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S100B and NSE serum levels in patients with Parkinson's disease

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Abstract

We evaluated S100B protein and neuron-specific enolase (NSE) serum levels in Parkinson's disease (PD) patients and their correlation with the severity of disease. The levels of S100B ($P=0.16$) and NSE ($P=0.39$) between PD and controls were similar. However, S100B levels correlated positively with the Hoehn and Yahr scale ($r=0.368$; $P=0.02$) and negatively with the Activities of Daily Living (ADL) scale ($r=-0.431$; $P=0.006$). Therefore, S100B and NSE may not have a diagnostic role in PD, but S100B may have a potential role as a marker of disease progression. The study of S100B may also contribute to elucidate the controversial role of glial cells in PD.

Keywords: Parkinson's disease; S100B protein; Neuron-specific enolase; Neurodegenerative disorders; Biochemical markers

1. Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). This decrease in neuronal number, and the associated massive depletion of striatal dopamine levels, produces the characteristic tremor, rigidity, and hypokinesia of the disorder [1–3]. Glial cells may participate in the pathophysiology of the disease. Indeed, glial cells can produce trophic factors, which may stimulate neuronal survival or, alternatively, they could produce toxic compounds, which may be involved in neuronal degeneration [4,5].

Clinical symptoms appear after at least 60% of neuronal loss has occurred in the SNpc [6]. The loss of dopaminergic neurons observed in post-mortem parkinsonian brains is associated with a significant glial reaction [7–9] and it is known that some dopaminergic neurons are more vulnerable than others to the pathologic process [4]. Moreover, glial cells surrounding dopaminergic neurons may be involved in this selective vulnerability, since there is an inverse relationship between the degree of neuronal loss in dopaminergic cell groups of the SNpc and the density of astroglial cells, suggesting that dopaminergic neurons are more prone to degenerate in areas with fewer astrocytes [10]. Some glial cells could play a neuroprotective role by metabolizing dopamine and scavenging oxygen free radicals present during dopamine metabolism, while others may be deleterious to dopaminergic neurons. This effect may be mediated by the production of nitric oxide (NO) and cytokines, which may in turn account for the oxidative stress in the SNpc of patients with PD [7]. There is no definitive ante morten diagnostic test for PD. Also, when the clinical diagnostic is done, there is no easy way to know the degree of neuronal cells loss. Therefore, a peripheral marker of PD would be of great interest to complement the clinic evaluation.

S100B is a calcium binding protein mostly produced and released by astrocytes in the central nervous system (CNS) [11]. It has intracellular functions, like modulation of cytoskeleton proteins and regulation of cellular cycles, and extracellular functions, which are dependent on concentration. At nanomolar concentrations, S100B has neurotrophic and gliotrophic actions, possibly having important roles in normal CNS development and recovery after injury.

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At micromolar concentrations, it may be toxic, producing neuronal and glial cell death by apoptosis [12]. Considering its prevalence in the CNS, several studies have been performed aimed at investigating its potential role as a peripheral biochemical marker of neuronal injury, possibly involving reactive gliosis, astrocytic death and/or blood-brain-barrier dysfunction [13]. Elevated cerebrospinal fluid (CSF) or serum S100B levels were found in several acute and chronic brain pathologies, including traumatic brain injury [14,15], stroke [16], Alzheimer's disease [17], schizophrenia [18], HTLV-I associated myelopathy [19] and systemic lupus erythematosus [20]. Of note, immunohistochemical studies in mice have shown that the expression of S100 protein is increased in glial cells after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment, a neurotoxin known to cause parkinsonism in humans and primates [21].

The neuron-specific enolase (NSE) is a cytoplasmatic glycolytic enzyme, whose isoform $\gamma\gamma$ is found in neurons, as well as in cells with neuroendocrine differentiation. Since NSE is not physiologically secreted, an increase of its serum and CSF levels is considered a marker of damage to neuronal cells, as reported in traumatic brain injury [15], stroke [22] and seizures [23]. Taken together, NSE and S100B protein could be considered, respectively, neuronal and glial peripheral markers of CNS pathologies. In the present work, we studied S100B and NSE serum levels in patients with PD to evaluate their potential use as peripheral markers of this disease. We also assessed the correlation between their serum levels and some clinical features.

