In conclusion, salivary CgA may be a useful and quantitative biochemical marker of the affective state, not only in moderate, but also in terminal ALS patients. Periodic salivary CgA measurements could have therapeutic implications for the QOL of these patients.

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Review Article

Bunina bodies in amyotrophic lateral sclerosis

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Bunina bodies, which are small eosinophilic intraneuronal inclusions in the remaining lower motor neurons, are generally considered to be a specific pathologic hallmark of amyotrophic lateral sclerosis (ALS). One year before a publication by Bunina, van Reeth *et al.* described similar intracytoplasmic inclusions in the anterior horn cells in a patient with Pick's dementia with atypical ALS. At present, only two proteins have been shown to be present in Bunina bodies, one is cystatin C and the other is transferrin. Bunina bodies consist of amorphous electron-dense material surrounded by tubular and vesicular structures on electron microscopy. Although the nature and significance of Bunina bodies in ALS are not yet clear, the bodies may be abnormal accumulations of unknown proteinous materials.

Key words: amyotrophic lateral sclerosis, Bunina body, cystatin C, motor neuron disease, transferrin.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is pathologically characterized by the presence of Bunina bodies, skein-like inclusions, Lewy body-like round inclusions, and basophilic inclusions in the remaining anterior horn cells in the spinal cord. Among them, Bunina bodies, which are small eosinophilic intraneuronal inclusions in the remaining lower motor neurons, are generally considered to be a specific pathologic hallmark of ALS. It is usually said that Bunina bodies were first described by Bunina in 1962 in two cases of familial ALS,[1] and later were observed in classical ALS patients and in ALS patients in Guam.[2] Although their morphological structures are well known, their nature, origin and significance remain unclear. Here, we review the

morphology of Bunina bodies based mainly on light microscopic, immunohistochemical and electron microscopic findings.[3–5]

HISTORICAL REVIEW OF BUNINA BODIES

In 1962, Bunina,[1] in the USSR, described the presence of intracellular inclusions in the motor neurons of the spinal cord and of the brain stem in two familial ALS cases, and she suspected that the inclusions might be a neurotrophic virus. One year before publication by Bunina, van Reeth *et al.*[6] described similar intracellular inclusions in the anterior horn cells in a patient with Pick's dementia with atypical ALS. The morphology of the inclusions in their paper seemed to be identical to Bunina-type inclusions, showing clear areas in the center and forming chain-like clusters. In 1963, Zil'ber *et al.*[7] described intracellular inclusions in anterior horn cells in monkeys that were given an intracerebral inoculation prepared from the spinal cords of ALS patients. Through arrangements made by the USSR–USA Scientific Exchanges Program,[2,8] Hirano[2] examined specimens from spinal cord of two human ALS cases and two experimental monkeys reported by Zil'ber *et al.*[7]; however, he did not disclose appreciable neuronal degeneration characteristic of ALS. Furthermore, Gibbs and Gajdusek[9] failed to produce neurological symptoms after inoculations with materials derived from several types of ALS. Hirano *et al.*[10] reported that similar eosinophilic inclusions were often found in Guamanians, familiar and classical ALS, and are not limited to any particular form of ALS. While it is not clear who first called these intracellular inclusions Bunina bodies, in 1967, Hirano *et al.*[10] used this term in their review of the pathologic findings in ALS. In 1977, Hart *et al.*[11] first described the ultrastructural features of Bunina bodies, and many researchers have subsequently investigated Bunina bodies. In 1993, Okamoto *et al.*[12] reported that Bunina bodies were immunostained with anticystatin C serum, and in 2006, Mizuno *et al.*[13] described that transferrin localizes in Bunina bodies.

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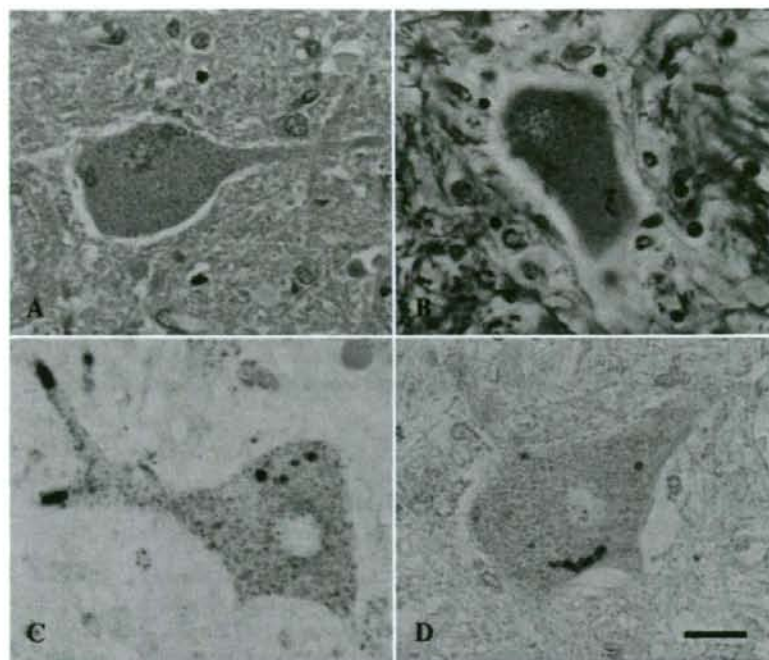
Fig. 1 Bunina bodies in the anterior horn cells in the lumbar cord of amyotrophic lateral sclerosis. (A) HE staining; (B) Klüver-Barrera staining; (C) cystatin C immunoreactivities are seen in the Bunina bodies in an anterior horn cell and its dendrites; (D) transferrin immunoreactivities are seen in the Bunina bodies. (Scale bar = 20 μm).

