Antioxidant status & lipid peroxidation in patients with rheumatoid arthritis

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Background & objectives: Rheumatoid arthritis (RA) is a debilitating, chronic multisystem disease with an unknown etiology. Recent findings indicate that increased oxidative stress and/or defective antioxidant status contribute to the etiology of RA. The present study was undertaken to examine the oxidant and antioxidant systems in patients with RA and healthy controls.

Methods: Twenty two patients with RA and 20 healthy volunteers were included in the study. Levels of malondialdehyde (MDA) and antioxidant vitamins (A, E, C) in serum samples were determined by high performance liquid chromatography (HPLC). Spectrophotometric methods were used to determine activity levels of antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), in erythrocytes.

Results: MDA levels in patients with RA were found to be significantly ($P<0.005$) higher than controls whereas levels of vitamins A, E, C and activities of GSH-Px, SOD were lower in the patients compared to controls ($P<0.005$ for SOD and antioxidant vitamins; $P<0.05$ for GSH-Px).

Interpretation & conclusion: There was an increased oxidative stress and a low antioxidant status in patients with RA. These changes are probably due to efforts for reducing lipid peroxidation and hence to lower tissue damage.

Key words Glutathione peroxidase - malondialdehyde - oxidative stress - rheumatoid arthritis - superoxide dismutase

Rheumatoid arthritis (RA) is a chronic, multisystem disease with an unknown etiology affecting about 1 per cent of the world’s population. RA is characterized by persistent inflammation in the synovial membranes of joints, associated with migration of activated phagocytes and other leukocytes into synovial and periarticular tissue. During phagocytosis, monocytes, neutrophils and macrophages generate superoxide radicals, hydrogen peroxide and the highly reactive hydroxyl radicals. These cytotoxic reactive oxygen species (ROS) may cause oxidative damage in the cells. Activated oxygen intermediates together with highly reactive radicals, such as the hydroxyl radicals, are able to destroy membrane lipids, proteins, deoxyribonucleic acid, hyaluronic acid, and cartilage. Enzymatic mechanisms include superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX). Vitamins A, C, E and glutathione are some of the major non-enzymic antioxidants in the body. Plasma concentrations of vitamin C, vitamin E and beta-carotene were found to be decreased in patients with RA.

Oxygen free radicals have been implicated as mediators of tissue damage in patients with RA. Hence, the aim of the present study was to assess the lipid peroxidation and antioxidant status of patients with RA.
Material & Methods

Patients for the study were selected from individuals attending the routine Rheumatology Clinic of Department of Internal Medicine at Firat Medical Center, Turkey during October 2001-April 2002. Criteria recommended by the American Rheumatism Association were used for the diagnosis of RA. The study protocol was approved by the ethics committee of the Firat university. Informed consent was obtained from all patients and controls.

A total of 22 patients with RA (10 males, 12 females, mean age 48.9 ± 12.3 yr, mean disease duration 11.7 ± 7.6 yr) were included into the study group. Twenty healthy volunteers (medical students, laboratory personnel and officials) were included as controls (8 males, 12 females, mean age 44.5±11.2 yr). None of the patients and controls were smokers or consuming alcohol or had any other chronic disease.

Blood samples collected from normal controls and patients were centrifuged at 3000 g for 5 min at +4°C and the plasma separated. Levels of MDA and antioxidant vitamins (A, E, C) were determined in plasma samples. After separating the plasma, erythrocytes were washed three times in 0.9 per cent NaCl solution and were haemolysed by dilution in water and stored at -20°C until used for measurement of SOD and GSH-Px activities. The haemoglobin (Hb) content of erythrocytes was determined by the cyanmethaemoglobin method.

SOD activity in erythrocytes was assayed using a kit (Randox, UK) following the manufacturer's instructions. In this method, xanthine and xanthine oxidase was used to generate SOD radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyl tetrazolium chloride to form a red formazan dye. SOD activity is measured by the degree of inhibition of this reaction, and expressed as U/g Hb.

GSH-Px activity levels were determined by the method of Paglia and Valentine using a commercially available kit (Randox, UK) and the activity levels were expressed as U/g Hb.

The quantification of vitamin A and E was done by the method of Catignani and Bieri and Miller et al., utilizing absorption spectra of 326 and 296 nm, respectively. HPLC separations were done at room temperature with a Cecil liquid chromatography system (UK) (Series 1100) consisting a sample injection valve (Cotati 7125) with a 20 µl sample loop, an ultra-violet (UV) spectrophotometric detector (Cecil 68174), integrator (HP 3395) and a Techsphere ODS-2 packed (5 µm particle and 80Å pore size) column [250×4.6 internal diameter (ID)] with a methanol: acetonitrile: chloroform (47: 42: 11, v/v) mobile phase at a flow rate of 1 ml/min.