2. Methods

2.1. Patients and controls

Serum was collected from 40 patients with PD from the Movement Disorders Clinic of the Hospital de Clínicas de Porto Alegre. Patients were examined by a neurologist and were diagnosed as having probable PD using as criteria the presence of three of four cardinal features (rest tremor, muscle rigidity, bradykinesia and asymmetric onset) and no alternative explanations for the findings [24]. All cases of secondary parkinsonism were excluded from the study. The clinical stage of PD was evaluated according to the classification of Hoehn and Yahr (H&Y) [25], in which higher values represent bilateral disease and higher impairment of balance; and the Schwab and England Activities of Daily Living (ADL) scale [26], in which smaller values correspond to higher degree of dependence. All 40 patients underwent the Mini-Mental Status examination of Folstein [27] and patients with a score <24 points were excluded. The exclusion criteria were any other neurological or psychiatric disease (except if associated with PD), cancer of any tissue, Chagas disease, heart disease, known tobacco or ethanol abuse and renal failure. It was decided not to withdraw the dopaminergic agonists before the study for ethical reasons. Other causes of parkinsonism were ruled out on the basis of clinical and laboratory exams. All subjects had normal structural computed tomography or magnetic resonance imaging of the brain. The patients were compared with 40 healthy individuals, selected from relatives of patients attending the outpatient clinic. The inclusion criteria were age and sex similar to patients. The exclusion criteria were the same as for patients. Drugs and comorbidities were controlled in both groups. All subjects gave informed consent. The study was approved by the medical ethics committee of the Hospital de Clínicas de Porto Alegre.

Blood samples (3 ml) from patients and controls were collected without anticoagulant and sera were frozen at -70 °C until analysed.

2.2. S100B protein

A quantitative monoclonal two-site immunoluminometric assay LIA-mat Sangtec 100 (BYK-Sangtec, Germany), was used for measuring S100B in all patients and controls. It is composed of three monoclonal antibodies specific to subunit β of S100 and a tracer antibody, which is bound to isoluminol. Oxidation of isoluminol is started by injection of an alkaline peroxide solution and catalyst solution. The immunological reaction is detected by light reaction [28].

2.3. NSE

The NSE serum levels were evaluated in 25 patients and 25 controls (randomly selected) by electrochemiluminescence assay ECLIA (Roche Diagnostics, USA). This is a quantitative method that uses a monoclonal antibody specific for NSE and labelled with a ruthenium complex which produces light emission.

2.4. Statistical analysis

Comparison between groups was made by using a parametric t test for NSE, and a non-parametric Mann Witney test for S100B. Correlations are presented with Pearson's coefficient, for NSE with age, time of disease and age of onset of PD, and with Spearman's coefficient for all S100B correlations and for the correlations of NSE with clinical scales. $P<0.05$ was considered statistically significant.

3. Results

The demographic, biochemical and clinical characteristics of patients and controls are depicted in Table 1. In controls, S100B levels did not vary with age

Table 1
Demographic, biochemical and clinical data of subjects evaluated

	Controls ($n=40$)	Patients ($n=40$)	P
Age (years)	62.9±10.5	63.8±10.7	0.69
Gender (male/female) (%)	67.5/32.5	67.5/32.5	1.00
Time of disease (years)*	NP	8.7±5.6	–
Age of onset (years)*	NP	54.9±11.0	–
Hoehn and Yahr Scale*	NP	2.6±1.2	–
ADL scale (%)*	NP	66.4±28.9	–
S100B (μg/l)**	0.122 (0.043/0.188)	0.074 (0.025/0.126)	0.16
NSE (μg/l)* ($n=25$)	6.6±1.8	6.4±1.5	0.39

NP=not performed; *mean±SD; **median (percentile 25/75).

($r=-0.101$; $P=0.50$), while they were significantly higher in females ($P=0.009$) (data not shown). There was no statistically significant difference between S100B serum levels of PD and controls (Table 1). In PD, S100B levels did not correlate with age ($r=0.334$; $P=0.14$), time of disease ($r=0.125$; $P=0.40$) or age of onset of disease ($r=0.106$; $P=0.52$), and there was no difference between genders ($P=0.09$) (data not shown). S100B levels presented a positive correlation with the H&Y scale (which increases with the severity of PD) ($r=0.368$; $P=0.02$) (Fig. 1A), and a negative correlation with the ADL scale (which decreases with the severity of PD) ($r=-0.431$; $P=0.006$) (Fig. 1B).

In controls, NSE levels correlated positively with age ($r=0.458$; $P=0.03$) and were significantly higher in males ($P=0.03$) (data not shown). There was no statistically significant difference between NSE levels of PD and controls (Table 1). In PD, NSE levels did not correlate with age ($r=0.033$; $P=0.88$), time of disease ($r=0.097$; $P=0.64$), age of onset of disease ($r=0.017$; $P=0.93$), H&Y scale ($r=0.250$; $P=0.23$) or ADL scale ($r=-0.374$; $P=0.07$), and there was no difference between genders ($P=0.07$) (data not shown).

