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Injury of the mammillothalamic tract in patients with subarachnoid hemorrhage: a diffusion tensor imaging study

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Abstract

Objective: Few studies have reported on injury of the mammillothalamic tract (MTT) in stroke patients. However, no study in patients with subarachnoid hemorrhage (SAH) has been reported. Using diffusion tensor tractography (DTT), we attempted to investigate injury of the MTT in patients with subarachnoid hemorrhage.

Methods: We recruited 16 patients with SAH and 15 control subjects. Diffusion tensor imaging (DTI) was obtained at 5.7 ± 1.5 weeks after onset and reconstruction of the MTT was performed using the probabilistic tractography method. The fractional anisotropy (FA) value and tract number of the MTT and the Mini-Mental State Examination (MMSE) score were determined. Values of FA and tract volume showing decrement of more than two standard deviations that of normal control were defined as abnormal.

Results: The FA value and tract volume in the patient group were significantly lower than those of the control group ($p < .05$). In addition, MMSE showed strong ($r = 0.67$, $p = 0.005$) positive correlation with tract volume without correlation with FA. In the individual analysis, 16 MTTs of 32 MTTs in 16 patients showed abnormalities of the MTT in terms of the FA value, the tract volume, or the presence of a reconstructed MTT. As a result, ten (62.5%) of 16 patients showed abnormality of the MTT in at least one hemisphere.

Conclusion: We found that patients with SAH showed injury of the MTT and this injury showed correlation with cognitive dysfunction. Our results suggest that evaluation of the MTT using DTI would be necessary for patients with cognitive dysfunction following SAH.

Keywords: Mammillothalamic tract, Subarachnoid hemorrhage, Diffusion tensor tractography

Strengths and limitations of this study

- This is the first study on the injury of the mammillothalamic tract (MTT) in patients with subarachnoid hemorrhage (SAH) using diffusion tensor imaging (DTI).
- The main strength of our study is to find the relation between MTT injury and cognitive function with SAH
- Limitation of our study is that fiber tracking of the MTT might be affected by artifact, such as an aneurysmal clip. Therefore, conduct of further studies to overcome this limitation would be necessary.

Introduction

The mammillothalamic tract (MTT) connects the mammillary body and the anterior thalamus as a part of the Papez circuit.¹ Because of the anatomical characteristics of the MTT, thin, short, and located deep within the brain, accurate estimation of the MTT has been difficult in the live human brain. However, diffusion tensor tractography (DTT), which is derived from diffusion tensor imaging (DTI), allows for reconstruction of the MTT in the human brain.² Therefore, accurate estimation of the MTT in terms of DTI parameters and three-dimensional configuration is now possible.² A few studies have reported on injury of the MTT in thalamic hemorrhage and thalamic infarct, however, so far, little is known about injury of the MTT.^{3 4}

Subarachnoid hemorrhage (SAH) is extravasation of blood into the subarachnoid space accompanied by various neurological sequelae in terms of memory, executive function, language, motor function, and cranial nerve function.⁵⁻¹⁶ In particular, memory deficit is known to be one of the most common sequelae of SAH.^{7 8 10 11 14} In the past, previous studies have suggested that the neurotoxic effects of blood, vasospasm, or increased intracranial pressure are pathogenic mechanisms of neural sequelae following SAH.^{5 7 17-23} After introduction of DTI, among the neural tracts associated with memory, injuries in the fornix and cingulum were reported in patients with SAH.²⁴ However, no study on neural injury of the MTT in patients with SAH has been reported.

In the current study, using DTT, we attempted to investigate injury of the MTT in patients with SAH.

Methods

Subjects

We recruited 16 patients with SAH (male: 9, female: 7, mean age: 51.6±13.3 years, range: 34~70 years) and 15 normal healthy control subjects (male: 7, female: 8, mean age: 48.1±15.1 years, range: 20~67 years) with no previous history of neurological, physical, or psychiatric illness for this study. Inclusion criteria for patients were as follows: (1) first ever stroke, (2) age 30~70 years, (3) hemorrhage in the subarachnoid space due to aneurismal rupture confirmed by a neuroradiologist, (4) DTI was scanned at a chronic stage (between 4 ~ 8 weeks) after onset, and (5) no hydrocephalus, intracerebral hemorrhage, or intraventricular hemorrhage. Severity of SAH was assessed according to Fisher CT grade.²⁵ Patients who showed any lesion along or around the MTT pathway between the mammillary body and thalamus were excluded. This study was conducted retrospectively and the study protocol was approved by the Institutional Review Board of a university hospital.

Figure 1

Clinical evaluation

Cognitive function was evaluated at the time of DTI scanning. The mini-mental state examination (MMSE) was used for assessment of cognitive impairment. The reliability and validity of the MMSE have been well established.²⁶

Diffusion tensor tractography

A 6-channel head coil on a 1.5 T Philips Gyroscan Intera (Philips, Ltd, Best, The Netherlands) with single-shot echo-planar imaging was used for acquisition of DTI data. For each of the 32 non-collinear diffusion sensitizing gradients, we acquired 67 contiguous slices