LIGHT MICROSCOPY

Bunina bodies are readily visualized with HE staining as bright pink, small, round, oval eosinophilic intraneuronal inclusions, sometimes showing clear areas in the center, and forming clusters[14-19] (Fig. 1A). They average 3–5 microns in diameter and the number varies in each neuron. The bodies are seen not only in the cytoplasm but also in the dendrites;[12,20] however, the bodies are not seen within the axoplasm. The bodies are bright purple granules in semi-thin sections stained with toluidine blue.[21]

Histochemical examinations showed that Bunina bodies are purple on phosphotungstic acid-hematoxylin (PTAH) staining, light blue on Klüver-Barrera staining (Fig. 1B), and red on Masson trichrome; however, they are negative for silver staining, PAS, Sudan black B and Congo red, and the bodies do not show autofluorescence and metachromasia for toluidine blue.[15,16] Tomonaga *et al.*[15] described that the bodies were red on methyl green-pyronine staining, but they were negative for methyl green-pyronine staining in our study.

Bunina bodies have been found in subtypes of ALS; however, the bodies were absent in a subset of familial ALS with posterior column involvement and in motor neuron disease with basophilic inclusions. Bunina bodies are present in almost all patients with sporadic ALS in our series, and Piao *et al.*[22] detected them in 88 cases from 102 autopsy cases of ALS. The bodies have been more fre-quently seen in the lumbar cord than in the cervical and thoracic cords, and are frequently seen in patients with relatively short disease duration, and ALS patients with dementia tend to have more and larger Bunina bodies in the lower motor neurons than do classical ALS patients.[3-5] Bunina bodies are mainly distributed in the motor neurons in the spinal cord and of the brain stem (nuclei ambiguus, hypoglossus, facial and motor trigeminus), and rarely in the Betz cells,[23,24] in the neurons of the oculomotor nuclei,[25] and Onuf's nuclei.[26,27] It has been reported that intracytoplasmic inclusions resembling or identical to Bunina bodies were observed in non-motor neurons of Clarke's column,[28-30] the intermediolateral nucleus[30] of the spinal cord, which consists of autonomic nerve cells, in the medullary reticular formation,[31,32] in the locus ceruleus,[33] and in neurons of the subthalamic neurons[34] in an unusual case of ALS.

IMMUNOHISTOCHEMISTRY

Despite immunohistochemical studies using many antibodies, at present, only two proteins have shown as present in Bunina bodies: one is cystatin C[12] and the other to be transferrin.[13] Other antibodies against neurofilament, tau, alpha- and beta-tubulin, microtubule-associated proteins, actin, myosin, desmin, synaptophysin, amyloid precursor protein, glial fibrillary acidic protein, alpha-synuclein[35] and p62[36] failed to demonstrate Bunina bodies.

Ubiquitin-positive inclusions such as skein-like inclusions or Lewy body-like/round inclusions are another hallmark of ALS; therefore, results of immmunorectivites of Bunina bodies against ubiquitin are important. Usually, Bunina bodies were negative for ubiquitin;[37] however, a few researchers described that a small percentage of Bunina bodies show positive immunoreactivites for ubiquitin.[21,38] Murayama *et al.*[21] described that antiubiquitin antibody recognized an ill-defined structure in or around some Bunina bodies (Bunina body-related structure). Recently, the TAR DNA-binding protein of 43 kDa (TDP-43), a nuclear protein that is involved in transcriptional repression and alternative splicing, was identified as a major component of neuronal intracytoplasmic inclusions in motor neurons in ALS, as well as in frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U).[39] Skein-like and Lewy body-like/round inclusions in ALS are positive for TDP-43; however, Bunina bodies are negative.[40] Bunina bodies were also negative for p62 of ubiquitin-related protein.[36]

Cystatin C, with a molecular weight of 13 260 Da, is a protein inhibitor of lysosomal cysteine protenases. Cystatin C is present in low concentrations in various extracellular fluids in humans, and is also present within human cortical neurons.[41,42] The main physiological function of cystatin C is local regulation of cystein proteinase. Immunohistochemical studies were performed with a polyclonal rabbit antiserum against human cystatin C and sections were stained by the adivin-biotin-peroxidase method.[12] Many small immunostained granules were scattered in almost all of the neurons and their dendrites in the spinal cord. Sequential staining of the same sections with HE and an anticystatin C antibody revealed that Bunina bodies were clearly labeled with anticystatin C antibody (Fig. 1C). The cytoplasmic and dendritic cystatin C-positive granules were reduced in number and size in the Bunina bodies-containing neurons compared to the normal-appearing neurons. Lewy body-like/round inclusions, skein-like inclusions and spheroids were not labeled with anticystatin C antibody.

Transferrin, an iron-binding protein, plays an important role in the transport and delivery of circulating ferric iron to the tissues. Mizuno *et al.*[13] examined transverse paraffin sections of lumbar spinal cords from 12 ALS cases, including two ALS with dementia and two ALS with basophilic inclusions, using antibodies to human transferrin. The results demonstrated that transferrin localized in Bunina bodies (Fig. 1D) and some of the basophilic inclusions. In contrast, skein-like inclusions, Lewy body-like inclusions, and round inclusions did not show obviously detectable transferrin immunoreactivities. Their findings suggest that although the mechanisms underlying transferrin accumulation in Bunina bodies and basophilic inclusions are unknown, transferrin could be involved in forming these inclusions.

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ELECTRON MICROSCOPY

The fine structures of Bunina bodies have been studied by a number of investigators with somewhat variable results.[3,11,43–46] Most authors seem to agree that they consist of amorphous electron-dense material surrounded by tubular and vesicular structures, and with a few central clear areas containing cytoplasmic components (Fig. 2). Tomonaga *et al.*[44] observed two different types of cytoplasmic inclusions, Bunina bodies and laminated cytoplasmic bodies, and they emphasized that both inclusions were closely related to the endoplasmic reticulum. Takahashi *et al.*[34] also observed similar findings in the subthalamic nuclei in an unusual ALS patient.

