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Clinical Study

Paraoxonase and arylesterase activity and total oxidative/anti-oxidative status in patients with idiopathic Parkinson's disease

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ABSTRACT

This study investigated serum paraoxonase (PON1) and arylesterase activity along with determination of oxidative status via measurement of total oxidant status (TOS), total anti-oxidant status (TAS) and oxidative stress index (OSI) in patients with Parkinson's disease (PD) and compared results with data from healthy controls. A total of 82 subjects, including 42 patients with idiopathic PD, newly diagnosed and untreated (24 men, 18 women, aged 47–66 years) and 40 healthy controls were enrolled in this study. We aimed to evaluate the oxidative status of PD patients via measurement of serum TOS and TAS and estimation of OSI using new automated methods. PON1 and arylesterase activities were measured spectrophotometrically. Serum total cholesterol, high density lipoprotein cholesterol, low density lipoprotein (LDL) cholesterol and triglyceride levels were measured using routine methods. TAS levels of PD patients were significantly lower than that of controls ($p < 0.05$). TOS levels of PD patients were higher than those of controls ($p < 0.05$). PON1 and arylesterase activities of PD were lower than those of controls ($p < 0.05$). Serum levels of total and LDL cholesterol were significantly reduced in PD patients. In conclusion, the presence of high TOS and OSI levels together with low levels of TAS in PD patients supports the important role of oxidative stress in the pathophysiology of PD. Since oxidative stress is involved in neurodegeneration, selecting anti-oxidants, metal chelators or other compounds boosting endogenous enzymatic and non-enzymatic defense mechanisms seems to be an obvious choice as treatment for PD.

1. Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by bradykinesia, tremor, progressive rigidity and postural instability, resulting from progressive degeneration of dopaminergic neurons in the substantia nigra of the midbrain. Both incidence and prevalence increase with age, with a prevalence of 0.3% and an estimated incidence of 8–18 per 100 000 person years [1]. Pathologically PD is characterized by loss of melanin-pigmented nigral neurons accompanied by depletion of dopamine in the striatum and the presence of Lewy bodies, which are deposits of specific cytoplasmic proteins such as ubiquitin, a-synuclein, and oxidized neurofilaments [2]. Similar to other neurodegenerative disorders, oxidative stress, inflammation, mitochondrial dysfunction, environmental factors and genetic predisposition may all be involved in PD [1,2]. Although oxidative stress plays a role in the pathogenesis of neuronal death, it is still not very clear whether the oxidative stress itself contributes to the onset of neurodegeneration or if it is a secondary manifestation of the neurodegenerative process [3].

Many cellular reactions utilize molecular oxygen for catalysis and energy production [4]. These reactions in turn produce reactive oxygen species (ROS) including superoxide anions, hydrogen peroxide, hydroxyl radicals, peroxy radicals and, in the presence of nitric oxide, reactive nitrogen species (RNS) such as peroxynitrite and nitro-tyrosyl radicals. While these reactive species are important for the execution of physiological functions, excessive production is detrimental to cell membranes and can cause cell death [5]. The rate of production and destruction of ROS is in a state of balance, known as oxidative balance. In cases where this oxidative balance is maintained, ROS may have no impact on the organism but in cases where this balance is tipped in favor of free radicals, oxidative stress develops [6]. The defense system protecting against free radical damage involves enzymatic and non-enzymatic anti-oxidant systems. The enzymatic system includes superoxide dismutase, glutathione peroxidase, catalase, aldehyde dehydrogenases, and sulfiredoxin. The non-enzymatic system includes naturally occurring anti-oxidants such as vitamin A (retinol), vitamin C (ascorbic acid), vitamin E (tocopherol), beta-carotene and glutathione, as well as polyphenol anti-oxidants like flavonoids [7,8]. Under certain conditions, the oxidative or anti-oxidative balance shifts towards oxidative stress as a result of increase in ROS and/or impairment in the anti-oxidant

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mechanisms [7]. The body's oxidant/anti-oxidant status can be ascertained by measuring the activity of individual molecules or enzymes; likewise the overall oxidant/anti-oxidant status can be assessed more easily by measuring total anti-oxidant status (TAS) [9] and total oxidant status (TOS) [10].