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4 parallel to the anterior commissure-posterior commissure line. Imaging parameters were as
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6 follows: acquisition matrix = 96×96 ; reconstructed to matrix = 128×128 ; field of view =
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8 $221 \times 221 \text{ mm}^2$; TR = 10,726 ms; TE = 76 ms; parallel imaging reduction factor (SENSE
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10 factor) = 2; EPI factor = 49; $b = 1000 \text{ s/mm}^2$; NEX = 1; and a slice thickness of 2.3 mm
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12 (acquired voxel size $1.73 \times 1.73 \times 2.3 \text{ mm}^3$). Affine multi-scale two-dimensional registration
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14 using the Oxford Centre for Functional Magnetic Resonance Imaging of Brain (FMRIB)
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16 Software Library (FSL; www.fmrib.ox.ac.uk/fsl) was used for removal of eddy current-
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18 induced image distortions.²⁷ Fiber tracking was performed using a probabilistic tractography
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20 method based on a multi-fiber model, and applied in the current study utilizing tractography
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22 routines implemented in FMRIB Diffusion (5000 streamline samples, 0.5 mm step lengths,
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24 curvature thresholds = 0.2).²⁷⁻²⁹ MTTs were determined by selection of fibers passing through
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26 three regions of interest (ROIs). Seed ROIs were placed on the mammillary body on the axial
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28 image.² Two target ROIs were drawn at the portion of the MTT area (between the portion of
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30 the fornix and the red nucleus in the anteroposterior direction) at about the bicommissural
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32 level and the portion of the anterior thalamus on the axial image.² Of 5000 samples generated
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34 from each seed voxel, results for each contact were the visualized threshold point at 5
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36 streamline through each voxel for analysis. Values of fractional anisotropy (FA) and tract
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38 volume of MTT were measured. Values of FA and tract volume showing decrement of more
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40 than two standard deviations that of normal control were defined as abnormal.
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47 **Statistical analysis**

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50 We used SPSS software (v.15.0; SPSS, Chicago, IL) for data analysis. Data on MTTs
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52 that were not reconstructed were excluded in statistical analysis. An independent t-test was
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54 used for determination of variances in the value of FA and tract volume between the patient
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56 and control group. Subsequently, using Pearson correlation, DTT parameters for FA and tract
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4 volume of the patient group were used in determination of correlation with MMSE.³⁰ The
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6 significant level of the *p* value was set at 0.05.
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Results

A summary of the demographic data for the patient and control groups is shown in Table 1. The artery distribution of aneurismal rupture for SAH in 16 patients was as follows: the anterior communicating artery: 11 patients (68.8%), the middle cerebral artery: three patients (18.8%), the posterior communicating artery: one patient (6.2%), and the anterior cerebral artery: one patient (6.2%). In addition, 12 patients (75%) patients underwent aneurysmal clipping and two patients underwent endovascular coiling (12.5%). The two remaining patients (12.5%) received conservative treatment. Average MMSE was 19.8 ± 8.8 and Fisher CT grade was 3.4 ± 0.5 . DTI scanning was performed at 5.7 ± 1.5 weeks (range: 4~8 weeks) after SAH onset.

Table 1

A summary of the results of DTT parameters for MTT in the patient and control groups is shown in Table 2. Values for FA and tract volume were significantly lower in the patient group than in the control group, respectively ($p < .05$). The tract volume of the MTT showed strong ($r = 0.67$, $p = 0.005$) positive correlation with MMSE, however, no correlation was observed between the FA value of MTT and MMSE ($r = 0.41$, $p = 0.11$).³⁰

Table 2

Figure 2 shows the results for incidence of injury of the MTT with DTT parameters. Among 32 hemispheres of 16 patients, the MTT was not reconstructed in five hemispheres of four patients (right hemisphere: two, left hemisphere: one, both hemispheres: one); in contrast,

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4 the MTTs were reconstructed in all 30 hemispheres of 15 control subjects. With regard to the
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6 FA and tract volume, nine MTTs in six patients (right hemisphere: one, left hemisphere: two,
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8 both hemispheres: three) and 13 MTTs in nine patients (right hemisphere: four, left
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10 hemisphere: one, both hemispheres: four) of 32 hemispheres in 16 patients revealed a
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12 decrement of more than two standard deviations, compared with control subjects. Six MTTs
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14 in five patients (right hemisphere: three, left hemisphere: one, both hemispheres: one) showed
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16 a decrement of more than two standard deviations in both FA and tract volume. As a result,
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18 16 MTTs (right hemisphere: two, left hemisphere: two, both hemispheres: six) of 32 MTTs in
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20 16 patients showed abnormalities of the MTT. Three patients in one hemisphere and one
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22 patient in both hemispheres whose MTT was not reconstructed belonged to these 16 patients.
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24 Consequently, in the individual analysis, the prevalence of MTT abnormality was 62.5% (10
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26 of 16 patients).
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Discussion

In the current study, we investigated injury of the MTT in patients with SAH using DTT. We obtained the following results: 1) the FA value and tract volume in the patient group were significantly lower than those of the control group, 2) MMSE showed strong ($r=0.67$, $p=0.005$) positive correlation with tract volume without correlation with FA value, 3) in the individual analysis, 16 MTTs of 32 MTTs in 16 patients showed abnormalities of the MTT in terms of the FA value, tract volume, or the presence of a reconstructed MTT. As a result, ten (62.5%) of 16 patients showed abnormality of the MTT in at least one hemisphere.

The FA value indicates the degree of directionality of water diffusion and has a range of zero (completely isotropic diffusion) to one (completely anisotropic diffusion). It represents the white matter organization: in detail, the degree of directionality and integrity of white matter microstructures such as axons, myelin, and microtubules.³¹ In contrast, tract volume, the number of voxels in a neural tract, reflects the neural fibers contained within a neural tract.³² Therefore, a decrease in the FA or tract volume indicates an injury of the neural tract. Consequently, our results showing that the FA value and tract volume in the patient group were significantly lower than those of the control group indicate injury of the MTT in the patient group.

Regarding the relation between MTT injury and cognition, MMSE showed strong positive correlation with the tract volume of the MTT without correlation with FA.³⁰ These results appear to indicate that the tract volume is more sensitive than the FA value for detection of injury of the MTT. The MMSE is the most widely used tool for screening cognitive dysfunction.³³ Because this study was conducted retrospectively, we could not employ detailed neuropsychological testing for evaluation of the function of the MTT.

