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Clinical Report

HTLV-I-associated myelopathy (HAM)/tropical spastic paraparesis (TSP) with amyotrophic lateral sclerosis-like manifestations

Toshio Matsuzaki[1,6], Masanori Nakagawa*[,1], Masahiro Nagai[3], Yasuyuki Nobuhara[1], Koichiro Usuku[2], Itsuro Higuchi[1], Koichi Takahashi[5], Takashi Moritoyo[1], Kimiyoshi Arimura[1], Shuji Izumo[3], Suminori Akiba[4] and Mitsuhiro Osame[1]

[1]Third Department of Internal Medicine, Kagoshima University Faculty of Medicine, Sakuragaoka 8-35-1, Kagoshima City, Kagoshima 890-8520, Japan; [2]Department of Medical Informatics, Kagoshima University Faculty of Medicine, Sakuragaoka 8-35-1, Kagoshima City, Kagoshima 890-8520, Japan; [3]Division of Molecular Pathology and Genetic Epidemiology, Center for Chronic Viral Diseases, Kagoshima University Faculty of Medicine, Sakuragaoka 8-35-1, Kagoshima City, Kagoshima 890-8520, Japan; [4]Department of Public Health, Kagoshima University Faculty of Medicine, Sakuragaoka 8-35-1, Kagoshima City, Kagoshima 890-8520, Japan; [5]Department of Neurology in Prefectural Hokusatsu Hospital, Miyahito 502-4, Okuchi City, Kagoshima 895-2526, Japan and [6]Department of Neurology in Okatsu Hospital, Masagohon-machi 3-95, Kagoshima City, Kagoshima 890-0067, Japan

To clarify the existence of HAM/TSP presenting amyotrophic lateral sclerosis (ALS)-like manifestations, we assayed HTLV-I proviral load in peripheral blood mononuclear cells (PBMC) in 15 patients with anti-HTLV-I antibody in serum and ALS-like manifestations (upper motor neuron involvement in at least one region and lower motor neuron involvement in at least two limbs) by quantitative PCR, and compared the proviral load with that of 233 HAM/TSP patients and of 213 HTLV-I carriers. Five of 15 patients with ALS-like manifestations had proviral loads as high as those in the 233 patients with HAM/TSP. Anti-HTLV-I antibody in cerebrospinal fluid (CSF) was present in all of five patients. The proviral load in the remaining 10 patients was similar to that in HTLV-I carriers. Four of five patients with a high proviral load met the diagnostic criterion of HAM/TSP except for lower motor neuron involvement. These four patients showed high neopterin levels in CSF. On the basis of HTLV-I proviral load in PBMC and the clinical symptoms, our tentative conclusion is that these four patients are HAM/TSP presenting ALS-like manifestations. *Journal of NeuroVirology* (2000) **6,** 544–548.

Keywords: HTLV-I; proviral load; ALS; motor neuron disease; neuropathy; myositis

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive nervous system disorder that causes degeneration of the lower and upper motor neurons. The clinical symptoms of ALS are characterized by muscular weakness with atrophy, hyper-reflexia, fasciculations and/or bulbar palsy (Subcommittee on Motor Neuron Diseases/Amyotrophic Lateral Sclerosis of the World Federation of Neurology Research Group on Neuromuscular Diseases and the El Escorial 'Clinical limits of amyotrophic lateral sclerosis' workshop contributors, 1994). Several etiological factors in ALS have been postulated, including geographic, environmental, toxic, viral and genetic factors (Gutman and Mitsumoto, 1996). The viral theory of ALS has been discussed (Rowland, 1991; Jubelt, 1991; Love, 1996; Karpati and Dalakas, 2000), because human immunodeficiency virus (HIV), human T-cell lymphotropic virus type I (HTLV-I) and enterovirus have been reported to be related to ALS (Arimura *et al*, 1989; Vernant *et al*, 1989; Sahashi *et al*, 1989; Kuroda and Sugihara, 1991; Lange, 1994; Westarp *et al*, 1995; Ferrante *et al*, 1995; Miller *et al*, 1999). Polioviruses and picorna-viruses are also related to ALS (Jubelt,

*Correspondence: M Nakagawa
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1992; Berger *et al*, 2000) with some conflicting results (Swanson *et al*, 1995), and retroviruses of mice have been found to cause both lymphoma and ALS (Gardner, 1991).

