autoantibodies will help clarify their pathogenesis. The proteomic analysis used in our study is a very useful tool for identifying several autoantigens reacting with autoantibodies at one time.

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ELSEVIER

Subacute Encephalopathy: Clinical Features, Laboratory Data, Neuroimaging, and Outcomes

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We sought to clarify the clinical, laboratory, neuroradiologic, and neurophysiologic features of the "subacute" subtype of encephalopathy. We retrospectively identified nine patients with subacute encephalopathy out of 97 patients diagnosed as manifesting acute encephalopathy. Neurologic symptoms, clinical course, laboratory data, neuroradiologic and electroencephalographic findings, and outcomes were reviewed through medical records. The median age of patients was 44 months (range, 28-156 months). The initial neurologic sign was a brief seizure in 4, a prolonged seizure in 3, delirious behavior in 1, and a loss of consciousness in 1. Loss of consciousness the next day was subtle in 4, and mild in 5. However, a worsening of consciousness was observed 3-7 days after onset. Laboratory data were unremarkable, and electroencephalography during the early phase found abnormalities in 4 of 7 patients. Magnetic resonance imaging revealed no abnormalities during the early phase, and mild cortical atrophy during the late phase. All but one patient had various degrees of neurologic sequelae. Subacute encephalopathy was characterized by a delayed worsening of neurologic symptoms, mild cortical atrophy on late magnetic resonance imaging, and poor neurologic outcomes. Recognition of this type of acute encephalopathy is important, and a method to promote early diagnosis is desirable.

Okumura A, Kidokoro H, Itomi K, Maruyama K, Kubota T, Kondo Y, Itomi S, Uemura N, Natsume J, Watanabe K, Morishima T. Subacute encephalopathy: Clinical features, laboratory data, neuroimaging, and outcomes. Pediatr Neurol 2008;38:111-117.

Introduction

Rapid deterioration in association with convulsions during a febrile illness is a common clinical manifestation of acute encephalopathy. Several new subtypes of acute encephalopathy were proposed based on their clinical, neuroradiologic, and laboratory findings. Acute necrotizing encephalopathy, as proposed by Mizuguchi, is characterized by symmetric lesions in the thalami and other brain regions [1]. There were several reports on mild subtypes of acute encephalopathy associated with transient splenial or white-matter lesions [2-4]. Subcortical white-matter lesions on diffusion-weighted images are characteristic in children with encephalopathy with prolonged seizures [5].

A unique subtype of acute encephalopathy, characterized by a relatively slow worsening of neurologic signs, has attracted the attention of pediatric neurologists in Japan [6-13]. According to these previous reports, the common neurologic sign at the outset is a seizure, and especially a prolonged one. The next day, patients may appear relatively well. Consciousness seems almost recovered, but slightly reduced responsiveness, am appearance of absent-mindedness, or subtle disorientation may be observed by parents or caregivers. Deterioration of consciousness, clustered seizures, and involuntary movements appear 3-7 days after the first seizure. Most patients have moderate to severe cognitive impairment. However, the clinical, laboratory, neuroradiologic, and neurophysiologic features of this subtype of encephalopathy are not yet fully understood. The aim of this study was to clarify the clinical, laboratory, neuroradiologic, and neurophysiologic features of the "subacute" subtype of encephalopathy, characterized by a relatively slow progression of neurologic symptoms.

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Table 1. Patient characteristics, clinical manifestations, and outcomes

Patient	Age (Months)	Sex	Prodromal Infection*	Initial Neurologic Signs	LOC on Next Day
1	45	F	URI (9)	LOC alone	Mild
2	156	M	NSFI (2)	Brief SZ	Subtle
3	28	M	Enterocolitis‡ (6)	Prolonged SZ	Mild
4	32	M	NSFI (0)	Delirious behavior	Subtle
5	33	F	URI (1)	Brief SZ	Subtle
6	37	M	NSFI (0)	Prolonged SZ	Mild
7	44	F	Influenza A (0)	Prolonged SZ	Mild
8	60	M	Influenza A (0)	Brief SZ	Mild
9	44	F	Influenza A (0)	Brief SZ	Subtle

* Numbers in parentheses indicate interval between prodromal illness and onset of encephalopathy.
† Numbers in parentheses indicate days after onset.
‡ Verotoxin-producing *Escherichia coli* were isolated from stool.
§ Oral tendency was also observed 20 days after onset.

Abbreviations:
F = Female
LOC = Loss of consciousness
M = Male
NSFI = Nonspecific febrile illness
SZ = Seizure
URI = Upper respiratory infection

Patients and Methods

We reviewed the hospital records of patients with acute encephalopathy who were admitted to the Department of Pediatrics in Nagoya University Hospital (Nagoya, Aichi, Japan) and its affiliated 12 hospitals between January 1998 and March 2005. We identified 97 patients with acute encephalopathy, as characterized by decreased consciousness with or without other neurologic signs lasting for >24 hours in children with infectious symptoms. We carefully excluded patients with sustained decreased consciousness after a febrile seizure, or those with delirious behavior without obvious reduced consciousness.

We assessed the detailed clinical course of each patient on the basis of medical records. We paid attention to initial neurologic signs, and the severity and time course of decreased consciousness. Nine (9%) of 97 patients fulfilled the following conditions: (1) mildly decreased consciousness on the day after onset, (2) deterioration of consciousness a few days after onset, and (3) no other cause of encephalopathy such as electrolyte derangement, metabolic abnormalities, or worsening of systemic diseases. These nine patients were the subjects of this study.

In this study, the initial neurologic signs were divided into the following four items: prolonged seizure, brief seizure, delirious behavior, and decreased consciousness alone. A prolonged seizure was defined as lasting for >20 minutes. A brief seizure was defined as those <20 minutes, irrespective of the number of seizures. Delirious behavior was defined as disoriented and incoherent action or speech lasting for >30 minutes. It may include visual hallucinations, irritability, fearful responses, and sensory misperception. Coma was defined as a condition in which a patient could not be aroused by maximal painful stimulation. This is consistent with a score of 3-5 in the Glasgow Coma Scale-Modified for Children or a score of 100-300 on the Japan Coma Scale. Semicoma was defined as a condition in which a patient could be aroused by painful stimulation. The loss of consciousness on the day after onset was milder than in most of these patients. Thus, we defined mild loss of consciousness as the condition in which a patient tended to be asleep but could be aroused without painful stimulation, and subtle loss of consciousness as the condition in which a patient remained awake but lacked spontaneity, or seemed absent-minded or slightly disoriented.

Laboratory data were also assessed through medical records. The following values were investigated: aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatinine kinase, glucose, ammonia, and cell counts and protein in cerebrospinal fluid. All findings of cranial computed tomography and magnetic resonance imaging were evaluated retrospectively by 17 pediatric neurologists who were unaware of patients' detailed clinical courses. Single-photon emission tomography and electroencephalograms were interpreted by pediatric neurologists in each hospital.

All patients were followed by pediatric neurologists for at least 1 year. The severity of cognitive impairment was defined as mild when intelligence or developmental quotient was between 50-70, moderate when it was between 30-50, and severe when it was <30. The severity of motor impairment was defined as mild when a patient could walk with or without support, moderate when a patient could seat oneself without support, and severe when a patient could not seat oneself.

Results

Patient Characteristics

Patient characteristics are summarized in Table 1. Their median age was 44 months (range, 28-156 months). No patient was <2 years of age. No patient had a family history of febrile seizures, epilepsy, or other neurologic disorders. One patient (patient 4) manifested mild cognitive impairment and focal epilepsy of unknown origin. Three (patients 7, 8, and 9) had a past history of febrile seizures. The remaining 5 patients had no history of neurologic disorders.

The pathogen of prodromal infection was identified in 4 patients. Influenza A infection was virologically proven in 3 patients. Verotoxin-producing *Escherichia coli* were isolated in a stool sample from patient 3. In this patient, there were no clinical or laboratory findings suggesting an association with hemolytic-uremic syndrome. Prodromal illness involved upper respiratory infection in 2 patients, and nonspecific febrile illness without respiratory, urinary,

Table 1. Continued

Most Severe LOC†	Sz During Subacute Phase†	Behavioral Abnormalities†	Cognitive Impairment	Motor Impairment
Coma (3)	None	Stereotypic movement (3)	None	None
Coma (4)	Brief SZ (6)	None	Moderate	Mild
Semicoma (4)	None	Stereotypic movement (4)	Severe	None
Semicoma (7)	Clustered brief SZs (5)	None	Severe	Moderate
Coma (3)	Clustered brief SZs (4)	Oral automatic movement (6)§	Moderate	Mild
Coma (3)	Prolonged SZ (7)	Oral tendency (18)	Severe	Mild
Coma (3)	None	Stereotypic movement (2)	Moderate	Mild
Coma (4)	None	Delirious behavior (3)	Mild	Mild
Coma (4)	Brief SZ (5)	None	None	Mild

or gastrointestinal signs in 3 patients. The interval between prodromal illness and onset of encephalopathy was <2 days in 7 patients. Theophylline had not been used in any of these patients before the onset of encephalopathy, whereas acetaminophen had been administered in 2 patients (patients 2 and 7).