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4 Therefore, conduct of further prospective studies, including specific neurophysiological tests
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6 for MTT function should be encouraged.
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10 The pathogenic mechanism of neural injury following SAH has not been elucidated
11 and few DTI studies have reported on this mechanism.^{15 34} In 2007, Liu et al. demonstrated
12 that SAH caused global mild vasogenic edema in white matter and deep gray matter by
13 measuring the apparent diffusion coefficient value in the subacute stage of SAH, which was
14 undetectable on T2-weighted and diffusion-weighted MR images.³⁴ In a recent study, Yeo et
15 al. [2012] reported injury of the CST at the midbrain in patients with SAH.¹⁵ This study
16 suggested that frequent occurrence of SAH into perimesencephalic cisterns could be ascribed
17 to injury of the CST, through mechanical (increased intracranial pressure or direct mass) or
18 chemical mechanisms (a blood clot itself can cause extensive damage).^{5 23 35} Considering that
19 the MTT is located in close proximity to a cistern, the MTT in patients with SAH appears to
20 be injured by mechanisms similar to those of CST injury at the midbrain.^{5 15 17 18 23}
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35 In conclusion, we found injury of the MTT in the patient group, compared with the
36 control group. In addition, in the individual analysis, 16 MTTs (50%) of 32 hemispheres
37 showed injury of the MTT and ten (62.5%) of 16 patients showed injury of the MTT in at
38 least in one hemisphere in terms of DTT parameters or the presence of a reconstructed MTT.
39 Our results suggest that evaluation of the MTT using DTI would be necessary for patients
40 with cognitive dysfunction following SAH. As for injury of the MTT, to the best of our
41 knowledge, one study was reported in patients with thalamic hemorrhage.³ Therefore, this is
42 the first DTT study on injury of the MTT in patients with SAH. However, several limitations
43 of DTI should be considered.^{36 37} First, DTI is a powerful anatomic imaging tool that can
44 demonstrate gross fiber architecture; however, reflection of all fibers, particularly small fibers,
45 can be difficult. Second, fiber tracking of the MTT might be affected by artifact, such as an
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4 aneurysmal clip. Therefore, conduct of further studies to overcome these limitations would be
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11 12 **Acknowledgement**

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22 **Contributors**

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24 Sung Ho Jang: Conceiving and designing the study, funding, data acquisition, manuscript
25 development and manuscript writing. Byung Yeon Choi: Acquisition and analysis of data,
26 Seong Ho Kim: Research design and data acquisition. Chul Hoon Chang: Research design
27 and data acquisition. Young Jin Jung: Research design and technical support. Hyeok Gyu
28 Kwon: Manuscript development, data acquisition, manuscript writing and manuscript
29 authorization.
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48 **Competing interests**

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50 None
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53 **Ethics approval**

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55 This study protocol was approved by the Institutional Review Board of the Yeungnam
56 university hospital.
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Figure legend

Fig 1. Diffusion tensor tractography (DTT) for the mammillothalamic tract (MTT). Brain CT shows subarachnoid hemorrhage and the MTTs in both hemispheres of a patient (34-year old male) are reconstructed between the mammillary body and the anterior thalamus. However, the right MTT (red) of the patient is thinned, compared with the left MTT (green) of the patients and normal control subjects.

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Fig. 2. The incidence of injury of the mammillothalamic tract

(FA: fractional anisotropy, MTT: mammillothalamic tract, TV: tract volume)

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Table 1. Demographic data for the patient and control groups.

	Patient group (<i>n</i> =16)	Control group (<i>n</i> =15)
Age (year)	51.6±13.3	48.1±15.1
Sex, male/female	9 / 7	7 / 8
Duration from onset (weeks)	5.7 ± 1.5	
Fisher's grading	3.4±0.5	
Ruptured artery (ACoA:ACA:MCA:PCA)	11:1:3:1	
Operation type (clipping:coiling:non)	12:2:2	
MMSE	19.8±8.8	

SAH: subarachnoid hemorrhage, ACoA: anterior communicating, ACA: anterior cerebral artery, MCA: middle cerebral artery, PCA: posterior communicating artery, MMSE: Mini-mental state examination

Table 2. Results of diffusion tensor tractography parameters of the mammillothalamic tract in the patient and control groups.

	Hemisphere	FA	<i>p</i>	Tract volume	<i>p</i>
Patient group	Right	0.36 (0.04)		69.08 (30.33)	
	Left	0.35 (0.04)		78.86 (28.80)	
	Both	0.36 (0.04)	.015*	74.15 (23.39)	.000*
Control group	Right	0.38 (0.04)		96.80 (22.7)	
	Left	0.37 (0.03)		99.73 (23.8)	
	Both	0.38 (0.03)		98.27 (22.9)	

Values represent mean (\pm standard deviation), FA: fractional anisotropy, *p*: An independent t-test for determination of variances in FA and tract volume between the patient and control groups.

