Materials and Methods

A two-part questionnaire sought to determine the number of cases and clinical features of the disease over a 2-year period, between January 2003-December 2004. The first part included six questions. The first question asked if the hospital had hospitalized cases of exanthem subitum-associated encephalitis/encephalopathy, because all patients with the disease are thought to be admitted to hospitals for intensive treatment. Exanthem subitum-associated encephalitis/encephalopathy was defined as exanthem subitum in patients with stupor or convulsion. To exclude exanthem subitum patients with severe forms of febrile convulsion, abnormal findings of radiologic examinations were required for diagnoses of patients with only convulsions to indicate exanthem subitum-associated encephalitis/encephalopathy. If a hospital reported a case of exanthem subitum-associated encephalitis, five questions were asked: (1) age of patients, (2) sex of patients, (3) presence or absence of human herpesvirus-6 virologic examinations and the examination methods used (significant increase in human herpesvirus-6 IgG antibodies, positive human herpesvirus-6 immunoglobulin M, positive human herpesvirus-6 DNA in serum, and human herpesvirus-6 isolation), (4) detection of human herpesvirus-6 DNA in cerebrospinal fluid via polymerase chain reaction, and (5) the patients' prognosis. The second part of the questionnaire was subsequently sent to hospitals that reported cases of exanthem subitum-associated encephalitis/encephalopathy, and focused on clinical features such as (1) a febrile period, (2) time of skin-rash appearance, (3) onset of neurologic signs, (4) cerebrospinal fluid findings, including the presence or absence of human herpesvirus-6 DNA, (5) electroencephalogram findings, (6) radiologic findings, including computed tomography and magnetic resonance images, (7) treatments, including antiviral drugs, steroids, and gamma-globulins, and (8) the patients' prognosis.

In March 2005, the first questionnaire was mailed to the chiefs of pediatric departments in the 3357 hospitals with pediatric wards throughout Japan. A second mailing was sent to nonresponders in August 2005 to increase survey reliability. In December 2005, the second part of the questionnaire was sent to hospitals that reported patients with exanthem subitum-associated encephalitis/encephalopathy in the first questionnaire.

Data analysis was performed by members of our laboratory. An association between seven clinical factors, including sex, age, onset of neurologic signs, presence of human herpesvirus-6 DNA in cerebrospinal fluid, abnormal electroencephalogram findings, abnormal computed tomography findings, and abnormal magnetic resonance imaging findings and neurologic sequelae, were examined via χ^2 test. The statistical analysis was performed using StatView software, version J-5.0 (distributed by HULINKS, Inc.). The protocol of this study was approved by the Review Board of Fujita Health University.

Results

Results of First Questionnaire

Of 3357 questionnaires sent to hospitals, 2357 (70.2%) were returned. Fifty-seven (2.4%) pediatric wards were closed, and seven questionnaires (0.3%) were returned without any information, leaving 2293 (68.3%) eligible for analysis. Eighty-six patients (45 male [52.3%] and 41 female ([47.7%]) with exanthem subitum-associated encephalitis/encephalopathy were reported at 61 hospitals (2.7%), and no patients with encephalitis/encephalopathy were reported at the remaining 2232 hospitals (97.3%). Seventy-seven (89.5%) of 86 patients were diagnosed with human herpesvirus-6 infection according to virologic examinations, e.g., viral isolation (1 patient), serologic assay (53 patients), and plasma polymerase chain reaction (34 patients). Eleven patients were diagnosed with human herpesvirus-6 infection using two different virologic examinations. Only nine patients (10.5%) were diagnosed with exanthem subitum according to typical clinical features of the illness. The mean age of patients was 14.0 ± 8.8 months S.D. The youngest patients were 3 months old, and a peak in patient numbers was evident at age 10 months. Eight patients were older than 24 months. A prognosis was available for 81 (94.2%) of 86 patients. Although 41 (50.6%) of the 81 patients manifested no sequelae, 38 (46.9%) of 81 patients manifested neurologic sequelae. Moreover, two fatal cases (2.5%) were reported. Details of neurologic sequelae were examined in the second questionnaire.

Results of Second Questionnaire

To collect more precise information, a second questionnaire was sent to hospitals that had reported patients with exanthem subitum-associated encephalitis/encephalopathy in the first questionnaire. Data for the second questionnaire were collected regarding 60 of the 86 patients, and several analyses were performed on the data gathered from these 60 patients. To predict the pathogenesis of the exanthem subitum-associated central nervous system manifestations, a time correlation between the onset of neurologic signs and the appearance of a skin rash was evaluated in 56 patients, because data on disease onset were not available for four patients. We defined the day when a skin rash appeared as day zero. Neurologic signs occurred before and after the appearance of a skin rash in 37 (66.1%) and 11 (19.6%) patients, respectively. One patient (1.8%) manifested neurologic signs when a skin rash appeared. No skin rashes were evident in seven patients (12.5%), diagnosed according to virologic examinations. Forty-three (71.7%) of the 60 patients manifested stupor and convulsion, whereas the remaining 17 patients (28.3%) manifested only convulsions during the observation period. However, abnormal magnetic resonance imaging findings were evident in these 17 patients.

Table 1. Summary of abnormal findings in radiologic examinations

Examination	Patients With Abnormal Findings/Patients Examined	Abnormal Findings	Number of Patients
CT	21/54 (38.9%)	Brain edema	14
		Low density area	
		Frontal and temporal lobe	3
		Left hemisphere	1
		Bilateral striatum region	1
MRI	34/50 (65.4%)	Hyperintense on diffusion image*	21
		Brain atrophy†	10
		Hyperintense on T_2-weighted image	9
		Brain edema	5
		Subdural effusion	3
		Hyperintense on FLAIR	2
		Hyperintense on T_1-weighted image	1
		Hypointense on T_1-weighted image	1

* Without corresponding apparent diffusion coefficient result.
† Brain atrophy was evident late in time course (later than 10 days after onset of illness) in 9 of 10 patients.

Abbreviations:
CT = Computed tomography
FLAIR = Fluid-attenuated inversion recovery
MRI = Magnetic resonance imaging

Cerebrospinal fluid findings were available for 53 patients. Pleocytosis (20 cells/μL, 15 cells/μL, 11 cells/μL, and 51 cells/μL) was evident in only 4 (7.5%) of 53 patients, and the remaining 49 patients (92.5%) demonstrated normal cell counts. Cerebrospinal fluid protein levels were within normal range (23.4 $\pm$ 14.6 mg/dL) in all patients. The presence of human herpesvirus-6 DNA in cerebrospinal fluid was examined in 39 (65.0%) of 60 patients via polymerase chain reaction. Human herpesvirus-6 DNA was detected in 21 (53.8%) of those 39 patients. Electroencephalogram findings in the acute phase of the illness (within 10 days after onset of illness) were available for 55 patients. Abnormal findings (31 patients with high-voltage slow waves, 4 patients with spikes, 7 patients with other findings, and 14 patients without abnormal findings) were evident in 42 (76.4 %) of 55 patients. Computed tomography and magnetic resonance imaging of the brain were performed in 54 and 50 patients, respectively. The radiologic findings are summarized in Table 1. Computed tomography was performed 3.0 $\pm$ 2.1 days after the onset of illness. Abnormal findings (14 patients with brain edema, five patients with low-density areas, and two patients with other findings) were evident in 21 (38.9%) of 54 patients. Magnetic resonance imaging was performed 8.7 $\pm$ 5.5 days after the onset of illness, and 34 (68.0%) of 50 patients manifested abnormal findings, including 21 patients with hyperintensity on a diffusion-weighted image (without the corresponding apparent diffusion coefficient result).

Antiviral drugs were administered to 31 (51.7%) of 60 patients. Acyclovir was used in 28 patients, ganciclovir in one patient, and a combination of both drugs in two patients. Thirty-three (55.0%) of 60 patients received steroid treatments, including three who received steroid pulse therapy. Immune globulin was administered to 19 (31.7%) of 60 patients, including seven patients with high-dose γ-globulin treatment.

Neurologic sequelae were evident in 32 (53.3%) of 60 patients. As shown in Table 2, cases of severe neurologic sequelae were reported, including nine patients with spastic quadriplegia, eight patients with mental retardation, and seven patients with hemiplegia. Two fatal cases were reported from two different hospitals.