Neurologic Signs and Clinical Courses

Neurologic signs and clinical courses are also listed in Table 1. The initial neurologic sign was a brief seizure in 4 patients, a prolonged seizure in 3, delirious behavior in 1, and loss of consciousness alone in 1. The severity of loss of consciousness on the next day of onset was subtle in 4 patients and mild in 5. However, worsening of consciousness was observed 3-7 days after onset. The most severe loss of consciousness was coma in 7 patients, and semicoma in 2.

During the subacute phase, seizures or behavioral abnormalities were present in all patients. Seizures were observed in 5 patients. A prolonged seizure was observed in 1 patient, clustered brief seizures in 2, and a single brief seizure in 2. Behavioral abnormalities were recognized in 6 patients. Stereotypic movements, such as purposeless hand movements, were recognized in 3 patients, oral tendency in 1, smacking-like oral automatic movements followed by an oral tendency in 1, and delirious behavior such as meaningless speech in 1.

Laboratory Data

In all but one patient, laboratory tests on admission revealed normal levels of aspartate aminotransferase, alanine aminotransferase, and creatinine kinase. One patient (patient 9) had mildly elevated levels of aspartate aminotransferase and alanine aminotransferase. Lactate dehydrogenase was mildly to moderately elevated in most patients on admission. These values reached peak levels on days 3-10 from the onset of encephalopathy. Marked elevations of aspartate aminotransferase (>200 IU/L) and alanine aminotransferase (>200 IU/L) were observed in 3 patients (patients 7-9), elevations of lactate dehydrogenase (>1000 IU/L) were observed in 3 (patients 5, 7, and 8), and elevations of creatinine kinase (>1000 IU/L) were observed in 2 (patients 2 and 6). Hyperammonemia, hypoglycemia, and metabolic or respiratory acidosis were not recognized in any patients. Cerebrospinal fluid analyses revealed mild pleocytosis with mildly increased protein levels in 3 patients (patients 1, 2, and 9).

Neuroradiologic Findings

Neuroradiologic findings are listed in Table 2. During the acute phase, computed tomography was performed in 6 patients, magnetic resonance imaging in 1, and both computed tomography and magnetic resonance imaging in 1. No abnormal findings were observed in these 8 patients.

During the subacute phase, when the worsening of consciousness had occurred, computed tomography was performed in 2 patients, magnetic resonance imaging in 3, and both computed tomography and magnetic resonance imaging in 3. No neuroradiologic examination was performed in 1 patient during this phase. Computed tomography demonstrated mild blurring of gray-white matter differentiation in the bilateral frontal areas of 2 patients (Fig 1). Magnetic resonance imaging revealed mild high

Table 2. Neuroradiologic findings

Patient	CT			MRI			SPECT
	Acute Phase	Subacute Phase	Late Phase	Acute Phase	Subacute Phase	Late Phase	
1	Normal (0)	Not performed	Not performed	Normal (1)	Normal (8)	Mild CA (40)	Not performed
2	Not done	Normal (7)	Normal (17)	Not performed	Normal (8)	Mild CA (33)	Hypoperfusion in bilateral F areas (11)
3	Normal (0)	Blurring of GWD in bilateral F areas (5)	Not performed	Not performed	HIA in bilateral F areas on T2WI/ FLAIR (14)	Mild CA (39)	Hypoperfusion in bilateral F-T areas (13)
4	Not done	Not performed	Not performed	Not performed	Normal (12)	Mild CA (34)	Diffuse hypoperfusion (20)
5	Normal (0)	Normal (4)	Not performed	Not performed	HIA in bilateral F areas on T2WI/ FLAIR (9)	Mild CA (23)	Hypoperfusion in bilateral F areas (22)
6	Normal (0)	Not performed	Not performed	Not performed	HIA in left T-P-O areas on DWI (9)	Mild CA (31)	Hypoperfusion in left T-P-O areas (16)
7	Normal (0)	Blurring of GWD in bilateral F areas (3)	Not performed	Not performed	Not performed	Mild CA (35)	Not performed
8	Normal (0)	Not performed	Mild CA (15)	Not performed	Not performed	Mild CA (21)	Not performed
9	Normal (0)	Normal (10)	Not performed	Normal (3)	Not performed	Normal (20)	Not performed

Numbers in parentheses indicate days after onset.

Abbreviations:
CA = Cortical atrophy
DWI = Diffusion-weighted images
F = Frontal
FLAIR = Fluid-attenuated inversion-recovery images
GWD = Gray-white matter differentiation
HIA = High-intensity area
O = Occipital
P = Parietal
T = Temporal
T2WI = T_2-weighted images

intensities in the bilateral frontal areas on T_2-weighted and fluid-attenuated inversion-recovery images in 2 patients (Fig 1), and marked high intensities in the left temporo-parieto-occipital area on diffusion-weighted images in 1 patient (Fig 2). No abnormalities were seen in 4 patients.

During the late phase, magnetic resonance imaging was performed in all 9 patients, and computed tomography was performed in 2 patients. Magnetic resonance imaging demonstrated mild cortical atrophy with widening of the extracerebral space and ventricles in all but one patient (Figs 1, 2). In 3 (patients 1, 2, and 8), previous neuroradiologic examinations did not indicate abnormal findings. In one patient (patient 9), neuroradiologic abnormalities were not observed throughout the clinical course.

Single-photon emission tomography was performed during the subacute or late phase in 5 patients. Four patients manifested marked hypoperfusion in the bilateral frontal areas (Fig 1), and one exhibited marked hypoperfusion in the left temporo-parieto-occipital area (Fig 2).

Electroencephalogram Findings

Electroencephalograms were recorded during the acute phase in 7 patients. Marked, generalized slowing was observed in 3 patients (patients 1, 5, and 9), and a slow basic rhythm in 1 patient (patient 3). However, electroencephalogram findings were unremarkable in 3 patients (patients 2, 6, and 8). An electroencephalogram during the subacute phase was performed in 8 patients, and revealed various degrees of abnormalities in all of them. Marked, generalized slowing was seen in 1 (patient 2), mild generalized slowing in 4 (patients 1, 3, 7, and 8), regional slowing in 2 (patients 5 and 6), and a slow basic rhythm in 1 (patient 4). Electroencephalograms during the late phase were performed in 6 patients (patients 1-6). Abnormalities in background activities were recognized in all of them, and paroxysmal discharges were observed in 3 (patients 3-5).

Treatments and Outcomes

Methylprednisolone pulse therapy was performed in 1 patient, intravenous dexamethasone therapy in 1, intravenous immunoglobulin therapy in 3, glycerol therapy in 3, and mannitol therapy in 1. In regard to anticonvulsants, diazepam was used in 6 patients, phenobarbital in 5, phenytoin in 2, carbamazepine in 2, valproate in 1, clonazepam in 1, and midazolam in 1. Mechanical ventilation was not necessary in any patients.