Because of the limited number of bodies and poor fixation, their precise nature and morphogenesis have not been clarified by electron microscopy. Therefore, serial sections of the anterior horns were observed by electron microscopy to disclose the detailed structure of Bunina bodies, and Okamoto *et al.*[3,4] observed a variety of features suggesting the process of Bunina body formation. Bunina bodies, though they seem to be complicated in structure, consist mainly of two elements, one amorphous material and the other tubular and vesicular structures. Large and typical Bunina bodies consist of electron-dense amorphous material surrounded by a few tubular and vesicular structures, sometimes with a central clear area containing 10 nm filaments and other cellular organelles (Fig. 2). There was no limiting membrane. These bodies with scant tubular and vesicular structures seem to represent the advanced stage of Bunina body formation. Bunina bodies surrounded by many tubular and vesicular structures may be in the earlier
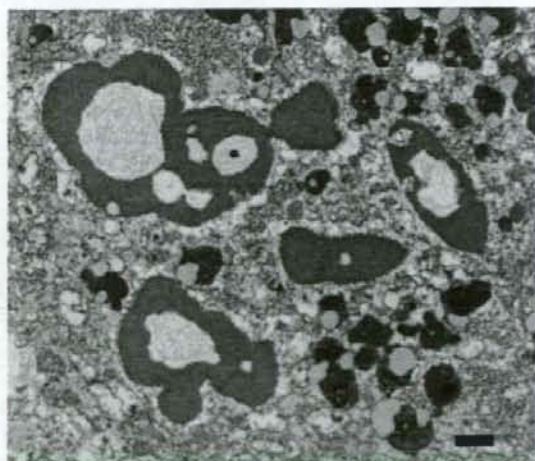

Fig. 2 Electron microscopic features of a typical Bunina body. (Scale bar = 2 μm).
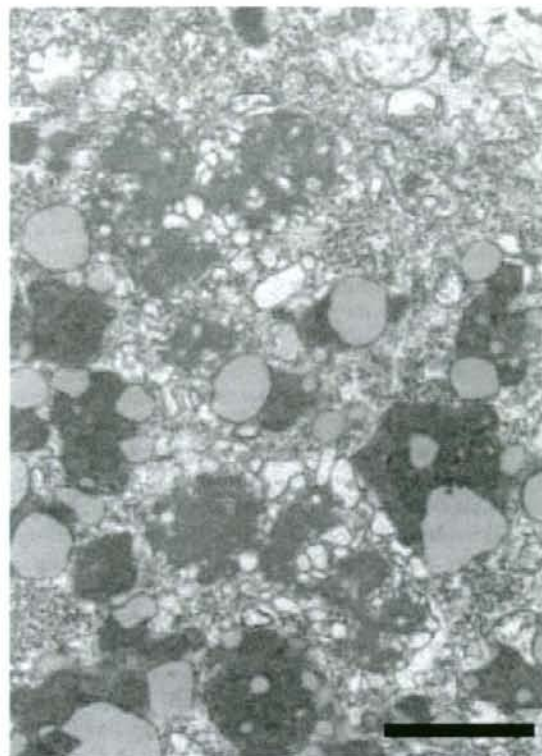
Fig. 3 Small Bunina bodies with numerous tubular and vesicular structures are seen among lipofuscin granules. (Scale bar = 2 μm).
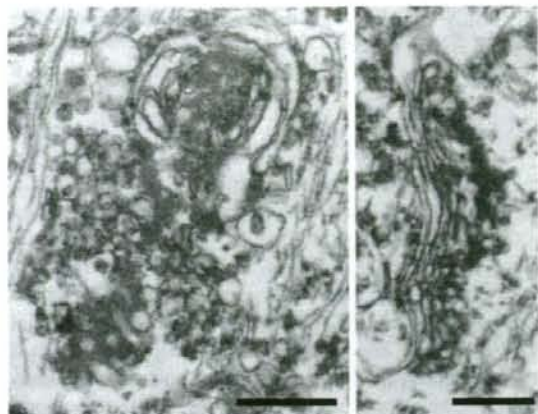

Fig. 4 Similar amorphous material are deposited in association with the Golgi apparatus (left and right). (Scale bar = 1 μm).

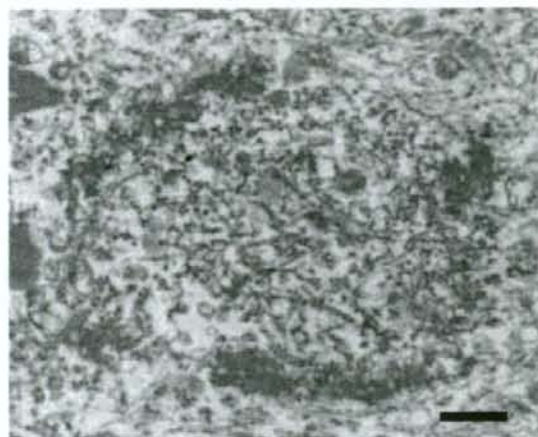
Fig. 5 Small elongated Bunina-like structures are seen around the Lewy body-like filamentous structures. (Scale bar = 2 μm).

stage (Fig. 3). Small Bunina bodies were scattered among lipofuscin granules (Fig. 3). Similar amorphous material was also deposited in association with the Golgi apparatus (Fig. 4). Okamoto *et al.*[3,4] suspect this is the earliest stage of Bunina body formation. Rarely, similar Bunina-like structures were seen around the Lewy-body like filamentous structures (Fig. 5). Laminated cytoplasmic bodies were very rarely observed in anterior horn cells, and no apparent transition between these bodies and Bunina bodies was observed.