* *p* < .05

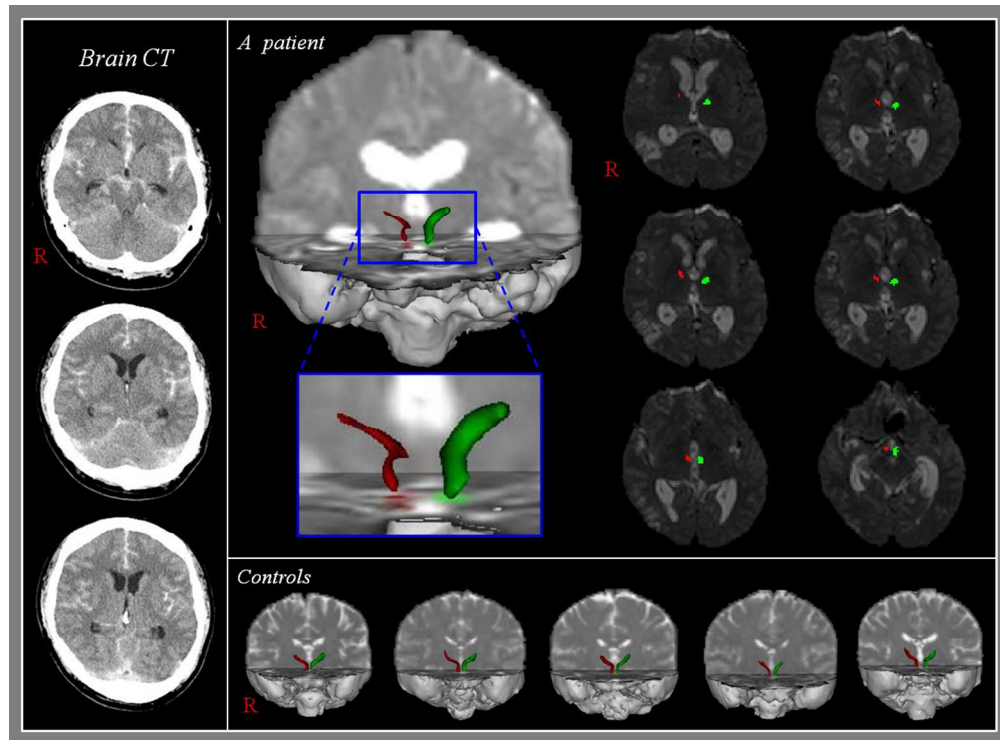


Fig 1. Diffusion tensor tractography (DTT) for the mammillothalamic tract (MTT). Brain CT shows subarachnoid hemorrhage and the MTTs in both hemispheres of a patient (34-year old male) are reconstructed between the mammillary body and the anterior thalamus. However, the right MTT (red) of the patient is thinned, compared with the left MTT (green) of the patients and normal control subjects. 148x109mm (300 x 300 DPI)

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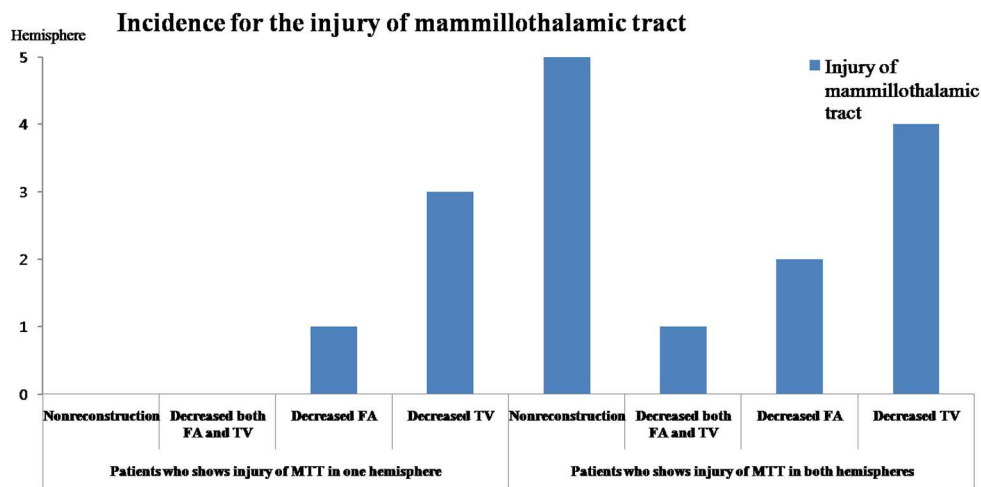


Fig. 2. The incidence of injury of the mammillothalamic tract (FA: fractional anisotropy, MTT: mammillothalamic tract, TV: tract volume)

216x109mm (300 x 300 DPI)

review only



CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	4
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	5
	2b	Specific objectives or hypotheses	5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	6-7
Participants	4a	Eligibility criteria for participants	6
	4b	Settings and locations where the data were collected	
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6-7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	6
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	6
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	6
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	

1			
2		assessing outcomes) and how	
3			
4		11b If relevant, description of the similarity of interventions	
5	Statistical methods	12a Statistical methods used to compare groups for primary and secondary outcomes	6
6		12b Methods for additional analyses, such as subgroup analyses and adjusted analyses	6
7			
8	Results		
9	Participant flow (a	13a For each group, the numbers of participants who were randomly assigned, received intended treatment, and	
10	diagram is strongly	were analysed for the primary outcome	
11	recommended)	13b For each group, losses and exclusions after randomisation, together with reasons	
12	Recruitment	14a Dates defining the periods of recruitment and follow-up	
13		14b Why the trial ended or was stopped	
14			
15	Baseline data	15 A table showing baseline demographic and clinical characteristics for each group	
16	Numbers analysed	16 For each group, number of participants (denominator) included in each analysis and whether the analysis was	
17		by original assigned groups	
18			
19	Outcomes and	17a For each primary and secondary outcome, results for each group, and the estimated effect size and its	9-10
20	estimation	precision (such as 95% confidence interval)	
21		17b For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
22	Ancillary analyses	18 Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing	9-10
23		pre-specified from exploratory	
24			
25	Harms	19 All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
26			
27	Discussion		
28	Limitations	20 Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	12-13
29	Generalisability	21 Generalisability (external validity, applicability) of the trial findings	
30	Interpretation	22 Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
31			
32	Other information		
33	Registration	23 Registration number and name of trial registry	
34	Protocol	24 Where the full trial protocol can be accessed, if available	
35	Funding	25 Sources of funding and other support (such as supply of drugs), role of funders	13
36			

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38 *We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also

39 recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials.

40 Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

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BMJ Open

Injury of the mammillothalamic tract in patients with subarachnoid hemorrhage: a retrospective diffusion tensor imaging study

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4 **Title: Injury of the mammillothalamic tract in patients with subarachnoid hemorrhage:**
5 **a retrospective diffusion tensor imaging study**
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8 **Running title: Injury of the mammillothalamic tract**
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Abstract

Objective: Few studies have reported on injury of the mammillothalamic tract (MTT) in stroke patients. However, no study in patients with subarachnoid hemorrhage (SAH) has been reported. Using diffusion tensor tractography (DTT), we attempted to investigate injury of the MTT in patients with subarachnoid hemorrhage.

Methods: We recruited 16 patients with SAH and 15 control subjects. Diffusion tensor imaging (DTI) was obtained at 5.7 ± 1.5 weeks after onset and reconstruction of the MTT was performed using the probabilistic tractography method. The fractional anisotropy (FA) value and tract number of the MTT and the Mini-Mental State Examination (MMSE) score were determined. Values of FA and tract volume showing decrement of more than two standard deviations that of normal control were defined as abnormal.

