10, for a total of 30. Disease severity may be classified on the basis of RAPID3 scores: >12 = high; 6.1–12 = moderate; 3.1–6 = low; ≤3 = remission (15).

Blood samples

Blood samples were obtained following an overnight fasting state. Samples were withdrawn from a cubital vein into blood tubes and immediately stored on ice at 4 °C. The serum was then separated from the cells by centrifugation at 3000 rpm for 10 min and they were analyzed.

Measurement of Total antioxidant status and total oxidant status

An automated measurement method by an automated analyzer (Siemens ADVIA 1200) as described earlier (16,17). In the measurement of TAS, absorbance of the colored dianisidyl radicals was monitored to determine the rates of Fenton reaction which initiates free-radical reactions with the production of a hydroxyl radical. Then, the antioxidative effect of sample against potent free-radical reactions was measured in terms of mmol Trolox equiv/L (Rel Assay Diagnostics, Gaziantep/TURKEY).In the measurement of TOS, intensity of the colored complex produced by the reaction of ferric ions, which were oxidized from ferrous iono-dianisidine complex due to presence of oxidants,with xylenol orange in an acidic medium was used to determine the total amount of oxidant molecules in the sample. The calibration of assay is performed with hydrogen peroxide. TOS values are expressed in terms of micromolar hydrogen peroxide (H2O2) equivalents per liter (μmol H2O2 equiv/L) (Rel Assay Diagnostics, Gaziantep/TURKEY). Oxidative stress index (OSI) was calculated by the following formula: OSI =TOS/TASx10 (18).

Catalase activity assay

Catalase (CAT) activity was assayed by a method described by Goth (19); 0.2 ml hemolysate was incubated in 1.0 ml substrate (65 lmol per H2O2 in 60 mmol/l sodium–potassium phosphate buffer, pH 7.4) at 37 degrees for 60 s. The enzymatic reaction was stopped with 1.0 ml of 32.4 mmol/l ammonium molybdate [(NH4)6MO7O24.4H2O], and the yellow complex of molybdate and H2O2 was measured at 405 nm against blank 3. One unit of CAT decomposes 1 lmol of H2O2/l min under these conditions. Results were expressed as kU/gHb, which was calculated as follows:

CAT(kU/gHb)= [A (Sample) – A(Blank)] / [A (Blank2) – A(Blank3)]

Blank 1 contained 1.0 ml substrate, 1 ml molybdate and 0.2 ml hemolysate; blank 2 contained 1.0 ml substrate, 1.0 ml molybdate, and 0.2 ml buffer; blank 3 contained 1.0 ml buffer, 1.0 ml molybdate, and 0.2 ml buffer.

Measurement of paraoxonase and arylesterase activities

Paraoxonase activity was determined using paraoxon as a substrate and measured by increases in the absorbance at 412nm due to the formation of 4-nitrophenol as already described (20). The activity was measured at 25ºC by adding 50µl of serum to 1ml Tris-HCl buffer (100mM at PH 8.0) containing 2mM CaCl2 and 5 mM of paraoxon. The rate of generation of 4-nitrophenol was determined at 412 nm. Enzymatic activity was calculated by using molar extinction coefficient 17 100 M-1 cm-1.

Arylesterase activity was measured using phenylacetate as a substrate. Serum was diluted 400 times in 100mM Tris-HCl buffer, pH = 8.0. The reaction mixture contained 2.0 mM phenylacetate (Sigma Chemical Co) and 2.0 mM CaCl2 in 100mM Tris-HCl buffer, pH = 8.0. Initial rates of hydrolysis were determined by following the increase of phenol concentration at 270 nm at 37 °C on a CE 7250 spectrophotometer (Cecil Instruments Limited, UK) (21). Enzyme activities were expressed in international units (U) or kilounits (kU) per 1 litre of sera.

Measurement of thiol level

The thiol level was estimated in tissue/plasma by the method of Hu (22). A 0.2-ml sample of 10% plasma/ tissue homogenate was mixed with water to make the volume up to 0.5 ml. Two milliliters of 0.3 M disodium hydrogen phosphate was added to each sample and 0.25 ml of DTNB reagent was added just before measuring the absorbance at 412 nm. Thiol groups were calculated using an absorptivity of 13,600 mmol/l.

Measurement of Myeloperoxidase Activity

Myeloperoxidase activity in the samples was analyzed according to Krawisz et al. (IU/mL) (23).

All the recruited subjects signed informed consent forms before participating in the study and the approval of the local Ethics Committee was obtained. All the subjects gave their consent to the random assignment to the groups.

The erythrocyte sedimentation rate (ESR) was measured through the Westergren method (mm/h) and the serum C-reactive protein (CRP) level was determined with the help of nephelometry (mg/dl).