BUNINA-LIKE INCLUSIONS

Ultrastructurally, Bunina-like inclusions were rarely observed in non-motor neurons in the olfactory bulb of aged humans,[47] in the cortical neurons in aged rats,[48] in the Betz cells of aged rhesus monkeys[49] and in the lumbar motor neurons in non-ALS patients.[50] Sasaki *et al.*[51] examined the ultrastructure of Betz cells of 17 non-ALS individuals and found electron-dense inclusion bodies in five more elderly cases,[51] and they suspected that the inclusions probably represent an age-related degenerative change. The inclu-

sions consisted of small electron-dense inclusions with vesicles and tubular structures and they resembled figures in the earliest stage of Bunina body formation in ALS.

IMMUNOELECTRON MICROSCOPY

Vibratome sections of 50-μ-thick and 10-μ-thick frozen sections, taken from 4% paraformaldehyde-fixed anterior horns of a patient with ALS and of a patient with non-ALS, were examined by immunoelectron microscopy for cystatin C[12]. Many small accumulations of immunoperoxidase reaction products were seen in the cytoplasm and dendrites of the anterior horn cells. Immunoperoxidase products were also present in Bunina bodies, especially in the
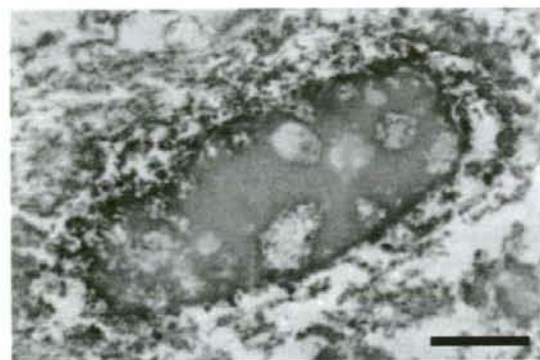
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Fig. 6 Immunoelectron microscopic finding of cystatin C expression in a Bunina body. The immunoperoxidase reaction products are seen predominately in the periphery of the Bunina body. (Scale bar = 2 μm).

periphery (Fig. 6). Compared to the routine electron microscopic features of Bunina bodies, immunoperoxidase products appeared mainly to be localized in the tubular and vesicular structures in the periphery of the bodies. The exact localization of cystatin C was not clear in the autopsied human spinal cord due to artifacts; therefore, Okamoto et al.[12] also performed immunoelectron microscopy on the lumbar cord of an adult cat by the same methods. In an adult cat, cystatin C was mainly localized in the medial aspects of the Golgi apparatus and in the lysosomes in the anterior horn cells of the spinal cord. Therefore, Okamoto et al.[12] speculated that Bunina bodies might represent an abnormal accumulation of unknown proteinous materials associated with the Golgi apparatus or endoplasmic reticulum.

DISCUSSION

Bunina[1] suspected that the inclusions might be a neurotrophic virus; however, electron microscopic studies did not support the possibility of viral origin. Several theories have been proposed based mainly on electron microscopic findings. Hart et al.[11] suggested that Bunina bodies were a special type of autophagic vacuole, probably arising from the mitochondria in which similar electron-dense masses were encountered. However, the electron-dense masses in the mitochondria were never large enough to occupy the entire mitochondrion, and laminated cytoplasmic bodies were very rarely seen in the anterior horn cells of ALS patients. Okamoto et al.[3,4] could not find any signs of transition from laminated cytoplasmic bodies to Bunina bodies. Chou[14] suggested that Bunina bodies were derived from amorphous conglomerated basophilic inclusions. Murayama et al.[21] described Bunina body-like structures

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with bundles of coated filaments and speculated that they may represent ubiquitinated precursor proteins that accumulated to form Bunina bodies. Using particle-induced X-ray emission spectrometry, Yoshida et al.[52] showed that aluminum strongly binds to the Bunina bodies as well as the rough endoplasmic reticulum, and they speculated that the Bunina bodies may be an end-product of nucleic acid dysmetabolism at the rough endoplasmic reticulum caused by aluminum along with magnesium depletion.

From many electron microscopic features of Bunina bodies, Okamoto et al.[3,4] speculated that amorphous material might develop around the tubular and vesicular structures in the early stage of Bunina body formation. The deposits may increase in amount with proliferation of vesicles and tubules, which may become embedded in cellular organelles and engulfed in some areas. These structures are devoid of ribosome attachment; therefore, it is suggested that they originate from smooth endoplasmic reticulum or the Golgi apparatus. However, Okamoto et al.[3,4] could not identify the origin from routine ultrastructural study because they have no specific marker available for smooth endoplasmic reticulum or the Golgi apparatus.

The Golgi apparatus has important functions in processing and transporting plasma membrane, lysosomal, and secreted proteins. Gonatas et al.[53] reported fragmentation and atrophy of the Golgi apparatus in the motor neurons in ALS patients using anti-MG160 antibody. MG160 is a conserved membrane sialoglycoprotein of the medial cisternae of the Golgi apparatus. Immunohistochemical study using anti-MG160 antibody disclosed that almost all anterior horn cells with Bunina bodies showed fragmentation of the Golgi apparatus; however, Bunina bodies themselves were not immunostained with anti-MG160 antibody.[54] Matsumoto et al.[55] also showed that Bunina bodies themselves were negative for Golgi apparatus.

Recently, ubiquitin immunoreactive lesions of FTLD-U and ALS have been defined by the presence of TDP-43, and these disorders can be subsumed into a single entity under the umbrella of TDP-43 proteinopathy.[39] However, Bunina bodies, one of the specific pathologic hallmarks of ALS, are negative for TDP-43.[40] The nature and significance of the Bunina bodies in ALS are not yet clear, and the fact that Bunina bodies appeared not only in the degenerating neurons but also in the normal-looking neurons suggests that Bunina bodies are an initial change or reaction of the motor neurons. Bunina bodies may represent primary and essential pathologic changes in the anterior horn cells in ALS, such as disordered protein metabolism, rather than secondary degenerative changes. More studies are needed to clarify the relationship between the cell organelles, such as the Golgi apparatus, and Bunina bodies, and to disclose the roles of cystatin C and transferrin in the pathogenesis of ALS.