Association Between Clinical Factors and Neurologic Sequelae or a Fatal Clinical Course

As described in Table 3, an association between six clinical factors and neurologic sequelae or a fatal clinical course was evaluated. No statistical association was evident between sex, age, or onset of neurologic signs and neurologic sequelae. Although 9 (42.9%) of 21 patients with positive human herpesvirus-6 DNA in cerebrospinal fluid manifested neurologic sequelae, 13 (72.2%) of 18 patients without human herpesvirus-6 DNA in their cerebrospinal fluid manifested neurologic sequelae. Thus, patients with human herpesvirus-6 DNA in their cerebrospinal fluid exhibited less frequent neurologic

Table 2. Summary of neurological sequelae

Neurologic Sequelae	Number of Patients
Spastic quadriplegia	9
Psychomotor retardation	8
Hemiplegia	7
Motor retardation	3
Epilepsy	2
Speech disturbance	2
Facial nerve paralysis	1

Table 3. Associations between six clinical factors and neurologic sequelae or fatal clinical course

Factors (n)	Neurological Sequelae (Yes/No)	*P* Value
Sex (60)		
Male	16/14	0.6023
Female	18/12	
Age (60)		
Infant	24/15	0.2994
Older than 12 months	10/11	
Onset of neurologic signs (49)		
Febrile period	21/16	0.1584
Exanthematous period	4/8	
HHV-6 DNA in CSF (39)		
Positive	9/12	0.0652
Negative	13/5	
Abnormal findings in CT (54)		
Yes	17/4	0.0097
No	15/18	
Abnormal findings in MRI (52)		
Yes	24/12	0.1201
No	7/9	

Abbreviations:
CSF = Cerebrospinal fluid
CT = Computed tomography
HHV-6 = Human herpesvirus-6
MRI = Magnetic resonance imaging

sequelae, although no statistical association was observed ($P = 0.0652$). Meanwhile, 17 (81.0%) of 21 patients with abnormal computed tomography findings manifested neurologic sequelae, whereas 15 (45.5%) of 33 patients without abnormal findings manifested sequelae, and the frequency of neurologic sequelae was significantly higher in patients with abnormal computed tomography findings than in those without abnormal findings ($P = 0.0097$). No statistical association was observed between abnormal electroencephalogram ($P = 0.1893$) or magnetic resonance imaging ($P = 0.1201$) finding and neurologic sequelae.

Discussion

After sending questionnaires to 3300 hospitals with pediatric wards, 2293 (69.5%) hospitals responded, and 86 patients with exanthem subitum-associated encephalitis were found during the 2-year observation period. Thus, the annual number of cases of this disease is estimated at 61.9 patients/year, under the assumption that the response from hospitals was independent of the frequency of patients. Because the response rate of the questionnaire was 70%, the assumption needs to be validated. Hashimoto et al. [24] compared the mean numbers of patients with intractable disease who were financially subsidized for treatment from responding departments with those from nonresponding departments. The ratio of the former to the latter was 1.0:1.1. This value suggests that the assumption might be sufficiently valid for nationwide epidemiologic surveys of intractable diseases in Japan.

Although this survey was designed to identify patients based on a clinical diagnosis of exanthem subitum, almost 90% of patients were diagnosed via virologic examinations, including seven patients (12.5%) with an atypical clinical course of exanthem subitum (without skin rash). It is possible that other etiologic agents such as human herpesvirus-7 [25,26] or enterovirus, which are considered agents for exanthem subitum-like illnesses, may have been involved in the 10% of patients without virologic examinations. Therefore, the annual number of patients with exanthem subitum-associated encephalitis/encephalopathy estimated in this survey may be slightly different from the annual number of patients with human herpesvirus-6 encephalitis/encephalopathy. To elucidate the precise frequency of the disease, future nationwide surveys for human herpesvirus-6 encephalitis/encephalopathy should be based on virologic examinations. In contrast to influenza virus infection, the annual number of patients with exanthem subitum is stable every year. Therefore, we think that almost 60 new cases of exanthem subitum-associated encephalitis/encephalopathy occur every year in Japan. At present, annual birthrate is almost 1,100,000/year in Japan. Moreover, according to the results of our epidemiologic study [27], most children are susceptible to human herpesvirus-6 infection. Therefore, if we hypothesize that most infants (approximately 1,100,000) contract human herpesvirus-6 infection every year, the incidence of exanthem subitum-associated encephalitis/encephalopathy would be estimated at 5.5 cases/100,000 exanthem subitum cases.

The most important finding in this survey is that the disease prognosis was unexpectedly poor. Nearly half of the patients manifested neurologic sequelae after exanthem subitum-associated encephalitis/encephalopathy. Furthermore, many patients manifested severe neurologic sequelae (Table 2), and an additional two cases were fatal. One patient with exanthem subitum-associated encephalitis [28] and a patient with human herpesvirus-6 encephalitis [29] manifested hemiplegia as neurologic sequelae. In addition to hemiplegia, several patients with spastic quadriplegia were also reported in our survey. Thus, paralysis is likely to be among the common and severe neurologic sequelae of exanthem subitum-associated encephalitis/encephalopathy. Although fatal cases of human herpesvirus-6 encephalitis were reported mainly in immunocompromised adult patients [30-33], three fatal cases of human herpesvirus-6 encephalitis in immunocompetent children have been reported to date [4,8,16]. Details on the two fatal cases reported in this survey are not clear. To clarify the pathogenesis of fatal cases, and therefore improve disease prognosis, a more in-depth analysis of patients with fatal exanthem subitum-associated encephalitis/encephalopathy is needed.

Predicting patient prognoses is important for clinicians. Neither patients' sex nor age was associated with the occurrence of neurologic sequelae. Insofar as it was demonstrated that human herpesvirus-6 viremia occurs during the febrile period of exanthem subitum, and that the virus isolation rate rapidly decreases after fever subsides [34],

the pathogenesis of human herpesvirus-6 encephalitis/encephalopathy may be different between patients with febrile-period onset and those with exanthematous-period onset. However, the onset of neurologic signs did not correlate with the occurrence of sequelae. The frequency of neurologic sequelae was higher in patients with abnormal computed tomography findings than in those with normal findings ($P = 0.0097$), suggesting that computed tomography is a useful procedure for predicting patients' prognoses. Magnetic resonance imaging is considered more sensitive than computed tomography for the detection of abnormal radiologic findings. However, magnetic resonance imaging did not predict the occurrence of neurologic sequelae, and abnormal findings were evident in 12 of 21 patients without neurologic sequelae, probably because of excess sensitivity.

Although specific findings of exanthem subitum-associated encephalitis/encephalopathy could be useful for patient management, no characteristic findings have been demonstrated to date. Examination of cerebrospinal fluid is useful in diagnosing central nervous system infections. In contrast to herpes simplex encephalitis, most patients exhibited normal cerebrospinal fluid in this survey. Detecting viral DNA in cerebrospinal fluid is important for determining the etiologic agent of the disease, and human herpesvirus-6 DNA was detected in 53.8% of patients in this survey. However, because polymerase chain reaction protocols and sensitivities are not standardized, further studies using a standardized polymerase chain reaction protocol are necessary to determine the detection rate of human herpesvirus-6 DNA in these patients. Recently, it was demonstrated that diffusion-weighted magnetic resonance imaging (without the corresponding apparent diffusion coefficient result) or fluid-attenuated inversion recovery magnetic resonance imaging is useful in the early detection of abnormal findings in encephalitis patients [35]. In this survey, abnormal findings in diffusion-weighted magnetic resonance imaging were reported for many patients, but these observations varied, without any specific findings. Furthermore, the mean time for performing magnetic resonance imaging was 8.7 days after onset of illness, which suggests that magnetic resonance imaging may be difficult to perform in the acute phase of the disease. To evaluate the reliability of radiologic examinations for detecting the characteristic findings of the disease, it is necessary to define a protocol for radiologic examinations, and to analyze a large number of cases prospectively. Moreover, details of radiologic findings should be examined in future surveys, to identify the characteristics of exanthem subitum-associated encephalitis/encephalopathy.

Although this study provides important information about exanthem subitum-associated encephalitis/encephalopathy (in particular, the unexpectedly high incidence of severe neurologic sequelae), it has several limitations because it was a questionnaire-based epidemiologic study. Although a severe case of encephalitis is easy to diagnose in exanthem subitum-associated encephalitis/encephalopathy, it may be difficult to distinguish between mild encephalitis/encephalopathy and atypical febrile seizures in a case with mild signs. Therefore, different thresholds for the diagnosis of mild exanthem subitum-associated encephalitis/encephalopathy among pediatricians might affect case reports. Moreover, the possibility of bias at the responding hospitals cannot be completely ruled out. To solve these problems, a future prospective study following exanthem subitum-associated encephalitis/encephalopathy within a small area (e.g., Aichi Prefecture) is necessary.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Predictors of a Prolonged Clinical Course in Adult Patients with Herpes Simplex Virus Encephalitis

Naoto Taira, Satoshi Kamei, Akihiko Morita, Masaki Ishihara, Kenji Miki, Hiroshi Shiota and Tomohiko Mizutani

Abstract

Objective Herpes simplex virus encephalitis (HSVE) patients occasionally follow a prolonged course despite standard antiviral treatment. The purpose of this study was to analyze clinical variables to identify predictors of a prolonged course.