Results: The FA value and tract volume in the patient group were significantly lower than those of the control group ($p < .05$). In addition, MMSE showed strong ($r = 0.67$, $p = 0.005$) positive correlation with tract volume without correlation with FA. In the individual analysis, 16 MTTs of 32 MTTs in 16 patients showed abnormalities of the MTT in terms of the FA value, the tract volume, or the presence of a reconstructed MTT. As a result, ten (62.5%) of 16 patients showed abnormality of the MTT in at least one hemisphere.

Conclusion: We found that patients with SAH showed injury of the MTT and this injury showed correlation with cognitive dysfunction.

Keywords: Mammillothalamic tract, Subarachnoid hemorrhage, Diffusion tensor tractography

Strengths and limitations of this study

- This is the first study on the injury of the mammillothalamic tract (MTT) in patients with subarachnoid hemorrhage (SAH) using diffusion tensor imaging (DTI).
- The main strength of our study is to find the relation between MTT injury and cognitive function with SAH
- Limitation of our study is that fiber tracking of the MTT might be affected by artifact, such as an aneurysmal clip. Therefore, conduct of further studies to overcome this limitation would be necessary.

Introduction

The mammillothalamic tract (MTT) connects the mammillary body and the anterior thalamus as a part of the Papez circuit.¹ Because of the anatomical characteristics of the MTT, thin, short, and located deep within the brain, accurate estimation of the MTT has been difficult in the live human brain. However, diffusion tensor tractography (DTT), which is derived from diffusion tensor imaging (DTI), allows for reconstruction of the MTT in the human brain.² Therefore, accurate estimation of the MTT in terms of DTI parameters and three-dimensional configuration is now possible.² A few studies have reported on injury of the MTT in thalamic hemorrhage and thalamic infarct, however, so far, little is known about injury of the MTT.^{3 4}

Subarachnoid hemorrhage (SAH) is extravasation of blood into the subarachnoid space accompanied by various neurological sequelae in terms of memory, executive function, language, motor function, and cranial nerve function.⁵⁻¹⁶ In particular, memory deficit is known to be one of the most common sequelae of SAH.^{7 8 10 11 14} In the past, previous studies have suggested that the neurotoxic effects of blood, vasospasm, or increased intracranial pressure are pathogenic mechanisms of neural sequelae following SAH.^{5 7 17-23} After introduction of DTI, among the neural tracts associated with memory, injuries in the fornix and cingulum were reported in patients with SAH.²⁴ However, no study on neural injury of the MTT in patients with SAH has been reported.

In the current study, using DTT, we attempted to investigate injury of the MTT in patients with SAH.

Methods

Subjects

We recruited 16 patients with SAH (male: 9, female: 7, mean age: 51.6±13.3 years, range: 34~70 years) and 15 normal healthy control subjects (male: 7, female: 8, mean age: 48.1±15.1 years, range: 20~67 years) with no previous history of neurological, physical, or psychiatric illness for this study. Inclusion criteria for patients were as follows: (1) first ever stroke, (2) age 30~70 years, (3) hemorrhage in the subarachnoid space due to aneurismal rupture confirmed by a neuroradiologist, (4) DTI was scanned at a chronic stage after onset, and (5) no hydrocephalus, intracerebral hemorrhage, or intraventricular hemorrhage. Severity of SAH was assessed according to the modified Fisher CT grade, World Federation of Neurosurgical Societies (WFNS) and Hijdra score.²⁵⁻²⁷ Patients who showed any lesion or artifact due to the clipping or coiling along or around the MTT pathway between the mammillary body and thalamus were excluded. This study was conducted retrospectively and the study protocol was approved by the Institutional Review Board of a university hospital.

Figure 1

Clinical evaluation

Cognitive function was evaluated at the time of DTI scanning. The mini-mental state examination (MMSE) was used for assessment of cognitive impairment. The reliability and validity of the MMSE have been well established.²⁸

Diffusion tensor tractography

A 6-channel head coil on a 1.5 T Philips Gyroscan Intera (Philips, Ltd, Best, The Netherlands) with single-shot echo-planar imaging was used for acquisition of DTI data. For each of the 32 non-collinear diffusion sensitizing gradients, we acquired 67 contiguous slices parallel to the anterior commissure-posterior commissure line. Imaging parameters were as follows: acquisition matrix = 96×96 ; reconstructed to matrix = 128×128 ; field of view = $221 \times 221 \text{ mm}^2$; TR = 10,726 ms; TE = 76 ms; parallel imaging reduction factor (SENSE factor) = 2; EPI factor = 49; $b = 1000 \text{ s/mm}^2$; NEX = 1; and a slice thickness of 2.3 mm (acquired voxel size $1.73 \times 1.73 \times 2.3 \text{ mm}^3$). Affine multi-scale two-dimensional registration using the Oxford Centre for Functional Magnetic Resonance Imaging of Brain (FMRIB) Software Library (FSL; www.fmrib.ox.ac.uk/fsl) was used for removal of eddy current-induced image distortions.²⁹ Fiber tracking was performed using a probabilistic tractography method based on a multi-fiber model, and applied in the current study utilizing tractography routines implemented in FMRIB Diffusion (5000 streamline samples, 0.5 mm step lengths, curvature thresholds = 0.2).²⁹⁻³¹ MTTs were determined by selection of fibers passing through three regions of interest (ROIs). Seed ROIs were placed on the mammillary body on the axial image.² Two target ROIs were drawn at the portion of the MTT area (between the portion of the fornix and the red nucleus in the anteroposterior direction) at about the bicommissural level and the portion of the anterior thalamus on the axial image.² Of 5000 samples generated from each seed voxel, results for each contact were the visualized threshold point at 5 streamline through each voxel for analysis. Values of fractional anisotropy (FA) and tract volume which was determined by counting the voxels of MTT were measured using MATLABTM (Matlab R2007b, The Mathworks, Natick, MA, USA). Values of FA and tract

