



HTLV-I proviral load correlates with progression of motor disability in HAM/TSP: Analysis of 239 HAM/TSP patients including 64 patients followed up for 10 years

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To clarify clinical and laboratory findings that may be related to the pathomechanism of HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP), we analyzed these findings in 239 patients with HAM/TSP, including 64 patients followed up for 10 years after their first examinations, with special interest in the HTLV-I proviral load in peripheral blood mononuclear cells (PBMCs). The proviral load in PBMCs did not differ in terms of modes of HTLV-I transmission. However, the proviral load in patients with age of disease onset greater than 65 years tended to be higher than those with a younger age of onset. In the 64 patients followed up for 10 years, the clinical symptoms deteriorated in 36 patients (56%), unchanged in 26 patients (41%), and improved in 2 patients (3%). HTLV-I proviral load also appeared to be related to the deterioration of motor disability in these patients. To our knowledge, the present study is the first longitudinal study concerning the relationship between the clinical course of HAM/TSP and HTLV-I proviral load. It is suggested that HTLV-I proviral load is related to the progression of motor disability and is an important factor to predict prognosis of patients with HAM/TSP. *Journal of NeuroVirology* (2001) 7, 228–234.

Keywords: HTLV-I; proviral load; HAM/TSP; epidemiology; long-term follow-up

Introduction

HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is characterized by slowly progressive spastic paraparesis and positive anti-HTLV-I antibody both in serum and in cerebrospinal fluid (CSF) (Gessain and Gout, 1992; Osame and McArthur, 1992; Nakagawa *et al*, 1995). Although the precise pathophysiology of HAM/TSP is not yet clear, interaction between virus infection and host immune

responses appears to be involved in the process (Usuku *et al*, 1988; Umehara *et al*, 1993; Ijichi *et al*, 1996; Jeffery *et al*, 1999). Increased HTLV-I replication in HAM/TSP evaluated by Southern blot analysis and quantitative PCR has been reported (Yoshida *et al*, 1989; Gessain *et al*, 1990; Kira *et al*, 1991; Wattel *et al*, 1992; Kubota *et al*, 1993; Kubota *et al*, 1994; Hashimoto *et al*, 1998; Nagai *et al*, 1998). These findings suggest that the increased HTLV-I replication is associated with pathogenesis of HAM/TSP, and it is important to reduce the HTLV-I proviral load for treatment of HAM/TSP. Our recent study clearly has shown that HTLV-I proviral load in peripheral blood mononuclear cells (PBMCs) is significantly higher in HAM/TSP than in HTLV-I carriers (Nagai *et al*, 1998). To clarify the relationship between the clinical and laboratory findings and HTLV-I proviral load

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in HAM/TSP, we retrospectively analyzed these findings in 239 patients with HAM/TSP including 64 patients followed up for 10 years after their first examinations.

Results

Mode of HTLV-I transmission and proviral load

The patients in Group I (181 patients) showed a shorter interval between the time of disease onset and inability to walk. The patients in Group II (19 patients) had short stature and slow progression of the disease. The interval time and the progression of the disease in Group III (39 patients) were midway between those of the previous 2 groups. The proviral load in PBMCs was not different among these 3 groups (Table 1).

Age of disease onset, age of patients, and proviral load

The proviral load ($1,180 \pm 991$ copies/ 10^4 PBMCs) in the group of the patients who had the disease onset at ages older than 65 (Group IV) tended to be higher than those (896 ± 724 copies/ 10^4 PBMCs) in the group who had the onset at younger age (Group V) ($P = 0.09$). Patients in Group IV, who had relatively higher proviral load, showed significantly higher progression rates of motor disability than those in Group V ($P = 0.04$) (Table 2). The proviral load was not correlated with the age of disease onset (Figure 1) or the age of patients at the sampling of PBMCs (before treatment) (Figure 2), although 2 patients with older age of disease onset showed the highest 2 proviral load of all the patients examined (Figure 1).