HTLV-I causes not only adult T-cell leukemia, but also a myelopathy known as HTLV-I associated myelopathy (HAM)/tropical spastic paraparesis (TSP) (Gessain *et al*, 1985; Osame *et al*, 1986). Clinical, pathological, immunological and molecular biological studies have revealed that some patients with HAM/TSP have not only myelopathy, but also other neurological and immunological disorders such as leukoencephalopathy, myositis, arthropathy, peripheral neuropathy and pulmonary alveolitis (Nakagawa *et al*, 1995). Several patients with HAM/TSP showed ALS-like manifestations because of the involvement of the lower and upper motor neurons (Arimura *et al*, 1989; Vernant *et al*, 1989; Sahashi *et al*, 1989; Kuroda and Sugihara, 1991; Lange, 1994; Nakagawa *et al*, 1995; Miller *et al*, 1999). Although coincidental association of ALS and HTLV-I infection is considered in areas in which HTLV-I is endemic, the possibility that HTLV-I causes HAM/TSP presenting ALS-like features also remains. Our previous study revealed that the proviral load in peripheral blood mononuclear cells (PBMC) was significantly higher in HAM than that in HTLV-I carriers (Nagai *et al*, 1998).

To determine the existence of a subgroup of HAM/TSP presenting ALS-like manifestations, we assayed a HTLV-I proviral load in 15 patients with ALS-like manifestations and anti-HTLV-I antibodies in serum.

Results

The HTLV-I proviral load in PBMC was 917 ± 755 (mean $\pm$ s.d.) copies/10^4 PBMC (median value: 701 copies, n=233) in HAM/TSP, and 120 ± 235 copies/10^4 PBMC (median value: 34 copies, n=213) in HTLV-I carriers ($P < 0.0001$) (Figure 1). In 15 patients with ALS-like manifestations and anti HTLV-I antibody, the proviral load was 546 ± 666 copies/10^4 PBMC (median value: 229 copies). These numbers were between those of HAM/TSP and HTLV-I carriers. On the bases of the proviral load in PBMC, we divided the 15 patients into two groups: five patients with proviral load higher than 590 copies/10^4 PBMC (590 copies/10^4 PBMC is the mean value (120)+2 s.d. (235×2) in HTLV-I carriers) and 10 patients with a proviral load lower than 590 copies/10^4 PBMC (Figure 1). In the five patients with the high proviral load, the average proviral load was 1278 ± 714 copies/10^4 PBMC (median value: 1145 copies/10^4 PBMC), which was as high as that in HAM/TSP (P=0.176). However, in 10 patients with a low proviral load, the load was 179 ± 129 copies/10^4 PBMC (median value: 166 copies/10^4 PBMC), which was a similar level as that in HTLV-I carriers (P=0.125) (Figure 1).

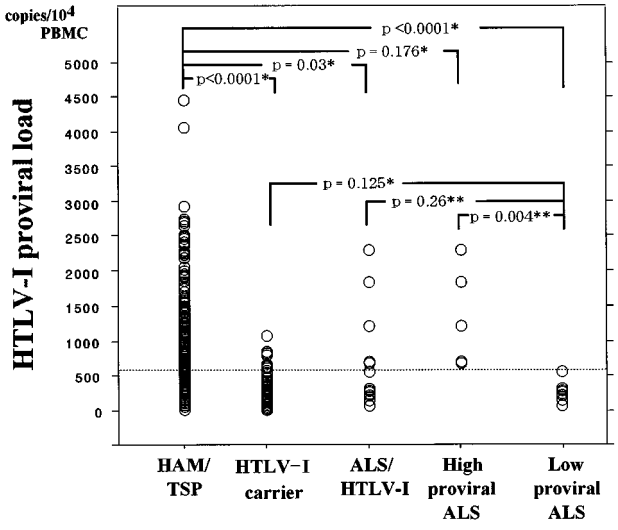

Figure 1 HTLV-I proviral load in PBMC in HAM/TSP, HTLV-I carriers and patients with ALS-like manifestations and positive anti-HTLV-I antibody in serum. *P* values were calculated using Welch's *t*-test* or Scheffé's F *post hoc* test**.

