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Metal changes in CSF and peripheral compartments of parkinsonian patients

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Abstract

Background: Involvement of metals in the risk of developing Parkinson's disease (PD) has been suggested. In the present study, concentration of metals in cerebrospinal fluid (CSF), blood, serum, urine and hair of 91 PD patients and 18 controls were compared.
Methods: Blood and hair were microwave digested, while CSF, serum and urine were water-diluted. Elements quantification was achieved by Inductively Coupled Plasma Atomic Emission Spectrometry and Sector Field Inductively Coupled Plasma Mass Spectrometry.
Results: Some metal imbalances in PD were observed: *i*), in CSF, lower Fe and Si; *ii*), in blood, higher Ca, Cu, Fe, Mg and Zn; *iii*), in serum, lower Al and Cu; *iv*), in urine, lower Al and Mn, higher Ca and Fe; and *v*), in hair, lower Fe. The ROC analysis suggested that blood Ca, Fe, Mg and Zn were the best discriminators between PD and controls. In addition, hair Ca and Mg were at least 1.5 times higher in females than in males of patients and controls. A decrement with age of patients in hair and urine Ca and, with less extent, in urine Si was observed. Magnesium concentration in CSF decreased with the duration and severity of the disease. Elements were not influenced by the type of antiparkinsonian therapy.
Conclusions: Variation in elements with the disease do not exclude their involvement in the neurodegeneration of PD.

Keywords: Metals; CSF; Blood; Urine; Serum; Hair; Parkinson's disease

1. Introduction

Clinical expression of Parkinson's disease (PD) seems to depend on the combination of multiple risk factors with environmental, occupational and genetic components. In particular, it has been reported that a combination of metals can act together to increase the risk of the disease [1]. Several investigations reported increased Fe levels in the substantia nigra of parkinsonian patients [2,3]. The potential pathogenicity of Fe in PD is related to its ability to promote redox reactions and free radicals generation and its selective binding to neuromelanin, producing Fe^{3+}-melanin complexes. These complexes may induce oxidative stress and death of dopaminergic neurons [4,5]. Increased levels of Al in the neurofibrillary tangles of patients with PD have also been reported [2]. Not alike Fe that normally accumulates in high concentration in different brain regions, Al is present in brain tissues in extremely small quantities suggesting that the intraneuronal-accumulation represents deposition from exogenous sources. Aluminium is not known to play any physiological role and does not promote free radical formation, but it has been demonstrated that in combination with Fe^{2+}, Al-salts markedly enhance iron-induced lipid peroxidation [6]. Manganese, as Fe, is a transition metal which is able to accept or donate single electrons and produce oxygen species and toxic cathecolamines; such behaviour seems to be the basis for Mn-induced neurodegeneration [7]. Moreover, brain permeability to Mn is higher than that to Fe and Zn and abnormal Mn concentrations in brain are reported to be associated with neurological disorders similar to PD [8]. The electron transfer chemistry of Cu makes it potentially a pro-oxidant agent and its high affinity with β-amyloid appears to contribute to neuropathological conditions such

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as Alzheimer's disease (AD) [9,10]. As regards Zn, it was found to be markedly increased in the substantia nigra of PD patients [11,12], but decreased in brain of AD patients [13]. The key role of Cu, Mn and Zn in the intracellular oxidative balance is also due to their participation in the superoxide dismutase enzymes which detoxify free radicals. Degeneration of the central nervous system (CNS) has also been found to be associated to irregular metabolism of Ca and Mg, producing an abnormally high brain Ca/Mg ratio [14]. Both elements appear to interact with Al; in fact some forms of dementia are related to a depletion of these metals due to the high intake of Al [15]. Finally, Si, as alumino-silicate complexes, accumulates in the neurofibrillary tangles of patients with neurological diseases [16,17], while, as silicic acid, seems to have a protective effect by binding Al and reducing its bio-availability [18].

