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ARTICLE

Circulating levels of neurotrophic factors are unchanged in patients with Parkinson's disease

Os níveis circulantes de fatores neurotróficos não estão alterados em pacientes com doença de Parkinson

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ABSTRACT

There is great evidence linking neurotrophic factor (NF) dysfunction with Parkinson's disease (PD) pathophysiology. This study was conducted to evaluate plasma levels of NFs and their possible associations with clinical symptoms in PD. For this purpose, 40 PD patients and 25 controls were subjected to a clinical evaluation and peripheral blood draw. Plasma levels of brain-derived neurotrophic factor (BDNF), pro-BDNF, neurotrophin 3, neurotrophin 4, nerve growth, glial cell line-derived neurotrophic factor and ciliary neurotrophic factor were measured by enzyme-linked immunosorbent assay. There was no significant difference between PD patients and controls regarding the plasma levels of the evaluated NFs. In addition, NF levels were not associated with disease duration, degree of motor or functional impairment, cognitive performance or severity of depressive symptoms. In conclusion, although NFs may play relevant roles in the pathophysiology of PD, the circulating levels of these molecules are not necessarily changed in patients with PD.

Keywords: Parkinson's disease; nerve growth factors; depression; biomarkers; cognition.

RESUMO

Há evidências de que alteracões nas ações exercidas por fatores neurotróficos (FNs) estejam associadas à fisiopatologia da doença de Parkinson (DP). O presente estudo foi conduzido para avaliar os níveis plasmáticos de FNs e suas possíveis associações com sintomas clínicos na DP. Para este fim, 40 pacientes com DP e 25 controles foram submetidos à avaliação clínica e coleta de sangue periférico. Os níveis plasmáticos do fator neurotrófico derivado do cérebro (BDNF), pro-BDNF, neurotrofina 3, neurotrofina 4, fator de crescimento do nervo, fator neurotrófico derivado da glia e fator neurotrófico ciliar foram avaliados por ensaio de imunoadsorção enzimática. Não houve diferença significativa entre pacientes com DP e controles quanto aos níveis plasmáticos dos FNs avaliados. Além disso, não encontramos associação entre os níveis dos FNs e duração da doença, grau de comprometimento motor ou funcional, desempenho cognitivo e gravidade dos sintomas depressivos. Em conclusão, embora os FNs possam desempenhar papéis relevantes na fisiopatologia da DP, os níveis circulantes dessas moléculas não estão necessariamente alterados em pacientes com DP.

Palavras-chave: doença de Parkinson; fatores de crescimento neural; depressão; biomarcadores; cognição.

Parkinson's disease (PD) is the second most frequent neurodegenerative disease and the leading cause of parkinsonism. Parkinsonism is defined by the presence of bradykinesia and at least one of the following symptoms: rigidity, resting tremor and postural instability. The pathophysiology PD is defined as the result of the loss of dopaminergic neurons in the substantia nigra *pars compacta* and the accumulation of alpha-synuclein aggregated in the remaining neurons[1]. The

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diagnosis of the disease is clinical, based on the presence of the cardinal motor symptoms and the exclusion of other causes of parkinsonism, including vascular and drug-induced parkinsonism. In recent years, several nonmotor symptoms have been recognized as major components of the disease[1,2].

Neurotrophic factors (NF[s]) are soluble polypeptides that are involved in the development, growing, functioning and regulation of neurons and neuron-supporting cells. They usually act through membrane-bound receptors with intrinsic tyrosine kinase activity, determining the activation of transcription factors and the expression of specific genes. These genes encode proteins involved in regulating neuronal survival, differentiation and plasticity[3,4]. Parkinson's disease is an age-related disease[5] and abnormal NF support during aging seems to play a major role in the pathophysiology of neurodegenerative diseases, such as Alzheimer's disease and PD.

Due to their intrinsic properties of promoting neuronal and glial cell regeneration, NFs became a subject of research in the treatment of neurodegenerative diseases. Interestingly, some drugs used clinically to treat Alzheimer's disease (memantine) and PD (levodopa, rasagiline, pramipexole, ropinirole) share the property of modulating NF levels in the brain regions involved in the pathophysiology of the respective disease[3]. In PD, although the strategies were successful in inducing protection of dopaminergic neurons *in vitro* and motor recovery in preclinical models of the disease, very limited success has been obtained in clinical studies[6].

