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Lipid peroxidation, some extracellular antioxidants, and antioxidant enzymes in serum of patients with rheumatoid arthritis

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Abstract The aims of our study were to assess whether the increased oxidative stress in inflamed joints is reflected by serum lipid peroxidation and also to check alterations in the levels of extracellular antioxidants and antioxidant enzyme activities in patients with rheumatoid arthritis. Serum malondialdehyde and ceruloplasmin levels and the activity of CuZn superoxide dismutase were higher, while transferrin levels and the activities of glutathione peroxidase and catalase were lower in patients \((n=37)\) than in healthy controls \((n=30)\). Disease activity score correlated positively with serum malondialdehyde level and CuZn superoxide dismutase activity. Probably, superoxide radicals in serum could be dismutated to produce hydrogen peroxide by increased CuZn superoxide dismutase activity, but hydrogen peroxide could not have been detoxified due to decreased activities of serum glutathione peroxidase and catalase. Hydrogen peroxide possibly converted to hydroxyl radical by iron due to lower transferrin level might have led to increased serum lipid peroxidation in patients with rheumatoid arthritis.

Keywords Rheumatoid arthritis · Serum · Malondialdehyde · Antioxidant enzymes · Disease activity score

Introduction

Rheumatoid arthritis (RA) is a chronic progressive autoimmune disorder characterized by symmetric, erosive synovitis and sometimes shows multisystem involvement [1, 2]. It affects about 1% of the population worldwide and may lead to progressive joint destruction, deformity, disability, and premature death [1, 2, 3].

The synovial fluid of the inflamed rheumatoid joint has been swarmed with inflammatory cells including activated neutrophils, which produce large amounts of superoxide radical \((O_2^-)\), hydrogen peroxide \((H_2O_2)\), and highly reactive hydroxyl radical \((OH^-)\) [4, 5]. Neutrophils from synovial fluids of patients with RA show enhanced \(O_2^-\) production, possibly because of their exposure to cytokines present in the synovial fluids [6, 7]. Ischemia and reperfusion during movements also contribute to the production of reactive oxygen species in the inflamed joint [8]. If not scavenged, these reactive species may lead to widespread lipid, protein, and DNA damage [9]. Babior suggests that reactive oxidants are important mediators in the pathogenesis of RA according to the studies related to oxidant damage and phagocyte function in these patients [10].

Cells have different antioxidant systems, including low molecular weight antioxidant molecules like glutathione [11] and various antioxidant enzymes, to defend themselves against free radical attacks [12]. Superoxide dismutase (SOD), the first line of defense against oxygen-derived free radicals, catalyzes the dismutation of the superoxide anion \((O_2^-)\) into hydrogen peroxide \((H_2O_2)\). \(H_2O_2\) can be transformed into \(H_2O\) and \(O_2\) by catalase. Glutathione peroxidase (GSH-Px) is a selenoprotein...
which reduces lipid or nonlipid hydroperoxides as well as H$_2$O$_2$ while oxidizing glutathione. Of the extracellular antioxidants, ceruloplasmin (CP) assists in the loading of iron onto transferrin (TF) [13]. TF binds iron so that iron cannot catalyze free radical damage [14].

Increased oxidant damage has been found in the synovial fluid of patients with RA [15, 16, 17]. Very rarely, no change [18] and generally increased lipid peroxidation in blood plasma [19, 20, 21] and erythrocytes [22] have also been reported in RA patients. However, the picture is not that clear in terms of the alterations in the levels of various antioxidants and antioxidant enzyme activities in blood of the patients with RA. Kose et al. determined increased CP and decreased TF plasma levels in RA patients [20], while Ashour et al. have reported decreased plasma CP levels in children with juvenile RA [23]. Increased [22, 24], decreased [25], or unaltered [18, 26] SOD activity, decreased [23] and unaltered [18, 22, 27] GSH-Px activity in serum or erythrocytes, and decreased [27] and unaltered [22, 26] catalase activity in erythrocytes of RA patients have been reported. As seen, the alterations in the antioxidant enzymes in the blood of patients with RA are inconsistent and, to our knowledge, no study reports serum catalase activity in these patients.

Hence, the aims of our study were to assess whether the increased oxidative stress in inflamed joints is reflected by serum lipid peroxidation, whether it is related with the severity of the disease, and also to check the alterations in the levels of some extracellular antioxidants and antioxidant enzyme activities in patients with RA.