ACKNOWLEDGMENTS

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□ **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

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

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 transferred 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-biotin-peroxidase 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/

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.
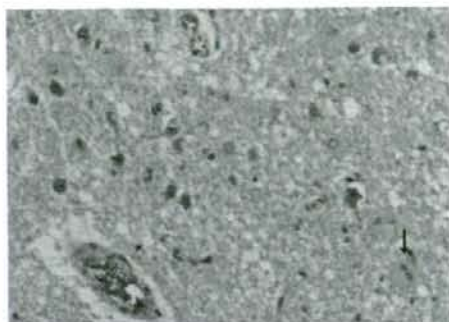

Figure 2. High magnification of right low corner of Figure 1. There were few anoxic changes in the remaining neurons, and binucleated astrocytes were rarely seen (binucleated astrocyte shown by the arrows was the same in Figs. 1 & 2). Hematoxylin and Eosin staining, ×200.
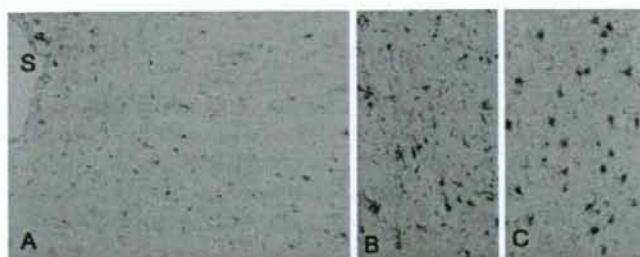

Figure 3. Insular cortex and claustrum in a same section of Case 1. There were a few CD68-positive microglia/macrophages in the insular cortex (A). However, CD68-positive microglia/macrophages (B) and GFAP-positive astrocytes (C) were abundant in the claustrum. S, subarachnoid space; A, ×100; B, ×200;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

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 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
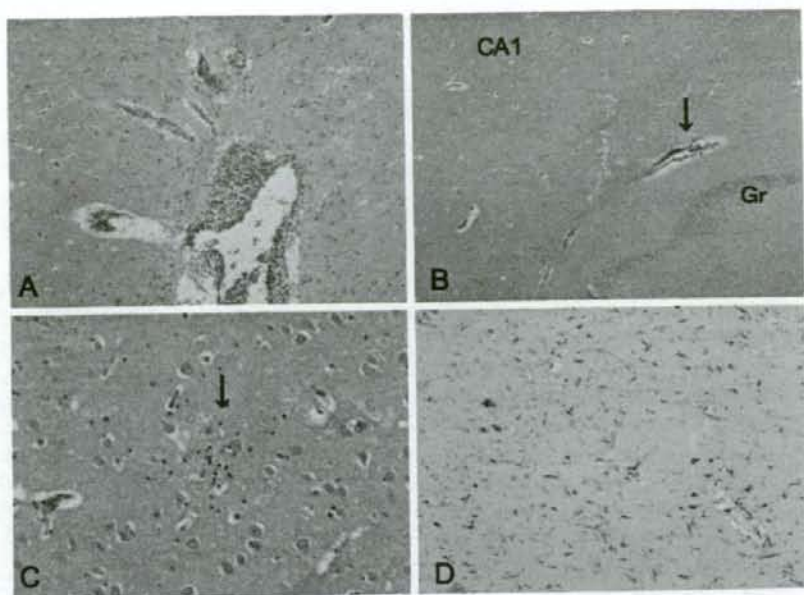
Figure 4. Lymphocytic infiltrations were seen in the subarachnoid spaces and in the perivascular spaces of the superficial cortices (A) and in the hippocampal areas (arrow, B) in Case 2. A few neuronophagia were seen in the CA1 area (arrow), and rod-shaped CD68-postive cells were abundant (D), but there 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.
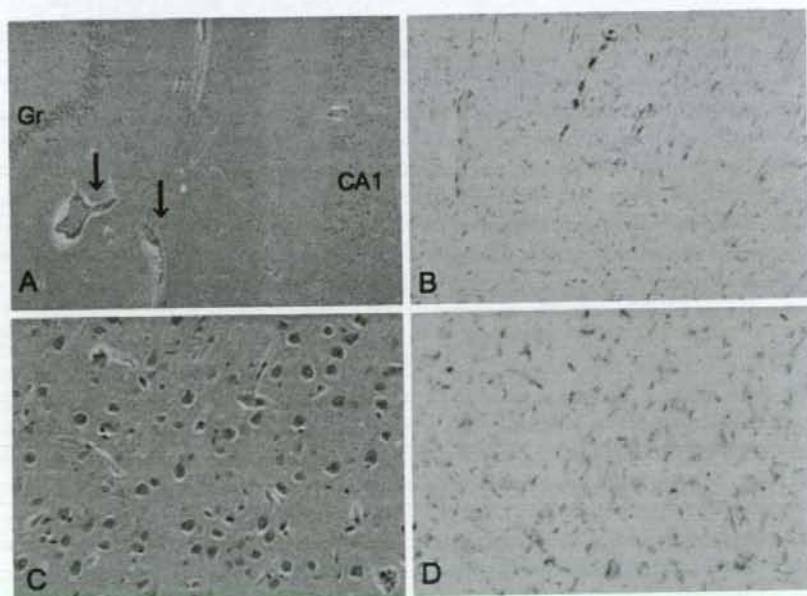
Figure 5. Perivascular lymphocytic infiltrations were seen in the molecular layers of the hippocampus (arrows, A), and CD68 positive microglia/macrophage were increased in the cortex (B) in Case 3. Neurons were well preserved in CA1 (C) with abundant CD68-positive cells (D). C and D were almost same areas in serial sections. Gr, granular cell layer; A, Hematoxylin and Eosin staining×40 ; B, ×200; C, Hematoxylin and Eosin staining×200; D, ×200.