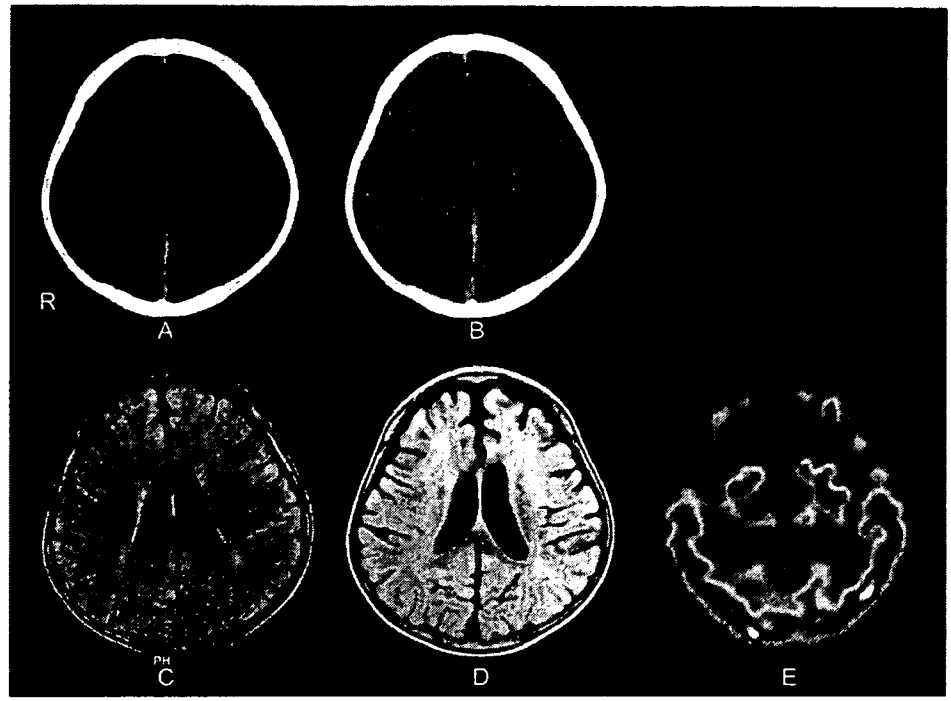

Figure 1. Neuroradiologic findings of patient 3. (A) Computed tomography on day of onset. No abnormal findings were recognized. (B) Computed tomography 5 days after onset. Mild blurring of gray-white matter differentiation was observed in the bilateral frontal areas. (C) Magnetic resonance imaging 14 days after onset (fluid-attenuated inversion recovery images, fast spin-echo, TR/TE/TI = 8000/120/1900 ms). Mild high intensities in the subcortical white matter were seen in the bilateral frontal areas. (D) Magnetic resonance imaging 39 days after onset (fluid-attenuated inversion recovery images, fast spin-echo, TR/TE/TI = 8000/120/2300 ms). Mild but diffuse cortical atrophy was observed. (E) Single-photon emission tomography 13 days after onset. Marked hypoperfusion was observed in the bilateral frontal areas.

The outcomes of patients are given in Table 1. No patients died, although all but one patient had various degrees of neurologic sequelae. Cognitive impairment was seen in 7 patients (severe in 3 patients, moderate in 3, and mild in 1), and motor impairment was seen in 7 (moderate in 1 patient, and mild in 6). In most patients, cognitive impairment was more prominent than motor impairment. The patient who had manifested mild cognitive impairment before the onset of encephalopathy (patient 4) had severe cognitive impairment with moderate motor impairment. The relationship between treatment and outcome could not be analyzed, because of the small number of patients and wide variety of treatments.

Discussion

Several authors proposed different names for acute encephalopathy syndromes with a relatively slow worsening of neurologic signs [6-13]. Although minor features are different among these syndromes, they have many points in common. Therefore, acute encephalopathy syndromes with a delayed worsening of consciousness should be integrated into one syndrome to avoid unnecessary confusion. To this end, we used the term "subacute" encephalopathy as a clinical entity to cover these syndromes, rather than devise a new name.

In the present study, we described the unique features of subacute encephalopathy. At the onset of encephalopathy, a prolonged or brief seizure was commonly observed, but some patients did not have a seizure at onset. The severity of decreased consciousness was invariably mild on the following day. Thereafter, worsening of consciousness began, and reached maximal severity 3-7 days after onset. Although neuroimaging was unremarkable at the outset, mild cortical atrophy was observed in most patients during the late phase. Most patients had moderate to severe

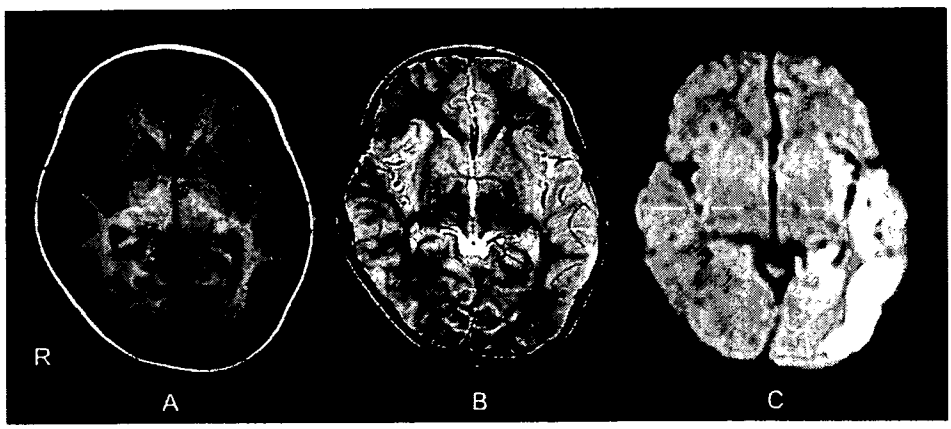

Figure 2. Neuroradiologic findings of patient 6: magnetic resonance imaging 9 days after onset. Subtle thickening of the cortex in the left temporo-parieto-occipital area was seen on T_1-weighted (A: fast spin-echo, TR/TE = 596/15 ms) and T_2-weighted (B, fast spin-echo, TR/TE = 4162/100 ms) images. Diffusion-weighted images (C: spin-echo echo-planar imaging, TR/TE = 2835/89 ms, b = 1000 sec/mm^2) demonstrated marked high intensities in the same region.

cognitive impairment, whereas motor impairment was relatively mild. Although these features are similar to those in previous reports [6-13], there are some differences between the patients in previous studies and ours.

One important difference involves the initial neurologic signs. A prolonged seizure or status epilepticus as the initial neurologic sign had been emphasized in this type of encephalopathy [5,10,12]. Yamanouchi and Mizuguchi included the presence of convulsive status epilepticus in the tentative diagnostic criteria of acute infantile encephalopathy predominantly affecting the frontal lobes [13]. However, a prolonged seizure was observed at the onset in only 3 of 9 patients in our cohort. Therefore, we think that the presence of a prolonged seizure or convulsive status epilepticus should not be stressed too strongly, although it is certainly an important neurologic sign at the onset.

Another prominent difference involves the localization of brain lesions. In previous studies, magnetic resonance imaging invariably revealed the involvement of the frontal lobes. Although this was the case in most of our patients, one of them (patient 6) exhibited a unilateral temporo-parieto-occipital lesion. In this patient, regional cerebral blood flow in the frontal lobes was not decreased. The localization of brain lesions will not be limited to the frontal lobes in patients with subacute encephalopathy. Age at onset was also different between our patients and those in previous studies. In previous studies, the age of patients was $\leq$3 years [9,12,13]. On the other hand, 6 of 9 patients were aged >36 months in this study. This finding indicates that subacute encephalopathy can be present in older children as well as in infants and young children.

The most important similarity between our study and previous studies involves poor neurologic outcomes despite a relatively mild loss of consciousness during the early phase. Although no patients were comatose on the next day after onset, moderate to severe cognitive impairment was present as a neurologic sequel in the majority of our patients. This was also the case with patients in previous studies [6-8,10-13]. We consider this to be the most prominent feature of subacute encephalopathy. We must be aware of the presence of this unique type of encephalopathy.

The pathophysiology of subacute encephalopathy remains unknown. Hypoxic brain injury is unlikely, because no patients had overt evidence of profound hypoxia. There have been several studies on the role of cytokines in the development of acute encephalopathy [14-17]. However, the role of cytokines in subacute encephalopathy is uncertain. Serum and cerebrospinal fluid levels of interleukin-6 were lower in patients with acute infantile encephalopathy predominantly affecting the frontal lobes than in patients with influenza-associated encephalopathy. A relatively slow worsening of neurologic signs may be suggestive of delayed neuronal loss caused by prolonged convulsions. Takanashi et al. suggested an accumulation of glutamate because of hyperactivity of glutamatergic neurons, using magnetic resonance spectroscopy in a child with prolonged febrile seizure with encephalopathy [5]. Apoptotic changes were also revealed in patients with acute encephalopathy [18]. However, these findings have not been investigated in children with subacute encephalopathy. Further multidisciplinary studies are necessary to clarify the pathogenesis of subacute encephalopathy.

The diagnostic value of neuroimaging in subacute encephalopathy remains unclear at present. Computed tomography or magnetic resonance imaging within a few days after the onset will be useless. Takanashi et al. indicated that magnetic resonance imaging within 2 days of the onset of encephalopathy revealed no acute lesions in children with prolonged febrile seizures with encephalopathy [5]. Computed tomography or conventional magnetic resonance imaging during the subacute phase will be of limited use. Computed tomography may reveal a blurring of gray-white matter differentiation, and magnetic resonance imaging may demonstrate mildly increased intensities in the subcortical white matter on T_2-weighted and fluid-attenuated inversion-recovery images. However, these changes can be subtle and overlooked. Diffusion-weighted imaging will be useful if it is performed during an appropriate period, as stated in previous studies [5,10,12].