Methods A series of 23 HSVE patients treated with acyclovir (ACV) during the acute stage were selected and divided into 2 groups: the non-prolonged group (n = 15), with improvement within 2 weeks after initial ACV treatment; and the prolonged group (n = 8), without improvement within 2 weeks. Differences in clinical variables, including age, duration from onset to initial ACV treatment, Glasgow coma scale (GCS) score, corticosteroid administration, detection of abnormal lesions on initial cranial computed tomography (CT) and magnetic resonance imaging, detection of periodic lateralized epileptiform discharges on electroencephalogram, and clinical outcome, were compared between the groups.

Results There were significant differences in GCS score, clinical outcome, and detection of lesions on CT between the non-prolonged and prolonged groups [$p = 0.021$, $p = 0.041$ (Mann-Whitney's U test), respectively, and $p = 0.027$ (Fisher's exact test)]. Four of the eight patients with a prolonged course had a poor outcome despite treatment with additional drugs.

Conclusion A lower GCS and a higher rate of lesions on CT were identified as predictors of a prolonged course for HSVE. These predictors are in accordance with the conventional predictors of poor outcome for HSVE. This study suggests that the initial ACV treatment was insufficient for HSVE patients with these predictors at the acute stage. The initial treatment may need to be modified for such patients.

Key words: outcome, prolonged, predictor, herpes simplex virus encephalitis

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Introduction

Herpes simplex virus (HSV) is a human herpes virus that can cause HSV encephalitis (HSVE), the most common, serious, sporadic, viral encephalitis in humans (1). HSVE patients who do not receive antiviral treatment have an extremely high mortality rate (about 70%), and fewer than 3% of survivors return to normal function (1, 2). Of the common central nervous system (CNS) viral infections, HSVE has a disproportionately high mortality compared with encephalitis due to other viruses. The introduction of acyclovir (ACV) has dramatically improved mortality and morbidity for patients with HSVE (2, 3); mortality rates for HSVE have decreased to 19 - 28% (2, 3). Although ACV treatment for HSVE is highly effective, the rate of poor outcome including advanced sequelae remains high, at 30 - 50%, and the rate of return to normal living is less than 50% (1-3). Thus, the morbidity and mortality remain significantly high for HSVE despite standard ACV treatment at the acute stage.

Conventional predictors of poor outcome for HSVE have been reported to include the following 5 factors: age over 30 years, duration of more than 4 days from onset to the initiation of antiviral treatment, Glasgow Coma Scale (GCS) score of 6 points or less, detection of abnormal lesions on

Division of Neurology, Department of Medicine, Nihon University School of Medicine, Tokyo
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Correspondence to Dr. Satoshi Kamei, skamei@med.nihon-u.ac.jp

cranial computed tomography (CT) at initiation of antiviral treatment, and the detection of more than 100 copies/mL of HSV-DNA by polymerase chain reaction (PCR) in the initial cerebrospinal fluid (CSF) (2, 4-6). Management of patients with these predictors of poor outcome is difficult, and some patients with HSVE follow a prolonged course even with appropriate ACV treatment. Thus, further improvements in therapeutic regimens are needed for patients with HSVE.

Several previous reports have described relapses of HSVE in pediatric (7-10) and adult patients (3, 11-15), but there have been no previous studies involving adult HSVE patients who had a prolonged course and did not improve significantly despite standard ACV treatment. Therefore, the details of the clinical course were studied in adult HSVE patients with a prolonged course. The present study is the first to evaluate the clinical predictors in HSVE patients with a prolonged course despite standard ACV treatment.

Patients and Methods

The subjects consisted of HSVE patients treated with ACV during the acute stage. A series of patients with HSVE were selected from among all HSVE patients admitted to Nihon University Itabashi Hospital in Tokyo, Japan, between 1996 and 2007. To evaluate predictors of a prolonged course in adult HSVE patients, diagnostic and therapeutic protocols were established in advance, as reported previously (16). In this diagnostic protocol, the etiological diagnosis of HSVE was based on positive results from the following three laboratory tests: nested or real-time PCR; specific intrathecal HSV antibody synthesis; and chemiluminescence assay (17). The patients were treated according to the clinical guideline of herpes simplex encephalitis in Japan, which consisted of intravenous ACV (30 mg/kg/day) for 14 days from the time of admission (18). The therapeutic protocol permitted the use of corticosteroids at the discretion of the patients' treating physicians, although it did not specify the dosage or duration of corticosteroid treatment. When used, corticosteroids were started at the same time that ACV treatment was started. The selected patients were treated according to our therapeutic protocol, which consisted of intravenous ACV for 14 days starting at the time of admission. HSVE patients who were not treated with ACV in the acute stage were excluded. The selected patients were divided into 2 groups: the non-prolonged group, which was defined as patients who showed improvement within 2 weeks after initiation of ACV treatment without deterioration; and the prolonged group, which was defined as patients without any neurological improvement at the time of completion of the administration of ACV for 14 days. When the standard treatment failed, the patients were given additional treatment, such as extended ACV treatment, or adenine arabinoside (Ara-A), or combination therapy with both ACV and Ara-A. The clinical, neuroradiological, and neurophysiological parameters were compared between these groups in order to identify the predictors of a prolonged course in HSVE patients.

To assess differences between the groups, several parameters were studied. Clinical parameters that were extracted from the medical records of adult HSVE patients were: 1) sex (m = male, f = female); 2) age (years); 3) presence or absence of a prolonged course (absent = 0, present = 1); 4) duration from onset of HSVE to initiation of ACV treatment (days); 5) GCS at the start of ACV treatment; 6) corticosteroid administration (given = 0, not given = 1); and 7) clinical outcome. The clinical outcome was classified into five groups as reported previously (19), and categorized as follows: complete recovery = 0; mild sequelae = 1, for patients with minor neuropsychological deficits; moderate sequelae = 2, for patients with limitations due to motor, speech, memory, or seizure disorders; severe sequelae = 3, for patients requiring supportive care; and death = 4. The clinical outcomes were assessed three months after the completion of ACV treatment in the same way as described previously (19).

Neuroradiological and neurophysiological parameters included: 8) detection of focal lesions on initial cranial CT (initial CT examinations were performed in all subjects within 24 hours after admission; absent = 0, present = 1); 9) detection of abnormal lesions on initial magnetic resonance imaging (MRI; absent = 0, present = 1); and 10) detection of periodic lateralized epileptiform discharges (PLEDs) on the initial electroencephalogram (EEG; absent = 0, present = 1).

All continuous variables are expressed as minimum, mean, median, and maximum, and differences between groups were assessed using Mann-Whitney's U test. All categorical variables are expressed as percentages, and differences were assessed using Fisher's exact test. Values of $p < 0.05$ were considered statistically significant. Statistical analyses were performed using SPSS™ for Windows software, version 15 (SPSS, Chicago, IL, USA).

All of the patients and/or their families gave their written informed consent to participate in the study according to a protocol approved by the Ethics Committee for Human Studies at Nihon University Itabashi Hospital.

Results

A series of 23 HSVE patients was selected from a total of 32 HSVE patients. The 23 patients included 14 men and 9 women, and their ages ranged from 17 to 77 years (average age, 46.4±19.9 years). Nine patients who were treated with Ara-A therapy and did not receive ACV during the acute stage were excluded. The clinical data, including treatments and clinical outcomes, of the 23 patients are shown in Table 1. The patients were divided into the non-prolonged group [n=15 (65.2%); patients 1 to 15 in Table 1] and the prolonged group [n = 8 (34.8%); patients 16 to 23 in Table 1]. The prolonged group patients received additional antiviral therapies: 1 patient was treated with Ara-A (patient 17); 3 patients were treated with extended ACV treatment (patients 18, 20, and 21); and 4 patients were treated with

Table 1. Clinical Data of the 23 Patients with Herpes Simplex Virus Encephalitis Included in the Present Study

Patient No.	Sex	Age (years)	Prolonged course	Days from onset to initial ACV	GCS score at initial ACV	Initial CSF leukocyte cell count (/μL)	Initial CSF protein (mg/dL)	Additional treatment	Corticosteroid administration	Detection of lesions on initial brain CT	Detection of abnormal lesions on initial brain MRI	Detection of PLEDs on EEG	Outcome score
A. Non-prolonged group													
1	m	74	0	2	6	52	33	None	0	1	1	1	2
2	m	19	0	10	7	149	120	None	0	1	1	1	0
3	f	20	0	3	10	9	33	None	0	0	0	1	1
4	f	24	0	4	12	362	40	None	1	0	0	0	0
5	f	34	0	5	15	820	398	None	1	0	0	0	0
6	m	30	0	10	10	16	88	Ara-A	0	0	1	0	0
7	f	53	0	7	11	458	189	Extend ACV	1	1	1	1	2
8	m	56	0	2	12	503	37	None	0	0	1	1	1
9	m	77	0	1	12	571	580	None	0	0	1	0	1
10	f	77	0	3	4	7	88	None	0	1	1	0	2
11	m	27	0	4	13	21	45	None	1	1	1	0	0
12	m	32	0	5	13	253	171	None	0	0	0	0	0
13	m	54	0	4	11	9	38	Extend ACV	0	0	1	0	1
14	f	76	0	6	6	7	24	Extend ACV	1	0	1	0	2
15	f	61	0	3	15	315	97	None	0	0	0	0	0
B. Prolonged group with good clinical outcome													
16	m	28	1	5	12	236	115	Extend ACV + Ara-A	1	0	1	0	0
17	m	17	1	4	7	139	104	Ara-A	0	1	1	1	1
18	f	28	1	5	3	4	77	Extend ACV	0	1	1	1	1
19	f	53	1	1	6	45	72	Extend ACV + Ara-A	0	1	1	1	1
C. Prolonged group with poor outcome													
20	m	66	1	5	3	56	85	Extend ACV	1	1	1	0	3
21	m	57	1	7	3	944	410	Extend ACV	1	1	1	0	4
22	m	59	1	2	12	5	40	Extend ACV + Ara-A	0	1	1	1	4
23	m	45	1	7	4	3	70	Extend ACV + Ara-A	1	1	1	1	4