Table 2 Clinical findings and HTLV-I proviral load in HAM/TSP patients with age of disease onset before or after 65 years old

	Group		P values
	IV ¹	V ²	
Number of patients	22	217	
Gender: male/female	7/15	64/153	
Age (year): mean \pm SD (range)	74.9 \pm 4.7 (67–82)	60.3 \pm 11.1 (27–82)	<0.0001
Age of disease onset	68.5 \pm 2.2 (65–72)	42.6 \pm 18.3 (3–64)	<0.0001
Disease progression rate ³ before treatment	2.2 \pm 1.8	0.6 \pm 1.4	0.04
HTLV-I proviral load (copies/ 10^4 PBMC) (median)	1180 \pm 991 (941)	896 \pm 724 (650)	0.09
Neopterin in CSF (normal <30 pmol/ml)	127 \pm 156	98 \pm 101	0.20
IgE in serum(u/ml) under 45 u/ml	55 \pm 469 60.0%	146 \pm 582 56.0%	0.49 0.81
Titers of anti-HTLV-I antibody (PA) 2 ⁿ in serum (median)	9–17 (14)	8–17 (13)	0.02
in CSF (median)	4–17 (9)	1–17 (7)	0.30
Abnormality in brain MRI (%)	73	52	0.02

¹Group IV: patients with age of disease onset at ≥ 65 years old.

²Group V: patients with age of disease onset at <65 years old.

³Disease progression rate = motor disability grade/duration of illness (grade/year).

Laboratory findings and proviral load

Proviral load in PBMC was correlated with neopterin levels in cerebrospinal fluid ($r = 0.367$, $P < 0.0001$) as previously reported by Nagai *et al*. However, neopterin levels in CSF were not different among

Table 1 Clinical features, laboratory findings and HTLV-I proviral load in 239 HAM/TSP patients with different modes of transmission

	All Patients	Group			P values		
		I ¹	II ²	III ³	I vs II	I vs III	II vs III
Number of patients	239	181	19	39			
Gender: male/female	71/168	53/128	1/18	10/29			
Age(year): mean \pm SD (range)	61.6 \pm 11.5 (27–82)	61.8 \pm 10.7 (27–82)	51.1 \pm 15.0 (27–77)	65.4 \pm 10.3 (38–82)	<0.001	0.08	<0.001
Age of disease onset	45.1 \pm 16.5 (3–72)	48.0 \pm 14.1 (18–72)	11.0 \pm 3.6 (3–15)	49.1 \pm 11.4 (21–70)	<0.001	0.60	<0.001
Disease progression rate ⁴ before treatment	1.2 \pm 1.5	1.3 \pm 2.8	0.2 \pm 0.13	0.8 \pm 1.2	0.06	0.24	0.37
HTLV-I proviral load (copies/ 10^4 PBMC) (median)	921 \pm 761 (694)	925 \pm 774 (715)	875 \pm 62 (650)	921 \pm 785 (646)	0.78	0.96	0.87
Neopterin in CSF (normal <30 pmol/ml)	107 \pm 106	104 \pm 111	103 \pm 73	121 \pm 96	0.98	0.57	0.74
IgE in serum(u/ml) under 45 u/ml	122 \pm 468 56.4%	136 \pm 550 49.3%	123 \pm 230 72.7%	74 \pm 107 59.0%	0.80 0.15	0.44 0.42	0.76 0.46
Titers of anti-HTLV-I antibody (PA) 2 ⁿ in serum (median)	8–17 (13)	8–17 (13)	8–18 (13)	8–17 (13)	0.47	0.23	0.17
in CSF (median)	1–17 (7)	1–17 (6)	4–16 (8)	2–17 (7)	0.12	0.93	0.19
Abnormality in brain MRI (%)	55	59	43	43	0.52	0.31	0.98

¹Group I: patients with adult onset (onset after 15 years old) and no history of blood transfusion before the onset of the disease.