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 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 auto-immune 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 infiltrates, 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

Callosal lesions and delirious behavior during febrile illness

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Abstract

We retrospectively reviewed electroencephalography and magnetic resonance imaging findings for 21 children exhibiting delirious behavior during febrile illness. Among these, five patients had transient callosal lesions with or without white matter lesions on diffusion-weighted images. We compared the clinical characteristics, duration, and components of delirious behavior, the duration and severity of reduced consciousness, and EEG findings among patients with or without callosal lesions. No significant differences were detected in these items according to the presence or absence of callosal lesions. Adding insight into the pathogenesis of this condition, our study revealed that callosal lesions are not uncommon in patients exhibiting delirious behavior during febrile illness.
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Keywords: Delirious behavior; Febrile illness; Callosal lesion; Diffusion-weighted images

1. Introduction

Delirious behavior is an important symptom of acute encephalopathy. In 2001, the Annual Report of the National Research Committee on Influenza-associated Encephalopathy in Japan stated that abnormal behavior was observed during the early period of the disease in 30 of 70 children who died of influenza-associated encephalitis/encephalopathy. However, children may show delirious behavior in association with high-fever even without acute encephalopathy. Our previous studies showed focal slowing of electroencephalographic activity and slight increases in interleukin (IL)-6 levels in patients with delirious behavior [1,2]. However, few

reports have been published regarding magnetic resonance imaging (MRI) abnormalities in patients with delirious behavior. Reversible callosal lesions are observed in patients with various diseases or conditions, including acute encephalopathy [3], epilepsy [4], cerebellitis [5], and convulsions with mild gastroenteritis [6]. We found that transient callosal lesions are observed in some children with delirious behavior. In this report, we describe another condition leading to transient callosal lesions.

2. Patients and methods

The subjects of this study were 21 consecutive children who fulfilled the following criteria: onset between January 2000 and September 2006, delirious behavior in association with febrile illness, and electroencephalography (EEG) and MRI within 72 h of the onset of

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neurological symptoms. Patients with bacterial meningitis or worsening of an underlying disease were excluded from the study. In Okazaki City Hospital, EEG was performed as soon as possible for children admitted who exhibited delirious behavior. MRI was performed when acute encephalopathy was suspected by attending pediatricians, although no patients were diagnosed with acute encephalopathy. To avoid unnecessary burden, MRI was not performed when the patient exhibited complete recovery of consciousness and a normal neurological examination within a few hours of the onset of delirious behavior.

Sixteen boys and five girls were examined, with a mean age of 6.6 years (range, 3.8–15 years). Convulsions were observed in three patients (14%). The causative pathogens of prodromal febrile illness were influenza in eight patients, mycoplasma in one patient, and mumps in one patient. The causative agent was not specified in the remaining 11 patients with non-specific febrile illness. All patients recovered without neurological sequelae.

Delirious behavior was diagnosed according to the diagnostic criteria of delirium in the Diagnostic and Statistical Manual of Mental Disorders [7]. Delirious behavior was noted under the following conditions: disturbance of consciousness with reduced ability to focus, sustain, or shift attention; a change in cognition or the development of perceptual disturbance; a disturbance developing over a short period of time (usually hours to days) and fluctuating throughout the day; and a disturbance unrelated to the direct physiological consequences of a general medical condition. We also investigated the presence or absence of the following components of delirious behavior in each event: visual hallucinations (e.g., "I see angels flying."), non-visual sensory misperceptions (e.g., "I wet my pants," without urinating), incoherent speech (e.g., meaningless answers to specific questions), emotional changes (e.g., crying loudly without reason), purposeless movements (e.g., pacing), impulsive behavior (e.g., suddenly running out the door or disobeying caregivers).

The severity of reduced consciousness upon admission was deemed mild when the patient remained awake, but seemed absent-minded or lacking in spontaneity, or moderate when the patient tended to sleep, but was arousable with verbal and/or tactile stimulation.

EEG was performed during both wakefulness and sleep, according to the 10–20 international methods. When a patient was not arousable, painful stimuli were applied to evaluate changes before and after stimuli. EEG findings during wakefulness or after painful stimuli were used to categorize patients into two groups: normal patients versus those showing focal slowing, which was defined as the insertion of high-voltage slow waves, primarily in the occipital regions, with relatively well preserved rhythmic alpha activities.

During MRI, conventional T1- and T2-weighted images, fluid-attenuated inversion recovery images, and diffusion-weighted images were obtained using a standard protocol. MRI findings were used to categorize patients into two groups: normal patients versus those showing callosal lesions, which were defined as abnormally high-intensity regions in the corpus callosum on T2- and/or diffusion-weighted images. Other types of MRI abnormalities were not observed in any patient.

Data were analyzed using the Mann–Whitney's U-test for numerical variables and the Fisher exact probability test for categorical variables. A p value of <0.05 was considered statistically significant.

3. Results

Callosal lesions were identified in 5 (24%) of 21 patients. Two patients showed additional white matter lesions, and the remaining three showed callosal lesions alone. Follow-up MRI was performed 4–12 days after admission, and all MRI were normal. The clinical course of two representative patients is briefly described below.

3.1. Patient 1

A previously healthy 8-year-old boy presented with unusual behavior and pyrexia beginning at the day before presentation. A nasal swab was positive for the influenza A antigen. The patient looked around restlessly and could not say his name or respond to questions in an appropriate manner. He had a brief generalized convulsion at 2 h post-admission. Although he was alert immediately after the convulsion, MRI was performed. High-intensity regions were observed in the genu and splenium of the corpus callosum on diffusion-weighted images (Fig. 1). Cerebrospinal fluid analysis and EEG showed no abnormal findings. After MRI, the patient showed further improvement in terms of consciousness, but then relapsed into delirious behavior 5 h later. He could not count his mother's fingers and repeatedly and continually uttered the word "no." In addition, his consciousness was mildly reduced. That night, he sat up every 10–15 min and uttered meaningless words or phrases. He did not seem to recognize his mother. The patient was treated with methylprednisolone pulse therapy. The following morning, the patient was alert and showed no delirious behavior, but he was unable to remember the events of the previous day. Delirious behavior did not recur. MRI performed 8 days after admission revealed no abnormal findings.