Based on the diagnostic criteria for ALS (Ross *et al*, 1998), the five patients with a high proviral load diagnosed as three definite, one probable and one suspected ALS. In addition to these ALS-like symptoms, spasticity in the lower legs, decreased vibration sense and autonomic dysfunction (urinary disturbance, constipation and sweating disturbance) were detected in the four of five patients in the high proviral load group, which was significantly different when compared with the patients with low proviral load and the patients without anti-HTLV-I antibody (Table 1). However, the incidence of these symptoms were not significantly different in comparison with that in typical HAM/TSP patients. In laboratory findings, anti-HTLV-I antibody titers in CSF were also significantly prominent in the high proviral load group. Of five patients with high proviral load, peripheral neuropathy was suspected in four patients electrophysiologically or by sural nerve biopsy, Sjögren syndrome in two patients and perivascular infiltration of $CD4^+$ or $CD8^+$ cells in the muscle biopsy of one patient. Two of three patients with a high proviral load showed improvement of clinical symptoms by prednisolone therapy; one patient was able to walk with a two-hand support, and the other showed a decrease in the lower leg spasticity. Only one patient with a high proviral load was unable to walk within 60 months after disease onset, whereas five patients with a low proviral load were unable to walk 21 months after disease onset during the study period (Table 1). Based on these clinical findings, at least four of five patients with a high proviral load met the diagnostic criterion of HAM/

Table 1 Comparison of clinical and laboratory findings between patients with ALS-like manifestations and high or low HTLV-I proviral load in PBMC, HTLV-I negative ALS and typical HAM/TSP.

	ALS-like HAM/TSP with high proviral load	*ALS-like HAM/TSP with low proviral load*	*ALS (HTLV-I negative)*	*Typical HAM/TSP*	P *values*
Number of patients	5	10	40	233	
HTLV-I proviral load (copies/10^4 PBMC)	1278±714	179±129	0	917±755	0.18
Men/women	1/4	8/2	23/17	70/163	0.63
Age at disease onset (years)	52.2	63.4	57.2	44.9	
Average interval between onset of disease and inability to walk (months)	60	21.2	31.1	167	
Number of patients unable to walk at the time studied	1	5	10	27	0.47
Effectiveness of prednisolone therapy	2/3	0/4	not done	116/142	0.46
Neurological findings					
Bulbar palsy	2/5	6/10	15/40	0/233	<0.001
Fasciculation in tongue	3/5	9/10	26/40	2/233	<0.001
Spasticity in lower legs	4/5	2/10	9/40	209/233	0.48
Positive Babinski sign	4/5	3/10	12/40	216/233	0.29
Sensory disturbance	4/5	6/10	7/40	187/233	0.99
Vibration sense less than 15 s	5/5	2/10	5/40	199/233	
Autonomic disturbance[a]	4/5	2/10	2/40	213/233	0.37
Laboratory findings in CSF					
Number of cells over 10 cells/μl	2/5	0/10	0/40	79/160	0.51
Protein over 45 mg/dl	3/5	7/10	3/40	44/176	0.078
Neopterin over 30 pmol/ml	4/5	2/9	4/40	143/171	0.83
IgG over 5 mg/dl	4/5	1/4	6/40	52/93	0.28
Titers of anti-HTLV-I antibody over 16×(PA)[b]	5/5	2/10	0/40	190/233	0.29
Abnormalities in nerve conduction study	4/5	4/10	2/40	11/218	<0.001

P values (ALS-like HAM/TSP with high proviral load *versus* typical HAM/TSP) were calculated using Fisher's exact probability test* or Student's *t*-test**. The values under 0.1 are shown. [a]Autonomic disturbance indicates urinary disturbance, constipation or sweating disturbance. [b]PA: particle agglutination method.

TSP (Osame, 1991) except for the lower motor neuron signs.

