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.

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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°C 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/
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-positive 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 epilepticus 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-methyl-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, Izuka 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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References


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