3.2. Patient 2

A previously healthy 7-year-old boy was admitted with a 2-day history of pyrexia, vomiting, and bilateral parotid swelling. Cerebrospinal fluid analysis showed cell counts of 84 cells/μl and 29 mg/dl protein. The

On admission

7 days later
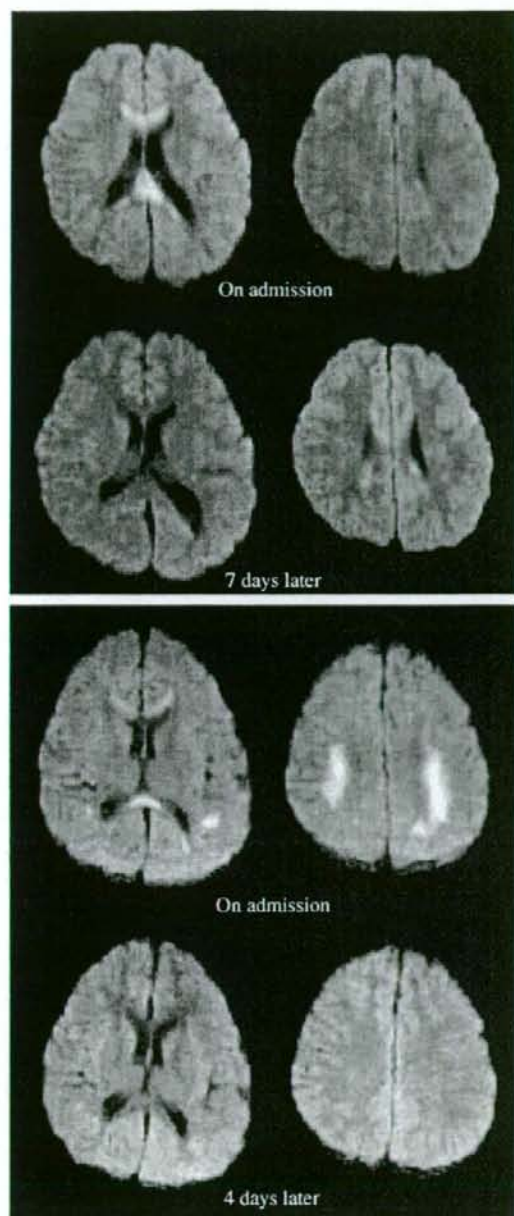
On admission

4 days later

Fig. 1. Upper: patient 1. Diffusion-weighted images revealed high-intensities were observed in the genu and splenium of the corpus callosum. Lower: patient 2. High-intensity areas were observed in the entire corpus callosum and centrum semiovale on diffusion-weighted images.

patient was diagnosed with mumps meningitis and delirious behavior was observed within a few hours of admission. The patient was unable to perform simple

calculations (e.g., $1 + 2$) and uttered meaningless or incoherent words and phrases. Mild loss of consciousness was also observed. MRI and EEG were performed on the day after admission. MRI revealed high-intensity regions throughout the corpus callosum and centrum semiovale on diffusion-weighted images (Fig. 1), and EEG indicated the insertion of high-voltage slow waves, primarily in the occipital regions. The patient was treated with intravenous gammaglobulin. Although the patient showed an almost complete recovery in consciousness on the following day, delirious behavior was observed intermittently until 2 days after admission. Thereafter, delirious behavior did not recur. MRI performed 5 days after admission demonstrated no abnormal findings.

Comparisons among patients with and those without callosal lesions (Table 1).

Age and sex did not differ between patients with and those without callosal lesions. The date of MRI was later in patients without callosal lesions, although the

Table 1
Comparison between patients with and without callosal lesions

	Callosal lesions ($N = 5$)	No callosal lesions ($N = 16$)	
Age (months)	88 (62–97)	61 (45–180)	NS
Sex (male:female)	4:1	12:4	NS
Date of MRI (days)	0 (0–2)	2 (0–3)	NS
Seizure	1 (20%)	2 (13%)	NS
Duration of DB			
≤ h	2 (40%)	8 (50%)	NS
$1 < $ ≤ 6 h	1 (20%)	6 (38%)	
$6 < $ ≤ 12 h	0	1 (6%)	
12 h<	2 (40%)	1 (6%)	
Components of DB			
Visual hallucination	0	7 (44%)	NS
Sensory misperception*	1 (20%)	1 (6%)	NS
Incoherent speech	3 (60%)	8 (50%)	NS
Emotional change	3 (60%)	4 (25%)	NS
Purposeless movement	1 (20%)	4 (25%)	NS
Impulsive behavior	0	1 (6%)	
Reduced consciousness			
Mild	5 (60%)	14 (88%)	NS
Moderate	0	2 (13%)	
Duration of reduced consciousness			
≤12 h	3 (60%)	5 (31%)	NS
$12 < $ ≤ 24 h	1 (20%)	8 (50%)	
$24 < $ ≤ 36 h	0	2 (13%)	
36 h<	1 (20%)	1 (6%)	
EEG findings			
Normal	2 (40%)	3 (19%)	NS
Focal slowing	3 (60%)	13 (81%)	
Minimal serum Na level (mEq/L)	132 (121–135) $N = 5$	138.5 (137–142) $N = 6$	$p = 0.0061$

DB, delirious behavior; NS, not significant.
* Sensory misperception does not include visual hallucination.

difference was not statistically significant compared to the date of MRI in patients with callosal lesions. MRI was performed 2 or 3 days after the onset of delirious behavior in 9 of 15 patients without callosal lesions, whereas it was performed within the first 2 days in 4 of 5 patients with callosal lesions. The duration and components of delirious behavior were not statistically different between patients with and those without callosal lesions, although patients with callosal lesions did not report visual hallucinations. No significant differences were observed in the duration or severity of reduced consciousness, or EEG findings, between patients with and those without callosal lesions. Minimal serum sodium levels were lower in patients with callosal lesions than in those without lesions, although serum sodium levels were not measured in the majority of patients without callosal lesions. Among patients with callosal lesions, no differences were observed in the duration or components of delirious behavior, or in the duration or severity of reduced consciousness. However, we were unable to perform a statistical analysis due to the small number of patients.

