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Antioxidant and inflammatory biomarkers for the identification of prodromal Parkinson's disease

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ABSTRACT

Objectives: We explored the role of oxidative stress and inflammatory molecules as potential Parkinson (PD) biomarkers and correlated biological with non-motor abnormalities (olfactory impairment and dysautonomia), in patients with idiopathic REM behavior disorder (iRBD) (prodromal PD) and established PD.
Methods: We recruited 11 iRBD and 15 patients with idiopathic PD (Hohen&Yahr 1–3, on L-DOPA and dopamine agonists combination therapy) and 12 age- and sex-matched controls (CTRL). We measured total olfactory score (TOS), autonomic function [deep breathing (DB), lying to standing (LS) and Valsalva manoeuvre (VM) ratios], blood reduced glutathione (Br-GSH), oxidative stress and inflammatory markers (neopterin).
Results: Anosmia was similarly prevalent in iRBD (36%) and PD (33%) patients, but absent in CTRL. Orthostatic hypotension was more common among iRBD (73%) and PD (60%) than in CTRL (25%). By univariable ordinal logistic regression, TOS, Br-GSH, LS and VM ratio worsened from CTRL to iRBD and PD groups. Only reduced Br-GSH levels ($p = 0.037$, OR = 0.994; 95%CI 0.988–1.000) were independently associated to PD. TOS correlated with Br-GSH ($R = 0.34$, $p = 0.037$), VM ratio ($R = 0.43$, $p = 0.015$), and neopterin (rho = 0.39, $p = 0.016$).
Conclusions: Reduced systemic antioxidant capacity is found in prodromal and overt PD and may represent, in association with olfactory loss and cardiovascular dysautonomia, a useful biomarker for an integrative, early diagnosis of PD.

1. Introduction

Early identification of Parkinson disease (PD) is a main challenge in neurological research. To date, the clinical diagnosis of PD is primarily based on the late onset of motor disorders, which manifest when 60–70% of the dopaminergic neurons are degenerated in the substantia nigra [1].

PD is now recognized as a more complex illness, encompassing non motor symptoms (NMS), such as sleep disturbances, sensory abnormalities, autonomic dysfunction, depression and cognitive decline [2], that may predate the onset of motor symptoms by many years and are currently being studied as features of prodromal PD [3]. Neuropathological changes related to NMS include findings of α-synuclein-containing Lewy bodies and Lewy neurites in the peripheral autonomic nervous system and neuronal loss in the dorsal motor nucleus of the vagal nerve, the olfactory bulb, and the lower brainstem nuclei that regulate REM sleep atonia [4]. These abnormalities might be responsible for the occurrence before the onset of parkinsonism of NMS such as dysautonomia, hyposmia, and REM sleep behavior disorders (iRBD), that have been prospectively linked to PD in population [5] and cohort studies [6], as recently reviewed [7,8].

Oxidative stress causes tissue injury and inflammation [9] and may play a role in PD. The autoxidation of dopamine in the dopaminergic neurons may produce reactive oxygen species (ROS). Mitochondrial dysfunction in nervous cells may increase oxidative stress by alteration of the respiratory chain, the activated microglia, responsible for chronic neuroiflammation, but also for ROS release after activation of specific enzyme, such as NADPH oxidase and inducible nitric oxide synthetase [10]. Elevated oxidative stress and a pro-inflammatory response occur early in the course of PD and contribute to exacerbate nigro-striatal degeneration [11].

Aims of our study were to explore the potential role of antioxidant and inflammatory biomarkers as novel tools for prodromal diagnostic evaluation of PD and to correlate biological findings with functional alterations, such as olfactory impairment and cardiovascular dysautonomia in patients with prodromal and established PD.

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2. Methods

2.1. Participants

We recruited eligible subjects aged ≥50 e ≤ 80 years consecutively referred between May 2014 and May 2015 by the Centre of Sleep Medicine (iRBD) and the Neurology outpatient clinic (PD) of our Hospital and age and gender-matched controls (CTRL) who attended our Institute for laboratory assessment.

Subjects presented none of the exclusion criteria listed below:

- other neurological disorder, major mental disorder, cognitive impairment (MMSE <26);
- previous myocardial infarction, heart failure, pacemaker, atrial fibrillation, beta-blockers;
- glomerular filtration rate < 15 mL/min;
- hepatic insufficiency
- long standing (>10 years) diabetes; subjects with hyperglycemia (serum glucose >100 and <126 mg/dL) without antidiabetic treatment could be enrolled

iRBD [12] had to meet the following criteria:

- repeated episodes of sleep-related vocalization and/or complex motor behaviors;
- video-polysomnographic (PSG) documentation of occurrence during REM sleep or history suggestive of dream enactment;
- polysomnographic observation of REM sleep without atonia;
- exclusion of other sleep disorder, medication, or substance use.