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4 volume showing decrement of more than two standard deviations that of normal control were
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6 defined as abnormal.
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9 **Statistical analysis**

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12 We used SPSS software (v.15.0; SPSS, Chicago, IL) for data analysis. Data on MTTs
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14 that were not reconstructed were excluded in statistical analysis. An independent t-test was
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16 used for determination of variances in the value of FA and tract volume between the patient
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18 and control group. Subsequently, using Pearson correlation, DTT parameters for FA and tract
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20 volume of the patient group were used in determination of correlation with MMSE.³² The
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22 significant level of the *p* value was set at 0.05.
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Results

A summary of the demographic data for the patient and control groups is shown in Table 1. The artery distribution of aneurismal rupture for SAH in 16 patients was as follows: the anterior communicating artery: 11 patients (68.8%), the middle cerebral artery: three patients (18.8%), the posterior communicating artery: one patient (6.2%), and the anterior cerebral artery: one patient (6.2%). In addition, 12 patients (75%) patients underwent aneurysmal clipping and two patients underwent endovascular coiling (12.5%). The two remaining patients (12.5%) received conservative treatment. Average MMSE was 19.8 ± 8.8 . Average modified Fisher CT grade, WFNS, and Hijdra score were 2.5 ± 0.9 , 2.1 ± 1.3 , and 15.7 ± 6.3 , respectively.²⁵⁻²⁸ DTI scanning was performed at 5.7 ± 1.5 weeks (range: 4~8 weeks) after SAH onset.

Table 1

A summary of the results of DTT parameters for MTT in the patient and control groups is shown in Table 2. Values for FA and tract volume were significantly lower in the patient group than in the control group, respectively ($p < .05$). The tract volume of the MTT showed strong ($r = 0.67$, $p = 0.005$) positive correlation with MMSE, however, no correlation was observed between the FA value of MTT and MMSE ($r = 0.41$, $p = 0.11$).³²

Table 2

Figure 2 shows the results for incidence of injury of the MTT with DTT parameters. Among 32 hemispheres of 16 patients, the MTT was not reconstructed in five hemispheres of four patients (right hemisphere: two, left hemisphere: one, both hemispheres: one); in contrast,

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4 the MTTs were reconstructed in all 30 hemispheres of 15 control subjects. With regard to the
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6 FA and tract volume, nine MTTs in six patients (right hemisphere: one, left hemisphere: two,
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8 both hemispheres: three) and 13 MTTs in nine patients (right hemisphere: four, left
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10 hemisphere: one, both hemispheres: four) of 32 hemispheres in 16 patients revealed a
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12 decrement of more than two standard deviations, compared with control subjects. Six MTTs
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14 in five patients (right hemisphere: three, left hemisphere: one, both hemispheres: one) showed
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16 a decrement of more than two standard deviations in both FA and tract volume. As a result,
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18 16 MTTs (right hemisphere: two, left hemisphere: two, both hemispheres: six) of 32 MTTs in
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20 16 patients showed abnormalities of the MTT. Three patients in one hemisphere and one
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22 patient in both hemispheres whose MTT was not reconstructed belonged to these 16 patients.
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24 Consequently, in the individual analysis, the prevalence of MTT abnormality was 62.5% (10
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26 of 16 patients).
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Discussion

In the current study, we investigated injury of the MTT in patients with SAH using DTT. We obtained the following results: 1) the FA value and tract volume in the patient group were significantly lower than those of the control group, 2) MMSE showed strong ($r=0.67$, $p=0.005$) positive correlation with tract volume without correlation with FA value, 3) in the individual analysis, 16 MTTs of 32 MTTs in 16 patients showed abnormalities of the MTT in terms of the FA value, tract volume, or the presence of a reconstructed MTT. As a result, ten (62.5%) of 16 patients showed abnormality of the MTT in at least one hemisphere. The FA value indicates the degree of directionality of water diffusion and has a range of zero (completely isotropic diffusion) to one (completely anisotropic diffusion). It represents the white matter organization: in detail, the degree of directionality and integrity of white matter microstructures such as axons, myelin, and microtubules.³³ In contrast, tract volume, the number of voxels in a neural tract, reflects the neural fibers contained within a neural tract.³⁴ Therefore, a decrease in the FA or tract volume indicates an injury of the neural tract. Consequently, our results showing that the FA value and tract volume in the patient group were significantly lower than those of the control group indicate injury of the MTT in the patient group. Regarding the relation between MTT injury and cognition, MMSE showed strong positive correlation with the tract volume of the MTT without correlation with FA.³² These results appear to indicate that the tract volume is more sensitive than the FA value for detection of injury of the MTT. The MMSE is the most widely used tool for screening cognitive dysfunction.³⁵ Because this study was conducted retrospectively, we could not employ detailed neuropsychological testing for evaluation of the function of the MTT. Therefore, conduct of further prospective studies, including specific neurophysiological tests

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4 for MTT function should be encouraged.
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7 The pathogenic mechanism of neural injury following SAH has not been elucidated and
8 few DTI studies have reported on this mechanism.^{15 36} In 2007, Liu et al. demonstrated that
9 SAH caused global mild vasogenic edema in white matter and deep gray matter by measuring
10 the apparent diffusion coefficient value in the subacute stage of SAH, which was
11 undetectable on T2-weighted and diffusion-weighted MR images.³⁶ In a recent study, Yeo et
12 al. [2012] reported injury of the CST at the midbrain in patients with SAH.¹⁵ This study
13 suggested that frequent occurrence of SAH into perimesencephalic cisterns could be ascribed
14 to injury of the CST, through mechanical (increased intracranial pressure or direct mass) or
15 chemical mechanisms (a blood clot itself can cause extensive damage).^{5 23 37} Considering that
16 the MTT is located in close proximity to a cistern, the MTT in patients with SAH appears to
17 be injured by mechanisms similar to those of CST injury at the midbrain.^{5 15 17 18 23}
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32 As for injury of the MTT, to the best of our knowledge, one study was reported in
33 patients with thalamic hemorrhage.³ Therefore, this is the first DTI study on injury of the
34 MTT in patients with SAH. However, several limitations of DTI should be considered.^{38 39}
35 First, DTI is a powerful anatomic imaging tool that can demonstrate gross fiber architecture;
36 however, reflection of all fibers, particularly small fibers, can be difficult. Second, fiber
37 tracking of the MTT might be affected by artifact, such as an aneurysmal clip. Third, we
38 investigated only injury of the MTT following SAH even though other tracts such as fornix,
39 cingulum, thalamocortical tract of Papez circuit might be also injured. Therefore, conduct of
40 further studies to overcome these limitations would be necessary.
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53 In conclusion, we found injury of the MTT in the patient group, compared with the
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4 control group. In addition, in the individual analysis, 16 MTTs (50%) of 32 hemispheres
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6 showed injury of the MTT and ten (62.5%) of 16 patients showed injury of the MTT in at
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8 least in one hemisphere in terms of DTT parameters or the presence of a reconstructed MTT.
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Contributorship statement

