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Short Communication

HTLV-I-associated myelopathy: Are ferritin, S100β protein, or guanine nucleotides CSF markers of disease?

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In southern Brazil, there is an endemic high prevalence foci of HTLV-I and HTLV-II infection. HTLV-infected individuals may develop HAM/TSP. Little is known about HAM/TSP pathogenesis and there is a lack of disease progression markers. This study investigated ferritin, S-100β protein, and guanine nucleotides (GN) concentrations in the CSF of 18 patients with HAM/TSP. In HAM/TSP patients, concentrations of ferritin and S100β were increased, whereas GMP was reduced. CSF ferritin, S100β, and GN are potential markers for HAM/TSP. *Journal of NeuroVirology* (2002) **8,** 64–67.

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Human T-lymphotropic virus type I and type II (HTLV-I and HTLV-II) are retroviruses belonging to the genus deltaretrovirus, linked to lymphoproliferative diseases such as adult T-cell leukemia/lymphoma (ATLL), caused by HTLV-I. Both are also responsible for immunomediated diseases, the prototype being HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Osame, 1990). The risk of development of myelopathy among HTLV-I-infected individuals seems to be 4% (Gessain and Gout, 1992). HTLV-II has been associated to sporadic cases of progressive myelopathy, although with low grades of disabilities and with sensitive ataxia (Hall *et al*, 1996).

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HTLV-I is a more cosmopolitan virus, with endemic areas in the south of Japan, central areas of Iran, Africa sub-Sahara, some Caribbean islands, southeast of the United States, and South America. HTLV-II is endemic in native populations of the central area of Africa (Pygmy) and America (amerindians), besides intravenous drug users from USA and Europe (Gessain and Gout, 1992). In Brazil, where the mandatory screening of HTLV-I/II began in 1994, the prevalence among blood donors demonstrates a wide regional variation, from 0.08% to 1.35%. The city of Porto Alegre, capital of the southernmost state of the country, Rio Grande do Sul, has a prevalence of 1.27% of infection for HTLV-I/II; HTLV-I represents 83%, and HTLV-II 17%, of the individuals infected by deltaretrovirus in Brazil (Ferreira *et al*, 1995; Menna-Barreto *et al*, 1995, 1996). In spite of the high prevalence of HAM/TSP in endemic areas, little is known concerning the mechanisms involved in the pathogenesis of the disease, and clinical practice lacks of disease progression markers.

High cerebrospinal fluid (CSF) ferritin concentrations have been detected in various pathological conditions associated to altered iron metabolism (i.e., reperfusion injury, inflammatory processes, hepatic disease, neoplasias, meningoencephalitis, cerebrovascular disease, and neurodegenerative disorders) (Sindic *et al*, 1981; Milman *et al*, 1993).

Protein S100β is a calcium-binding protein synthesized essentially in astroglial and Schwann cells in the CNS. High concentrations of S100β protein have been found in patients with different neurological pathologies (i.e., Alzheimer's, multiple sclerosis) or injuries (i.e., head injury) and indicate damage to neural cells (Green *et al*,1997; Ingebrigtsen *et al*, 1999; Regner *et al*, 2001). Guanine nucleotides (GN) are involved in numerous intracellular functions (i.e., transmembrane signaling, guidance of vesicular traffic within cells). However, recent evidence also points to extracellular actions of GN in the CNS. GN, particularly GMP, are physiologically present in pharmacologically relevant concentrations in human CSF (Regner *et al*, 1997) and have been implicated in neuroprotection (Malcon *et al*, 1997; Regner *et al*, 1998; Schmidt *et al*, 2000).

To predict HAM/TSP after HTLV infection, there is a need of sensitive markers of disease progression and understanding of mechanisms associated to pathogenesis of HAM/TSP. This study investigated ferritin, S-100β protein, and guanine nucleotides concentrations in the CSF of HTLV-infected individuals with HAM/TSP.

Since 1994, we have been following up 251 HAM/TSP patients. In this study, we investigated 18 consecutive patients aged 34 to 68 (mean 48) years (9 male, 9 female) with HAM/TSP. Approval of the study protocol was granted by Pontificial Catholic University Medical Research Ethics Board. Patients studied had HTLV-I-infection diagnosis confirmed by Western Blot analysis in serum and CSF. HAM/TSP diagnosis was established through clinical neurological investigation (Osame, 1990). HAM/TSP degrees of disability were estimated using the EDSS scale (Kurtzke, 1983) (Table 1). As a control group, 14 patients aged 23 to 64 (mean 42) years (7 male, 7 female) suffering from back pain were included in the study. The myelograms and clinical data were analyzed and only patients with no evidence of pathology involving the CNS were examined.