Discussion

HAM/TSP patients with ALS-like manifestations have been reported (Arimura *et al*, 1989; Vernant *et al*, 1989; Sahashi *et al*, 1989; Kuroda and Sugihara, 1991; Lange, 1994; Westarp *et al*, 1995; Ferrante *et al*, 1995; Miller *et al*, 1999), however, there were no reliable laboratory markers to distinguish patients with HAM/TSP and ALS-like manifestations from ALS patients just as HTLV-I carriers. Our previous study revealed that the proviral load in PBMC was significantly higher in HAM/TSP than that in HTLV-I carriers (Nagai *et al*, 1998). Therefore, we analyzed the proviral load in 15 patients with anti-HTLV-I antibody in serum and ALS-like manifestations that met the revised criteria for earlier diagnosis of ALS (Ross *et al*, 1998), although some patients showed other symptoms that were listed as the exclusion criteria for ALS.

Five of 15 patients showed HTLV-I proviral loads as high as those in HAM/TSP. In addition, four of five patients had high titers of anti-HTLV-I antibodies in both serum and CSF, high neopterin and unusual complications of ALS such as peripheral neuropathy, perivascular infiltration of $CD4^+$ or $CD8^+$ cells in biopsied muscle, and Sjögren syndrome. These complications have been reported in association with HAM/TSP and in HTLV-I carriers (Nakagawa *et al*, 1995; Kanazawa *et al*, 1993; Higuchi *et al*, 1993), but were considered rare in ALS patients. This multiple organ involvement was a characteristic feature of these patients with ALS-like manifestations and high HTLV-I proviral load. Therefore, the present study suggests that these four of five patients with high HTLV-I proviral load and ALS-like manifestations are classified as a subgroup of HAM/TSP, although coincidental association of ALS and HTLV-I infection is still considered in some of these patients in areas in which HTLV-I is endemic. The remaining 10 patients with ALS-like manifestations and low proviral load may be considered as HTLV-I carriers with ALS.

Autopsy findings of one HAM/TSP patient with ALS-like manifestations were reported by Kuroda and Sugihara (Kuroda and Sugihara, 1991). Prominent infiltration of inflammatory cells was found not only in the patient's pyramidal tracts and anterior horn cells, but also in all the degenerative areas. A similar pathology may be present in the five patients with a high proviral load and ALS-like manifesta-

tions. Unfortunately, we have not obtained autopsy cases of ALS-like manifestations with high HTLV-I proviral load.

Consequently, our tentative conclusion is that there may exist a subgroup of HAM/TSP presenting ALS-like manifestations, and that HTLV-I proviral load in PBMC has some value in the diagnosis of patients with ALS-like manifestations with a variant form of HAM/TSP, or being HTLV-I carriers alone. Further detailed studies are required to confirm this conclusion and to clarify the pathophysiology of HAM/TSP presenting ALS-like manifestations.

Materials and methods

All studies were performed with informed consent. From a series of patients admitted to the Third Department of Internal Medicine in Kagoshima University Hospital, 15 patients who had upper motor neuron involvement in at least one region and lower motor neuron involvement in at least two limbs that met the diagnostic criteria for ALS (Ross *et al*, 1998), and positive anti-HTLV-I antibody in serum, were selected. Anti-HTLV-I antibodies were detected using the particle agglutination (PA) method (Fujirebio Inc., Tokyo, Japan). HTLV-I proviral load in PBMC that was stocked before treatment was assayed by quantitative PCR using ABI PRISM 7700™ (Nagai *et al*, 1998) and compared with the load in 233 HAM/TSP patients and in 213 HTLV-I carriers.

Muscle biopsy was performed in seven of 15 patients. Sural nerve biopsy was performed in two patients because peripheral nerve involvement was suspected clinically and electrophysiologically. Correlations between the viral load and clinical, laboratory and electrophysiological findings in the 15 patients were analyzed statistically. We also compared the clinical findings in the 15 patients and 40 ALS patients without HTLV-I antibody.

Statistical significance of results was calculated by Welch's *t*-test, Student's *t*-test, Fisher's exact probability test, or Scheffé's F *post hoc* test using StatView™ software. We set the level of statistical significance for the *P* value at less than 0.05.

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