Sung Ho Jang: Conceiving and designing the study, funding, data acquisition, manuscript development and manuscript writing. Byung Yeon Choi: Acquisition and analysis of data, Seong Ho Kim: Research design and data acquisition. Chul Hoon Chang: Research design and data acquisition. Young Jin Jung: Research design and technical support. Hyeok Gyu Kwon: Manuscript development, data acquisition, manuscript writing and manuscript authorization.

Competing interests

None

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Data sharing statement

No additional data are available.

Ethics approval

This study protocol was approved by the Institutional Review Board of the Yeungnam university hospital.

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Table 1. Demographic data for the patient and control groups.

	Patient group (<i>n</i> =16)	Control group (<i>n</i> =15)
Age (year)	51.6±13.3	48.1±15.1
Sex, male/female	9 / 7	7 / 8
Duration from onset (weeks)	5.7 ± 1.5	
Modified Fisher grade	2.5 ± 0.9	
WFNS	2.1 ± 1.3	
Hijdra score	15.7 ± 6.3	
Ruptured artery (ACoA:ACA:MCA:PCA)	11:1:3:1	
Operation type (clipping:coiling:non)	12:2:2	
MMSE	19.8±8.8	

WFNS: World Federation of Neurosurgical Societies, ACoA: anterior communicating, ACA: anterior cerebral artery, MCA: middle cerebral artery, PCA: posterior communicating artery, MMSE: Mini-mental state examination

Table 2. Results of diffusion tensor tractography parameters of the mammillothalamic tract in the patient and control groups.

	Hemisphere	FA	<i>p</i>	Tract volume	<i>p</i>
Patient group	Right	0.36 (0.04, 0.36)		69.08 (30.33, 72.00)	
	Left	0.35 (0.04, 0.36)		78.86 (28.80, 80.50)	
	Both	0.36 (0.04, 0.36)	.015*	74.15 (23.39, 74.00)	.000*
Control group	Right	0.38 (0.04, 0.38)		96.80 (22.7, 96.00)	
	Left	0.37 (0.03, 0.38)		99.73 (23.8, 103.00)	
	Both	0.38 (0.03, 0.38)		98.27 (22.9, 98.50)	

Values represent mean (\pm standard deviation, median value), FA: fractional anisotropy, *p*: An independent t-test for determination of variances in FA and tract volume between the patient and control groups.

* *p* < .05

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4 **Figure legend**
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7 **Fig 1.** Diffusion tensor tractography (DTT) for the mammillothalamic tract (MTT). Brain CT
8 shows subarachnoid hemorrhage and the MTTs in both hemispheres of a patient (34-year old
9 male) are reconstructed between the mammillary body and the anterior thalamus. However,
10 the right MTT (red) of the patient is thinned, compared with the left MTT (green) of the
11 patients and normal control subjects.
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19 **Fig. 2.** The incidence of injury of the mammillothalamic tract
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22 (FA: fractional anisotropy, MTT: mammillothalamic tract, TV: tract volume)
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4 **Title: Injury of the mammillothalamic tract in patients with subarachnoid hemorrhage:**
5 **a retrospective diffusion tensor imaging study**
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8 **Running title: Injury of the mammillothalamic tract**
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For peer review only

Abstract

Objective: Few studies have reported on injury of the mammillothalamic tract (MTT) in stroke patients. However, no study in patients with subarachnoid hemorrhage (SAH) has been reported. Using diffusion tensor tractography (DTT), we attempted to investigate injury of the MTT in patients with subarachnoid hemorrhage.

Methods: We recruited 16 patients with SAH and 15 control subjects. Diffusion tensor imaging (DTI) was obtained at 5.7 ± 1.5 weeks after onset and reconstruction of the MTT was performed using the probabilistic tractography method. The fractional anisotropy (FA) value and tract number of the MTT and the Mini-Mental State Examination (MMSE) score were determined. Values of FA and tract volume showing decrement of more than two standard deviations that of normal control were defined as abnormal.

Results: The FA value and tract volume in the patient group were significantly lower than those of the control group ($p < .05$). In addition, MMSE showed strong ($r = 0.67$, $p = 0.005$) positive correlation with tract volume without correlation with FA. In the individual analysis, 16 MTTs of 32 MTTs in 16 patients showed abnormalities of the MTT in terms of the FA value, the tract volume, or the presence of a reconstructed MTT. As a result, ten (62.5%) of 16 patients showed abnormality of the MTT in at least one hemisphere.

Conclusion: We found that patients with SAH showed injury of the MTT and this injury showed correlation with cognitive dysfunction. ~~Our results suggest that evaluation of the MTT using DTI would be necessary for patients with cognitive dysfunction following SAH.~~

Keywords: Mammillothalamic tract, Subarachnoid hemorrhage, Diffusion tensor tractography

Strengths and limitations of this study

- This is the first study on the injury of the mammillothalamic tract (MTT) in patients with subarachnoid hemorrhage (SAH) using diffusion tensor imaging (DTI).
- The main strength of our study is to find the relation between MTT injury and cognitive function with SAH
- Limitation of our study is that fiber tracking of the MTT might be affected by artifact, such as an aneurysmal clip. Therefore, conduct of further studies to overcome this limitation would be necessary.