4. Discussion

Our study revealed that reversible callosal lesions are not uncommon in patients with delirious behavior during febrile illness. The duration or components of delirious behavior and the severity of reduced consciousness did not differ between patients with and those without callosal lesions. However, callosal lesions may provide insight into the pathomechanism of delirious behavior in children with febrile illness, although it may be different among patients.

Although the pathogenesis of callosal lesions is unclear, several explanations have been proposed. Initially, high-signal intensities and reduced apparent diffusion coefficients observed in several metabolic disorders, such as Canavan disease, metachromatic leukodystrophy, and phenylkenuria, were interpreted as evidence that intramyelinic edema results in reduced water diffusion [8,9]. The separation of myelin layers is thought to reduce water diffusion. However, Takanashi et al. [10] reported reduced water diffusion in the corpus callosum of a newborn with mild asphyxia. These results suggest that callosal lesions are unrelated to myelin because myelination is not present in the corpus callosum before 2 months of age. The study proposed that inflammatory infiltrates may cause reduced water diffusion. In the present study, patients with callosal lesions also showed low serum sodium levels. In previous studies, transient callosal lesions were observed in association with hyponatremia [11]. Takanashi et al. recently reported that serum sodium levels were lower in patients with clinically mild encephalitis/encephalopathy with a reversible splenial lesion than those with upper respiratory infec-

tion, febrile seizures, or other types of acute encephalopathy [12]. These observations suggest that hyponatremia will be closely related to the pathogenesis of callosal lesion, although whether hyponatremia is a cause or a consequence of callosal lesions is unclear. Our findings suggest that callosal lesions may represent widespread but mild abnormalities in commissural fibers that pass through the corpus callosum. These fibers are widely distributed throughout both hemispheres, although the density of these fibers is relatively low outside the corpus callosum. Diffusion abnormalities in the hemispheres may result in subthreshold effects due to the low density of the affected fibers. In contrast, these fibers are tightly packed in the corpus callosum. Thus, subtle abnormalities in the diffusion properties of the affected fibers may result in accumulation beyond the threshold.

The pathophysiology of delirious behavior remains poorly understood. EEG and neuropsychological studies have revealed generalized disruption of higher cortical functions in adults showing delirious behavior [13]. Currently, the leading hypotheses for the pathogenesis of delirium focus on the roles of neurotransmission and inflammation. Cholinergic deficiency and dopaminergic excess contribute to delirium in adults [14]. Cytokines, including IL-1, IL-6, and tumor necrosis factor (TNF)-α, may also be related to delirious behavior by increasing the permeability of the blood–brain barrier and altering neurotransmission [14,15]. Our previous studies revealed a mild increase in serum IL-6 levels in children showing delirious behavior [2].

The presence of callosal lesions indicates an abnormality in commissural fibers, although its pathogenesis remains unclear. The commissural fibers connect corresponding areas in the bilateral cerebral hemispheres. Therefore, abnormalities in the commissural fibers may result in the disconnection of the bilateral hemispheres, leading to the disruption of higher cortical function. We propose that callosal lesions are a representative finding in disorders of integrated brain function.

Callosal lesions were not observed in the majority of children exhibiting delirious behavior, although the duration or components of delirious behavior did not differ between patients with and those without callosal lesions. One possible explanation may be the duration of the callosal lesion. Previous studies have revealed that callosal lesions may disappear within a few days to weeks of their appearance [3,6,16]. In our cohort, MRI was performed relatively late in patients who did not show callosal lesions, although a statistically significant difference was not detected. Thus, callosal lesions may have been missed in these patients. Another explanation is that abnormalities in the commissural fibers may be too subtle to detect on diffusion-weighted images. More precise analyses using diffusion tensor imaging may reveal more minute changes in the diffusion properties of commissural fibers.

In summary, transient callosal lesions were observed in 5 of 21 patients exhibiting delirious behavior during febrile illness. In contrast, the duration and components of delirious behavior did not differ between patients with and those without callosal lesions. These findings may help to clarify the pathophysiology of delirious behavior in children with febrile illness.

Acknowledgement

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

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We sought to clarify the clinical, laboratory, neurora-
diologic, and neurophysiologic features of the "sub-
acute" 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 electroencepha-
lographic 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. Labora-
tory data were unremarkable, and electroencephalogra-
phy during the early phase found abnormalities in 4 of 7
patients. Magnetic resonance imaging revealed no abnor-
malities 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 reso-
nance imaging, and poor neurologic outcomes. Recogni-
tion of this type of acute encephalopathy is important,
and a method to promote early diagnosis is desirable.
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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 necrotiz-
ing encephalopathy, as proposed by Mizuguchi, is charac-
terized 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 le-
sions on diffusion-weighted images are characteristic in
children with encephalopathy with prolonged seizures [5].

A unique subtype of acute encephalopathy, character-
ized 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 recov-
ered, but slightly reduced responsiveness, am appearance
of absent-mindedness, or subtle disorientation may be
observed by parents or caregivers. Deterioration of con-
sciousness, 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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