The early diagnosis of subacute encephalopathy is of clinical interest. However, no useful clues have been delineated for such an early diagnosis. Neuroimaging during the early phase will not be useful, as explained above. Laboratory data will not be helpful, because the abnormalities in laboratory data are non-specific and are not pathogomotic in children with subacute encephalopathy. We think that electroencephalograms may be useful in some patients, because electroencephalograms indicated abnormalities in 4 of 7 patients in whom they were performed during the acute phase. Yamanouchi et al. reported that electroencephalograms were recorded at onset in 6 of 9 patients with acute infantile encephalopathy predominantly affecting the frontal lobes, and revealed slow background activity [12]. We think that careful evaluation of consciousness will be also useful for early recognition of subacute encephalopathy. Maegaki et al. reported on acute encephalopathy with a biphasic clinical course, whose clinical features were very similar to those of subacute encephalopathy, in 6 of 16 patients with grade II or III loss of consciousness according to the Japan Coma Scale, 12 hours after onset [8]. Therefore, a combination of electroencephalograms and a precise neurologic examination focusing on responsiveness and wakefulness will be a diagnostic clue in regard to subacute encephalopathy.

In conclusion, "subacute" encephalopathy, characterized by a delayed worsening of neurologic signs, is a distinct subtype of acute encephalopathy. Early recognition is not easy, because the initial neurologic signs are varied, and laboratory data and neuroimaging are unremarkable during the acute phase. Magnetic resonance imaging during the late phase reveals mild cortical atrophy, and single-photon emission tomography indicates hypoperfusion

in the affected regions. The neurologic outcome is poor in most patients. Recognition of subacute encephalopathy is important, and a method to promote early diagnosis is desirable.

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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

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Abstract

We report three rare autopsied cases; one was non-herpetic acute limbic encephalitis (NHALE) and two were so-called acute juvenile female non-herpetic encephalitis (AJFNHE). 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. The pathomechanism of NHALE and AJFNHE is obscure and an 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.

Clinical Findings

Case 1

Four days after fever onset in September 1985, a 43-year-old Japanese woman developed grand mal seizures, which expanded to status epilepticus and the patient was trans-

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ferred to the Geriatric Research Institute and Hospital. At the admission, she showed status epilepticus and several anticonvulsants were not effective and she was controlled under respirator. CSF examinations showed cells 16/mm3, protein 73 mg/dl, glucose 100 mg/dl. EEG showed periodic sharp waves. Brain CT 15 days after the onset showed low densities in the bilateral medial regions of the temporal lobes, however MRI could not be examined at that time. Viral titers in CSF were unremarkable including herpes simplex virus. She died 28 days after the onset.

Case 2

Maeda et al (14) previously reported this patient in a Japanese language journal in 1974, and we reexamined the case pathologically. Three days after common cold-like symptoms in March 1970, a 32-year-old Japanese woman developed confusion, abnormal behavior and automatism. Ten days after the onset, she refused to eat and showed urinary incontinence, forced laughing, tic-like involuntary movement and high fever, and was transferred to our hospital 13 days after onset. Her consciousness was drowsy, then myoclonus and grand mal seizures appeared 17 days after the onset. Status epilepticus and decerebrate posture persisted for 10 days. On admission to Gunma University Hospital, CSF examinations showed cells 67/mm3, protein 25 mg/dl, glucose 75 mg/dl. Virus titers were not examined. EEG showed diffuse high delta activities with 5-6 c/s sporadic theta waves in the parietal regions. She died 26 days after the onset.

Case 3

Eleven days after fever onset and perioral eruptions in September 2003, a 27-year-old Japanese woman developed visual hallucinations and depressive state, and was admitted to the Department of Neurology, Nagoya University Hospital. She showed a moderately high fever, intermittent grand mal seizure without apparent motor palsy. Laboratory data were as follows. Serum CK 2,234 IU/l, TSH 18.09 μU/ml, fT3 2.45 pg/ml, fT4 0.84 ng/dl, anti-thyroid peroxidase antibody 97.36 U/ml and anti-thyroglobulin antibody 14.42 U/ml. Serum autoantibody against alpha-enolase was negative. CSF examinations showed cells 14 /mm3, protein 24 mg/dl, glucose 66 mg/dl. Viral titers in CSF were unremarkable including herpes simplex virus. MRI studies were unremarkable. Pelvic CT was also unremarkable. Steroid pulse therapy was not effective. Generalized seizures were continued, and pancytopenia, septic shock were added. She died of multiple organ failure 50 days after the onset.

Materials and Methods

We examined the brains of the three patients described above and 13 brains of control patients from the Geriatrics Research Institute and Hospital. Ten controls showing hippocampal sclerosis were selected from among 320 serial autopsies files, and patient ages ranged from 54 to 90 years, and survival durations ranged from 17 days to 10 months after acute respiratory failure. And another 3 cases without pathologic cerebral changes including hippocampus were also examined. In all cases, the autopsies were performed in accordance with established procedures and the samples were used in this study after obtaining informed consent from the family of each patient.

Brains were fixed in 4% paraformaldehyde in phosphate-buffered solution (PBS) (pH 7.4) and multiple sections including the hippocampus were embedded in paraffin. Five micrometer thick sections were examined by H-E and K-B staining, and were also immunostained, which was carried out using a polyclonal rabbit anti-GFAP antibody (1 : 1,000, Dako, Denmark), monoclonal mouse anti-phosphorylated neurofilament (SM1 31) (1 : 10,000, Sternberger, USA), monoclonal mouse anti-synaptophysin antibody (1 : 200, Chemicon, USA), polyclonal rabbit anti-herpes simplex virus type 1 (HSV-1) antibody (1 : 800, Dako, Denmark), monoclonal mouse anti-human CD68 antibody (1 : 200, Dako, Denmark). CD68 antibody labels macrophages and other members of monoclonal phagocytes. For enhancement, autoclave treatment for 5 minutes was performed for synaptophysin and CD68. Sections were blocked in normal serum for 30 minutes at room temperature, then labeled with the first antibody at 4℃ overnight, washed in PBS for 30 minutes, incubated with the second antibody provided by Histofine SAB-PO kit (Nichrei, Japan), washed in PBS for 30 minutes, and finally visualized by the avidin-biotonperoxidase method.

Pathological Findings

Case 1

Brain weight was 1,190 g, and macroscopic findings were unremarkable. Microscopically, there were no lymphocyte infiltrations in the meninges or brain parenchyma, and there were no infarcts or demyelination either. Neurons in the CA 1 (15) were markedly lost, and astrocytic gliosis, spongiosis (Fig. 1), however, there were no anoxic changes in the remaining neurons (Fig. 2), and binucleated astrocytes were rarely seen (Fig. 2). Hippocampal granular neurons were also lost with astrocyte proliferations. There were no neuronophagia or perivascular lymphocytic infiltrations in the hippocampal areas. CD68 immunostaining showed increased microglia/macrophages in the hippocampal areas. HSV-1 immunostaining was negative, and synaptophysin were relatively well preserved. Astrocyte proliferations and microglia/macrophage infiltrations were not apparent in the cerebrum (Fig. 3A), however those changes were clearly present in the claustrum (Fig. 3B, 3C) and mildly in the basal ganglia.

There was no tumor in the general organs including ovary.

Case 2

Brain weight was 1,200 g and the only macroscopically

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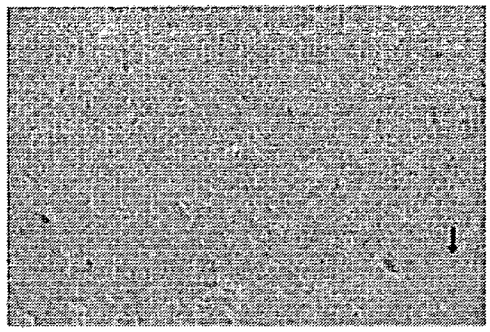

Figure 1. Low magnification of hippocampal CA1 area in Case 1. Neuronal loss with astrocyte proliferations and spongiosis were apparent in CA1. Perivascular lymphocytic infiltrations were not observed. Hematoxylin and Eosin staining, ×40.

