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Clinical Study

Serum paraoxonase and arylesterase activity and oxidative status in patients with multiple sclerosis

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ABSTRACT

The aim of this study was to investigate serum paraoxonase and arylesterase activities, and to determine oxidative status via the measurement of total oxidant status (TOS), total antioxidant status (TAS) and the oxidative stress index (OSI) in patients with relapsing-remitting multiple sclerosis (RRMS). Results were compared with data from healthy controls. A total of 60 subjects, including 30 newly diagnosed and untreated patients with RRMS (20 females, 10 males, 18–40 years of age) and 30 healthy controls (20 female, 10 male 20–40 years of age) were enrolled in this study. The oxidative status of the RRMS patients was measured by TOS, TAS and estimation of the OSI was made by a new automated method. Paraoxonase (PON1) and arylesterase activities were measured spectrophotometrically. TAS levels of RRMS patients were significantly lower than that of controls ($p < 0.05$). TOS levels of RRMS patients were higher than that of controls ($p < 0.05$). PON1 and arylesterase activities of RRMS patients were lower, but not significantly, than those of controls ($p > 0.05$). There was no correlation between serum PON1 activity and OSİ in patients with RRMS ($p > 0.05$). Hypercholesterolemia was not observed in multiple sclerosis patients. In conclusion, although the mechanism underlying the significant reduction of TAS levels of multiple sclerosis patients compared with those of controls is unknown, the results imply that endogenous antioxidants may have been exhausted by increased oxidative stress and we believe that additional antioxidant treatment might be beneficial for these patients.

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory, immune-mediated, demyelinating neurodegenerative disease of the central nervous system (CNS) that is caused by the interaction of genetic and environmental components.[1] The incidence and prevalence of MS have been increasing recently worldwide; its pathogenesis is still unknown.[2] Although different mechanisms may contribute to demyelination and neurodegeneration in MS, it has recently become clear that mitochondrial injury, and subsequent energy failure, is a major factor driving tissue injury.[3] Oxidative damage has an important role of in the pathogenesis of demyelination and neurodegeneration in MS lesions.[2,3] Reactive oxygen species (ROS) are thought to play an important role in a variety of physiologic and pathophysiologic processes. Increased oxidative stress may play an important role in disease mechanisms.[4,5] ROS have been implicated in the progression of cancer, cardiovascular disease and neurodegenerative and neuroinflammatory disorders, such as MS.[6] In the last decade there has been a major interest in the involvement of ROS in MS pathogenesis and evidence is emerging that free radicals play a key role in various processes underlying MS pathology.[3,6] Oxidative stress has been described as an oxidant–antioxidant imbalance, occurring when the net amount of oxidant exceeds the antioxidant capacity.[4,5] The ratio of total oxidative status (TOS) level to total antioxidant status (TAS) level is described by the oxidative stress index (OSI). This has been used as a new marker of the degree of oxidative stress.[7,8] To our knowledge, no data are available in the literature to show whether increased OSI plays a role in the pathogenesis of MS. Therefore, we investigated various markers including TAS and TOS, in the serum of patients with MS.

Paraoxonase (PON1), a 43–45 kDa glycoprotein, is synthesized mainly in the liver. It hydrolyzes organophosphates such as pesticides, neurotoxins and arylesters.[9] Paraoxonase is high-density lipoprotein–associated esterase/lactonase thought to play a role in the antioxidant and anti-inflammatory properties exerted by high-density lipoprotein (HDL). Many investigations have provided considerable evidence for PON1 anti-atherogenicity.[9–11] Studies have shown that PON1 inhibits oxidation of HDL and the low-density lipoproteins (LDL) that preserve HDL function, increases cellular cholesterol efflux from macrophages, ameliorates the

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effects of oxidized LDL, and decreases lipid peroxides in atherosclerotic lesions.[9] There are two polymorphisms in the PON1 coding region: leucine/methionine at position 55 (M55L) and glutamine/arginine at position 192 (Q192R). These polymorphisms are associated with a number of pathophysiologic conditions, including coronary artery disease, Parkinson's disease, stroke, familial hypercholesterolemia, type 2 diabetes mellitus, late-onset Alzheimer's disease and reduced bone mass in postmenopausal women.[9,11] In various trials, it has been shown that paraoxonase prevents oxidative stress by inhibiting the oxidation of cell membrane lipids induced by ROS which develop in acute and chronic inflammation. It has been reported that paraoxonase activity may change during inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, Behcet's disease, psoriasis and inflammatory bowel disease.[9,11] Varying results have been obtained in studies of PON1 activity in patients with MS.[12,13]

The aim of this study was to investigate the relationship between serum paraoxonase and arylesterase activities, total oxidant status (TOS), total antioxidant status (TAS) and the oxidative stress index (OSI) in patients with MS.

