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Clinica Chimica Acta 351 (2005) 143–148

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S100B and NSE serum concentrations in Machado Joseph disease

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Received 25 May 2004; received in revised form 23 August 2004; accepted 23 August 2004

Abstract

Background: NSE and S100B are considered as neuronal and glial peripheral markers of central nervous system pathologies, respectively. We evaluated the potential use of S100B and NSE serum concentrations as peripheral markers of symptomatic patients with Machado Joseph disease (MJD).
Methods: We measured S100B and NSE peripheral concentrations of 22 MJD patients and compared with healthy subjects concentrations. The correlations of both markers with CAG repeat size, age of onset, disease duration, and the scores of the Extended Disability Status Scale of Kurtzke, Unified Parkinson's Disease Rating Scale, and the Montgomery-Asberg depression rating scale were also assessed.
Results: S100B serum concentrations between control and MJD subjects were not statistically different, whereas NSE serum concentrations were higher in MJD patients than in control subjects (p=0.00001). S100B presented a moderate correlation with disease duration and depression score, whereas NSE presented a moderate correlation with depression score and a good negative correlation with EDSS score.
Conclusions: Symptomatic MJD patients present increased concentrations of NSE and normal concentrations of S100B in blood.

Keywords: S100B; NSE; Machado-Joseph disease; Brain markers

1. Introduction

Machado-Joseph disease (MJD) is an autosomal dominant neurodegenerative disorder caused by an expansion of CAG trinucleotide repeats in the MJD gene (chromosomal locus 14q24.3-q31) [1–5]. Normal alleles vary from 12 to 43 repeats, whereas expanded

Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; EDSS, extended disability status scale of kurtzke; MJD, machado joseph disease; NSE, neuron specific enolase; SD, standard deviation; UPDRS, unified Parkinson's disease rating scale.

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doi:10.1016/j.cccn.2004.08.010

alleles vary between 56 and 86 repeats [6]. The CAG repeat size exhibits an inverse correlation with age at onset and is also partially correlated with several clinical manifestations, although the causative chain of events at cellular level is not yet understood [7].

Disease manifestations usually start during adulthood, with a mean±SD age at onset of 32±12 years, among Brazilian patients and descents of Flores Island of Azorean archipelago [8], or 37±14, among Portuguese and descents of Sao Miguel, in the same islands [9]. The wide range of clinical manifestations include: gait and limb ataxia; dysarthria and dysphagia; pyramidal syndrome, supranuclear, progressive external ophthalmoplegia; extrapyramidal signs, including dystonia, rigidity and bradykinesia; a lower motor neuron disease, with fasciculations and amyotrophy; loss of tactile, algesic and vibration senses; eyelid retration, contraction fasciculations, loss of weight and a sleep disorder [9,10]. Patients will become confined to a wheelchair and will later be beddriden. The median survival time after onset is 17 years [8]. No therapy was established until now.

Neuropathologic lesions are widespread in MJD. There is extensive neuronal cell loss and gliosis in Clarke's columns, dentate nucleus, pontine nuclei, and vestibular nuclei [11,12]. There is moderate to severe involvement of substantia nigra, anterior horn cells, and motor cranial nerve nuclei [11,12]. Variable degrees of involvement of the striatum, subthalamic nucleus, and globus pallidus have also been reported [7,12,13].

S100B is a calcium binding protein mostly produced and released by astrocytes in the central nervous system (CNS), where it exerts neurotrophic and gliotrophic actions [14]. Considering its predominance in CNS, several studies have been performed in order to investigate its potential role as a peripheral biochemical marker of neural injury, possibly involving reactive gliosis, astrocytic death and/or blood–brain-barrier dysfunction. Accordingly, increased cerebrospinal fluid (CSF) and/or serum S100B concentrations were found in several acute and chronic pathologies, including those involved in traumatic brain injury [15], stroke [16], Alzheimer's disease [17], schizophrenia [18], HTLV-I associated myelopathy [19] and systemic lupus erythematosus [20]. The neuron-specific enolase (NSE) is a cytoplasmatic glycolytic enzyme, whose γγ isoform is found in neurons and cells with neuroendocrine differentiation, as well as in tumors originated from them. Since NSE is not physiologically secreted, an increase of its serum and CSF concentrations can be associated with structural damage to neuronal cells, as reported in traumatic brain injury [21], stroke [22] and seizures [23]. Taking 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 symptomatic patients with MJD to evaluate their potential use as peripheral markers of this disease. We also assessed the correlation between their serum levels and CAG repeat size, disease duration, and functional and mental status.