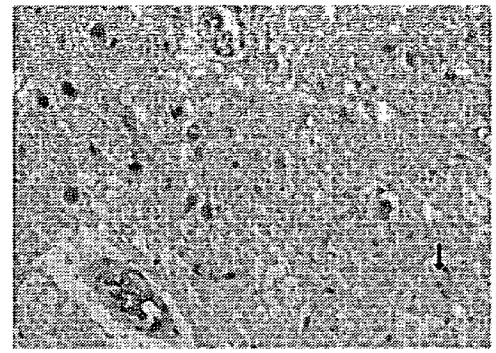

Figure 2. High magnification of right low corner of Figure 1. There were few anoxic changes in the remaining neurons, and binucleated astrocytes were rarely seen (binucleated astrocyte shown by the arrows was the same in Figs. 1 & 2). Hematoxylin and Eosin staining, ×200.

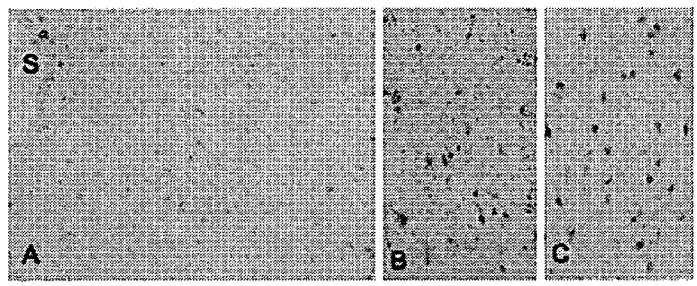

Figure 3. Insular cortex and claustrum in a same section of Case 1. There were a few CD68-positive microglia/macrophages in the insular cortex (A). However, CD68-positive microglia/macrophages (B) and GFAP-positive astrocytes (C) were abundant in the claustrum. S, subarachnoid space; A, ×100; B, ×200; C, ×200.

abnormal finding was brain swelling. There was no necrosis or bleeding. Mild lymphocytic infiltrations were observed in the subarachnoid spaces throughout in the cortices, brain stem and cerebellum (Fig. 4A, 4B). In the parenchyma, perivascular lymphocytic infiltrations were also seen in the superficial layers of the cortices (Fig. 4A), in the basal ganglia and in the Ammon's horns (Fig. 4B). In the Ammon's horns, neurons were relatively well preserved and there was no gliosis but limited neuronophagia was seen in the CA1 area (Fig. 4C). Microglia/macrophage infiltrations were apparent (Fig. 4D); however, there was no gliosis in those areas. Hippocampal granular neurons were well preserved. Diffuse microglia/macrophage infiltrations were observed throughout in the cerebral cortices. HSV-1 immunostaining was negative. Bilateral soybean-sized cysts were seen in the ovary, however histological examinations did not show teratoma.

Case 3

Brain weight was 1,276 g and the macroscopic findings were unremarkable. Histologically, the brain showed slight edematous and many small pericapillary bleeding, however, there was no necrosis, vasculitis or intranuclear inclusion. Mild lymphocytic infiltrations were seen around the small vessels in the cortices (Fig. 5A) and in the subarachnoid spaces. Lymphocytic infiltrations were somewhat predominant in the frontal lobe, however mild lymphocytic infiltrations were also seen in the basal ganglia, brain stem and cerebellum. Microglia/macrophages diffusely infiltrated the cerebral cortices (Fig. 5B). Neurons in the hippocampal areas were well preserved (Fig. 5C), and microglia/macrophages were diffusely infiltrated in the hippocampal areas (Fig. 5D) without gliosis. HSV-1 immunostaining was negative.

Hippocampal sclerosis

In our 10 patients with hippocampal sclerosis, many remaining neurons in CA1 areas showed anoxic features such as eosinophilic atrophic changes in the earlier stages, and marked neuronal loss with gliosis in the advanced stages.

Discussion

Because many previously reported cases of NHALE have shown a rather favorable prognosis, only a few autopsied patients have been reported. Mochizuki et al (8) reported a

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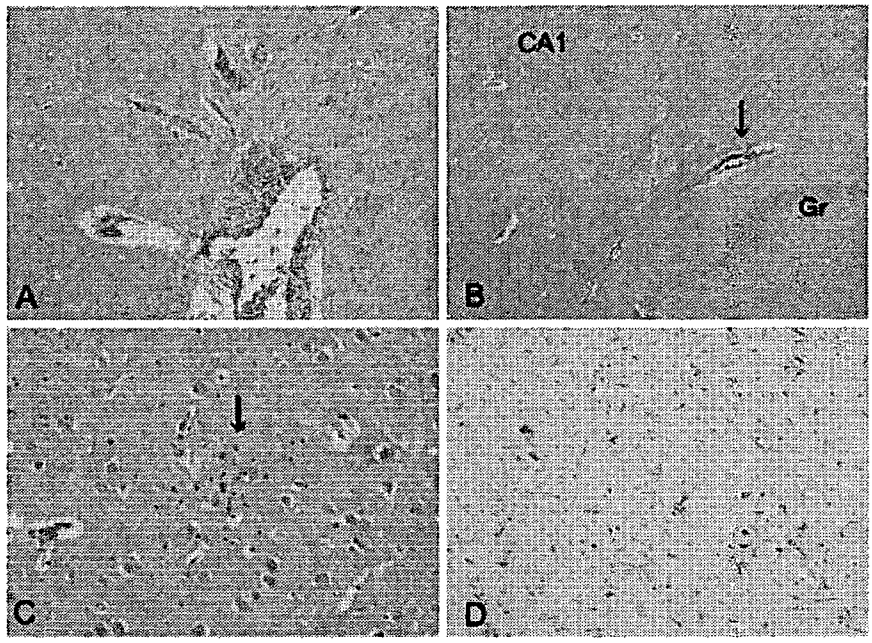

Figure 4. Lymphocytic infiltrations were seen in the subarachnoid spaces and in the perivascular spaces of the superficial cortices (A) and in the hippocampal areas (arrow, B) in Case 2. A few neuronophagia were seen in the CA1 area (arrow), and rod-shaped CD68-postive cells were abundant (D), but there were few GFAP-positive astrocytes (not shown). C and D were almost same areas in serial sections. Gr: granular cell layer. A, Hematoxylin and Eosin staining×100; B, ×40; C, ×200; D, Hematoxylin and Eosin staining×200.

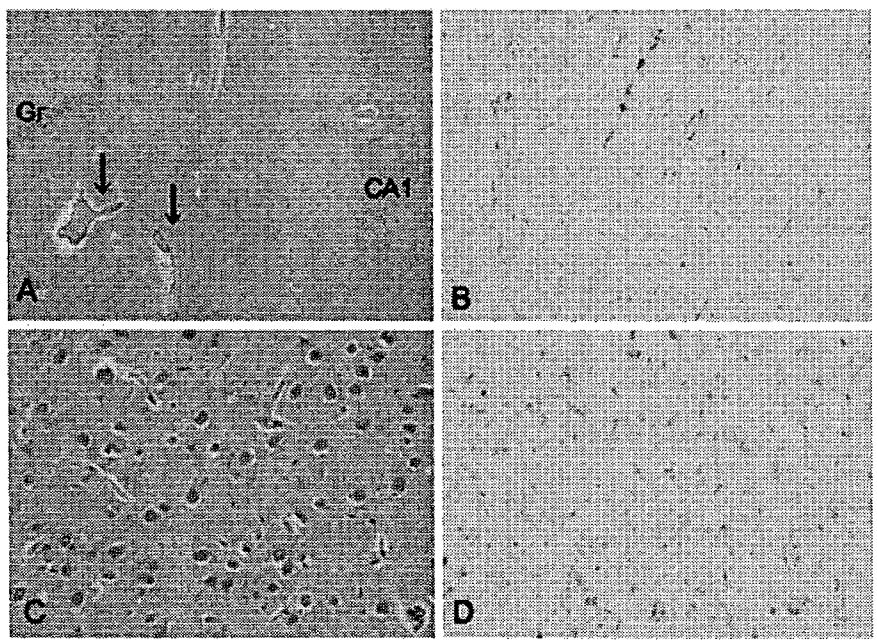

Figure 5. Perivascular lymphocytic infiltrations were seen in the molecular layers of the hippocampus (arrows, A), and CD68 positive microglia/macrophage were increased in the cortex (B) in Case 3. Neurons were well preserved in CA1 (C) with abundant CD68-positive cells (D). C and D were almost same areas in serial sections. Gr, granular cell layer; A, Hematoxylin and Eosin staining×40 ; B, ×200; C, Hematoxylin and Eosin staining×200; D, ×200.

