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Features of amygdala in patients with mesial temporal lobe epilepsy and hippocampal sclerosis: An MRI volumetric and histopathological study

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ABSTRACT

Objective: It is well-known that there is a correlation between the neuropathological grade of hippocampal sclerosis (HS) and neuroradiological atrophy of the hippocampus in mesial temporal lobe epilepsy (mTLE) patients. However, there is no strict definition or criterion regarding neuron loss and atrophy of the amygdala neighboring the hippocampus. We examined the relationship between HS and neuronal loss in the amygdala.
Materials and methods: Nineteen mTLE patients with neuropathological proof of HS were assigned to Group A, while seven mTLE patients without HS were assigned to Group B. We used FreeSurfer software to measure amygdala volume automatically based on pre-operation magnetic resonance images. Neurons observed using Klüver-Barrera (KB) staining in resected amygdala tissue were counted. and the extent of immunostaining with stress marker antibodies was semiquantitatively evaluated.
Results: There was no significant difference in amygdala volume between the two groups (Group A: 1.41 ± 0.24; Group B: 1.41 ± 0.29 cm^3; p = 0.98), nor in the neuron cellularity of resected amygdala specimens (Group A: 3.98 ± 0.97; Group B: 3.67 ± 0.67 $10 \times {}^{-4}$ number of neurons/μm^2; p = 0.40). However, the HSP70 level, representing acute stress against epilepsy, in Group A patients was significantly larger than that in Group B. There was no significant difference in the level of Bcl-2, which is known as a protein that inhibits cell death, between the two groups.
Conclusions: Neuronal loss and volume loss in the amygdala may not necessarily follow hippocampal sclerosis. From the analysis of stress proteins, epileptic attacks are as likely to damage the amygdala as the hippocampus but do not lead to neuronal death in the amygdala.

1. Introduction

Hippocampal sclerosis (HS) is the most common pathological characteristic identified in patients with mesial temporal lobe epilepsy (mTLE) who undergo surgery. Histopathologically, HS is characterized by segmental pyramidal neuron loss in CA1, CA3, and CA4, whereas neurons in CA2 are relatively seizure-resistant. Some longitudinal magnetic resonance imaging (MRI) studies have provided evidence that chronic seizures result in progressive hippocampal atrophy. A retrospective cross-sectional MRI study suggested a close correlation between hippocampal atrophy and the histopathological grades of the resected hippocampus (Fuerst et al., 2001). On the other hand, there is limited available data regarding neuronal loss in the amygdala associated with HS (Miller et al., 1994; Bernasconi et al., 2003). The involvement of the amygdala in epilepsy has not been explicitly established to date in relation to HS and mTLE. In this study, we investigated whether mTLE patients with and without HS experienced decreases in volume and cellularity of the amygdala and sought to understand the role of the amygdala in epileptic networks in mTLE.

2. Materials and methods

2.1. Subjects

This study investigated 26 patients (14 men, 12 women) who underwent anterior temporal lobectomies with amygdalo-

Abbreviations: HS, hippocampal sclerosis; mTLE, mesial temporal epilepsy; ATL+ AH, anterior temporal lobectomies with amygdalo hippocampectomies; TLEs, mesial temporal epilepsies; sGTC, secondary generalized tonic-clonic seizures; LI, laterality index

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Table 1
Clinical profiles of 26 patients with temporal lobe epilepsy.
Abbreviations;: sGTC, secondary generalized tonic-clonic seizures; AEDs, anti-epileptic drugs; FC, febrile convulsion; SE, subdural electrodes; HHI, hippocampal hyper-intensity; HA, hippocampal atrophy; HS, hippocampal sclerosis; DNT, Dysembryoplastic neuroepithelial tumor; CA, cavernous angioma.

No.	Age (y) /sex	Epilepsyduration (y)	Side	sGTC (±)	AEDs	FC (±)	MRI Lesion	SE (±)	Engel's classification	histopathology (Watson's grade)
1	10/M	6	Rt.	−	2	+	Rt.HHI	−	I	HS grade 4
2	14/M	4	Lt.	−	3	−	Lt. HHI	−	I	HS grade 3
3	14/F	1	Rt.	−	3	+	Rt. HA, HHI	−	I	HS grade 3
4	15/M	9	Rt.	−	3	+	Rt. HHI	−	I	HS grade 4
5	15/M	14	Lt.	−	3	−	Lt. HA, HHI	−	I	HS grade 4
6	20/F	9	Rt.	+	2	−	Bil. HHI	−	I	HS grade 3
7	23/F	6	Rt.	−	4	+	Rt. HA, HHI	−	I	HS grade 3
8	23/M	20	Rt.	+	2	−	Rt. HHI	−	I	HS grade 3
9	24/F	10	Rt.	+	3	−	Rt. HA	+	III	HS grade 3
10	27/M	24	Rt.	+	4	−	Rt. HA	−	I	HS grade 4
11	28/M	19	Rt.	−	2	−	Rt. HA, HHI	−	I	HS grade 3
12	32/F	5	Lt.	−	3	−	Lt. HA, HHI	−	I	HS grade 4
13	32/F	15	Rt.	−	2	−	Bil. HHI	+	I	HS grade 3
14	34/M	24	Rt.	+	2	−	Bil. HHI	+	I	HS grade 2
15	36/F	7	Rt.	+	2	+	Rt. HA, HHI	−	I	HS grade 4
16	39/M	24	Lt.	+	1	−	Lt. HA, HHI	−	I	HS grade 4
17	42/M	27	Lt.	+	2	−	not particular	+	I	HS grade 3
18	42/M	2	Lt.	−	2	−	Bil. HHI	−	II	HS grade 4
19	42/F	14	Lt.	−	3	−	Lt. HA, HHI	−	II	HS grade 4
20	10/F	1	Rt.	−	1	−	DNT	+	I	DNT
21	12/F	8	Lt.	−	3	−	not particular	+	I	no significant
22	15/M	8	Lt.	−	4	−	not particular	+	I	no significant
23	20/M	1	Rt.	−	3	−	Tumor	−	I	Ependymoma
24	25/M	8	Rt.	−	3	−	not particular	+	I	no significant
25	51/F	8	Lt.	+	4	−	Lt. HHI	+	III	post encephalitis
26	52/F	12	Rt.	−	3	−	CA	−	I	CA