2. Patients and methods

2.1. Subjects

Twenty-two molecularly confirmed MJD patients (11 men and 11 women) were studied. The MJD polymorphic expanded regions were analyzed as previously described [24]. All patients were submitted to complete physical and neurological examinations before the study. Blood from healthy blood donors from both sexes in the same age range was collected as control. Serum was obtained by blood centrifugation at 3000×*g* for 5 min, frozen immediately and stored at −70 °C until analyses. All control and patient subjects gave informed consent for the study. None of the subjects had melanoma, cardiac disease, inflammatory disease, or neurologic disease other than MJD.

2.2. Functional and mental analyses

Patients were functionally assessed using the Extended Disability Status Scale of Kurtzke (EDSS) [25] and the Unified Parkinson's Disease Rating Scale (UPDRS) [26]. The Montgomery-Asberg depression rating scale was used to evaluate depressive symptoms [27].

2.3. S100B assay

Serum S100B protein was measured in duplicates by a monoclonal immunoluminometric assay (LIA-

Table 1
Clinical and molecular data of MJD patients

Variable	Median	Interquartile (25–75)
Age (years)	50.5	35.5–54.5
Age of onset (years)	31.5	21.5–37.7
Disease duration (years)	7.5	5.2–11.0
CAG expansion	76	73.2–77.0
Depression	9	6.0–13.5
EDSS	4	3.5–6.0
UPDRS	43.5	39.5–55.7

mat® BYK-Sangtec®100, Dietzembach, Germany) in a Lumat LB9507 luminometer (EG&G Berthold). The procedure was performed as previously described [28]. S100B standard curve was linear up to 20 μg/l and the CV was within 5%.

2.4. NSE assay

Serum NSE was measured using an eletrochemiluminescent assay provided by Roche Diagnostics®, Indianapolis, IN. Is a double sandwich assay that use an antibody anti-NSE bound with ruthenium (luminescent label). The reaction and quantification were performed by Elecsys-2010 (Roche). Since NSE is also present in blood cells, no hemolyzed sample was used. The assay was carried out in duplicate and the CV was within 5%.

2.5. Statistical analysis

Since S100B and NSE serum concentrations were approximately normal distributed, and the Levene test showed homogeneity of variance between case and control groups, comparison of serum S100B or NSE concentrations between MJD and control subjects was performed by Student's *t*-test. Correlations between S100B or NSE serum concentrations with EDSS, UPDRS and depression scores were assessed by Spearman correlation test, since the scales were considered non parametric variables. Correlations between S100B or NSE serum concentrations with age of onset, disease duration and CAG expansion were assessed by Pearson correlation test, once they were considered as parametric variables. Statistical significance was defined as $p<0.05$.

3. Results

The clinical and molecular data of MJD patients are shown in Table 1. S100B serum concentrations in control (mean±SD: 0.082±0.042 μg/l) and MJD (mean±SD: 0.108±0.073 μg/l) subjects were not statistically different, as shown in Fig. 1. However, NSE serum concentrations difference between control (mean±SD: 4.65±1.80 ng/ml) and MJD (mean±SD: 8.05±4.20 ng/ml) subjects achieved statistical significance ($p=0.00001$), as shown in Fig. 2. The correlation of NSE or S100B serum concentrations and clinical and molecular parameters of MJD patients are shown in Table 2. S100B presented a moderate correlation with the disease duration and depression score, whereas NSE presented a moderate correlation with depression score and a good negative correlation

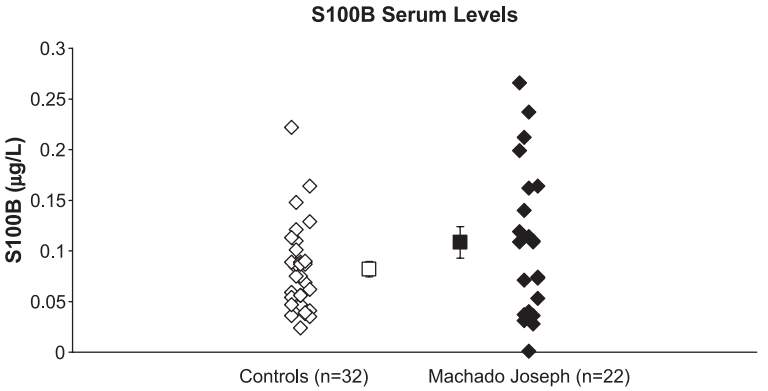

Fig. 1. Serum S100B concentrations in MJD patients and healthy subjects showed by individual values. The squares represent means±SEM. There was no statistically significant difference between groups.