CSF (3 ml) was collected during myelography (controls) or diagnostic lumbar punctures (HTLV infection). CSF samples were centrifuged at 2000 g for 5 min to eliminate cells and other insoluble material, and individually stored at -70°C. CSF samples were clear, pH 7.4, and protein concentration of approximately 0.3 mg/ml. On the day of assays, CSF samples were thawed and ferritin, S100β protein, or nucleotide concentrations were determined. Ferritin concentrations were determined by the Stratus Ferritin Fluorometric Enzyme Imunnoassay (DADE®, USA), a rapid automated technique, sensitive to nanogram levels of ferritin. S100β protein CSF concentrations were analyzed with a commercially available two-site immunoradiometric assay kit (LIA-mat® Sangtec® Medical AB, Bromma, Sweden). Samples were analyzed in triplicate. A value of S100β protein of 1.81 μg/L or more was considered pathological. Nucleotide (AMP, GMP, ADP, GDP, ATP, and GTP) concentrations were determined by reverse-phase HPLC system (Shimadzu, Japan) equipped with a Shim-pack WAX® column (Shimadzu, Japan).

Ferritin and S100β protein concentrations were markedly increased in HTLV-infected patients (Table 2). Guanine and adenine nucleotide concentrations had distinct profiles among control and HTLV-infected groups. GMP was notably reduced in HTLV-infected patients (Table 2). There were significant correlations (*Spearman's correlation coeficient*) between the markers (S100β, ferritin,

Table 1 Characteristics and CSF parameters of HTLV-infected subjects

	Subjects						*CSF parameters*			
Case no.	*WB-HTLV serum*	*HIV*	*Clinical status*	*EDSS*	*Age/sex*	*Ethnic origin*	*WB-HTLV CSF*	*Cell count*	*Protein*	*IgG-index*
1	I	—	TSP	2.0	34/f	Caucasian	HTLV-I	1	51	0.45
2	I	—	TSP	1.5	51/f	Caucasian	HTLV-I	4	37	0.45
3	I	—	TSP	1.5	45/m	Afro-american	HTLV-I	10	58	0.45
4	I	—	TSP	2.0	68/m	Afro-american	HTLV-I	2	36	0.37
5	I	—	TSP	2.0	59/f	Caucasian	HTLV-I	5	27	1.10
6	I	—	TSP	1.5	50/f	Caucasian	HTLV-I	7	47	0.62
7	I	—	TSP	2.0	42/m	Caucasian	HTLV-I	21	50	0.80
8	I	—	TSP	6.5	55/f	Caucasian	HTLV-I	6	56	0.89
9	I	—	TSP	2.5	54/m	Caucasian	HTLV-I	3	86	0.52
10	I	—	TSP	3.0	34/f	Afro-american	HTLV-I	1	33	0.51
11	I	—	TSP	4.0	46/m	Caucasian	HTLV-I	5	45	0.45
12	I	—	TSP	3.5	49/f	Afro-american	HTLV-I	12	37	1.70
13	I	—	TSP	6.0	51/f	Caucasian	HTLV-I	35	81	1.10
14	I	—	TSP	3.5	58/f	Caucasian	HTLV-I	10	40	1.10

WB: Western blot.
EDSS: expanded disability status scale (Kurtzke, 1983).
CSF normal values: cells <4/mm; protein <40 mg/dl; IgG-index <0.7.

Table 2 Characteristics and CSF markers concentrations in control and HAM/TSP patients

Features	*Control*	*HAM/TSP*
Age (years) (mean ± SD)	42.1 ± 12.4	49.7 ± 9.3
Ferritin (μg/L) (mean±SE)	1.2±0.2	5.4 ± 0.2
S100β (μg/L) (mean±SE)	1.7 ± 0.1	3.6 ± 0.6
Guanine nucleotides (μM) (mean±SE)		
GMP	86.3 ± 18.5	0.5 ± 0.2
GDP	6.9 ± 3.6	n . d.
GTP	n . d.	n . d.
Adenine nucleotides (μM) (mean±SE)		
AMP	7.5 ± 2.4	3.9 ± 1.2
ADP	6.6 ± 2.7	n . d.
ATP	2.0 ± 1.9	7.9 ± 3.1

The data represent markers concentration values in individual samples of CSF collected from control and HAM/TSP patients (both sex).
n.d.: not detected in the samples.

and GMP) and EDSS scale scores (the latter establishes HAM/TSP degrees of disability), as follows: $r = +0.653$ ($P < 0.004$) for S100β; $r = +0.565$ ($P < 0.001$) for ferritin; and $r = -0.8147$ ($P < 0.001$) for GMP (Figure 1).