The calculations were performed using the Statistical Package for Social Sciences for Windows software version 16.0 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to confirm that data within the ranges of normal distribution in both groups. A non-parametric test was employed for the variables outside the normal distribution. The comparison of the data between the groups was carried out through the independent-samples t test. Statistical significance was based on a value of $p < 0.05$ with a 95% confidence interval.

RESULTS

Employed the medication of DMARD 37 patients with RA and 61 healthy controls were included in the study (Figure 1), and clinical examination and investigations were performed and disease activity was assessed.

Peripheral blood samples were used for all the assays. Demographic information of people which involve the study has been expressed in Table 1.

Table 1. Demographic and clinical data of patients with rheumatoid arthritis

	Patients (n=37)	Controls (n=61)	P
Age	48.6±11.22	44.22±13.42	>0.05
Gender (Female/male)	24/13	41/20	>0.05
BMI	29.30±5.06	27.26±3.09	>0.05

Laboratory findings and RAPID3 of the patients and control group has been expressed in Table 2.

Table 2. Laboratory findings and RAPID3 of the patients and controls OKSIDAN ARTCAK

	Patients (n=37)	Controls (n=61)	p
ESR (mm/h)	31.3±11.02	9.4±6.15	<0.05
CRP (mg/L)	2.1±1.27	1.2±0.92	<0.05
TOS (mol H2O2/L)	3.72±0.89	3.85±1.94	>0.05
TAS (meq Troloks/L)	4.54±0.47	4.54±0.55	>0.05
OSI	82.74±21.80	84.38±36.61	>0.05
PON (U/L)AO	87.24±57.18	84.38±52.56	>0.05
PONaCl (U/L)AO	228.18±118.44	226.47±115.68	>0.05
ARE (U/L)AO	137.97±21.07	146.21±19.37	>0.05
MPO (IU/mL)O	581.00±607.62	574.30±943.31	>0.05
CAT (kU/gHb)AO	27.89±24.95	24.65±30.26	>0.05
THIOL (mmol/l)AO	0.24±0.02	0.29±0.03	<0.05
RAPID3	3.69±1.40	3.12±1.60	>0.05

ESR- erytrocyte sedimentation rate. CRP- C-reactive protein, TAS- total antioxidant status, TOS- total oxidative status, OSI- Oxidative stress index, PON- paraoxonase, PONaCl- paraoxonase NaCl, ARE- arylesterase, MPX- myeloperoxidase, CAT-catalase.

Significant correlations among parameters in the patients group has been expressed in Table 3.

Table 3. Significant correlations among parameters in the patients group

	R	p
RAPID3-THIOL	-0.383	0.004
RAPID3-CAT	-0.463	0.0
RAPID3- MPO	-0.440	0.001
RAPID3 –TOS	-0.319	0.018
RAPID3-ARE	-0.359	0.007
RAPID3-OSI	-0.315	0.019

TAS- total antioxidant status, TOS- total oxidative status, OSI- Oxidative stress index, PON- paraoxonase, PONaCl- paraoxonase NaCl, ARE- arylesterase, MPX- myeloperoxidase, CAT-catalase.