The extractions of vitamin C and MDA were done following the method of Cerhata et al. The supernatant was filtered and vitamin C level was determined using the method of Tavazzi et al. and MDA level by the method of Karatas et al. by HPLC utilizing a column (250×3.9 ID) packed with Tocnopak C18 reversed-phase material (UK) (10µm particle size) For vitamin C 3.7 mM phosphate buffer, pH 4.0 was used as mobile phase with a flow rate of 1 ml/min while for MDA the mobile phase was 30 mM KH2PO4 buffer, pH 4 with H3PO4 and methanol at a flow rate of 1.5 ml/min.

All chemicals and reagents used were of analytical grade and were purchased from Merck Chemical Co., Germany.

The SPSS software (Chicago, IL, USA) was used for statistical analyses. Differences in various parameters between the two groups were analyzed for significance using the Mann-Whitney U-test. Statistical significance was defined as \( P < 0.05 \).

Results & Discussion

The recovery rates were found to be 98.2 per cent for vitamin A, 99.5 per cent for vitamin E, 97 per cent for vitamin C and 98.8 per cent for MDA.

MDA levels were found to be significantly \( P < 0.005 \) elevated in the patients with RA compared to the controls (Table). This is in agreement with other studies where higher MDA levels have been reported in patients with RA. Oliveri et al., reported no change in lipid peroxidation but generally increased lipid peroxidation in serum, plasma and erythrocytes has been reported.
Decreased SOD and GSH-Px activity levels in patients with RA may indicate a degradation of these antioxidant enzymes by free radicals during detoxification processes. Decrease in antioxidant vitamin levels may be related to their role in these antioxidant processes. It is known that in rheumatic diseases, especially in RA, Cu levels are increased whereas Zn levels are decreased27-29. Decreased SOD activity levels may be due to disturbance in Cu and/or Zn levels. It appears that increased levels of superoxide and other radicals are not detoxified in patients with RA due to decreased efficiency of antioxidant enzymatic and non-enzymatic mechanisms, and may act as mediators of tissue damage.

Acknowledgment

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References


### Table

Antioxidant vitamins (A, E and C) and MDA in plasma and erythrocyte antioxidant enzyme activities (SOD and GSH-Px) in patients with RA and controls

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=20)</th>
<th>Patients with RA (n=22)</th>
</tr>
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<tbody>
<tr>
<td>Vitamin A (µg/dl)</td>
<td>77.2±12.2</td>
<td>53.6±8.1**</td>
</tr>
<tr>
<td>Vitamin E (µg/ml)</td>
<td>8.3±1.7</td>
<td>6.0±1.4**</td>
</tr>
<tr>
<td>Vitamin C (µg/ml)</td>
<td>8.8±2.1</td>
<td>6.2±1.7**</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>1.5±0.1</td>
<td>4.6±0.8**</td>
</tr>
<tr>
<td>SOD (U/gHb)</td>
<td>1530±263</td>
<td>985±181**</td>
</tr>
<tr>
<td>GSH-Px (U/gHb)</td>
<td>45.9±15.3</td>
<td>36.4±9.8*</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>13.2±1.1</td>
<td>11.9±1.3*</td>
</tr>
</tbody>
</table>

MDA, malondialdehyde; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; Data are mean±SD. *P*<0.05, **P*<0.005 compared to controls.

Antioxidant enzyme (SOD and GSH-Px) activities were found to be significantly lower in patients compared to controls (*P*<0.005 and *P*<0.05, respectively) in the present study. There are controversial reports on erythrocyte SOD and GSH-Px activities in patients with RA, as increased1,17,19,21, unaltered22 or decreased1,23 SOD activity has been reported. Similarly, decreased1,23 or unaltered19,24 GSH-Px activity in serum or erythrocytes have also been reported. Erythrocyte SOD activity levels were found to be lower in patients with RA than healthy controls1,7,25 and these results are in agreement with our findings. Akyol et al18 reported that SOD levels in erythrocytes of patients with RA are not different from controls, but they found low SOD activity levels in plasma.

We found significantly (*P*<0.005) lower antioxidant vitamin (A, E, C) levels in patients with RA compared to the controls. In patients with RA, low plasma concentrations of vitamin E26, vitamin C2, beta-carotene and vitamin A8 have been reported. The results of the present study are similar to these findings.

The mean haemoglobin levels in the patients was also found to be significantly (*P*<0.05) lower than that of the healthy controls (Table). Levels of Hb are also reported to be low in earlier studies1. Akyol et al18 found no differences between Hb values of rheumatic patients and healthy controls.


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