Evidence linking NF dysfunction with PD came from postmortem studies that reported reduced levels or expression of brain-derived neurotrophic factor (BDNF)[7,8], nerve growth factor (NGF)[7], glial cell line-derived neurotrophic factor (GDNF)[8] and ciliary neurotrophic factor (CNTF)[8] in the substantia nigra of people who suffered from PD. Moreover, circulating levels of NGF[9] and BDNF[10-14] were also found to be altered in the circulation of patients with PD.

Given the relevance of NFs in PD, the aim of this work was to evaluate plasma levels of NFs and their possible associations with clinical symptoms in PD.

METHODS

Participants and clinical evaluation

This study was conducted in the same cohort of patients as the study by Rocha et al.[15], and therefore included 40 patients diagnosed with PD and a group of 25 control participants of comparable age, sex, educational level and body mass index (BMI). We followed the methods of Rocha et al.[15]. The diagnosis of PD was based on the UK Brain Bank criteria[1]. Patients were recruited from the Movement Disorders outpatient clinic, Santa Casa de Belo Horizonte Hospital, Belo Horizonte, Brazil. Control participants were recruited from the local community. Participants 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), significant sensory impairment and active infectious or autoimmune diseases in the previous four weeks. In addition, individuals who had used corticosteroids, anti-inflammatories or antibiotics in the four weeks prior to the study were excluded. All participants provided written informed consent before admission to the study. The Research Ethics Committee of the *Universidade Federal de Minas Gerais*, Brazil approved this study.

All patients were evaluated with the Unified Parkinson's Disease Rating Scale (UPDRS)[16], which assesses different signs and symptoms of PD. The UPDRS scores were obtained in the "on" state of the disease. The modified Hoehn and Yahr staging scale was used to establish the stage of PD[17]. The modified Schwab and England activities of daily living scale was used to assess the daily routines of PD patients[16]. All individuals were subjected to a cognitive examination, which included the Mini-Mental Status Examination[18] adapted for the elderly Brazilian population[19]. The Mini-Mental Status Examination is a brief test for cognitive screening, comprising items from different domains such as orientation, attention, memory and language. Since impairment in executive functioning is the most common cognitive deficit in PD patients, the Frontal Assessment Battery was also used[20,21]. This is a brief assessment tool that evaluates executive functioning and consists of six sub-tests exploring cognitive processes related to the frontal lobes: conceptualization, mental flexibility, motor programming, sensitivity to interference, inhibitory control and environmental autonomy. In addition, all participants were evaluated using the Beck's Depression Inventory, a self-rating instrument for depressive symptoms comprising 21 items, each ranging from 0 to 3, according to the severity of symptoms[22]. The Beck's Depression Inventory has been validated as a tool for depression screening and diagnosis in PD[23,24].

Assessment of neurotrophic factors

Ten milliliters of blood were drawn by venipuncture in vacuum tubes containing heparin (Vacuplast, Huangyn, China) on the same day as the clinical assessment. In order to rule out any confounding factors caused by circadian rhythm, all samples were collected at the same time of the day, between 14:00-16:00. The whole blood samples were kept at room temperature and used within two hours of having been drawn. These samples were then centrifuged at 1,700 g for 10 min, 4°C, twice. The plasma was collected and stored at -70°C until assayed.

Plasma levels of BDNF, pro-BDNF, GDNF, NGF, CNTF, neurotrophin (NT)3 and NT4 were measured by enzyme-linked immunosorbent assay according to the procedures supplied by the manufacturer (DuoSet, R&D Systems, Minneapolis, MN, USA). The assays were performed in duplicate, blinded

to the clinical status of the participants. Concentrations are expressed as pg/mL. Lower detection limits for all analyzed molecules were 10 pg/mL.