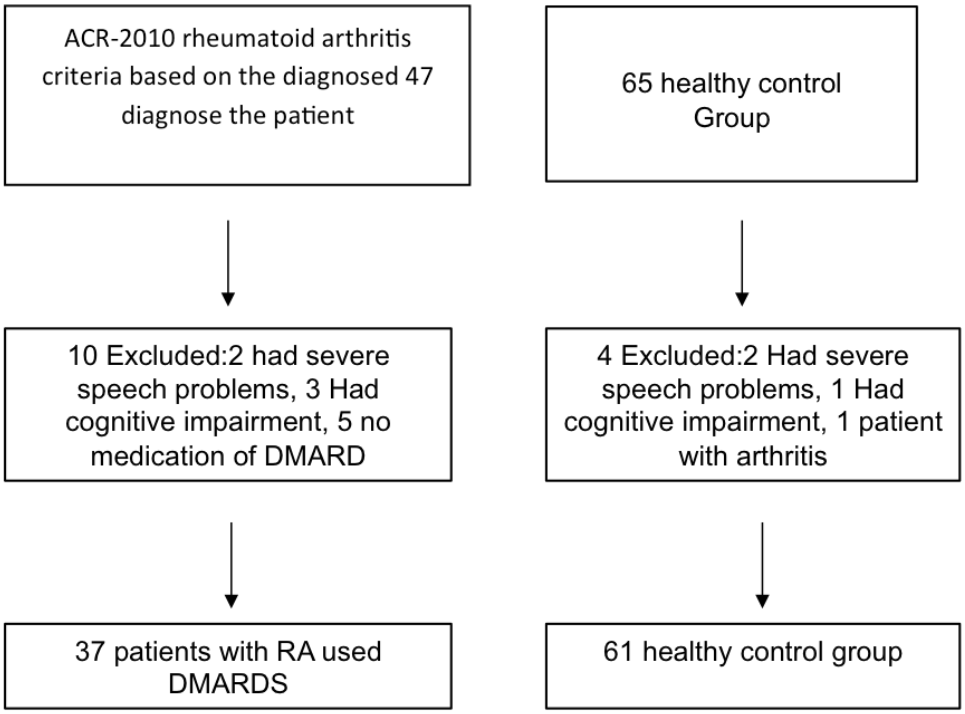

Figure 1. Study flowchart.

CONCLUSION

In our study, ESR and CRP values has been found significantly high levels in RA group when we compare with control group ($p<0.05$). Thiol values which means anti-oxidant parameters, has been found low level according to control group ($p<0.05$).

A negative correlation was found between the RAPID 3 and the thiol, ARE, OSI, CAT, MPO, TOS (Table 3).

Imbalance in the human oxidative / antioxidative status leads to oxidative stress, which is involved in ageing and number of disorders: vascular diseases (e.g. atherosclerosis, stroke, reperfusion damage), neurodegenerative diseases (e.g. Parkinson's, Alzheimer's, multiple sclerosis), lung diseases (e.g. pneumonia), eye diseases (e.g. cataract, retinopathy), autoimmune diseases (e.g. rheumatoid arthritis) (24,25).

Present study indicates increased oxidative stress, which is reflected by increased lipid peroxidation in peripheral blood of patients with RA. These results are also in accordance with the earlier studies (26).

In the current study the lipid peroxidation product i.e. erythrocyte lipid peroxidation products levels have been raised significantly in erythrocytes of the patients with rheumatoid arthritis. Raise in erythrocyte lipid peroxidation products could be because of increased generation of reactive oxygen species (ROS) due to the excessive oxidative damage generated in these patients. These oxygen species in turn can oxidize many other significant biomolecules including membrane lipids. Related reports of elevated erythrocyte lipid peroxidation products levels have been reported in patients with rheumatic disease (27).

The decline in the levels of these non-enzymatic antioxidant parameters may be because of the improved turnover, for preventing oxidative damage in these patients suggesting an improved defense against oxidant damage in Rheumatoid Arthritis. Related reports of increased malondialdehyde (MDA-marker of oxidative stress) levels have been reported in patients with rheumatoid arthritis (28).

In the study of Altindag and et al. paraoxonase and arylesterase activities were importantly lower in patients with RA, lipid hydroperoxide (LOOH) levels were significantly higher in

patients with RA than in healthy controls. In patients with RA, serum TOS was higher and serum TAS was lower when compared with those of healthy controls. PON was negatively correlated with LOOH, ARE was positively correlated with TAS, LOOH was negatively correlated with TAS (29). In our study, according to the ARE values were lower in the control group. ARE TAS significant difference compared to control group according to statistically control group. ARE blood values and a negative correlation between RAPID 3 (RA disease activity) was determined (Table 3).

Catalase, play an significant role in the protection of erythrocytes from oxidative stress. Lowered activities of red cell catalase have been reported in patients with rheumatoid arthritis (30). In the current study, we have observed a significant reduce in the catalase activity in patients with rheumatoid arthritis compared to controls. Catalase is the enzyme, which protects the cells from the accumulation of hydrogen peroxide by dismutating it to form water and oxygen or by using it as an oxidant in which it workings as a peroxidase (31). Related reports of declined catalase activity were experiential in rheumatoid arthritis by Kerimova et al (32). However others have reported a raise in plasma catalase activity in patients with rheumatoid arthritis when compared to controls (33). CAT levels in our study are increase in plasma catalase activity in patients with rheumatoid arthritis when compared to controls too. CAT levels in our study are statistically no significant differences compared to the control group.

Thiol groups play an significant role in a diversity of activities of cell membranes. Paderson Lane and et al. search the thiol levels of surface-thiols and glutathione levels of leukocystes in their study. The thiol levels of surface-thiols and glutathione levels of leukocystes levels found importantly lower than control group, in RA group (34). Thiol levels in our study are consistent with the literature. There are statistically significant differences compared to the control group.

Particularly, plasma MPO concentration is significantly higher in patients with RA (35), an inflammatory autoimmune disease most frequently affecting the synovial membrane of flexible joints (36). MPO levels in our study are not consistent with the literature. There are statistically no important differences compared to the control group.

In summary, our work was mostly similar to the oxidative parameters control group. We think that this is connected to DMARD use.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with human participants performed by any of the authors.

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