²Group II: patients whose ages of onset were before 15 years old and who had no history of blood transfusion.

³Group III: patients who had history of blood transfusion before the onset of the disease.

⁴Disease progression rate = motor disability grade/duration of illness (grade/year).

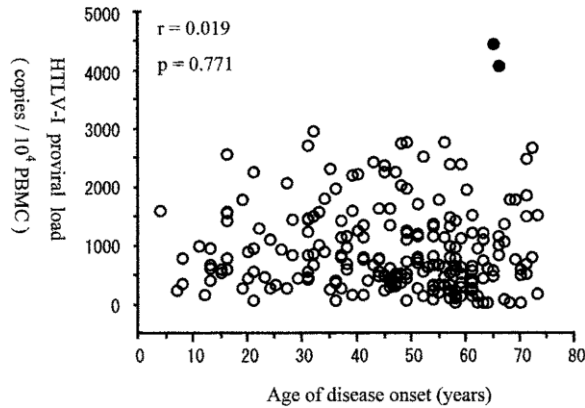


Figure 1 Correlation between HTLV-I proviral load and the age of disease onset. Although two patients (closed circles) with older age (>65 years old) of disease onset showed the highest two proviral load of all the patients examined, there was no significant correlation between these factors.

these 5 groups (Table 1, 2). The titers for anti-HTLV-I antibody in CSF were correlated with the proviral load (Figure 3), but the titers in serum (Figure 4), serum IgE levels, and the incidence of brain MRI abnormalities were not correlated with the proviral load.

Proviral load and the 10 year follow-up study

During the 10 years after the first treatments, the clinical symptoms deteriorated in 36 of 64 patients (56%), unchanged in 26 (46%) (Table 3), and improved in 2 (3%). HTLV-I proviral load was not different between deteriorated and unchanged groups. However, the proviral load was decreased immediately after corticosteroid therapy, but had been gradually increased in 25 patients during the latter half of the 10-year treatments, even when they had received other immunomodulation therapies (Nakagawa *et al*, 1996) (Figure 5). Among 25 patients, 17 patients showed high HTLV-I proviral load (540 or more copies/ 10^4

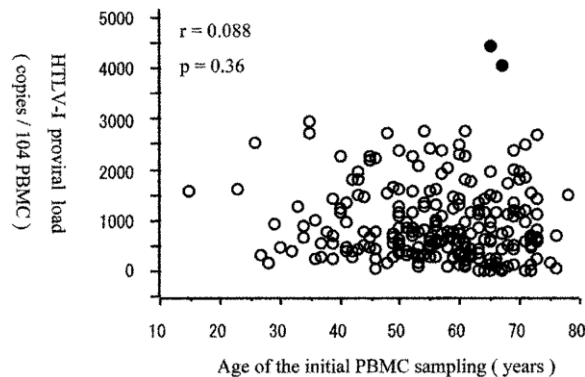


Figure 2 Correlation between HTLV-I proviral load and the age of the initial PBMC sampling. There was no significant correlation between these factors. Two patients (closed circles) showed the highest two proviral load of all the patients examined as indicated in Figure 1.

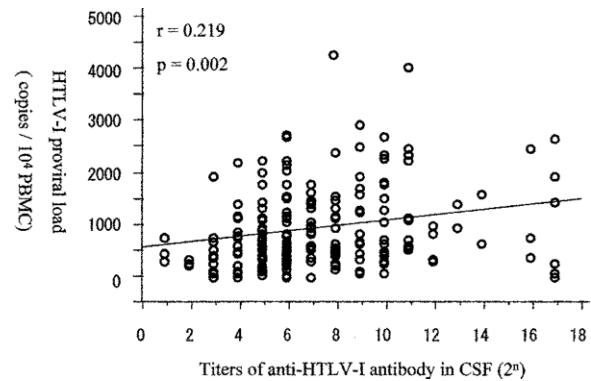


Figure 3 Correlation between HTLV-I proviral load and the titers of anti-HTLV-I antibody in CSF. There was a correlation between these factors.