GCS, Glasgow coma scale; CT, computed tomography; MRI, magnetic resonance imaging; ACV, acyclovir; Ara-A, adenine-arabinoside; PLEDs, periodic lateralized epileptiform discharges; EEG, electroencephalogram; m, male; f, female; Corticosteroid administration: 0 = given; 1 = not given; Detection of lesions on initial brain CT and MRI: 0 = absent; 1 = present; Detection of PLEDs on EEG: 0 = absent; 1 = present; Outcome score: 0 = complete recovery; 1 = mild sequelae; 2 = moderate sequelae; 3 = severe sequelae; 4 = death; Prolonged course: 0 = absent; 1 = present; Additional treatment gives the name of the antiviral drug that was administered after ACV treatment for 14 days. Extend ACV indicates extension of the duration of ACV administration.

both (patients 16, 19, 22, and 23). Of these 8 patients, 2 (patients 22 and 23) of the 4 patients who received both additional treatments after initial ACV died, and 2 (patients 20 and 21) of the 3 patients with extended ACV treatment had poor outcomes (Table 1). Despite the administration of additional antiviral treatments, the clinical outcome was poor in 50% (4 patients, Nos. 20 to 23) of prolonged group patients, including 3 patients (patients 21 to 23) who died. Patient 21 died of HSVE, and patients 22 and 23 died of multiple organ failure. However, the 4 remaining patients (patients 16 to 19) had a good outcome.

Differences in the clinical characteristics between the non-prolonged and prolonged groups and the results of the statistical analyses are shown in Table 2. The mean GCS score at the time of the initial ACV treatment was 6.3 in the prolonged group and 10.5 in the non-prolonged group; the GCS score was significantly lower in the prolonged group than in the non-prolonged group ($p = 0.021$, Mann-Whitney's U test). The rate of abnormal lesions on cranial CT at the time of the initial ACV treatment was 87.5% in the prolonged group and 33.3% in the non-prolonged group; thus, abnormal lesions on cranial CT were significantly more common in the prolonged group than in the non-prolonged group ($p = 0.027$, Fisher's exact test). The mean clinical outcome score was 2.25 in the prolonged group and 0.8 in the non-prolonged group; thus, the clinical outcome score was significantly worse in the prolonged group than in the non-prolonged group ($p = 0.041$, Mann-Whitney's U test). No other variables showed significant differences between the groups. The prolonged group was thus characterized as having a lower GCS score at the start of ACV treatment, a higher rate of abnormal lesions on initial cranial CT, and a poorer outcome score.

Discussion

In the present study, compared to HSVE patients in the non-prolonged group, HSVE patients in the prolonged group had a lower GCS score at the start of ACV treatment, a higher rate of abnormal lesions on initial cranial CT, and a poorer clinical outcome score. A lower GCS score and a higher rate of abnormal lesions on initial cranial CT in the

Table 2. Baseline Clinical Characteristics by Patient Group (Prolonged or Non-prolonged Course)

	Prolonged course (n = 8)	Non-prolonged course (n = 15)	p
(1) Male (%)	75.0	53.3	1.00
(2) Age (minimum, mean, median, and maximum: years)	17.0, 44.1, 49.0, 66.0	19.0, 47.6, 53.0, 77.0	0.693
(3) Days from onset to initiation of ACV (minimum, mean, median, and maximum)	1.0, 4.5, 5.0, 7.0	1.0, 4.6, 4.0, 10.0	0.776
(4) GCS score at initiation of ACV (minimum, mean, median, and maximum)	3.0, 6.3, 5.0, 12.0	4.0, 10.5, 11.0, 15.0	0.021*
(5) Corticosteroid administration (%)	50.0	66.7	0.657
(6) Detection of lesions on initial CT (%)	87.5	33.3	0.027*
(7) Detection of lesions on initial MRI (%)	100.0	66.7	0.112
(8) Detection of PLEDs on EEG (%)	62.5	33.3	0.221
(9) Clinical outcome score (0 = complete recovery; 1 = mild sequelae; 2 = moderate sequelae; 3 = severe sequelae; 4 = death) (minimum, mean, median, and maximum)	0, 2.25, 2.0, 4.0	0, 0.8, 1.0, 2.0	0.041*

GCS, Glasgow coma scale; CT, computed tomography; MRI, magnetic resonance imaging; PLEDs, periodic lateralized epileptiform discharges; EEG, electroencephalogram. Continuous variables were compared using Mann-Whitney's U test. Categorical variables were compared using Fisher's exact test. *$p < 0.05$

prolonged group are also in accordance with the conventional predictors of poor outcome for HSVE (2, 4, 5). In the prolonged group, the average GCS score was 5.5 with a poor outcome and 7.0 with a good outcome. The GCS score of the prolonged group with a poor outcome tended to be lower than that with a good outcome. 87.5% of prolonged group patients had abnormal lesions on CT.

According to a previous study, abnormal lesions, such as a low density lesion in the temporal lobe, became distinct and spread as the clinical features progressed (20). Therefore, the prolonged group already had progression during the period of initial treatment compared to the non-prolonged group. MRI is reported to be a useful tool and superior to CT for early detection of abnormal lesions (21), and most HSVE patients in the present study showed abnormal lesions on MRI; this may explain why the detection rate of lesions on MRI was not significantly different between the two groups.

The therapeutic management of HSVE has been established (22), but it is still considered to be unsatisfactory in some patients. HSVE patients occasionally show a relapse or have a prolonged course; patients who had a relapse were not included in the present study. With respect to relapse, several studies have been reported (3, 7-15). In the previous studies, the frequency of relapse after HSVE ranged from 5% to 26% (8-10), with pediatric patients having higher relapse rates. In a report of a series of 27 children with acute HSVE, 7 patients (26%) had a relapse of HSVE, and 5 of the 7 improved with repeated, high-dose ACV (30 - 45 mg/kg/day) treatment, although 3 of the 5 patients developed moderate to severe sequelae (9). In other reports, 4 of 32 (13%) adult HSVE patients had a relapse. These 4 patients were treated with additional ACV (30 mg/kg /day) at the time of relapse, but 3 of the 4 patients developed moderate to severe sequelae (15). According to these studies, a low initial dose of ACV has been reported to be frequently associated with HSVE relapse (9, 15). Although the duration of the original ACV trial (2) was 10 days, most physicians currently continue therapy for 14 - 21 days to reduce the risk of relapse (3, 14, 22). A recently published clinician's guide for HSVE (22) recommends the continuous administration of ACV if HSV-DNA is still detected on PCR at the end of initial ACV treatment.

In contrast to relapses, HSVE patients with a prolonged course have not been studied previously. In the present study, 8 of 23 HSVE patients had a prolonged course despite standard ACV treatment (30 mg/kg/day for 14 days) at the time of admission, and additional treatment with antivi-

ral drugs (ACV, Ara-A, or both) was ineffective in 4 (patients 20 to 23 in Table 1) of the 8 patients, though it was effective in the remaining 4 (patients 16 to 19 in Table 1).

The pathophysiological findings associated with prolonged HSVE remain unclear. Prolongation of HSVE was considered to be introduced by insufficient HSV inhibition, secondary encephalitis, or both. Insufficient HSV inhibition resulted from an insufficient ACV dose and/or the presence of ACV-resistant HSV. Considering that 8 out of 23 patients had a prolonged course despite initial standard ACV treatment in the present study (18), this initial treatment might have been insufficient to inhibit HSV infection for patients with a prolonged course. On the other hand, it has been reported that levels of pro-inflammatory cytokines in the CSF of HSVE patients were high (23), and HSV was not always detected by PCR at the time of relapse in the CSF of relapsed pediatric patients with HSVE (9). These reports suggested that prolongation of HSVE might be also caused by secondary encephalitis based on the host immune response. In the present study, corticosteroid treatment was given to 50% of prolonged group patients and 66.7% of non-prolonged group patients. Corticosteroid treatment was not significantly different between the groups, but 10 of 14 patients given corticosteroids did not have a prolonged course. Therefore, corticosteroid treatment may have a beneficial protective effect against secondary encephalopathy based on the host immune response in HSVE (23). Since our previous retrospective study showed that corticosteroid treatment improved the outcome of HSVE (16), this suggested the need for further investigation to determine whether corticosteroid treatment had the potential to protect against HSVE progression.