Most of the literature is based on metal imbalances in the CNS by means of the analysis of a variety of post-mortem tissues. Studies of elemental alteration in human fluids of living PD patients are few and provide partial and contradictory information. In particular, cerebrospinal fluid (CSF) studies are infrequent, although the CSF composition could be an indirect reflection of brain metabolic activity. For example, high Cu and normal Fe concentrations were found in the CSF of PD patients [19]; this was only partially supported by other studies, where normal levels of CSF Cu, Fe and Mn were reported [20,21]. Normal levels of serum Cu and Fe [21,22] were observed in PD, but other studies suggested abnormalities in serum Cu and clear reductions of serum Fe [23,24]. Low levels of Zn in the CSF of PD patients were also found [21] but in most of the literature no difference in Zn of patients with neurodegeneration, nor in CSF neither in blood nor serum, was reported [25–27].

In this context, the aim of this study was to get data about Al, Ca, Cu, Fe, Mg, Mn, Si, and Zn concentrations in CSF, blood, serum, urine and hair of a large population of PD patients in order to evaluate, in comparison with age-matched controls, whether alteration in their levels could be either a co-factor of risk for developing PD or a marker of the disease.

2. Methods

2.1. Study population

Ninety-one patients (64 males and 27 females, mean age of 65.5±9.7 years) with PD diagnosed according to the London Brain Bank Criteria [28] were examined. The mean duration of the disease was 4.6±4.1 years and the Hoehn and Yahr phase range was 1–3 [29]. Approximately 25% of patients were not undergoing treatment with anti-parkinsonian drugs, while the remaining were being treated with dopaminergic agonists, L-DOPA or a combination of both. Eighteen (10 males and 8 females) age-matched controls with a mean age of 63.3±13.8 years, who were not affected by any central neurological disorder, were also examined. Subjects were interviewed in order to obtain detailed information on family, dietary habits, lifestyle and personal medical history. Exclusion criteria applied to both groups were: liver or renal failure, cardiological and respiratory disorders, diseases causing malabsorption, intake of vitamin or mineral supplements, active infections, assumption of thyroid hormones or lithium, vegetarian dietary habits; assumption of psychoactive drugs except for anti-parkinsonism medications; artificial metallic body parts. The study was approved by the Ethics Committee of the Neuroscience Department of Tor Vergata University, Rome.

2.2. Sample collection, treatment and analysis

Blood, urine and CSF were collected after fasting overnight and drug abstention. All the plastic collection containers were thoroughly cleaned with a mixture of 10% of HNO_3 (ultrapure grade, Carlo Erba, Milan, Italy) and repeatedly rinsed with deionized water (EASY-pure, PBI, Milan, Italy). The anticoagulants were not used to avoid metal contamination and all samples were kept at −20 °C till they were treated. Samples treatment consisted in an acid-assisted microwave (MW) digestion of blood by means of an ETHOS MEGA II MW oven (FKV, Bergamo, Italy) and in a dilution of urine, serum and CSF with high purity deionized water. Details of sampling procedures and pre-treatment of body fluids are previously reported [30]. Hair samples, cut from the sub-occipital zone of the head, were subjected to an accurate and standardized washing procedure to eliminate external contaminations caused by environmental dust and dirt, sweat and desquamation of the epidermis, as well as detergents and cosmetics and then they were MW digested [31]. After cooling, the samples were made up to 20 ml with deionized water.

Calcium, Cu, Fe, Mg, Si and Zn in all specimens were quantified by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). An Optima 3100 XL spectrometer (Perkin Elmer, Norwalk, CT, USA) was utilized and the spectral lines adopted for the analysis were (λ in nm): Ca, 393.3; Cu, 324.7; Fe, 259.9; Mg, 279.5; Si, 251.6 and Zn, 213.8. Aluminium and Mn quantifications were carried out by Sector Field Inductively Coupled Plasma Mass Spectrometry (SF-ICP-MS), using the ELEMENT model from Thermo Finnigan (Bremen, Germany) in the medium resolution mode (*i.e.*, 3000 m/Δm) at masses 27 and 55, respectively. In both cases, the standard addition approach was adopted for calibrations.

2.3. Statistical analysis

Data were checked for normality by the Kolmogorov–Smirnov test. The statistical analysis used the SPSS statistical package (SPSS, Chicago, IL, USA) and included the Mann–Whitney *U*-test, the calculation of Spearman's correlation coefficients (ρ) and the receiver operating