Environmental factors may influence PD risk [11]. One often-discussed risk factor is pesticide exposure [12]. Paraoxonase 1 (PON1), a 43–45 kDa glycoprotein, is synthesized mainly in the liver, which hydrolyzes organophosphates such as pesticides, neurotoxins, and arylesters [11,12]. The paraoxonase (PON) gene family consists of three members – PON1, PON2, and PON3 – located adjacent to each other on the long arm of chromosome 7 in humans (q21.3–q22.1) [13]. These three human PON genes share approximately 60% identity at the amino acid level and approximately 70% identity at the nucleotide level [14]. PON is a high-density lipoprotein (HDL)-associated esterase/lactonase implicated in the anti-oxidant and anti-inflammatory properties exerted by HDL. Many investigations have provided considerable evidence for PON1 anti-atherogenicity [13,14]. Studies have shown that PON1 inhibits oxidation of HDL and low-density lipoproteins (LDL) that preserve HDL function, increases cellular cholesterol efflux from macrophages, ameliorates effects of oxidized LDL, and decreases lipid peroxides in atherosclerotic lesions [13,14]. 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 pathophysiological conditions, including coronary artery disease, PD, stroke, familial hypercholesterolemia, type 2 diabetes mellitus, late-onset Alzheimer's disease and reduced bone mass in post-menopausal women [12–14]. The activity of PON1 in patients with PD has been very different to results obtained in studies on populations without PD [11,15,16].

The aim of this study was to investigate the relationship between serum paraoxonase and arylesterase activity, TOS, TAS and oxidative stress index (OSI) in patients with PD.

2. Methods

2.1. Subjects

This study was conducted at the Neurology Clinic of Recep Tayyip Erdoğan University School of Medicine, Turkey. Forty-two newly diagnosed and untreated idiopathic PD patients (24 men, 18 women, mean age 59.3 ± 4.9 years [standard deviation], range 47–66) were included in this study. The PD patients were diagnosed according to the Parkinson's Disease Society Brain Bank clinical diagnostic criteria for idiopathic PD [17]. Severity ratings for PD between 0 and 5 used the Hoehn and Yahr scale, and all patients were between Hoehn and Yahr stage 1 and 2.

The procedures were in accordance with the revised form of the Declaration of Helsinki 2008 and all participants signed an informed consent form. The study protocol was approved by the local Ethical Committee. The control group consisted of 40 healthy individuals (24 men, 16 women, mean age 57.0 ± 4.9 years [standard deviation], range 45–67). All subjects were informed about the study. Body mass index (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 determination of oxidative status via measurement of TOS, TAS and OSI. Serum total cholesterol, HDL cholesterol and triglyceride levels were measured with routine methods (Architect C1600; Abbott Laboratories, Abbott Park, IL, USA). LDL was calculated using the Friedewald formula.

2.3. Measurement of paraoxonase and arylesterase activity

Paraoxonase activity was measured in absence (basal activity) and presence of NaCl (salt-stimulated activity) [18]. Briefly, the rate of paraoxon hydrolysis was measured by the increase of absorbance at 412 nm at 25 °C. The amount of generated p-nitrophenol 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 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 produced phenol, 1310 M^{-1} cm^{-1}. One unit of arylesterase activity was defined as 1 mmol phenol generated/minute under the above conditions and expressed as U/L serum. Phenotype distribution of paraoxonase was determined in 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 [19].

2.4. Measurement of the TOS

Serum TOS was determined using a novel automated measurement method previously described [9]. 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 color, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules in the sample. The assay was calibrated with hydrogen peroxide and results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equiv./L). The assay has excellent precision with error values lower than 2%.

2.5. Measurement of the TAS

Serum TAS was determined using an automated measurement method previously described [10]. Briefly, potent free radical reactions were initiated with the production of a hydroxyl radical via the Fenton reaction and the rate of reactions was monitored by following the absorbance of colored dianisidyl radicals. Using this method, the anti-oxidative effect of the sample against potent free radical reactions, which were initiated by synthesized hydroxyl radical, was measured. This method was applied to an automated analyzer (Architect C1600; Abbott Laboratories). 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 OSI

The OSI was calculated by dividing the TOS by the TAS, that is, OSI = (TOS, μmol H_2O_2 equiv./L)/(TAS, μmol Trolox equiv./L).