The prolonged group patients had a poorer clinical outcome than the non-prolonged group patients. Therefore, initial antiviral treatment may need to be modified in patients who have the predictors for a prolonged course, and this may improve their clinical outcome. The possible modifications of the initial antiviral treatment may include high-dose ACV treatment or combination ACV and Ara-A therapy in patients with the predictors of a prolonged course.

In conclusion, 2 predictors of a prolonged course were identified in HSVE patients: a lower GCS score at the start of antiviral treatment and a higher rate of abnormal lesions on initial CT. Since the number of patients was small in the present study, further investigation is required to assess the predictors of clinical outcome using multivariate analysis in a larger number of HSVE patients.

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Cerebrospinal fluid levels of cytokines in non-herpetic acute limbic encephalitis: Comparison with herpes simplex encephalitis

Takashi Ichiyama [a,*], Hiroshi Shoji [b], Yukitoshi Takahashi [c], Takeshi Matsushige [a], Madoka Kajimoto [a], Takashi Inuzuka [d], Susumu Furukawa [a]

[a] Department of Pediatrics, Yamaguchi University Graduate School of Medicine, 1-1-1 Minamikogushi, Ube, Yamaguchi 755-8505, Japan
[b] International University of Health and Welfare, Ohkawa, Japan
[c] National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, Shizuoka, Japan
[d] Department of Neurology and Geriatrics, Gifu University Graduate School of Medicine, Gifu, Japan

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ABSTRACT

Background: Recently, non-herpetic acute limbic encephalitis (NHALE) was identified as a new subgroup of limbic encephalitis. The immunological pathophysiology of NHALE is still unclear. *Methods:* We measured the concentrations of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), IL-4, IL-6, IL-10, and soluble TNF receptor 1 (sTNFR1) in the cerebrospinal fluid (CSF) of 15 patients with NHALE and 13 with herpes simplex encephalitis (HSE) by cytometric bead array or ELISA. *Results:* The CSF concentrations of IL-6 in patients with NHALE and IFN-γ, IL-6, IL-10, and sTNFR1 in HSE patients were significantly higher than those of controls ($p < 0.001$, $p = 0.004$, $p < 0.001$, $p = 0.018$, and $p < 0.001$, respectively). There were significant correlations among CSF IL-6, IL-10, and sTNFR1 levels in HSE patients. The CSF concentrations of IFN-γ and sTNFR1 levels of patients with HSE were significantly higher than those with NHALE ($p = 0.001$ and $p = 0.002$, respectively). *Conclusions:* CSF cytokine levels in NHALE were relatively low compared with those in HSE. These results may be related to the favorable prognosis of NHALE.

1. Introduction

In Japan, non-herpetic acute limbic encephalitis (NHALE) was identified as a new subgroup of limbic encephalitis [1–3]. The clinical picture of NHALE is similar to that of herpes simplex encephalitis (HSE). However, the disease is not caused by herpes simplex virus (HSV) infection or a paraneoplastic disease process. Many previously reported patients with NHALE had a rather favorable neurological prognosis compared to those with HSE [2,4]. There have been a few reports on the autopsy cases with NHALE [4,5]. These reports demonstrated that there were neuronal loss and severe gliosis with inflammatory cell infiltrations in the hippocampus and amygdala. The pathogenesis of NHALE is still unclear.

To investigate the immunological pathogenesis of NHALE, we determined the cerebrospinal fluid (CSF) concentrations of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), IL-4, IL-6, IL-10, and soluble TNF receptor 1 (sTNFR1) as cytokines related to inflammation in patients with NHALE and HSE.

2. Patients and methods

Informed consent was obtained from the families of the patients and controls enrolled in this study.

2.1. NHALE

CSF samples were obtained from 15 patients with NHALE (five males and 10 females, aged from 12 to 82 years; median, 35 years) admitted to Yamaguchi University Hospital and seven collaborating research hospitals from July 1999 to February 2008 (Tables 1 and 2). The criteria for the diagnosis of NHALE were: (1) acute or subacute onset neurological disorder with limbic-associated symptoms, such as amnesia, delirium, panic, anxiety, excitation, etc., (2) negative HSV DNA in CSF by the nested polymerase chain reaction (PCR) and negative HSV antibodies in CSF determined by the enzyme-linked immunosorbent assay (ELISA), (3) lesions of the temporal lobe, especially hippocampi and amygdalae, on magnetic resonance imaging (MRI) (Fig. 1), (4) absence of malignancy, (5) no bacteria or fungi in CSF culture, and (6) the exclusion of all other neurological, vascular, metabolic, endocrine, toxic, and drug-induced disorders. CSF samples obtained during the acute stage were stored at −70 °C.

* Corresponding author. Fax: +81 836 22 2257.
E-mail address: ichiyama@yamaguchi-u.ac.jp (T. Ichiyama).

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2.2. HSE

CSF samples were obtained from 13 patients with HSE (eight males and five females, aged from 13 to 76 years; median, 61 years) admitted to Yamaguchi University Hospital and two collaborating research hospitals from October 2000 to December 2005 (Table 1). The diagnosis was based on the demonstration of HSV DNA in the CSF by nested PCR. CSF samples during acute stage were stored at −70 °C.

2.3. Control subjects

The control subjects for the CSF levels of the cytokines were 19 afebrile and non-infectious patients with neurological disorders, such as epilepsy, dementia, etc. (11 males and eight females, aged from 13 to 79 years; median, 55 years), as shown in Table 1. CSF samples were obtained from them on routine analysis and they all had normal CSF cell counts.

2.4. Clinical data

The clinical data including age, gender, clinical symptoms on admission, CSF findings at the time of specimen collection, MRI findings during the acute stage, and clinical outcomes in patients with NHALE and HSE were investigated. The outcomes were defined as follows: (1) normal resolution, (2) mild sequelae, (3) severe sequelae necessitating help with daily life activities, and (4) death [6].

2.5. Determination of cytokine concentrations

The concentrations of CSF IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10 were measured with a cytometric bead array (CBA) kit (BD PharMingen, San Diego, CA, USA) according to the manufacturer's manual, as previously described [7–9], with modification of the data analysis using GraphPad Prism software (GraphPad Prism Software, San Diego, CA, USA). Briefly, each series of beads exhibiting discrete fluorescence intensities is coated with a monoclonal antibody against a single cytokine, and a mixture of six series of beads can detect six cytokines in one sample. A secondary phycoerythrin-conjugated monoclonal antibody stains the beads proportionally to the amount of bound cytokine. After fluorescence intensity calibration and electronic color compensation procedures, standard and test samples were analyzed with a FACScan flow cytometer equipped with CellQuest software (BD PharMingen). The lower detection limits for IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10 were 7.1, 2.8, 2.6, 2.6, 2.5, and 2.8 pg/ml, respectively.

The CSF concentrations of sTNFR1 were determined with a sTNFR1 ELISA kit (Bender Medsystems, Vienna, Austria), as described previously [10]. The lower detection limit for sTNFR1 was 0.05 ng/ml.

2.6. Statistical analysis

All data were log transformed to obtain an approximately normal distribution. The differences in the results between groups were analyzed with a *t*-test and the χ^2 test, and those with a *p*-value of less than 0.05 were considered significant. Correlations were analyzed using Pearson's coefficient correlation. Analyses and calculations were performed using SPSS-12.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Clinical characteristics

Clinical data of patients with NHALE are shown in Tables 1 and 2. There were no significant differences in age or gender among patients with NHALE and HSE and controls (median age, 35, 61, and 55 years, respectively). The CSF cell counts of patients with NHALE were lower than those with HSE ($p = 0.015$, 9/μl vs. 32/μl as a median). The CSF protein levels of patients with NHALE were less than those with HSE ($p = 0.003$, 33 vs. 50 mg/dl as a median). Of the 15 patients with NHALE, 9 (67%) had mild sequelae and 6 (33%) survived without sequelae. Of the 13 patients with HSE, 1 (8%) died and 12 (92%) experienced disability (54% had severe and 38% had mild sequelae).

Table 1
Clinical data of patients with NHALE, HSE, and controls

	NHALE $N = 15$	HSE $N = 13$	Control subjects $N = 19$
Age (median, range)	35 yr, 11–82 yr	61 yr, 13–76 yr	55 yr, 13–79 yr
Sex (male: female)	5:10	8:5	11:8
Comorbid conditions	–	–	Epilepsy, 9; dementia, 5; psychosis, 4; Tic, 1
Prognosis	Normal, 6; mild sequelae, 9	Mild sequelae, 5; severe sequelae, 7; death, 1	–

NHALE, non-herpetic acute limbic encephalitis; HSE, herpes simplex encephalitis.