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59-year-old woman with disturbance of consciousness, uncontrolled generalized seizures, and abnormal MRI signals in the bilateral medial temporal lobe and along the lateral part of the putamen. She died 12 days after onset. Autopsy examination demonstrated scattered foci consisting of neuronal loss, neuronophagia and some perivascular lymphocytic infiltrations in the hippocampus and amygdala. However, there was no hemorrhagic necrosis in the brain and HSV was also immunohistologically negative. They suggested that their patient showed neuropathological changes of NHALE as a possible new clinicopathological entity. Another similar patient was reported in an abstract form. Briefly, Maki et al (16) reported a 53-year-old woman who died 36 days after the onset of illness and showed abnormal MRI findings in the hippocampus and amygdala. She developed generalized seizures and status epileptics and finally multiple organ failure. Autopsy disclosed marked neuronal loss and gliosis mainly in the CA1 areas and amygdala without lymphocytic infiltrations and necrosis in the brain.

Our Case 1 is similar to the two patients described above with regard to clinical features and pathological findings mainly limited to the hippocampal areas. Classical hippocampal sclerosis in which neuronal loss is most severe in CA1 accompanied by gliosis may be induced by many causes, such as epilepsy, stroke, cardiopulmonary arrest, encephalitis and neurodegenerative diseases (17-19). In our Case 1 and that reported by Maki et al (16), the pathology was similar to hippocampal sclerosis without inflammatory changes, however the pathomechanism remains obscure. One possibility is that the two patients showed more prolonged courses than the case of Mochizuki et al (8), so the inflammations might be subsided. The second possibility is that the hippocampal lesions were caused by severe seizures. Misumi et al (20) reported a 30-year-old man with sudden onset seizure showing abnormal MRI signal in the right medial temporal lobe, and brain biopsy showed edema without specific abnormalities and they suggested that secondary brain edema induced by seizure must be considered. Seizure-induced transient brain edema is not rare in the temporal lobe, and these findings may reflect transient cytotoxic and vasogenic edema induced by seizure (21-24). The majority of NHALE patients showed severe generalized seizures or status epilepticus, so we must carefully consider this possibility when abnormal MRI findings are seen in the medial regions of the temporal lobe. In our 10 patients with hippocampal sclerosis, many remaining neurons in CA1 areas showed anoxic features such as eosinophilic atrophic changes. However, the remaining neurons in CA1 of our Case 1 did not show such eosinophilic atrophic changes, therefore the hippocampal changes may not be simply caused by anoxia. More studies are needed to consider the pathogenesis of the hippocampal lesions.

Claustrum frequently showed abnormal MRI findings in NHALE cases (11, our cases: data not shown), and astrocytic proliferations and microglia/macrophage infiltrations observed in the claustrum in our Case 1 may correlate with those abnormal MRI findings.

Kamei (12) proposed a new clinical entity named acute juvenile female non-herpetic encephalitis (AJFNHE), and the characteristics of AJFNHE were defined as follows: 1) a clinical profile of encephalitis with psychosis, disturbance of consciousness, and/or convulsion, 2) progression to coma and status epilepticus, 3) a prolonged clinical course, 4) a relatively good long-term outcome despite a severe clinical course in the acute stage, 5) a predilection for juvenile females, 6) a lack of abnormal intensity on cranial MRI, 7) negative data for HSV infection. Clinically, our Cases 2 and 3 were almost consistent with AJFNHE criteria, however no MRI was done in Case 2. Case 3 showed hypothyroid laboratory data with positive anti-thyroid peroxidase and anti-thyroglobulin antibodies, therefore we must differentiate Hashimoto's encephalopathy. Hashimoto's encephalopathy has been recognized as rare clinical entities and characterized by progressive or fluctuating neurological symptoms, and response to corticosteroid treatment is universally excellent (25, 26). Postmortem examination demonstrated mild perivascular lymphocytic infiltration throughout the brain and leptomeninges plus diffuse gliosis of gray matter in the cortex and basal ganglia, and to a lesser extent, the parenchymal white matter (25). Recently, Fujii et al (27) reported that autoantibodies against the amino terminal of alpha-enolase are a useful diagnostic marker for Hashimoto's encephalopathy. Clinical courses with untreatable status epilepticus, the lack of a steroid therapy and the absence of autoantibody against alpha-enolase may be different from those in Hashimoto's encephalopathy.

Our Cases 2 and 3 differed from Case 1 with regard to 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 and in the parenchyma. HSV infections were ruled out because of the lack of hemorrhagic necrosis, intranuclear inclusions and negative HSV on the immunohistological study. These mild inflammatory changes with diffuse microglia/macrophages activation in the brain might be the main pathological findings in our Cases 2 and 3, and the pathological findings suggest the mild viral infectious or postinfectious state in the CNS. Relationship between NHALE and AJFNHE is obscure, however both diseases seem to be different in some points. Especially, NHALE showed more limited pathology in the limbic system, whereas AJFNHE showed widespread pathology with microglia/macrophage activation. N-methyl-D-aspartate glutamate receptor epsilon 2 (GluR ε2) is frequently found in the serum and CSF in both disorders, suggesting an autoimmune mechanism (10, 28). Recently, Dalmau et al (3) reported paraneoplastic anti-N-methy-D-aspartate (NMDA) receptor encephalitis associated ovarian teratoma. Tumor resection and immunotherapy resulted in improvement or full recovery of eight of nine patients. Two of three patients without tumor resection died of neurological deterioration. Two autopsies showed extensive microgliosis, rare T-cell in-

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filtrates, and neuronal degeneration predominantly involving, but not restricted to the hippocampus. Similar extensive microgliosis were also seen in our Cases 2 and 3. We have to collect and examine many autopsied patients to order to clarify the pathomechanism. More recently, Iizuka et al (29) reported that 4 Japanese women diagnosed with AJFNHE showed positive against antibodies to NR1/NR2 heteromers of NMDA receptor in serum or CSF, and their findings indicate that majorities of AJFNHE in Japan may anti-NMDA receptor encephalitis.

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Original article

Serum levels of cytokines and EEG findings in children with influenza associated with mild neurological complications

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Abstract

We studied the relation among serum cytokine levels, EEG changes, and mild neurological complications (delirium and febrile seizure) in children with influenza. The serum levels of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and soluble tumor necrosis factor receptor-1 (sTNFR-1) were measured in 27 children with proven influenza infection with mild neurological complications (10 patients with delirium and 17 with febrile seizures) and seven control children. EEG was recorded in 14 children with neurological complications. EEG showed focal slowing in four of nine patients with delirium and in four of five with febrile seizures. Generalized slowing was observed in one patient with delirium. The median serum IL-6 level was 31.2 ± 15.1 pg/ml (range, 7.5–64.5 pg/ml) in the delirium group, 42.3 ± 44.0 pg/ml (range, 8.0–196.0 pg/ml) in the febrile seizure group, and 15.4 ± 7.0 pg/ml (range, 7.2–28.0 pg/ml) in the control group. Serum TNF-α and sTNFR-1 levels were not different among three groups. Mild neurological complications associated with influenza were related to the mildly abnormal serum IL-6 levels and EEG findings. The combination of these parameters will be useful for early diagnosis and differentiation of neurological complications in children with influenza. Further studies will be necessary for investigating that IL-6 has the diagnostic value for differentiation between severe encephalopathy and mild neurological complications in children with influenza.

Keywords: Interleukin-6; Influenza; Electroencephalography

1. Introduction

Influenza-associated encephalopathy often results in death or neurological sequelae. Early recognition of influenza-associated encephalopathy is desirable in order to improve the outcome of the patients. Delirium is sometimes seen during the early stage of influenza-associated encephalopathy, before consciousness is markedly reduced. It can be an alarming sign and a clue to an early diagnosis. However, febrile delirium is often observed in children without encephalopathy [1–5]. It would be helpful if we could distinguish patients with delirium evolving into encephalopathy from those without encephalopathy.

The mechanism of delirium is poorly understood. Several reports have examined the relationship between influenza-associated encephalopathy and cytokines [6–11]. However, few studies have examined the relationship between cytokines and mild neurological complications such as delirium and febrile seizure. Therefore, we

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studied the relation among serum cytokine levels, electroencephalogram (EEG) changes, and neurological complications in children with influenza.

2. Patients and methods

The study subjects were 27 children with proven influenza infection with mild neurological complications who consecutively visited Okazaki City Hospital during the winters of 2002–2004. The control patients were seven patients with proven influenza without neurological complications who agreed to participate in the study. Influenza antigen was detected from pharyngeal swabs in all patients. Blood was sampled for measuring cytokines as soon as possible after the influenza infection was confirmed.

The serum levels of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and soluble tumor necrosis factor receptor-1 (sTNFR-1) were measured within 48 h after the onset of fever in all but two children. When the children had neurological complications, cytokines were measured within 24 h after their occurrence. The concentrations of serum IL-6 were measured using a two-step sandwich-type chemiluminescent enzyme immunoassay; serum TNF-α and sTNFR-1 were measured using a quantitative sandwich-type enzyme-linked immunosorbent assay.