2. Methods

2.1. Subjects

This study was conducted at the Recep Tayyip Erdogan University Medical Faculty, Department of Neurology and Clinical Biochemistry. The study consisted of 30 patients with newly diagnosed and untreated relapsing-remitting multiple sclerosis RRMS (20 females and 10 males, 18–40 years of age, mean age 29.2 ± 5.5 years). The diagnosis was based on the patient's history, a physical examination, and MRI findings using the 2005 McDonald criteria.[14] All patients scored between 0.5 and 1 on the Expanded Disability Status Scale (EDSS). Data was collected prospectively. The procedures were in accordance with the revised form of the Helsinki Declaration 2008 and all participants signed an informed consent form. The study protocol was approved by the local Ethical Committee (Date: 24 Feb 2012 Decision No: 18). The control group consisted of 30 healthy individuals (20 females and 10 males, 20–40 years of age, mean age of 28.7 ± 4.7 years). Body mass index (BMI; weight/height^2) was obtained through height and weight measurements using a wall-mounted ruler and a digital scale.

2.2. Blood sample collection

After overnight fasting, peripheral venous blood samples were taken from patients and controls into empty tubes. After coagulation, samples were immediately separated from the cells by centrifugation at 3000 *g* for 10 minutes, and then stored at −80 °C until further analysis of paraoxonase and arylesterase activities, along with the determination of oxidative status via measurements of TOS and TAS. Serum total cholesterol, HDL cholesterol and triglyceride levels were measured with photometric methods (Architect C1600, Abbott Laboratories, Abbott Park, Chicago, Illinois, USA) whereas LDL was calculated using the Friedewald's formula.[15]

2.3. Measurement of paraoxonase and arylesterase activities

Paraoxonase activity was measured in the absence (basal activity), and presence, of NaCl (salt-stimulated activity).[16] Briefly, the rate of paraoxon hydrolysis was measured by the increase in absorbance at 412 nm at 25 °C. The amount of *p*-nitrophenol generated was calculated from the molar absorptivity coefficient at pH 8, which was 17,100 M^{-1} cm^{-1} Paraoxonase activity was expressed as U/L serum. Phenylacetate was used as a substrate to measure arylesterase activity. The reaction was initiated by the addition of serum and the increase in absorbance was read at 270 nm. Blanks were included to correct spontaneous hydrolysis of phenylacetate. Enzymatic activity was calculated from the molar absorptivity coefficient of the resultant phenol, 1310 M^{-1} cm^{-1}. One unit of arylesterase activity was defined as 1 μmol phenol generated per minute under the above conditions and expressed as U/L serum. The phenotype distribution of paraoxonase was determined in the presence of 1 mol/L NaCl (salt-stimulated paraoxonase). The ratio of salt-stimulated paraoxonase activity to arylesterase activity was used to assign individuals to one of the three possible phenotypes.[17]

2.4. Measurement of total oxidant status

The TOS of serum was determined using a novel automated method.[7] Oxidants present in the sample oxidize ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. Ferric ion reacts with xylenol orange in an acidic medium to produce a colored complex. The intensity of the color, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules in the sample. The assay is calibrated with hydrogen peroxide and results are expressed in terms of micromolar hydrogen peroxide (H_2O_2) equivalents per liter (μmol H_2O_2 equiv/L).

2.5. Measurement of total antioxidant status

The TAS of serum was determined using an automated measurement method.[8] Briefly, potent free-radical reactions were initiated with the production of a hydroxyl radical via the Fenton reaction, and the rates of reactions were monitored by following the absorbance of colored dianisidyl radicals. Using this method, the antioxidative effect of the sample against potent free-radical reactions, which were initiated by the synthesized hydroxyl radical, was measured. The method was performed by an automated analyzer (Architect C1600). Both intra- and inter-assay coefficients of variations were lower than 3%. Data were expressed as μmol equiv/L Trolox (Hoffman-LaRoche, Basel, Switzerland).

2.6. Calculation of the oxidative stress index

Oxidative stress index (OSI) was calculated by dividing the total oxidant status (TOS) by the total antioxidant status (TAS):

OSI = (TOS, μmol H_2O_2 equiv/L)/(TAS, μmol Trolox equiv/L)

2.7. Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences version 15.0, for Windows (SPSS, Chicago, IL, USA). Data were expressed as mean ± standard deviation. The normality of the distribution for all variables was assessed by the Kolmogorov–Smirnov test. Student's *t*-test was used for normally distributed variables and the Mann–Whitney *U*-test was used for non-parametric variables. Relationships between variables were analyzed by Pearson or Spearman correlation analysis according to the distribution type of the variable. A value of $p < 0.05$ was considered to be statistically significant.

3. Results

The demographic and clinical data of the subjects are summarized in Table 1. There were no significant differences in age, BMI or serum lipids between patients with RRMS and healthy controls.