Table 2
Clinical characteristics of the 15 patients with non-herpetic acute limbic encephalitis

No./age/gender	Main symptoms on admission	Lesions on MRI	CSF findings		Neurological prognosis
			Cell (μl)	Protein (mg/dl)	
1/34 yr/M	Amnesia, delirium	Bilateral temporal lobes	12	39	Normal
2/73 yr/F	Somnolence, convulsion	Bilateral temporal lobes	32	24	Normal
3/35 yr/M	Amnesia, convulsion	Bilateral temporal lobes	9	39	Mild amnesia
4/11 yr/M	Convulsion, delirium	Right temporal lobe	187	33	Intellectual impairment
5/18 yr/F	Convulsion	Bilateral temporal lobes	39	31	Normal
6/49 yr/F	Amnesia, convulsion	Bilateral temporal lobes	42	50	Amnesia, psychopathy
7/31 yr/F	Convulsion	Bilateral temporal lobes	0	27	Epilepsy
8/47 yr/F	Insomnia, convulsion	Bilateral temporal lobes	9	47	Normal
9/82 yr/F	Amnesia, fugue	Bilateral temporal lobes	1	39	Amnesia
10/67 yr/M	Convulsion, delirium	Bilateral temporal lobes	1	35	Amnesia
11/75 yr/F	Convulsion, enuresis	Bilateral temporal lobes	0	32	Amnesia, psychopathy
12/51 yr/M	Amnesia, convulsion	Bilateral temporal lobes	0	28	Amnesia
13/14 yr/F	Panic	Left temporal lobe	121	27	Normal
14/19 yr/F	Excitation, convulsion	Bilateral temporal lobes	8	48	Intellectual impairment, epilepsy
15/12 yr/F	Anxiety, insomnia	Bilateral temporal lobes	14	25	Normal

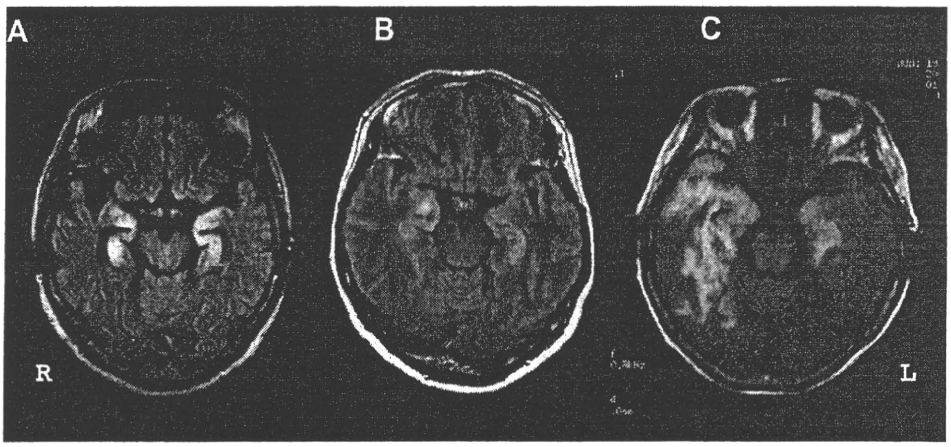

Fig. 1. FLAIR MRI of Patient 2 (A), Patient 6 (B), and Patient 9 (C) demonstrated high signal intensity lesions in the bilateral temporal lobes.

3.2. CSF concentrations of cytokines

In patients with NHALE, the CSF concentrations of IL-6 were significantly higher than those of controls ($p < 0.001$), but those of IFN-γ, TNF-α, IL-2, IL-4, IL-10, or sTNFR1 were not (Fig. 2).

In patients with HSE, the CSF concentrations of IFN-γ, IL-6, IL-10, and sTNFR1 were significantly higher than those of controls ($p = 0.004$, $p < 0.001$, $p = 0.018$, and $p < 0.001$, respectively), but those of TNF-α, IL-2, or IL-4 were not (Fig. 2). There were significant correlations among CSF IL-6, IL-10, and sTNFR1 levels in HSE patients (IL-6 and IL-10, $p = 0.008$; IL-6 and sTNFR1, $p < 0.001$; IL-10 and sTNFR1, $p = 0.030$) (Fig. 3).

The CSF concentrations of IFN-γ and sTNFR1 levels of patients with HSE were significantly higher than those with NHALE ($p = 0.001$, and $p = 0.002$, respectively) (Fig. 2).

4. Discussion

Main lesions in NHALE were in the bilateral temporal lobes, especially the hippocampus and amygdala, similar to those in HSE. However, HSV DNA or anti-HSV antibodies were not detected in the CSF of patients with NHALE. Previous reports on autopsy cases of NHALE revealed that HSV-1 or -2 were not detected in the brain [4,5]. Therefore, NHALE has been identified as a new type of encephalitis, especially in Japan [1–4]. Several autoantibodies, including those against the *N*-methyl-D-aspartate glutamate receptor and voltage-gated potassium channel, were detected in patients with NHALE [4,11–14]. Moreover, patients with limbic encephalitis associated with autoimmune disease, including Hashimoto's disease, Sjögren's syndrome, and systemic lupus erythematosus, have been reported [15–17]. These previous studies suggest that NHALE is immune-mediated encephalitis.

The clinical outcomes of patients with NHALE were relatively favorable compared with those with HSE. Moreover, CSF cell counts and protein concentrations of patients with NHALE were significantly less and lower than those with HSE, suggesting that inflammation in the CNS in NHALE is milder than that in HSE. In this study, we demonstrated CSF cytokine profiles of NHALE compared with HSE. In patients with NHALE, the CSF concentrations of IL-6 were significantly higher than those of controls, but those of IFN-γ, TNF-α, IL-2, IL-4, IL-10, or sTNFR1 were not. IL-6 is well-known as a cytokine that plays important roles in inflammatory re-

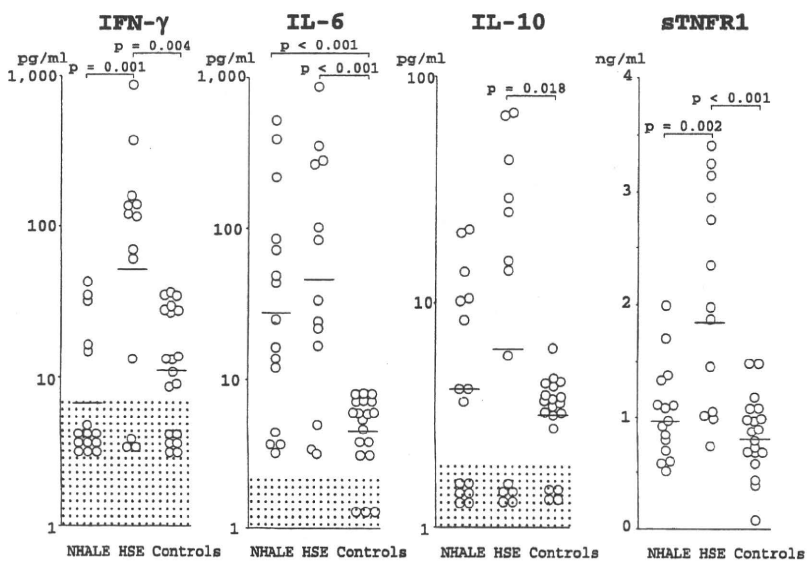

Fig. 2. The CSF concentrations of IFN-γ, IL-6, IL-10, and sTNFR1 in patients with NHALE, HSE, and controls. Horizontal lines indicate geometric means. Shaded areas indicate values below the detection limits.

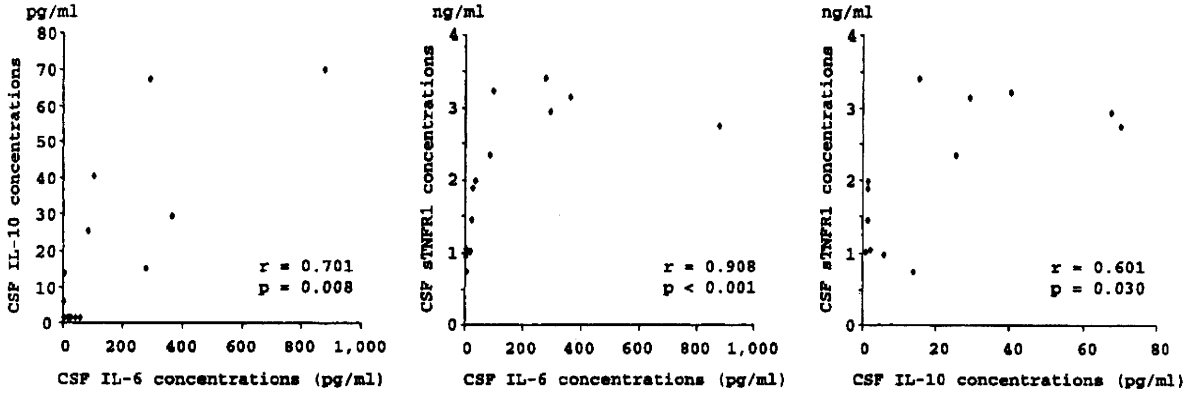

Fig. 3. The relationship among CSF IL-6, IL-10, and sTNFR1 concentrations in patients with HSE. *r*, Pearson's coefficient.

sponses [18,19]. Our results suggested that NHALE involves mild inflammation modified by IL-6 in the central nervous system (CNS). IFN-γ, which is produced by NK cells and $CD8^+$ and Th1 type $CD4^+$ T lymphocytes, plays an important role in host defense against viral infection, and inhibits viral replication [20]. We previously demonstrated that CSF IFN-γ levels were elevated in CNS disorders due to direct viral invasion, such as viral meningitis and HSE [2,21,22], but not in immune-mediated CNS disorders, such as acute disseminated encephalomyelitis, influenza-associated encephalopathy, acute encephalopathy following prolonged febrile seizures, and hemolytic uremic syndrome with encephalopathy [23–26]. Taking our findings into consideration, NHALE without elevated IFN-γ levels in the CSF in this study is not caused by direct viral infection.