Statistical analysis

Association between dichotomous variables was assessed with Fisher's exact test. All variables were tested for Gaussian distribution by the Shapiro-Wilk normality test. The two groups (patients vs. controls) were compared using the Mann-Whitney U or Student's t tests when non-normally or normally distributed, respectively. Spearman's correlation analyses were performed to examine the relationship between clinical variables and plasma levels of the NFs. All statistical tests were two-tailed and were performed using a significance level of $\alpha = 0.05$. Statistical analyses were performed using SPSS software version 22.0 (SPSS Inc., Chicago, IL, USA) as well as GraphPad Prism 5.0 for Windows™ (GraphPad Software, Inc., La Jolla, CA, USA)

RESULTS

Sociodemographic and clinical results

This study included 40 patients with PD and 25 controls whose clinical and demographic characteristics are shown in Table 1. Patients with PD and the controls did not differ with respect to age, sex, educational level and BMI. The control individuals showed better cognitive performance than patients with PD, as demonstrated by the Mini-Mental Status Examination scores. In addition, PD patients were worse than controls in the programming task of the Frontal Assessment Battery. Patients with PD also had higher scores on the Beck's Depression Inventory compared with controls. This result indicates that patients with PD experience more depressive symptoms than individuals who are not diagnosed with PD.

The clinical features of PD are presented in Table 2. Patients with PD exhibited mild to moderate motor impairment as evidenced by the UPDRS, with a median Hoehn and Yahr staging of 2% and Schwab and England activities of daily living median of 80%. These parameters are compatible with non-advanced PD. The great majority of PD patients included in this study (92.5%) were taking levodopa.

Plasma levels of NFs

There was no significant difference between PD patients and controls regarding the plasma levels of the evaluated NFs (Figure). The NF levels obtained for both the patients with PD and the controls are provided in Table 3.

The NF levels were not associated with disease duration or with the degree of motor or functional impairment, as assessed by the UPDRS. Among controls, higher levels of BDNF were associated with lower severity of depressive symptoms, as assessed by the Beck's Depression Inventory (rho = -0.547, p = 0.005). The same association was not found in patients with PD.

Table 1. Clinical (non-motor) and demographic features of participants included in the assessment of neurotrophic factors.

Variable	Patients with PD (n = 40)	Controls (n = 25)	p-value
Sex (female/male)	13/27	6/19	0.58[a]
Age in years (mean ± SD)	68.71 ± 10.07	65.23 ± 8.75	0.20[b]
Body mass index. Kg/m^2 (mean ± SD)	26.02 ± 3.73	27.64 ± 3.71	0.09[c]
Educational level in years (mean ± SD)	4.72 ± 2.87	6.72 ± 5.37	0.16[b]
MMSE [mean ± SD (median)]	24.00 ± 3.99 (25)	27.00 ± 3.57 (29)	0.001[b]
FAB [mean ± SD (median)]	11.49 ± 2.99 (12)	12.32 ± 3.67 (13)	0.28[b]
Conceptualization	1.23 ± 1.01 (1)	1.64 ± 1.11 (2)	0.12[b]
Mental flexibility	1.82 ± 1.10 (2)	2.08 ± 1.04 (2)	0.34[b]
Programming	1.74 ± 0.91 (2)	2.24 ± 0.83 (2)	0.04[b]
Sensitivity to interference	2.26 ± 0.94 (3)	1.84 ± 1.25 (2)	0.21[b]
Inhibitory control	1.41 ± 0.88 (1)	1.52 ± 1.09 (1)	0.73[b]
Environmental autonomy	3.00 ± 0.00 (3)	3.00 ± 0.00 (3)	1.00[b]
BDI [mean ± SD (median)]	8.64 ± 7.58 (6)	2.76 ± 3.35 (1)	< 0.001[b]
Drugs in use (frequency in %)			
Antihypertensive	55	48	0.62[a]
Antidiabetic	10	20	0.29[a]
Hypolipidemic	10	24	0.17[a]
Levothyroxine	10	4	0.64[a]
Antidepressants	20	12	0.51[a]

PD: Parkinson's disease; SD: standard deviation; FAB: frontal assessment battery; MMSE: mini-mental state evaluation; BDI: Beck's depression inventory; [a]: Fisher's exact test; [b]: Mann-Whitney test; [c]: Student's *t* test.

Table 2. Clinical features (motor) of patients with Parkinson's disease included in the dosage of neurotrophic factors.