Group A: patients 1–19; Group B: patients 20–26.
Abbreviations;: sGTC, secondary generalized tonic-clonic seizures; AEDs, anti-epileptic drugs; FC, febrile convulsion; SE, subdural electrodes; HHI, hippocampal hyper-intensity; HA, hippocampal atrophy; HS, hippocampal sclerosis; DNT, Dysembryoplastic neuroepithelial tumor; CA, cavernous angioma.

hippocampectomies (ATL+ AH) for intractable drug-resistant mTLE. All patients were preoperatively evaluated by MRI, long-term scalp video-EEG monitoring, and intracranial-EEG if necessary. Interictal and ictal EEG findings and seizure semiology were consistent with temporal lobe epilepsies (TLEs). Neuropsychological tests were performed on all patients and the intracarotid sodium amobarbital procedure (ISAP; the Wada test) was performed as part of the preoperative routine.

Patients were divided into two groups based on whether there was neuropathological proof of HS (Group A) or not (Group B). Nineteen patients (eleven men, eight women) were assigned to Group A and seven (three men, four women) to Group B.

The mean age of Group A at surgery was 26.9 ± 10.3 (10–42) years, and mean duration of epilepsy was 12.6 ± 8.1 (1–27) years. Seizure frequencies varied from 0.3 to 30 times per month. Specimens were resected from the right hemisphere of 12 of the 19 patients in Group A. The patients in Group B underwent ATL +AH based on a diagnosis of brain tumor or TLE with no neuropathological HS. Three patients in Group B had a tumor (ependymoma, cavernous angioma or dysembryoplastic neuroepithelial tumor), three had TLE without radiological or neuropathological detection of lesions, and the final patient had a history of encephalitis. In the three patients with tumors, the tumors did not involve the amygdala, according to the MRI findings. The mean age at surgery for Group B was 26.4 ± 16.5 (10–52) years, and the mean duration of epilepsy was 6.6 ± 3.8 (1–12) years. Seizure frequencies varied from 0.3 to 120 times per month. Specimens were resected from the right hemisphere of four of the seven patients in Group B.

We do not have autopsy controls. The protocols for this study were approved by the Nishi-Niigata Chuo National Hospital Ethics Committee. Informed consent for research obtained at pre-operation in all cases.

2.2. Clinical features

Descriptions of clinical seizure characteristics were obtained from patients' clinical history charts and long-term video-EEG recordings. Clinical data, including age at surgery, sex, duration from seizure onset, presence of secondary generalized tonic-clonic seizures (sGTC), number of anti-epileptic drugs, and history of febrile seizures were investigated. Postoperative seizure outcomes were evaluated six months after surgery. We classified seizure outcome according to Engel's classification: I, free from seizure (excluding auras) since surgery; II, seizure frequency up to two times per year; III, reduction in seizure frequency greater than 75%; and IV, reduction in seizure frequency less than 75%. Clinical features of the patients in both groups are summarized in Table 1.

Duration from seizure onset in patients in Group A (12.6 ± 8.1 years) was significantly longer than in Group B (6.6 ± 3.8 years, $p < 0.05$). There were more patients with a history of febrile seizures in Group A (5/19 patients) than in Group B (0/7 patients, $p < 0.05$). The groups did not differ in other clinical features, including age, sex, number of patients with sGTC, number of anti-epileptic drugs at operation, and rates of Engel' s classification I.

2.3. MRI volumetry

To assess the volume of the amygdala, we performed quantitative morphometric analysis of T1-weighted MRI data using automated segmentation and a probabilistic region-of-interest labeling technique (FreeSurfer software version 5.3.0 https://surfer.nmr.mgh.harvard.edu). Image processing included removal of non-brain tissues with a hybrid watershed, surface deformation procedure, automated Talairach transformation, and segmentation of the subcortical white and gray matter (Fig. 1a). To examine the laterality of amygdala volume, the laterality index (LI) was calculated as follows: (operated side−-contralateral side)/(operated side + contralateral side).