Patients with idiopathic PD, confirmed by a neurologist with expertise in movement disorders (EFNS/MDS-ES 2013 guidelines), had to be in Hohen & Yahr stage ≥1 and ≤3 and on L-DOPA and dopamine agonists combination therapy, titrated to maintenance doses since at least 1 month.

We excluded PD patients with any of the following:

- genetic etiology or familial clustering;
- atypical or secondary parkinsonism;
- history of cerebrovascular events;
- treatment with COMT inhibitors.

The study was approved by Niguarda Hospital Ethics Committee. All patients expressed their written informed consent to participate.

2.2. Study measurements

Eligible subjects underwent: medical and drug history taking; physical and neurological examination; iRBD screening question [13] olfactory and autonomic function testing; fasting blood and urine sampling. PD subjects were administered the Movement Disorder Society-UPDRS Parts I-II (nMEDL and MEDL scores) and underwent functional testing on medications.

2.3. Biochemical assessments

Glutathione (GSH), the most important endogenous scavenger, was assessed in total and reduced form and in plasma and blood samples by high performance liquid chromatographic (HPLC) [14]. Plasma malondialdehyde (MDA), a marker of lipid peroxidation, was assayed by a commercial kit (Chromsystems, Germany) in HPLC with fluorescence detection. Commercial ELISA kits were used for plasma assay of 8-hydroxy-2-deoxyguanosine (8-OHdG), index of oxidative DNA damage (Trevigen, Gaithersburg, MD, USA); 3-nitrotyrosine (3-NT), a stable end product of peroxynitrite oxidation (Hycult Biotech (Uden, The Nederland)); the inflammatory cytokines tumor necrosis factor alpha (TNFα) (Cayman Chemical Company, Ann Arbor, MI, USA) and interleukin 1-beta (IL1β), Boster Immunoleader (Pleasanton, CA, USA). Urine neopterin levels, a sensitive marker of cellular-mediated inflammation, were measured by an isocratic HPLC method and normalized by urine creatinine concentrations [15].

2.4. Functional testing

Olfactory function was assessed by the Sniffin' Sticks Extended Test (Burghart, Medizintechnik, GmbH, Wedel, Germany) [16]. The olfactory threshold is the minimum concentration of an odorant (n-butanol) that can be detected by a subject when presented with 16 different dilutions in felt tip pens. Olfactory discrimination assesses the ability to discriminate between different odorants in 16 different triplets. Olfactory identification evaluates the ability to correctly identify an odorant among four possible odors for each of 16 trials. The total olfactory score (TOS) was calculated as sum of the 3 sub-scores for olfactory threshold, discrimination and identification and reclassified as normal olfaction (between 31 and 48), hyposmia (between 16 and 30) and anosmia (≤15).

Autonomic function [17] was tested in the supine position at a comfortable ambient temperature. Heart rate was recorded via standard 12-lead electrocardiogram (Norav PC ECG-1002). Blood pressure was measured non-invasively by a manual sphygmomanometer.

2.4.1. Deep breathing (DB)

After 10-minute rest, subjects performed 1 minute DB (6 inspiratory and expiratory cycles of 5 sec each) during continuous ECG recording. The DB expiration/inspiration ratio was calculated as ratio of averages of the three longest RR intervals during expiration and the three shortest RR intervals during inspiration.

2.4.2. Lying to standing (LS)

After 10-minute rest, patients were instructed to stand up and remain standing for 5 min. Changes in systolic/diastolic blood pressure and heart rate were assessed after 1 and 5 minutes standing from the supine position. Orthostatic hypotension (OH) was defined as a drop ≥ 20 mm Hg in systolic and/or ≥10 mm Hg in diastolic blood pressure. The LS 30/15 ratio was the ratio between the longest RR interval measured between the 25th and 35th beat after active standing and the shortest RR interval between the 10th and 20th beat.

2.4.3. Valsalva manoeuvre (VM)

Patients in the sitting position were instructed to blow into a tube connected to a manometer to maintain the pressure at 40 mm Hg for 15 s, during continuous heart rate and blood pressure monitoring. VM ratio was the ratio of the shortest RR interval (tachycardia) during expiration to the longest RR interval (bradycardia) after expiration.

2.5. Statistical analysis

Data are presented as median (interquartile range) or frequency (percentage). Categorical variables were compared by the chi-square test. Pearson's R correlation coefficient or Spearman's rho index were used to correlate continuous clinical, biochemical and functional variables (age, symptom duration, nMEDL, MEDL, GSH, Neopt, MDA, 3-NT, 8-OHdG, TNFα, IL1β, TOS, LS 30:15 ratio, VM ratio, DB-ratio). Associations with the dependent variable “group” were tested by univariable ordinal logistic regression; variables with $p < 0.10$ were entered in a multivariable model. A p value <0.05 was considered significant. Statistical analyses were carried out with the Statistical Package for the Social Sciences (SPSS Inc., Chicago, Illinois, USA), version 17.0 for Windows.