PBMCs). Twelve of 17 patients showed deterioration of muscle strength with a hazard ratio of 4.4 (Table 4).

Discussion

HAM/TSP is characterized by chronic inflammatory myelopathy associated with HTLV-I infection (Gessain and Gout, 1992; Matsuoka *et al*, 1998; Osame and McArthur, 1992; Umehara *et al*, 1993). In chronic virus infections, the virus load is an important factor of disease onset, severity, and prognosis. As the increased HTLV-I replication in HAM/TSP has been reported using several methods (Yoshida *et al*, 1989; Gessain *et al*, 1990; Kira *et al*, 1991; Wattel *et al*, 1992; Kubota *et al*, 1993; Hashimoto *et al*, 1998), some new approaches have been proposed to reduce the proviral load in HAM/TSP. However, these treatments have not demonstrated the consistent clinical benefits (Lehky *et al*, 1998; Taylor *et al*, 1999).

The precise quantification of the HTLV-I proviral load in PBMCs in a large number of HAM/TSP patients has been reported by Nagai *et al* (1998). Their analysis of proviral load in PBMCs in 202 HAM/TSP patients and 243 asymptomatic HTLV-I carriers show significantly higher proviral load in HAM/TSP

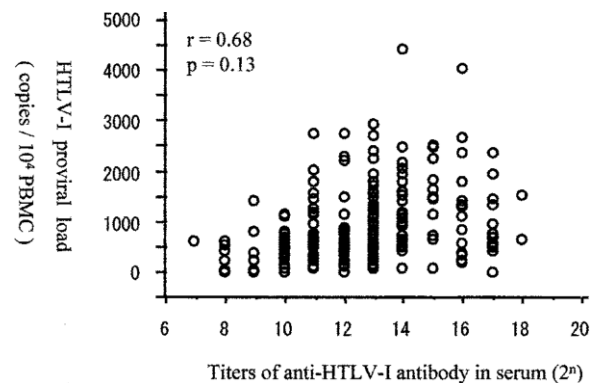


Figure 4 Correlation between HTLV-I proviral load and the titers of anti-HTLV-I antibody in serum. There was no correlation between these factors.

Table 3 Ten-year follow-up study after first treatment (1st Tr) in 62 patients* with HAM/TSP

	Deteriorated group ¹	Unchanged group ²
Number of patients	36 (56.3%)	26 (40.6%)
Male/Female	12/24	10/16
Age (year): mean ± SD (range)	61.7 ± 11.8 (38–80)	64.0 ± 10.6 (43–79)
Age of disease onset	42.1 ± 17.8 (6–78)	40.6 ± 17.5 (15–72)
Duration of illness	10.6 ± 10.1	18.3 ± 16.4
Patients with mother-to-child transmission	18/36 (50.0%)	12/26 (46.2%)
Onset after blood transfusion	6/36 (16.7%)	4/26 (15.3%)
Disease progression rate ³ after 1st Tr (grade/year)	0.61 ± 0.6	0.28 ± 0.2
$P = 0.015$		
Titers of anti-HTLV-I antibody(2 ⁿ)		
in serum before 1st Tr	9–18	9–18
5 years after 1st Tr	11–17	13–17
10 years after 1st Tr	10–17	10–17
In CSF before 1st Tr	4–12	1–10
5 years after 1st Tr	4–10	7
HTLV-I proviral load before 1st Tr (means ± SD copies/10 ⁴ PBMC)	941 ± 534 (n = 16)	868 ± 609 (n = 8)
Neopterin in CSF (means ± SD pmol/ml; normal <30 pmol/ml)	166 ± 170 (n = 16)	106 ± 127 (n = 8)

¹Deteriorated group: patients who showed deterioration of motor disability one grade or more after first examination.