**Materials and methods**

Reduced glutathione (GSH), NADPH, glutathione disulfide reductase, H$_2$O$_2$, xanthine, xanthine oxidase, CuCl$_2$, nitroblue tetrazolium, trichloroacetic acid, and thiobarbituric acid were obtained from Sigma (St. Louis, Mo., USA). All other chemicals and reagents used were of the highest quality available.

Thirty-seven patients suffering from RA for 2–10 years (14 men, 23 women, mean age 52 ± 11 years) and 30 healthy subjects (14 men, 16 women, mean age 40 ± 12 years) were recruited for the study after giving their informed consent. The study was performed in accordance with the ethics standards laid down in the Declaration of Helsinki. All the patients examined met the American Rheumatism Association criteria for RA [1] and were taking nonsteroidal anti-inflammatory drugs. None of the subjects was taking alcohol or had intestinal absorption defects and showed any clinical or laboratory signs of liver disease, diabetes mellitus, thyroid disease, infectious disease, or coronary artery disease. Control subjects were healthy medical students, laboratory personnel, and those attending a health screening program.

Erythrocyte sedimentation rate (ESR) was determined according to the Westergreen method using anticoagulant containing whole blood. Venous blood was collected in vacutainers without additive, allowed to clot for 30 min at room temperature, and centrifuged at 3000 G for 5 min to get serum. Serum aliquots were stored at −80°C until biochemical analyses. Hemolyzed samples were excluded.

Malondialdehyde (MDA) levels were measured by the spectrophotometric method of Beuge and Aust [28]. Briefly, serum is mixed with two volumes of a stock solution of 15% (w/v) trichloroacetic acid, 0.375% (w/v) thiobarbituric acid, and 0.25 N hydrochloric acid. The combination of serum and stock solution was heated for 30 min in a boiling water bath. After cooling, the precipitate was removed by centrifugation, and the absorbance of the supernatant was determined at 535 nm. CuZn superoxide dismutase (CuZn-SOD) activity was determined by the inhibition of the reduction of nitroblue tetrazolium by superoxide anion radicals, which are produced by the xanthine-xanthine oxidase system [29]. GSH-Px activity was measured by coupled spectrophotometric assay at 340 nm from the oxidation of NADPH in the presence of H$_2$O$_2$ used as substrate [30]. Catalase activity was determined by measuring the rate of decay of H$_2$O$_2$ absorbance at 240 nm [31]. Biochemical measurements were carried out at room temperature using a CE341 spectrophotometer (Cecil, UK). Serum C-reactive protein (CRP), CP, and TF levels were determined by nephelometric method (Beckman Array 360 Protein System, USA).

Disease activity score (DAS) was calculated using the following formula as described [32, 33]:

$$DAS = 0.53938 \sqrt{RAI} + 0.06465 \times SW + 0.330 \times \ln ESR + 0.224$$

where RAI is the Ritchie articular index, SW the number of swollen joints, and ESR the erythrocyte sedimentation rate.

Student’s t test was used to compare the group means. Correlations between variables were determined by linear regression analysis. P values less than 0.05 were considered significant. SPSS for Windows (version 8.0.0) was used for statistical analyses.

**Results**

Erythrocyte sedimentation rate and C-reactive protein levels were higher in patients with RA than in healthy subjects, as characteristic laboratory findings of the disease [34] (Table 1). Disease activity scores of the patients are also shown in Table 1.

Lipid peroxidation as measured by serum MDA level was higher (91%) in patients with RA than in healthy control subjects (Table 2).

Of the antioxidant enzymes studied, the activity of serum SOD was higher (77%), while the activities of GSH-Px (31%) and catalase (14%) were lower in patients with RA than in healthy control subjects (Table 2).

Of the extracellular antioxidants determined in our study, the serum CP level was higher (70%), while the TF level was lower (29%) in patients with RA than in healthy controls (Table 2). There was a positive correlation between serum CP and TF levels in the healthy control subjects (correlation coefficient 0.45, P < 0.05).

Disease activity scores correlated positively with serum MDA level (correlation coefficient 0.42, P < 0.01) (Fig. 1) and SOD activity (correlation coefficient 0.38, P < 0.05) in patients with RA. However, no other correlation could be found among the parameters in healthy controls and patients with RA.

**Discussion**

Although no change in lipid peroxidation has very rarely been reported [18], higher lipid peroxidation in plasma [19, 20, 21] and erythrocytes [22] and also increased levels of 8-oxo-7-hydrodeoxyguanosine in lymphocyte