In PD, S100B and NSE correlated positively ($r=0.423$; $P=0.03$), while in controls there was no correlation ($r=-0.370$; $P=0.09$) (data not shown).

4. Discussion

Although increased serum levels of S100B and NSE have been found in many disorders that involve neuronal death [14–20], in our study there was no significant difference between serum levels of S100B and NSE when comparing overall groups of patients and controls. This result could be due to the low volume of neuronal death present in PD, which is limited essentially to small areas of the brain such as the SNpc.

Since the temporal pattern of neuronal death in the PD patients is unknown, it is difficult to evaluate the quantitative loss of neurons during the progression of the disease. Here, we did not find any correlation of NSE levels with time of disease. As NSE is considered a marker of neuronal death, it seems that if the neuronal loss in PD vary with time, the serum concentration of this marker cannot detect this correlation.

In the present study, serum S100B levels correlated significantly with the H&Y and the ADL scales, indicating that the severity of the disease was associated with the levels of S100B. However, this correlation occurred mainly because patients with higher degree of severity presented S100B levels similar to controls, while patients with clinically less severe degree presented lower levels than controls (data not shown). This may point that PD patients have lower levels of S100B protein in the beginning of the disease, a fact which remains to be explained. As S100B has neurotrophic and gliotrophic actions, possibly having important roles in normal CNS development and recovery after injury, individuals with reduced levels could be more susceptible to some conditions such as PD. The increase of

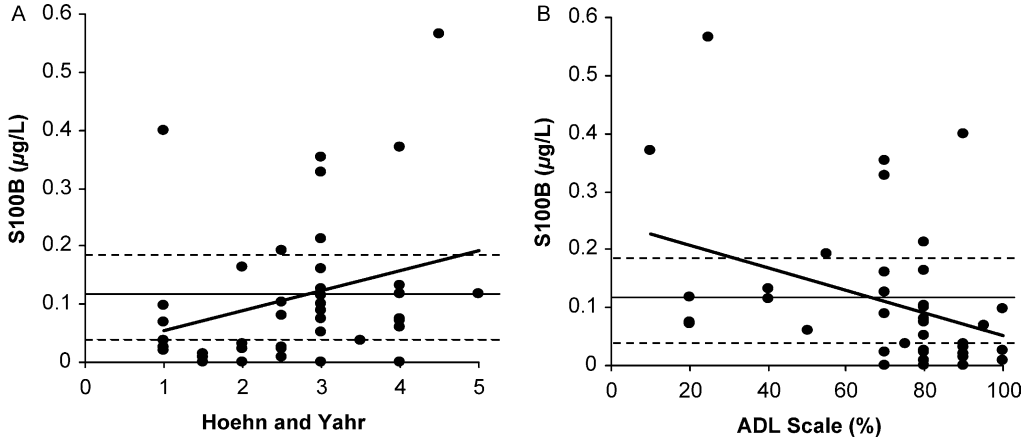

Fig. 1. Correlation between serum levels of S100B protein and scales of severity of Parkinson's disease. (A) Hoehn and Yahr scale ($n=40$; $r=0.368$; $P=0.02$); (B) ADL scale ($n=40$; $r=-0.431$; $P=0.006$). The three horizontal lines represent median (central line), percentile 25 (lower line) and percentile 75 (upper line) of S100B serum levels in controls.

this protein levels with the increase of the PD severity could be a consequence of neural damage of other brain regions, which occurs with the progression of PD. This could lead to a larger reactive astrogliosis with consequent increase in serum S100B levels. Moreover, patients in the worst clinical condition are receiving higher doses of dopamine agonists and/or levodopa, which could also contribute to the increase of S100B levels.

The role of glial cells in PD is not well determined. It is supposed that they have neuroprotective roles in PD by producing neurotrophic factors and metabolising oxygen free radicals [29]. On the other hand, glial cells have been implicated in the pathophysiology of PD experimental models by increasing iNOS expression after an initial injury [30]. In neural cell cultures, S100B protein induces neuronal death through induction of astrocytic iNOS and generation of NO [31].

In conclusion, it seems that NSE and S100B do not have a diagnostic role as peripheral markers in PD, but serum S100B may have a potential role as a marker of the degree of severity of this disease. Further studies are needed to appraise the contribution of S100B in clinical evaluation of PD and the role of astrocytes in PD.

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