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Circulating levels of adipokines in Parkinson's disease ☆

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ABSTRACT

Introduction: Adipokines are adipocyte-derived secretory factors, which have functions in satiety, energetic homeostasis, insulin sensitivity, vascular disease and also immune response. Parkinson's disease (PD) has been associated with unintended weight loss and reduced prevalence of cardiovascular risk factors. In addition, inflammation has been proposed as one of the factors contributing to PD pathophysiology. Therefore, we sought to investigate if adipokine levels – adiponectin, leptin and resistin – are altered in PD patients. Also, we aimed to evaluate association between adipokine levels and clinical variables in PD.
Methods: Forty PD patients and twenty-five age-, gender- and body mass index-matched controls were enrolled in this study. Peripheral blood was drawn and plasma levels of adiponectin, leptin and resistin were measured by Enzyme-Linked Immunosorbent Assay.
Results: There was no significant difference between PD patients and controls regarding plasma levels of the evaluated adipokines. In PD patients, higher leptin levels were associated with increased age and body mass index. No other correlation was found between adipokine levels and clinical or demographic data.
Conclusions: Although adipokines play important roles in inflammation, it seems that they are not implicated in the inflammatory response associated with PD.

1. Introduction

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder worldwide and the main cause of parkinsonism, a highly disabling syndrome [1]. Studies have concordantly shown that PD patients present lower body weights in comparison with age-matched subjects. The unintended weight loss can even precede the motor signs required for clinical diagnosis in PD. Several factors have been proposed to explain this weight change, including anorexia, dysphagia, reduced sense of smell and taste, altered gastrointestinal motility and absorption and increased energy requirements [2]. However, the mechanisms underlying the weight loss in PD are still unknown. Besides the unintended weight loss, PD has also been associated with reduced prevalence of cardiovascular risk factors, such as arterial hypertension, diabetes and dyslipidemia [3]. Here again the mechanisms involved have not been completely elucidated.

The cause of neurodegeneration observed in PD is not fully understood. Inflammation has been proposed as one of the factors contributing to the onset and progression of neuronal death in PD. *Postmortem*, epidemiological, genetic and immunological studies in humans and animal models have supported this hypothesis [4]. Activated microglial cells were found within the *substantia nigra pars compacta* (SNPc) of PD patients [5]. Increased levels of proinflammatory cytokines [6–8], human leukocyte antigen (HLA)-DR-positive microglia and infiltrating CD8 + cells [5] were reported in PD brains. Analyses of blood have also demonstrated that levels of inflammatory cytokines such as interleukin (IL)-6 [9–11], tumor necrosis factor (TNF)-α [11] and its soluble receptor sTNFR1 [12], IL-2 [11,13], interferon (IFN)-γ [11], IL-1β [14] and IL-10 [11,15] are increased in PD patients compared to age-related control individuals, indicating a peripheral proinflammatory status.

Among several inflammatory molecules, adipokines are of great interest since they are involved not only in inflammation but also in other physiological processes. Adipokines are adipocyte-derived secretory factors, which have functions in satiety, energetic homeostasis, insulin sensitivity, vascular disease and also immune response. Leptin, adiponectin and resistin are typical examples of adipokines [16,17]. The physiological function of leptin involves appetite control through

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the central nervous system [18]. It is generally accepted that leptin acts as a proinflammatory adipokine, since it induces the production of TNF-α and IL-6 by monocytes [19] and enhances the mRNA expression of CC-chemokines, including CCL3, CCL4 and CCL5 [20]. In contrast to leptin, adiponectin levels are decreased in obesity and it appears to have anti-inflammatory effects, resulting in TNF-α and IFN-γ decrease and IL-10 increase [21,22]. Adiponectin levels are also decreased in insulin resistance [23]. Unlike leptin and adiponectin, the role of resistin in human physiology and inflammation is more controversial. Resistin has at first been suggested as an adipokine upregulated during weight gain, impairing insulin sensitivity, which provided a direct connection between adiposity and insulin resistance [24,25]. This role, however, seems to be limited to rodents [26]. In addition, resistin synthesis is restricted to adipocytes in mice, whereas in humans resistin is mainly produced by leukocytes [16]. Nonetheless, the proinflammatory properties of resistin in human mononuclear cells are evident, since resistin promotes the expression of TNF-α and IL-6 by these cells [27].

Since weight loss, reduced prevalence of cardiovascular risk factors and peripheral proinflammatory status are described in PD, and the adipokines are strongly associated with all these processes, studies have hypothesized that adipokine levels are altered in PD patients. Until now however the few studies about this topic failed to show any alteration in adipokine levels in PD. Since the literature about adipokines in PD is still scarce, this study was designed to investigate adipokine levels – adiponectin, leptin and resistin – in PD patients and compare them to control individuals. Also, we aimed to evaluate association between adipokine levels and clinical variables in PD.