Variables	Patients with PD (n = 40)
Length of illness in years [mean ± SD (range)]	5.45 ± 4.13 (0.4–18)
UPDRS [mean ± SD (range)]	51.82 ± 25.27 (11–105)
UPDRS I [mean ± SD (range)]	3.36 ± 2.96 (0–11)
UPDRS II [mean ± SD (range)]	14.08 ± 7.14 (2–31)
UPDRS III [mean ± SD (range)]	34.56 ± 18.43 (8–69)
H&Y [mean ± SD (range)]	2.44 ± 0.69 (1–4)
S&E in % [average ± SD (range)]	77.95 ± 11.96 (50–100)
Drugs in use [N (frequency in %)]	
Levodopa	37 (92.50)
Pramipexole	20 (50.00)
Entacapone	7 (17.50)
Amantadine	11 (27.50)

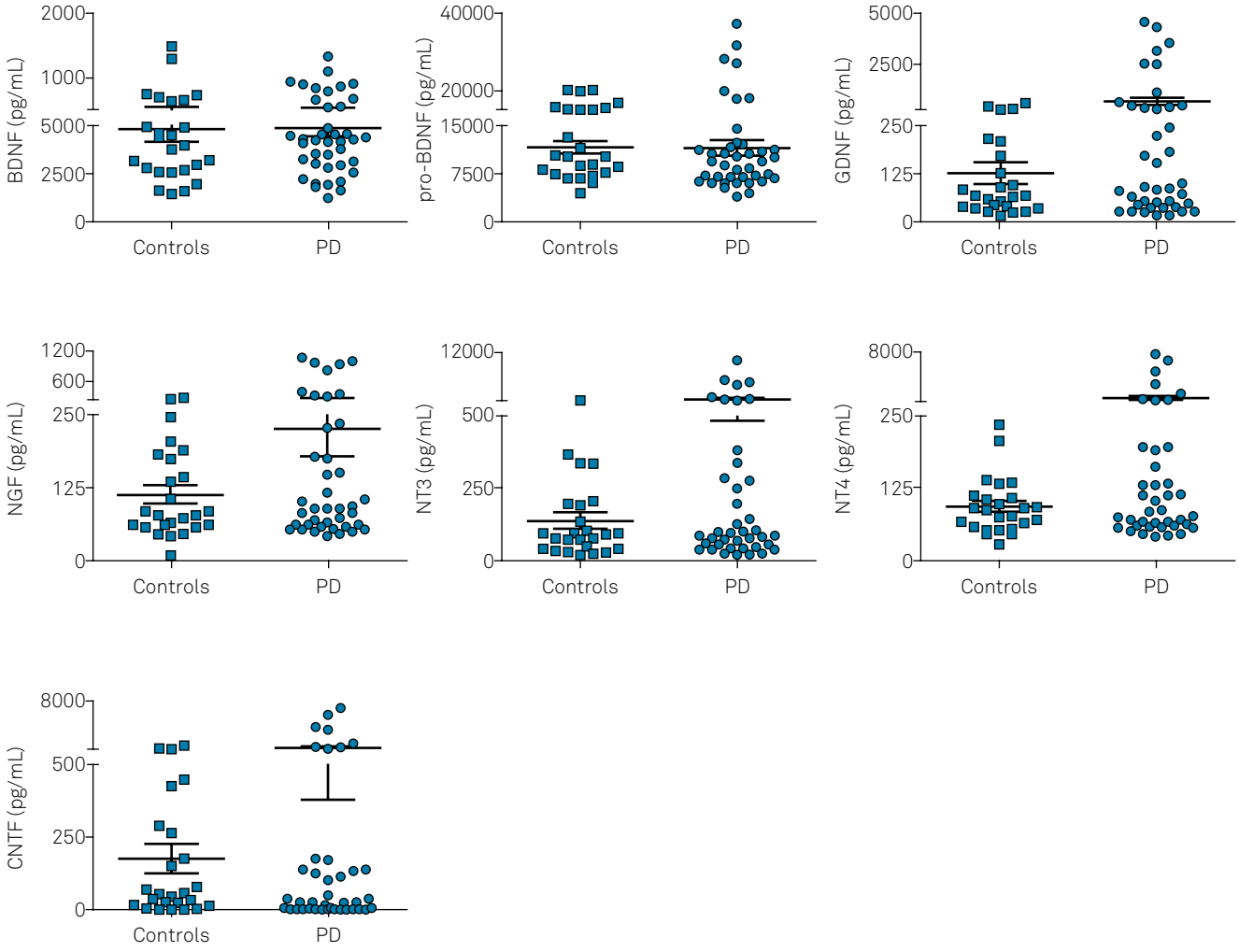
PD: Parkinson's disease; SD: standard deviation; UPDRS: unified Parkinson's disease rating scale; H&Y: Hoehn and Yahr staging scale; S&E: Schwab and England activities of daily living scale.