²Unchanged group: patients who showed same motor disability grade as that observed in the first examination.

³Disease progression rate = motor disability grade/duration of observation.

patients than in HTLV-I carriers and higher proviral load in female patients. These results also revealed the correlation between HTLV-I proviral load and neopterin levels in CSF. In the present study, we examined the relation between proviral load and the clinical and laboratory findings in a larger number of HAM/TSP patients and found that the proviral load was related to the disease onset and the progression rate of motor disability, but not to the mode of HTLV-I transmission. The proviral load in the patients who had the disease onset at ages older than

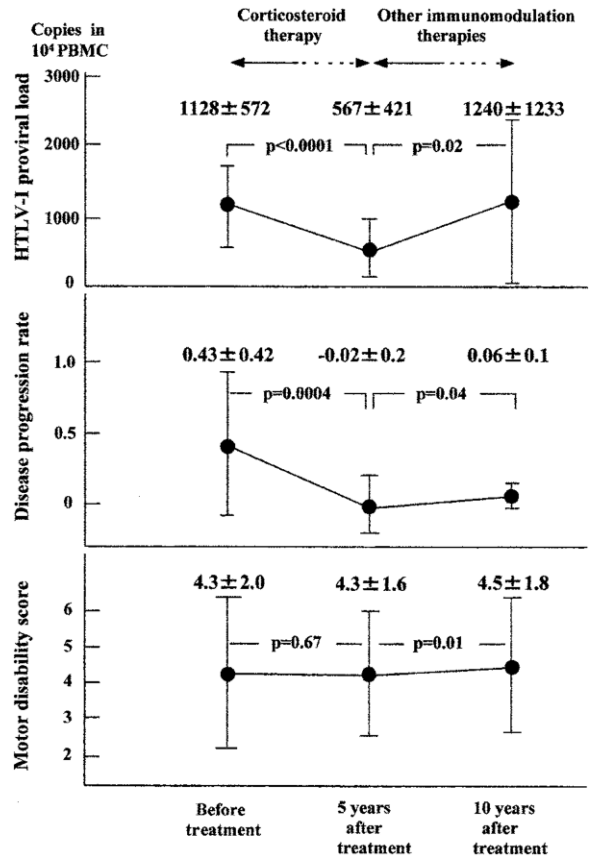


Figure 5 Changes in HTLV-I proviral load, disease progression rates and motor disability scores in 25 patients with HAM/TSP followed up for 10 years, who showed no significant increase of the proviral load during 10 years, although it was fluctuated by the treatments. The corticosteroid therapy mainly consisted of oral prednisolone at daily doses of 5–20 mg. Other immunomodulation therapies included erythromycin, salazosulfapyridine, high-dose vitamin C, interferon- α , pentoxifylline and fosfomycin.

65 tended to be higher than that in the patients who had the onset at younger age, although the difference was not significant. Some patients with older age of disease onset showed the highest proviral load in all of the patients examined. These findings suggested that some patients with older age (>65 years old) of disease onset and high proviral load had higher thresholds of the proviral load for the disease onset, whereas the others with low proviral load had

Table 4 Clinical course and HTLV-I proviral load in 25 patients with HAM/TSP followed up for 10 years, who showed no significant increase of the proviral load during 10 years, though it was fluctuated by the treatments

HTLV-I proviral load (copies/10 ⁴ PBMC)	HTLV-I proviral load		Hazard ratio	95% CI	P value
	<540	≥540*			
No. of Patients enrolled	8	17			
Deteriorated motor disability**	4	12	1.7	0.5–5.2	0.37
Deteriorated urinary disturbance**	3	8	1.4	0.4–5.2	0.63
Deteriorated muscle weakness**	2	12	4.4	1.2–20.8	0.03

*Median HTLV-I proviral load in HAM/TSP reported by Nagai et al.