In patients with HSE, the CSF concentrations of IFN-γ, IL-6, IL-10, and sTNFR1 were significantly higher than those with controls. Our present data that CSF IFN-γ levels were elevated in HSE were consistent with a previous study [2]. There were significant correlations among CSF IL-6, IL-10, and sTNFR1 levels in HSE patients. In addition, CSF sTNFR1 levels in HSE were significantly higher than those in NHALE. TNF-α increases blood–brain vascular permeability, injures vascular endothelial cells, and induces the necrosis of myelin and oligodendrocytes [29–31]. Previous studies have suggested that TNF-α mediates the pathogenesis of acute encephalitis/encephalopathy [23,32–35]. It is believed that sTNFR reflects the true biological activity of TNF-α [36–38]. CSF sTNFR1 levels are related to the neurological prognosis in bacterial meningitis and acute encephalopathy/encephalitis [10,33]. CSF sTNFR1 levels may reflect the neurological outcome in HSE. IL-10 as an anti-inflammatory cytokine decreases the production of IL-1, IL-6, and TNF-α induced by an endotoxin or bacteria [27,28]. Therefore we suggest that IL-10 is induced in the CNS to modulate pro-inflammatory cytokine-mediated inflammation in the CNS of patients with HSE. Patients with HSE showed elevated pro-inflammatory and anti-inflammatory cytokines in the CSF, suggesting that there was severe inflammation in the CNS of these patients.

In conclusion, the CSF concentrations of IL-6 in patients with NHALE and IFN-γ, IL-6, IL-10, and sTNFR1 in HSE patients were significantly higher than those in controls. Patients with HSE had many elevated cytokines in the CSF, but those with NHALE showed only an elevated CSF level of IL-6. These findings may be related to the fact that the clinical outcome of NHALE is relatively favorable compared with that of HSE.

Acknowledgments

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Brief Communication

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Steroid-Responsive Chronic Cerebellitis With Positive Glutamate Receptor δ2 Antibody

Masaya Kubota, MD, PhD, and Yukitoshi Takahashi, MD, PhD

We report the clinical course of a 4-year-old girl with chronic cerebellitis (onset 2 days after diphtheria-pertussis-tetanus vaccination at 1 year and 7 months old) associated with anti-glutamate receptor δ2 antibody, who improved dramatically with steroid therapy (methylprednisolone pulse therapy plus oral prednisolone). Recently, it has been reported that the anti-glutamate receptor δ2 selectively expressed at the post-synaptic site of parallel fiber–Purkinje cell synapses has an important role in cerebellar function in the developing brain. The present case suggests that anti-glutamate receptor δ2 antibody plays a primary role in an immune-mediated process causing chronic cerebellar symptoms, and the lesion site seems to be localized to the parallel fiber–Purkinje cell synapse. Because the cerebellum is strongly involved in language acquisition as well as motor development, treatment must facilitate time for language learning while reducing the side effects of the corticosteroid therapy.

Keywords: chronic cerebellitis; glutamate receptor δ2

Because postinfectious acute cerebellar ataxia in childhood is generally benign and self-limited, no specific treatment is required. However, in some cases unlike acute cerebellar ataxia, unusual long-term cerebellar symptoms persist and therapeutic intervention is necessary. Recently, extensive molecular genetic studies have revealed the essential role of glutamate receptor δ2 in cerebellar functions, and glutamate receptor δ2 mutant mice or mice treated with specific antibody to glutamate receptor δ2 showed impairments in various cerebellar functions.[1–6] At present, the clinical relevance of glutamate receptor δ2 dysfunction is not fully understood. We herein report the clinical course of a 4-year-old girl with chronic cerebellitis associated with anti-glutamate receptor δ2 antibody who improved dramatically with steroid treatment, despite the absence of any therapy for 1 year and 8 months after onset.

From the Department of Pediatrics (Pediatric Neurology), Metropolitan Hachioji Children's Hospital, Hachioji-city, Tokyo, Japan.

Address correspondence to: Masaya Kubota, MD, PhD, Department of Pediatrics (Pediatric Neurology), Metropolitan Hachioji Children's Hospital, Hachioji-city, Tokyo, Japan. e-mail: mkmegped@opal.plala.or.jp.

Kubota M, Takahashi Y. Steroid-responsive chronic cerebellitis with positive glutamate receptor δ2 antibody. *J Child Neurol.* 2008;23:228-230.

Case Report

This 4-year-old Korean girl had been quite healthy with normal psychomotor development until nystagmus and ataxic gait appeared at the age of 1 year and 7 months. Her family history was unremarkable. Two days later, she showed an unstable wide-based gait and could not sit steadily. She was given a diphtheria-pertussis-tetanus vaccination 2 days before onset of the cerebellar symptoms. Despite the diagnosis of acute cerebellar ataxia, the symptoms gradually worsened. A study of the cerebrospinal fluid showed cell count of 20/μL (15 lymphocytes), protein 10 mg/dL, and glucose 60 mg/dL. Two weeks after onset, prednisolone (1 mg/kg weight) was given every day for 2 weeks, but the symptoms persisted. Thereafter, the patient was not given any medication for 1 year and 8 months.

When she was admitted to our hospital for further evaluation at the age of 2 years and 9 months, she could not stand or walk without help due to cerebellar ataxia and could not speak at all. Excessive salivation was noticed. No definite nystagmus was observed. Basically, muscle tone of the extremities was hypotonic and intentional tremor of the upper extremities was observed. Deep tendon reflexes were induced normally, and no pathological reflexes were evident. On brain magnetic resonance imaging (MRI) and single-photon emission computed tomography (SPECT),

Table 1. Anti-Glutamate Receptor δ2 and ε2 Antibody Before and After Steroid Introduction (at 2 y and 9 mo, and 3 y and 9 mo)

	IgG-ε2	IgM-ε2	IgG-δ2	IgM-δ2
Cerebrospinal fluid (2 y and 9 mo)	–	–	–	+
Cerebrospinal fluid (3 y and 9 mo)	–	–	+	+
Serum (2 y and 9 mo)	–	–	–	–
Serum (3 y and 9 mo)	–	–	–	–

no structural or metabolic abnormalities were demonstrated. Urine vanilmandelic acid and homovanillic acid were within normal limits, and no abnormal mass was found on an abdominal ultrasound study. As shown in Table 1, anti-glutamate receptor δ2–immunoglobulin (Ig) M in cerebrospinal fluid was found to be positive and anti-glutamate receptor δ2-IgG was still negative. At that time, serum (simultaneously studied serum) anti-glutamate receptor-IgM and anti-glutamate receptor-IgG were negative. (For details regarding the detection of anti-glutamate receptor-IgM and -IgG, see the Methods section of Takahashi et al.[7]) Because the production of anti-glutamate receptor δ2 antibody in the central nervous system might be closely related to the patient's cerebellar symptoms, we selected corticosteroid therapy, with a high-dose methylprednisolone as a first-line treatment (at the age of 3 years and 3 months). Three weeks after the methylprednisolone pulse therapy (30 mg/kg weight divided for 3 days), the parents recognized a gradual improvement in truncal ataxia, resulting in walking with help more smoothly than before, and excess salivation disappeared. Subsequently, we started oral prednisolone (1 mg/kg weight, on alternate days) for further improvement. Surprisingly, 2 months later, she could walk without help and could eat food with a spoon by herself. Five months after the beginning of corticosteroid therapy, the truncal and limb ataxia almost disappeared so that she could walk fast and steadily. Concerning articulation, she could imitate a single phoneme or limited words and occasionally began to speak some words 6 months after therapy. One year later (at the age of 4 years and 2 months), she could run steadily and walk up stairs well, and began to speak two-word sentences. At present (4 years and 8 months of age), she has begun to speak intelligible Japanese and Korean. Then we started to taper the dose of corticosteroid. Thus, corticosteroid therapy improved the long-term cerebellar symptoms without any adverse effects, despite the treatment interval of 1 year and 8 months. At the age of 3 years and 9 months, anti-glutamate receptor δ2-IgM in the cerebrospinal fluid was found to be still positive and anti-glutamate receptor δ2-IgG had converted to positive (Table 1). Anti-glutamate receptor ε2 antibody was not detected in cerebrospinal fluid or serum.