The children with mild neurological complications were divided into two patient groups. The delirium group (group De) contained the patients with delirious behavior, such as fear, hallucinations, and disorientation, and recovering consciousness within 24 h. Some of these patients also had seizures. The febrile seizure group (group FS) was defined as patients with febrile seizures without delirium or impaired consciousness. The control group consisted of seven patients without neurological complications.

In addition, EEG and measurement of serum cytokines were performed in two patients with influenza-associated encephalopathy during the same period. Encephalopathy was defined as impaired consciousness lasting for more than 24 h with or without other neurological symptoms such as seizures. Those with vascular, metabolic, endocrine, or toxic disorders were not included into encephalopathy. Because the number of patients with encephalopathy was small, we excluded these patients from statistical analyses.

EEGs were recorded 4–72 h after the appearance of neurological complications. EEG findings during wakefulness were classified into three categories (Fig. 1): normal, focal slowing defined as the insertion of high-voltage slow waves mainly in the occipital regions with preserved rhythmic alpha activities, and generalized slowing defined as generalized high-voltage slow waves without rhythmic alpha activity. In all of the patients with generalized slowing, EEG findings were not altered by opening and closing the eyes.

This study was approved by the Ethics Committee of Okazaki City Hospital. Written informed consent was obtained from the parents of each child.

Statistical analysis was performed using StatView software. With regard to the patient characteristics, the Kruskal–Wallis test was used for numerical variables and the χ-square test was used for categorical variables. The Kruskal–Wallis test was also used to analyze the serum cytokine concentration among three groups. If significant differences were found, Tukey's test was performed as a post hoc test. A p value below 0.05 was considered statistically significant.

3. Results

The characteristics of the patients are summarized in Table 1. Ten patients were categorized in group De and 17 in group FS. In group De, four of them also had a febrile seizure. Although there was no significant difference, the majority of the children in group De was male and used antipyretics before they developed a neurological complication. The manifestations of febrile delirium included meaningless speech, periodic crying without an apparent trigger, blank eyes, and hallucinations, such as "My mother has grown a beard", "My sister is running around", and "These are not human hands".

EEGs were obtained in nine patients in group De and five in group FS (Table 1). In 11 cases, the EEG was recorded within 36 h after the neurological complication appeared. In four patients with both delirium and a seizure, EEGs were recorded 10–33 h after the appearance of a seizure. Focal slowing was seen in four patients each in groups De and FS. Generalized slowing was observed one patient in group De. A 2-year-old boy in group De whose EEG showed generalized slowing had a seizure after he developed delirious behavior. Impaired consciousness lasted for 5 h. EEG was recorded 36 h or more after the onset of neurological complications in three of five patients with normal EEG.

The relation between neurological complication and the serum cytokine levels is shown in Fig. 2. The median serum IL-6 level was 31.2 ± 15.1 pg/ml (range, 7.5–64.5 pg/ml) in group De, 42.3 ± 44.0 pg/ml (range, 8.0–196.0 pg/ml) in group FS, and 15.4 ± 7.0 pg/ml (range, 7.2–28.0 pg/ml) in the control group. The serum IL-6 level differed significantly between group De and the control group and between group FS and the control group (each $p < 0.05$). The serum IL-6 level reached 196 pg/ml in one child in group FS. His seizure was a generalized one lasting 5 min, and no other neurological abnormalities were observed.

Serum TNF-α level was less than 5 pg/ml in most patients in the De, FS, and control groups. A remark-

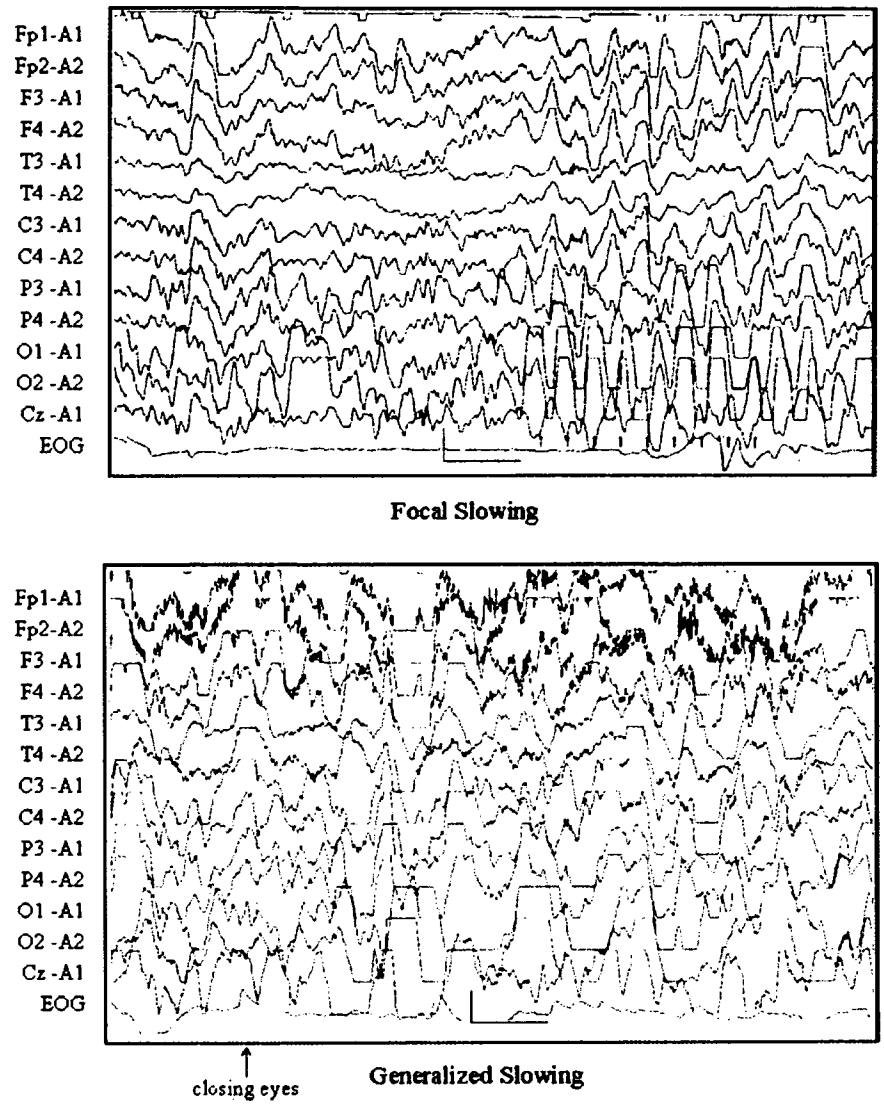

Fig. 1. EEG findings. (Upper) Focal slowing. Insertion of slow waves in the occipital regions with preserved rhythmic alpha activities was observed. (Lower) Generalized slowing. Generalized high-voltage slow waves without rhythmic alpha activities were recognized.

Table 1
The clinical characteristics, EEG findings, and serum IL-6 levels of the patients

	Delirium ($n = 10$)	Febrile seizure ($n = 17$)	Control ($n = 7$)
Age (years)	2–6 (4.3)	0.75–7 (2.7)	0.25–10 (3.2)
Sex (M:F)	9:1	11:6	2:5
Body temperature (°C)	38.4–41.0 (40.0)	38.9–41.0 (40.0)	37.8–40.0 (39.1)
Interval from the onset of fever (h)	0–36 (20.6)	0–25 (11.7)	
Seizure	4 (40%)	17 (100%)	0
Use of antipyretics	7 (70%)	4 (24%)	2 (29%)
Use of antiviral drug	5 (50%)	6 (35%)	1 (14%)
EEG findings			
Generalized slowing	1	0	
Focal slowing	4	4	
Normal	4	1	

The values were presented as range (median).