**Refer to the Materials and methods section in the text for criteria of these findings.

lower thresholds or other genetic factors that made the individuals more susceptible to HAM/TSP. In the HAM/TSP patients with low proviral load, the declining of the immune system with age may have some important roles for the disease onset (Nakagawa *et al*, 1995). Host immune systems that are restricted by HLA in HAM/TSP (Jeffery *et al*, 1999) could regulate the threshold of the proviral load for the disease onset.

The reason why the late-onset group had higher proviral load was obscure. Although increases in anti-HTLV-I antibody titers with age have been suspected (Kaplan *et al*, 1990), no longitudinal study with a large numbers of patients had previously been carried out to confirm that anti-HTLV-I antibody titers and HTLV-I proviral load increase with age in HTLV-I carriers and HAM/TSP. Previous studies have reported that the proviral load is correlated with soluble interleukin-2 receptor levels, but not with age or sex (Wattel *et al*, 1992; Ono *et al*, 1998; Etoh *et al*, 1999). These studies are also cross-sectional but not a longitudinal study. Our 10-year follow-up study of 25 HAM/TSP patients, which was a longitudinal study but of small size, had showed no significant increase of the proviral load in the 10 years, although the proviral load fluctuated with treatments for HAM/TSP. Therefore, the increased HTLV-I proviral load in the patients with older age (>65 years old) of disease onset might be influenced by some factors other than age. Because most of the studies applied a cross-sectional analysis of the viral load in HAM/TSP, the longitudinal study to evaluate a large number of HAM/TSP and HTLV-I carriers was critical to address the previously noted issues.

Some previous studies have reported that the proviral load correlated with the titers for anti-HTLV-I antibody in serum (Ono *et al*, 1998; Etoh *et al*, 1999; Ureta-Vidal *et al*, 1999), but some have showed only the weak correlation (Ono *et al*, 1998). In the present study, we found the correlation between the titers in CSF and the proviral load, but we found no correlation between the titers in serum and the proviral load. These conflicting results in the serum titers might be due to the ages of subjects studied; the previous two studies used subjects younger than ours (Etoh *et al*, 1999; Ureta-Vidal *et al*, 1999). As the titers in CSF appeared to reflect the proviral load more than those in the serum, at least in HAM/TSP in the present study, the titers in CSF might be a better and more easily available marker to evaluate and predict the disease situation than those in serum.

The 10-year follow-up study showed that HTLV-I proviral load correlated with the disease progression, especially with muscle weakness, and suggested that corticosteroid therapy was effective, but other immunomodulation therapies were not, to reduce the HTLV-I proviral load or to stop disease progression. Taylor *et al* (1999) have reported that lamivudine has dramatically decreased the HTLV-I proviral load in 5 HAM/TSP patients who are relatively younger

(47-years-old on average) and have higher anti-HTLV-I antibody titers (2^{14} – 2^{18}) than those in the present study. The clinical improvement, however, was seen only in the patients with recent-onset HAM/TSP and the effect persisted only during the treatment with lamivudine. Based on these previous studies and at present studies, we thought that the proviral load might not be directly and immediately related to the clinical symptoms in HAM/TSP patients, but could instead be related to the disease onset and the progression of HAM/TSP over a long duration. Therefore, it is important to measure the proviral load in HAM/TSP patients at least once a year with the clinical evaluation of the disease severity to predict the disease prognosis; the patients with high proviral load should be treated to reduce the proviral load with corticosteroid or other medication. The large-scale and long-duration follow-up study for HAM/TSP is necessary to establish the reliable prediction of the disease prognosis through parameters including the proviral load.

In conclusion, the present study is the first longitudinal study concerning the relationship between clinical course of HAM/TSP and HTLV-I proviral load, and suggests that the proviral load is related to the disease onset, the progression of motor disability, and some clinical aspects in HAM/TSP patients. Therefore, it is crucial to reduce the proviral load in HAM/TSP patients to protect them from disease progression and to prevent the onset of disease in HTLV-I carriers.