Several investigators have pointed potential biomarkers for HAM/TSP progression in HTLV I-infected patients. Among these biomarkers are proviral load and CSF neopterin. Increased levels of HTLV-I proviral load in peripheral blood mononuclear cells (PBMC) have been found to predispose to HAM/TSP and other HTLV-I-associated inflammatory disorders, ranging from 10- to 100-fold higher than in asymptomatic carriers (Nagai *et al*, 1998). Additionally, neopterin, which is a by-product of guanosine triphosphate (GTP) metabolism, has been shown to be a useful biochemical marker of the cellular immune response, reflecting the degree of T-cell activation in a variety of systemic disorders (Griffin *et al*, 1991; Nomoto *et al*, 1991; Ali *et al*, 1992). Elevated levels of neopterin were detected in the CSF of HTLV-I-infected patients developing HAM/TSP disorders (Griffin *et al*, 1991; Nomoto *et al*, 1991; Ali *et al*, 1992). Moreover, Nagai *et al*. (1998) demonstrated a significant correlation between the proviral load and the concentration of neopterin in CSF of HAM/TSP patients.

The observed ferritin increase in HAM/TSP may be associated to altered immune responses and oxidative stress, whereas elevated S100β protein concentration was expected, because high concentrations have been clearly related to neural damage. The distinct nucleotide concentrations found in patients with HAM/TSP may be related to altered purine metabolism, because similar hypothesis has been previously proposed for multiple sclerosis (Hooper *et al*, 2000). GMP has been implicated in neuroprotection (Malcon *et al*, 1997; Regner *et al*, 1998; Schmidt *et al*, 2000). Hence, its remarkable reduction in HAM/TSP patients may be related to a decline in endogenous protective mechanisms. Ferritin, S100β protein, and GN may represent potential biochemical markers for HAM/TSP, and, to our knowledge, there are no previous reports of their determination in the CSF of HTLV-infected patients. Additionally, further investigation of these compounds in HTLV infection may contribute to the elucidation of some of the mechanisms involved in progression to HAM/TSP.

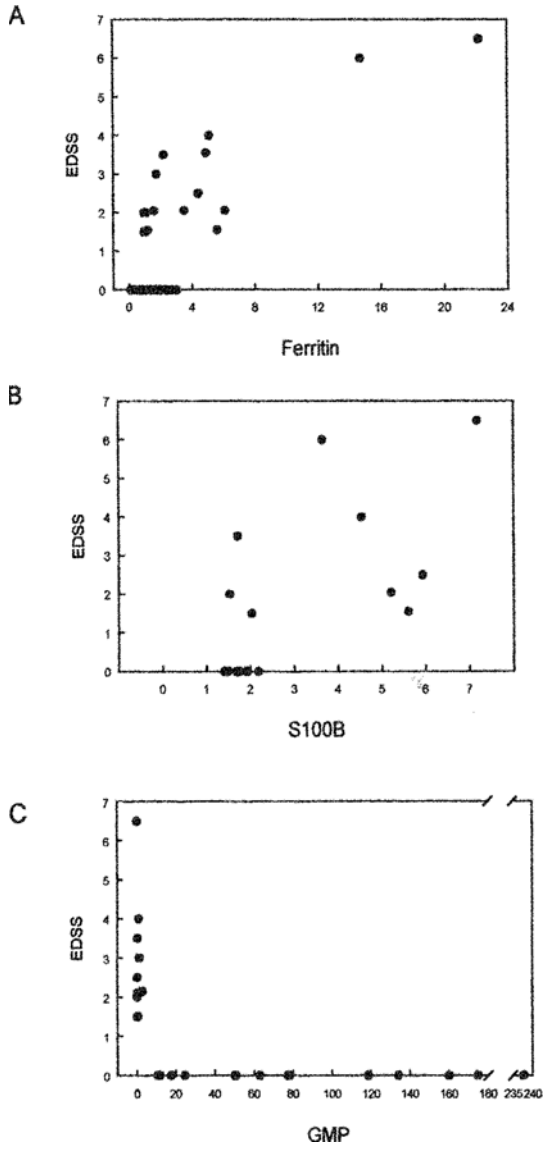

Figure 1 Correlations between CSF ferritin (**A**), S100β (**B**) and GMP (**C**) levels and EDSS scale scores. Data represent individual values of ferritin (**A**), S100β (**B**), and GMP (**C**) concentrations in the CSF of control (EDSS = 0) and HAM/TSP patients (EDSS > 0). There were significant correlations between the markers (S100β, ferritin, and GMP) and EDSS scale scores, as follows: $r = +0.653$ ($P < 0.004$) for S100β; $r = +0.565$ ($P < 0.001$) for ferritin; and $r = -0.8147$ ($P < 0.001$) for GMP. (*Spearman's correlation coefficient.*)

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