2. Methods

2.1. Subjects

This study included 40 PD patients diagnosed according to the United Kingdom PD Brain Bank criteria [28] and a group of 25 controls matched by age, gender, body mass index (BMI) and educational level. Patients were recruited from the outpatient movement disorders clinic of the 'Santa Casa de Belo Horizonte' Hospital, Belo Horizonte, Brazil. Controls were recruited from the local community. Participants reported no weight changes in the three months preceding the study and were excluded if they had undergone previous neurosurgery or if they had any other neurological disorder and/or cognitive decline (*i.e.*, delirium or dementia, according to the DSM-IV criteria), significant sensory impairment and infectious or autoimmune diseases in activity in the previous 4 weeks. In addition, individuals who had used corticosteroids, anti-inflammatories or antibiotics in the 4 weeks prior to the study were excluded. All subjects provided written informed consent before admission to the study. This study was approved by the Research Ethics Committee of the *Universidade Federal de Minas Gerais*, Brazil. An overview of the demographic and clinical characteristics of the sample enrolled in this study is given in Table 1. PD patients and controls did not differ in age, gender, BMI index, educational level and use of medications (except for those used for PD treatment).

2.2. Clinical evaluation

All patients were evaluated with the Unified Parkinson's Disease Rating Scale (UPDRS) [29] that assesses different signs and symptomatic dimensions of PD. The UPDRS scores were obtained in the "on" state. In order to estimate the PD disease staging, we applied the modified Hoehn and Yahr Staging Scale (HY) [30]. Also, the modified Schwab and England Activities of Daily Living Scale (S&E) assessed disability in performing activities of daily living in PD patients [29].

All individuals were subjected to cognitive examination, which included the Mini-Mental State Examination (MMSE) [31] adapted for the Brazilian elderly population [32]. The MMSE is a brief battery for cognitive screening, comprising 30 items from different domains such as orientation, attention, memory and language [31]. It is a traditional test used to assess overall cognitive performance and to screen for dementia.

2.3. Adipokine assessment

Ten milliliters of blood was drawn by venipuncture in vacuum tubes containing heparin (Vacuplast, Huangyan, China) on the same day of the clinical assessment. In order to rule out any confounding factors caused by circadian rhythm and fasting/feeding conditions, all samples were collected at the same time of the day, 4 h after the last meal. The whole blood samples were kept at room temperature and used within 2 h after being drawn. These samples were then centrifuged at 3000 *g* for 10 min, 4 °C, twice. The plasma was collected and stored at −70 °C until assayed.

Plasma levels of leptin, adiponectin and resistin were measured by Enzyme-Linked Immunosorbent Assay (ELISA) according to the procedures supplied by the manufacturer (DuoSet, R&D Systems, Minneapolis, MN, USA). All samples were assayed in duplicate and analyses were performed blinded. Lower detection limits for all analyzed adipokines were 5 pg/mL. Concentrations are expressed as pg/mL.

2.4. Statistical analysis

Association between dichotomous variables was assessed by Fisher's exact test. All variables were tested for Gaussian distribution by the Kolmogorov–Smirnov normality test. Two groups (patients *vs.* controls) were compared by Mann–Whitney or Student's *t* tests when non-normally or normally distributed, respectively. Leptin levels are gender-dependent [33] and are higher in women than in men even

Table 1
Demographic and non-motor features of Parkinson's disease (PD) patients and control subjects.

	PD patients (n = 40)	Control subjects (n = 25)	*P* value
Gender (female/male)	13/27	6/19	0.58[a]
Age in years (mean ± SD)	68.71 ± 10.07	65.23 ± 8.75	0.16[c]
Body mass index in kg/m^2 (mean ± SD)	26.02 ± 3.73	27.64 ± 3.71	0.09[c]
Educational level in years [mean ± SD (median)]	4.72 ± 2.87 (4)	6.72 ± 5.37 (6)	0.16[b]
MMSE [mean ± SD (median)]	24.00 ± 3.99 (25)	27.00 ± 3.57 (29)	0.001[b]
Medication in use (frequency in %)			
Anti-hypertensive (%)	55.00	48.00	0.62[a]
Anti-diabetic (%)	10.00	20.00	0.29[a]
Hypolipidemic (%)	10.00	24.00	0.17[a]
Levothyroxine (%)	10.00	4.00	0.64[a]
Antidepressants (%)	20.00	12.00	0.51[a]

Abbreviations: PD = Parkinson's disease; SD = standard deviation; MMSE = Mini-Mental State Examination.
[a] Fisher's exact test.
[b] Mann–Whitney test.
[c] Student's *t* test.