Materials and methods

Patients Between January 1, 1986 and December 31, 1998, 317 patients with HAM/TSP were evaluated in our department. The clinical features, laboratory findings, and HTLV-I proviral load obtained before treatment in 239 of 317 patients were analyzed and databased as previously reported (Nakagawa *et al*, 1995).

Patient subgroups Based on the history of blood transfusion and the age of disease onset, 239 patients were divided into 3 groups. Group I (Adult onset group): 181 patients with adult onset (ages of onset after age 15) and no history of blood transfusion before the onset of disease, Group II (Childhood onset group): 19 patients whose ages of onset were younger than 15 years and who had no history of blood transfusion before the onset of disease, Group III (Blood transfusion group): 39 patients who had history of blood transfusion before the onset of disease. In addition to these 3 groups, we divided 239 patients into 2 other groups based on the age of disease onset regardless of the history of blood transfusion; Group IV (Late onset group): 22 patients who had disease onset after age 65, Group V (Younger onset group): 217 patients who had disease onset before age 65.

Table 5 Motor disability grade, urinary disturbance score, manual muscle test score and disease progression rate in HAM/TSP

A) Motor disability grade	B) Urinary disturbance score
Grade Motor disability	0: Normal
0 Normal gait and running	1: Slightly residual urine
1 Normal gait and runs slowly	2: Strained urination
2 Abnormal gait (staggering or spastic)	3: Needs urinary catheter into the bladder
3 Abnormal gait and unable to run	C) Manual muscle test score
4 Needs support while using stairs but walks without assistance	0: Absence of a muscle contraction
5 Needs one-hand support in walking	1: Trace of a contraction
6 Needs two-hand support in walking	2: Fair
7 Unable to walk but can crawl with hands and knees	3: Poor
8 Unable to crawl but can turn sideways in bed	4: Good
9 Unable to turn sideways but can move toes	5: Normal
10 Completely bedridden	E) Disease progression rate after treatment: (grade/year) (Motor disability grade after treatment) –(the grade before treatment)
D) Disease progression rate before treatment: (grade/year)	
Motor disability grade before treatment	
Duration of illness (years)	Duration of observation (years)

Ten-year follow-up study We analyzed the clinical and laboratory findings obtained from 64 of 239 patients in a 10-year follow-up study. We divided the 64 patients into 3 groups based on their clinical course: Deteriorated group: patients who showed deterioration of motor disability 1 grade or more during the 10 years; Unchanged group: patients who had the same motor disability grade as that obtained in the first examination; Improved group: patients who showed improvements in their motor disability grades.

Assay of Anti-HTLV-I antibodies and HTLV-I proviral load: Anti-HTLV-I antibodies in serum and CSF were detected using enzyme-linked immunosorbent assay (ELISA) and particle agglutination (PA) methods (Fijirebio Inc, Tokyo, Japan) for all the patients (Osame *et al*, 1987). The HTLV-I proviral load in PBMCs was assayed in 239 of 317 patients by the methods of quantitative PCR using the ABI PRISM 7700TM sequence detection system before treatment (Nagai *et al*, 1998). The HTLV-I proviral load in 25 of 64 patients followed for 10 years was assayed at 3 time points—before treatment, after corticosteroid therapy, and 10 years after the first examination.

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Evaluation of clinical severity Motor disability grade, urinary disturbance score, manual muscle test score, and disease progression rate were evaluated based on the criteria shown in Table 5.

Statistical analysis Demographic and clinical characteristics were compared using one-way ANOVA and chi-square tests. In survival analysis, maximum likelihood estimates of hazards ratios and 95% confidence intervals were calculated using the Cox proportional hazards models. All *P* values presented were two-sided.

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