Lymphocyte Stimulation Test by Glutamate Receptor δ2 and Diphtheria-Pertussis-Tetanus Vaccine

The patient's peripheral blood mononuclear cells were cultured in the presence of D33 (cell line expressing glutamate receptor δ2 subunits) alone and 6250 × diluted diphtheria-pertussis-tetanus vaccine alone, and in the presence of D33 and 6250 × diluted diphtheria-pertussis-tetanus vaccine at the age of 4 years. Responses were assessed by [^{3}H]thymidine incorporation in lymphocytes. Results were expressed as counts per minute (cpm) and as stimulation indexes (= cpm of cultures with drug/cpm of cultures without drug). Results were considered positive when the stimulation index was higher than 2. As shown in Table 2, stimulation index for the mixture of D33 (glutamate receptor δ2 subunits) and diphtheria-pertussis-tetanus vaccine was 5.17, whereas stimulation index for diphtheria-pertussis-tetanus vaccine alone was 2.7 and stimulation index for D33 alone was 1.32.

Discussion

Because the long-term cerebellar symptoms in our case differed from simple acute cerebellar ataxia, we diagnosed her as having chronic cerebellitis associated with anti-glutamate receptor δ2 antibody. Since Takahashi et al[7] reported opsoclonus-myoclonus syndrome with positive anti-glutamate receptor δ2 antibody, our case may be categorized in the broad opsoclonus-myoclonus syndrome spectrum, but the main symptoms in our case were limb and truncal ataxia and language developmental delay without apparent myoclonic movement in ocular and limb muscle.

The present case suggests that anti-glutamate receptor δ2 antibody plays a primary role in the pathogenesis of chronic cerebellar ataxia; furthermore, the lesion was functional and not destructive, because a dramatic improvement in cerebellar symptoms was brought about by corticosteroid therapy, despite the absence of any therapy for 1 year and 8 months after onset. The absence of structural and metabolic abnormality in MRI and SPECT also supports this idea. The anti-glutamate receptor δ2 antibody was generated exclusively in the central nervous system because the antibody was positive only in the cerebrospinal fluid before and after corticosteroid introduction. In cases with persistent cerebellar symptoms, the presence of anti-glutamate receptor δ2 antibody in cerebrospinal fluid should be checked.

The glutamate receptor δ2 was selectively expressed at the postsynaptic site of parallel fiber–Purkinje cell synapses,[1] and glutamate receptor δ2 mutant mice showed impairments in long-term depression at these synapses,[2] motor learning,[3,5] stabilization of the parallel fiber–Purkinje cell synapse,[2,4] and refinement of climbing fiber innervation

Table 2. Results of Lymphocyte Stimulation Test by Glutamate Receptor δ2 and Diphtheria-Pertussis-Tetanus Vaccine at the Age of 4

	Control	Phytohemagglutinin	D33 (glutamate receptor δ2 subunit) (400 μg)	6250 × diluted diphtheria-pertussis-tetanus	D33 (glutamate receptor δ2 subunits) (400 μg) + 6250 × diluted diphtheria-pertussis-tetanus
Count per minute	320	83 453	423	863	1654
Stimulation index			1.32	2.7	5.17

to Purkinje cells.[2] In addition to these developmental abnormalities, Hirai et al[6] showed that application of an antibody specific for glutamate receptor δ2 to cultured Purkinje cells induced α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor endocytosis, attenuated synaptic transmission and abrogated long-term depression; moreover, adult mice treated with this antibody revealed cerebellar dysfunction without apparent morphological changes in Purkinje cells.

Taken together with these basic data, and positive anti-glutamate receptor δ2 antibody and dramatic effect of corticosteroid therapy on cerebellar symptoms in our case, the lesion site seems to be localized at the parallel fiber– Purkinje cell synapse.

Sugiyama et al[8] reported a similar case of chronic cerebellitis associated with anti-glutamate receptor δ2 antibody but the cerebellar symptoms fluctuated, despite a high-dose intravenous immunoglobulin and corticosteroid pulse therapy. The long-term use of corticosteroids may be necessary to suppress the disease process, because short-term corticosteroid therapy 2 weeks after the onset in our patient was not effective for the cerebellar symptoms.

The lymphocyte stimulation test showed that the mixture of D33 (glutamate receptor δ2 subunits) and diphtheria-pertussis-tetanus vaccine activated lymphocytes more intensely than D33 (glutamate receptor δ2 subunits) or diphtheria-pertussis-tetanus vaccine alone. Lymphocytes stimulated by the lymphocyte stimulation test are usually T cells. Although we could not confirm a subset of stimulated T cells (CD4+ or CD8+) by glutamate receptor δ2, activated effector T cells that could invade the central nervous system beyond the blood-brain barrier definitively exist in peripheral blood circulation. We speculate that these activated T cells are produced by cross-reaction using molecular mimicry after a diphtheria-pertussis-tetanus vaccination and play an important role in the subsequent onset of chronic cerebellitis.

On the retarded language development in our case, we must not regard it as a simple dysarthria but as a learning problem in which the cerebellum is strongly involved. The internal model was introduced to extend the cerebellar functions in voluntary movement, perception, and language. Ito[9,10] maintains the hypothesis that reorganization of the neuronal circuit by error-driven induction of long-term depression constitutes the major memory and learning mechanisms of the cerebellum. Therefore, treatment must facilitate time for language learning while reducing the side effects of corticosteroid therapy.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Neuropathological Studies of Patients with Possible Non-Herpetic Acute Limbic Encephalitis and So-called Acute Juvenile Female Non-Herpetic Encephalitis

Koichi Okamoto[1], Tsuneo Yamazaki[1], Haruhiko Banno[2], Gen Sobue[2], Mari Yoshida[3] and Masamitsu Takatama[4]

Abstract

Objective This study was to clarify the neuropathological findings of non-herpetic acute limbic encephalitis (NHALE) and so-called acute juvenile female non-herpetic encephalitis (AJFNHE).

Methods We examined three rare autopsied cases consisting of probable one NHALE and two AJFNHLE. For comparison, we also studied 10 autopsied cases of hippocampal sclerosis mainly caused by anoxia.

Results In NHALE, neuronal loss with gliosis and microglia/macrophage infiltrations were mainly seen in the CA1 areas in the hippocampus. However, there were no apparent anoxic neuronal changes in the remaining neurons in the CA1, and astrocyte proliferations and microglia/macrophage infiltrations were also observed in the claustrum, while these were mildly present in the basal ganglia. In AJFNHE, pathological findings differed from those of NHALE with regard of the absence of limited pathology in the limbic system, microglia/macrophages widely infiltrated the brain including the hippocampal areas and mild lymphocytic infiltrations were observed in the subarachnoid spaces as well as in the parenchyma.

Conclusions The pathomechanism of NHALE and AJFNHE is obscure and autoimmune theory is proposed, however we must collect and examine many autopsied cases in order to clarify the pathomechanism.

Key words: non-herpetic acute limbic encephalitis, acute juvenile female non-herpetic encephalitis, hippocampal sclerosis

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Introduction

Many diseases affect the limbic system, and limbic encephalitis (LE) is usually classified into paraneoplastic LE, LE by viral infections, LE associated with autoimmune disease such as LE with antibody against voltage-gated potassium channels, and LE of unknown etiology (1-6). Non-herpetic acute limbic encephalitis (NHALE) is regarded as a new subgroup of LE (7-9). Patients with NHALE differ from those with herpes simplex encephalitis in terms of the lack of evidence of herpes simplex virus (HSV) and showed magnetic resonance imaging (MRI) findings localized to the limbic system such as bilateral hippocampi and amygdalae (7, 8, 10, 11). However, similar patients with so-called acute juvenile female non-herpetic LE (AJFNHLE) without abnormal MRI findings in the limbic systems have also been reported mainly in Japan (12, 13). The relationship between NHALE and AJFNHLE are equivocal because autopsied patients have very rarely been reported. Here, we describe three autopsied cases consisting of probable one NHALE and two AJFNHLE. For comparison, we also studied 10 autopsied cases of hippocampal sclerosis mainly caused by anoxia.

[1]Department of Neurology, Gunma University Graduate School of medicine, Maebashi, [2]Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya, [3]Department of Neuropathology, Institute for Medical Science of Aging, Aichi Medical University, Aichi and [4]Geriatric Research Institute and Hospital, Maebashi

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Correspondence to Dr. Koichi Okamoto, okamotok@med.gunma-u.ac.jp