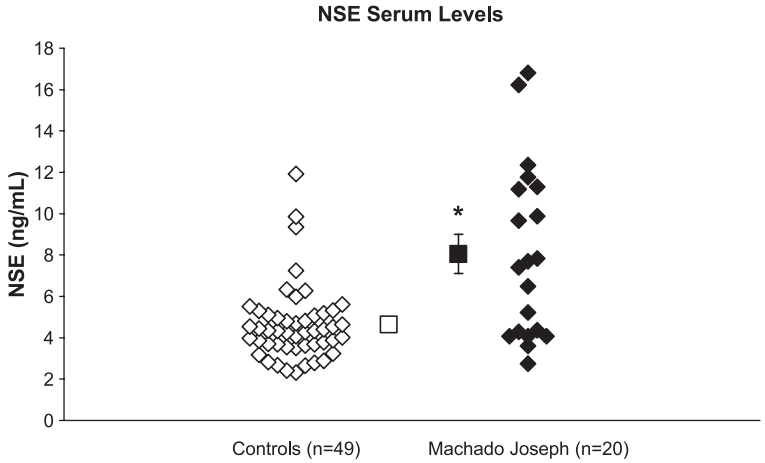

Fig. 2. Serum NSE concentrations in MJD patients and healthy subjects showed by individual values. The squares represent means±SEM. *Denotes a statistically significant difference between groups (p=0.00001).

with EDSS score. S100B and NSE serum concentrations in MJD patients were not correlated with each other (data not shown).

4. Discussion

Biochemical markers have been studied as a potential tool to detect and assess the extent and intensity of CNS injury, and could be useful in the diagnosis and prognostic of patients. In this context, measurement of peripheral concentrations of S100B and NSE, which are studied as glial and neuronal markers in several CNS injuries respectively, have been shown of inestimable value. However, it is important to take into account that these proteins are not entirely specific for the CNS, since S100B is also present in other tissues, such as adipose tissue, bladder, colon and melanoma cells, and NSE is also present in erythrocytes and platelets. Thus caution must be exercised with regards to sampling and the inclusion and exclusion of criteria.

Concerning MJD, the present work showed that symptomatic patients presented normal concentrations of S100B protein in blood. Moreover, serum S100B concentration was not correlated with molecular parameters and was not or only moderately correlated with clinical evaluation. However, these results do not completely discard a putative role of peripheral S100B in MJD. In fact, previous works demonstrated that in chronic disorders such as schizophrenia and Alzheimer's disease, the S100B concentrations are increased in the early stages of the disease, which appears to be the period where the initiation of neurodegenerative process occurs [29,30]. The MJD patients evaluated here had a median of 7.5 years of disease duration, and it is possible that the initial degenerative processes that could lead to an increase in the S100B concentrations have been lost at this time.

Table 2
Correlations between S100B or NSE serum concentrations and clinical and molecular parameters of MJD patients

Variable	CAG expansion	Age of onset	Disease duration	Depression	EDSS	UPDRS
S100B						
Correlation	−0.372	0.381	0.452	0.461	0.029	−0.140
Significance	0.088	0.080	0.035*	0.031*	0.933	0.665
NSE						
Correlation	0.055	−0.058	−0.243	0.447	−0.729	−0.284
Significance	0.817	0.809	0.303	0.048*	0.011*	0.372

It has been reported that when reactive gliosis is present, the responsive astrocytes increase their S100B secretion. In spite of post-mortem neuropathological studies have demonstrated the presence of gliosis in different CNS regions of the MJD patients, in the present work we were not able to consolidate the role of peripheral S100B concentrations as a feasible marker of chronic gliosis, in spite of its moderate positive correlation with disease duration.

Neuronal loss has been described as the prominent feature in pathological examination of the CNS in MJD [11–13]. Our results demonstrated a significant increase in serum NSE concentrations in our group of MJD patients, in comparison to healthy subjects, which could be related to neuronal loss. Importantly, besides this significant difference, the individual values in both groups were within the range specified as being physiologic by the manufacturer (up to 16 ng/ml). Thus, the mild increase of NSE blood concentrations in symptomatic MJD patients could be interpreted as consequence of a slow continuous neuronal loss process. The negative correlation with EDSS score is curious, since higher scores represent more physical disability. This could reinforce the hypothesis that patients with less physical disability have more neurons in degenerative process, whereas more impaired patients, although having more previous neuronal loss, possess less ongoing neuronal degeneration. Curiously, as S100B protein, NSE concentrations were also moderately correlated to depression score, but the relevance of this finding should be better characterized in future studies.

Acknowledgements

This work was supported by CNPq and CAPES, Brazil.

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