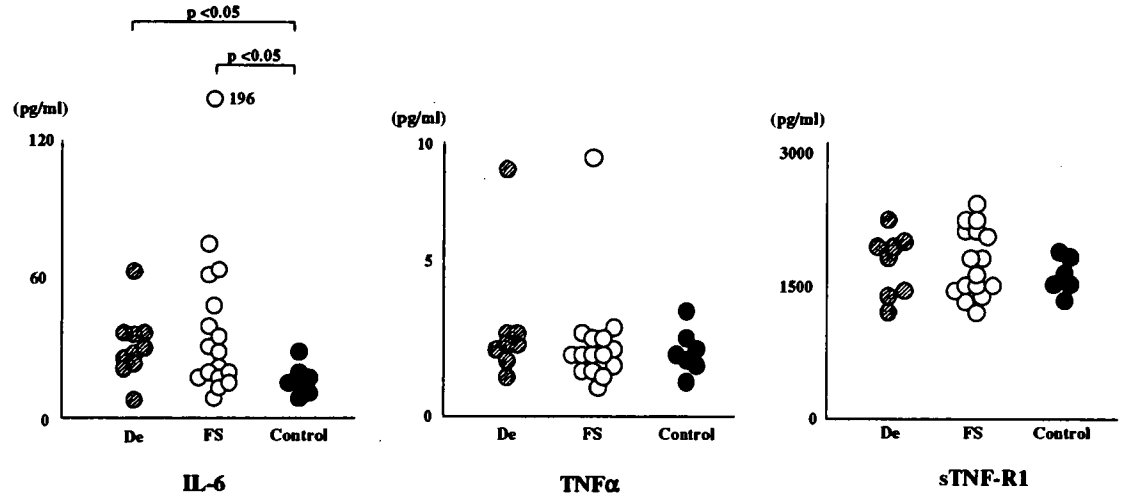

Fig. 2. Neurological complications and serum cytokines levels. The serum IL-6 level differed significantly between group De and the control group and between group FS and the control group (each $p < 0.05$). De, patients with delirium; FS, patients with febrile seizures.

able elevation of serum sTNFR-1 levels was not observed in any patients. There were no significant differences in the serum TNF-α and sTNF-R1 levels among these groups.

As to patients with encephalopathy, one of them was a 1-year-old boy with clustered seizures followed by delirium and impaired consciousness. EEG showed generalized slowing and serum IL-6 level was high (117 pg/ml). TNF-α and sTNF-R1 were not measured. The other was a 4-year-old boy who had a prolonged seizure as the first neurological manifestation. EEG revealed generalized slowing. He developed deep coma and severe brain edema within 5 days after admission, and died 3 weeks later. His serum IL-6 level was markedly elevated to 262 pg/ml, whereas sTNF-R1 was not increased (2570 pg/ml). TNF-α was not measured.

The relation among the severity of the EEG abnormality, the serum IL-6 concentration, and neurological complications is shown in Fig. 3. Although there was no statistical difference among the three groups, serum IL-6 level was the highest in infants with generalized slowing (median 117 pg/ml, range 38.4–262 pg/ml) and was the lowest in those with normal EEG (median 28.3 pg/ml, range 7.5–38.4 pg/ml). Serum IL-6 level was in-between in infants with focal slowing (median 32 pg/ml, range 14.8–75.4 pg/ml).

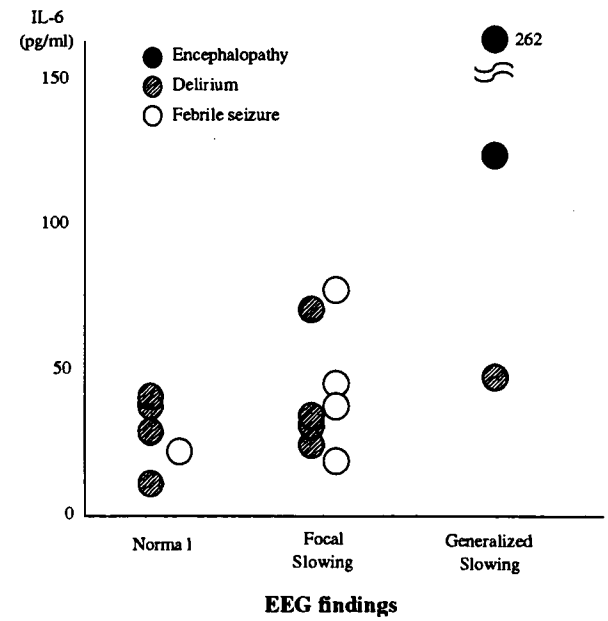

Fig. 3. The relation among the serum IL-6 levels, EEG findings, and neurological complications.

4. Discussion

The pathophysiology of hypercytokinemia in influenza-associated encephalopathy is poorly understood. It has been postulated that mucosal influenza infection triggers hypercytokinemia, followed by the activation of brain astrocytes and microglia. This may result in increased production of cytokines in the brain [14,15]. IL-6 is thought to be an indicator of the severity of influenza-associated encephalopathy [6–9]. TNF-α is a potent tissue-damaging cytokine and is elevated in patients with influenza-associated encephalopathy with poor prognosis [6,8,9]. TNF-α can be underestimated because it is unstable. On the other hand, the concentration of sTNF-R1, an inhibitor of TNF-α, reflects the bioactivity of TNF-α [16,17]. We chose to study these cytokines because they are thought to participate in the pathogenesis of neurological complications associated with influenza.

Our study revealed that IL-6 was mildly elevated in some children with delirium and febrile seizures. There have been a few reports that serum IL-6 is increased slightly in febrile seizures [18–20], whereas there have been no reports of the cytokine levels in febrile delirium. This suggests that IL-6 participates in the development of febrile delirium, as well as in influenza-associated encephalopathy. Delirium usually appears when patients have a high fever. Therefore, one can postulate that it results from intrinsic neuronal responses to elevated body temperature or immune responses to influenza. However, our previous study showed that some patients had delirium even after fever subsided with the use of antipyretics [4,21]. Therefore, we consider that humoral changes involving cytokines are more closely related to delirium than to high body temperature itself, although little is known of the pathophysiology of delirium during febrile illness. A mild elevation of cytokines might cause relatively mild neurological symptoms, such as convulsions and delirium.

Delirium can be observed during the very early phase of influenza-associated encephalopathy and can be an alarming sign. However, delirium is observed in some febrile children without encephalopathy. We previously demonstrated that the EEG was useful for distinguishing between children with encephalopathy and those with delirium without encephalopathy [4]. Unfortunately, an EEG is not always available, and its interpretation is not always easy. Therefore, the serum IL-6 level can be helpful for the diagnostic evaluation of patients with neurological complications.

In this study, some children with febrile delirium exhibited mild EEG changes, in agreement with previous reports [3,4]. We reported that the EEG showed slow waves in the occipital regions in 13 of 15 children with febrile delirium [4]. Similar EEG findings have been reported in children [3] and even in adults with febrile delirium [12,13]. It is interesting that similar EEG changes were also observed in some children with a febrile seizure. We observed focal slowing in four of five children with a febrile seizure. This implies that mild EEG changes, such as focal slowing, are common and non-specific in febrile children with mild neurological complications. Conversely, generalized slowing was observed in only three patients, two of whom were diagnosed with encephalopathy. Therefore, generalized slowing on EEG is a clue to the early diagnosis of encephalopathy in children with influenza or other febrile illness. On the other hand, four children with delirium had normal EEG. EEG was recorded 72 h after the appearance of delirium in two of them. EEG abnormalities in these patients may have been mild, if present, and have disappeared before EEG was undertaken. This indirectly suggests that an elevation of serum IL-6 levels was mild in patients with mild EEG abnormalities. However, EEG immediately after an appearance of delirium will be desirable in order to obtain its optimal diagnostic value.

The results of our study suggest that the combination of EEG and serum IL-6 level may enhance a diagnostic value. Patients with delirium or febrile seizure had mild EEG abnormalities and a mild increase in the serum IL-6. Patients with encephalopathy showed severe EEG abnormalities and markedly elevated serum IL-6. We might be able to predict a poor outcome, when a patient shows both severe EEG abnormalities and a markedly elevated serum IL-6 level.

In this study, patients with delirium were grouped together whether they had had a febrile seizure or not. This is because the differentiation between delirium with and without encephalopathy will be quite important apart from the presence or absence of a febrile seizure. In addition, our previous study suggested that clinical features and EEG findings in patients with delirious behavior were not different between those with and without a febrile seizure [4]. EEGs in our patients were recorded at least 4 h after a febrile seizure. Therefore, EEGs were not likely to be affected by a seizure.

The major shortcoming of this study is the insufficient statistical analysis of the serum cytokine levels, EEG findings, and neurological complications, although there were significant differences in the serum IL-6 level between the control group and groups De and FS. The number of patients was small and interval from the onset of symptom to EEG or blood sampling was not uniform. Our study is the first to suggest a correlation involving the serum cytokine levels, EEG findings, and mild neurological complications. Further studies with more patients with encephalopathy are necessary to clarify this relationship.

In conclusion, mild neurological complications associated with influenza were related to the mildly abnormal serum IL-6 levels and EEG findings. The combination of these parameters will be useful for the early diagnosis and differentiation of neurological complications in children with influenza. Further studies will be necessary for investigating that IL-6 has the diagnostic value for differentiation between severe encephalopathy and mild neurological complications in children with influenza.

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