DISCUSSION

Despite several studies using different approaches having pointed out a key role of NFs in PD, we found that circulating levels of NFs (BDNF, pro-BDNF, NGF, CTNF, GDNF, NT3 and NT4) were not changed in PD patients when compared with BMI-, sex- and age-matched controls.

It is worth noting a significant dispersion in the levels of NFs, mainly in the PD group. This dispersion might explain the divergence from previous studies. For example, a series of studies found lower circulating levels of NFs in PD compared with controls[10-14]. Increased levels of NFs have also been described in serum[25] and cerebrospinal fluid (CSF)[26] of PD patients.

The dispersion in the levels of NFs might be explained by individual characteristics such as disease stage, medical comorbidities, physical activity, medications in use, disease phenotype, among others. Physical activity has been extensively linked to changes in NF levels. Not only do BDNF levels increase, but motor symptoms may also decrease in response



BDNF: brain-derived neurotrophic factor; CNTF: ciliary neurotrophic factor; GDNF: Glial cell line-derived neurotrophic factor; NGF: nerve growth factor; NT: neurotrophin; PD: Parkinson's disease.

Figure. Plasma concentrations of neurotrophic factors. Patients with Parkinson's disease and controls showed no statistically significant difference in the plasma levels of neurotrophic factors evaluated.

Table 3. Plasma concentrations of neurotrophic factors evaluated in patients with Parkinson's disease and controls.

Neurotrophic factor	Patients with PD (n = 40)	Controls (n = 25)	p-value
BDNF	4878.22 ± 2786.02 (4256.89)	4810.83 ± 3269.54 (4025.40)	0.69[a]
Pro-BDNF	11526.15 ± 7599.66 (9423.39)	11638.26 ± 4738.97 (10237.25)	0.30[a]
NGF	225.94 ± 297.01 (88.40)	112.78 ± 76.87 (76.56)	0.38[a]
GDNF	661.2 ± 1242 (88.78)	127.4 ± 139.3 (68.67)	0.22[a]
CNTF	630.33 ± 1585.85 (25.94)	176.24 ± 254.66 (53.58)	0.40[a]
NT3	799.00 ± 1988.73 (92.55)	140.34 ± 145.35 (92.55)	0.40[a]
NT4	697.91 ± 1768.08 (86.67)	94.09 ± 48.49 (87.68)	0.32[a]

Results are given in pg/mL [mean ± standard deviation (median)]. PD: Parkinson's disease; BDNF: brain-derived neurotrophic factor; NGF: nerve growth factor; GDNF: glial cell line-derived neurotrophic factor; CNTF: ciliary neurotrophic factor; NT: neurotrophin; [a]: Mann-Whitney Test.

to physical activity[25,27,28]. Indeed, physical activity has been proposed as a therapeutic intervention to ameliorate PD symptoms and delay PD progression. The existing data suggest an association between the increase in serum levels of BDNF and the beneficial effects of physical activity in PD[6].

Regarding the disease phenotype (i.e., predominant clinical presentation), lower BDNF levels have been associated with a greater severity of depressive symptoms[13] and cognitive impairment[14]. Corroborating these results, lower CSF levels of BNDF have also been associated with depression[29], and higher CSF BDNF levels with better cognitive performance in PD[30]. Conversely, our independent cohort of PD patients showed that BDNF levels correlated positively with the duration of the disease and the severity of motor symptoms[10]. We hypothesized that lower BDNF levels in early stages of the disease may be associated with pathogenic mechanisms of PD. The increase of BDNF levels with the progression of the disease may be a compensatory mechanism in more advanced stages of PD[10].

We are aware of the limitations of our study, including the sample size and the cross-sectional design of the study. The lack of information about physical activity is an important limitation for the interpretation of our results. In addition, all patients were medicated and the observed findings might also be influenced by their ongoing treatment. In contrast, the strict exclusion criteria, the selection of controls with comparable age, sex and BMI, and the comprehensive clinical evaluation can be regarded as strengths of the study.

In conclusion, although NFs may play relevant roles in the pathophysiology of PD, we did not find changes in the circulating levels of these molecules. Several factors can influence the circulating levels of NFs, and these need to be controlled to obtain meaningful pathophysiological information in PD.

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