Introduction

The mammillothalamic tract (MTT) connects the mammillary body and the anterior thalamus as a part of the Papez circuit.¹ Because of the anatomical characteristics of the MTT, thin, short, and located deep within the brain, accurate estimation of the MTT has been difficult in the live human brain. However, diffusion tensor tractography (DTT), which is derived from diffusion tensor imaging (DTI), allows for reconstruction of the MTT in the human brain.² Therefore, accurate estimation of the MTT in terms of DTI parameters and three-dimensional configuration is now possible.² A few studies have reported on injury of the MTT in thalamic hemorrhage and thalamic infarct, however, so far, little is known about injury of the MTT.^{3 4}

Subarachnoid hemorrhage (SAH) is extravasation of blood into the subarachnoid space accompanied by various neurological sequelae in terms of memory, executive function, language, motor function, and cranial nerve function.⁵⁻¹⁶ In particular, memory deficit is known to be one of the most common sequelae of SAH.^{7 8 10 11 14} In the past, previous studies have suggested that the neurotoxic effects of blood, vasospasm, or increased intracranial pressure are pathogenic mechanisms of neural sequelae following SAH.^{5 7 17-23} After introduction of DTI, among the neural tracts associated with memory, injuries in the fornix and cingulum were reported in patients with SAH.²⁴ However, no study on neural injury of the MTT in patients with SAH has been reported.

In the current study, using DTT, we attempted to investigate injury of the MTT in patients with SAH.

Methods

Subjects

We recruited 16 patients with SAH (male: 9, female: 7, mean age: 51.6±13.3 years, range: 34~70 years) and 15 normal healthy control subjects (male: 7, female: 8, mean age: 48.1±15.1 years, range: 20~67 years) with no previous history of neurological, physical, or psychiatric illness for this study. Inclusion criteria for patients were as follows: (1) first ever stroke, (2) age 30~70 years, (3) hemorrhage in the subarachnoid space due to aneurismal rupture confirmed by a neuroradiologist, (4) DTI was scanned at a chronic stage (~~between 4~8 weeks~~) after onset, and (5) no hydrocephalus, intracerebral hemorrhage, or intraventricular hemorrhage. Severity of SAH was assessed according to the modified Fisher CT grade, World Federation of Neurosurgical Societies (WFNS) and Hijdra score.²⁵⁻²⁷ Patients who showed any lesion or artifact due to the clipping or coiling along or around the MTT pathway between the mammillary body and thalamus were excluded. This study was conducted retrospectively and the study protocol was approved by the Institutional Review Board of a university hospital.

Figure 1

Clinical evaluation

Cognitive function was evaluated at the time of DTI scanning. The mini-mental state examination (MMSE) was used for assessment of cognitive impairment. The reliability and validity of the MMSE have been well established.^{28,26}

Diffusion tensor tractography

A 6-channel head coil on a 1.5 T Philips Gyroscan Intera (Philips, Ltd, Best, The Netherlands) with single-shot echo-planar imaging was used for acquisition of DTI data. For each of the 32 non-collinear diffusion sensitizing gradients, we acquired 67 contiguous slices parallel to the anterior commissure-posterior commissure line. Imaging parameters were as follows: acquisition matrix = 96×96 ; reconstructed to matrix = 128×128 ; field of view = $221 \times 221 \text{ mm}^2$; TR = 10,726 ms; TE = 76 ms; parallel imaging reduction factor (SENSE factor) = 2; EPI factor = 49; $b = 1000 \text{ s/mm}^2$; NEX = 1; and a slice thickness of 2.3 mm (acquired voxel size $1.73 \times 1.73 \times 2.3 \text{ mm}^3$). Affine multi-scale two-dimensional registration using the Oxford Centre for Functional Magnetic Resonance Imaging of Brain (FMRIB) Software Library (FSL; www.fmrib.ox.ac.uk/fsl) was used for removal of eddy current-induced image distortions.^{29,27} Fiber tracking was performed using a probabilistic tractography method based on a multi-fiber model, and applied in the current study utilizing tractography routines implemented in FMRIB Diffusion (5000 streamline samples, 0.5 mm step lengths, curvature thresholds = 0.2).^{29-31,27-29} MTTs were determined by selection of fibers passing through three regions of interest (ROIs). Seed ROIs were placed on the mammillary body on the axial image.² Two target ROIs were drawn at the portion of the MTT area (between the portion of the fornix and the red nucleus in the anteroposterior direction) at about the bicommissural level and the portion of the anterior thalamus on the axial image.² Of 5000 samples generated from each seed voxel, results for each contact were the visualized threshold point at 5 streamline through each voxel for analysis. Values of fractional anisotropy (FA) and tract volume which was determined by counting the voxels of MTT were measured using MATLABTM (Matlab R2007b, The Mathworks, Natick, MA,

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4 | USA). Values of FA and tract volume showing decrement of more than two standard
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6 deviations that of normal control were defined as abnormal.
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9 **Statistical analysis**

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12 We used SPSS software (v.15.0; SPSS, Chicago, IL) for data analysis. Data on MTTs
13 that were not reconstructed were excluded in statistical analysis. An independent t-test was
14 used for determination of variances in the value of FA and tract volume between the patient
15 and control group. Subsequently, using Pearson correlation, DTT parameters for FA and tract
16 volume of the patient group were used in determination of correlation with MMSE.³²³⁰ The
17 significant level of the *p* value was set at 0.05.
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Results

A summary of the demographic data for the patient and control groups is shown in Table 1. The artery distribution of aneurismal rupture for SAH in 16 patients was as follows: the anterior communicating artery: 11 patients (68.8%), the middle cerebral artery: three patients (18.8%), the posterior communicating artery: one patient (6.2%), and the anterior cerebral artery: one patient (6.2%). In addition, 12 patients (75%) patients underwent aneurysmal clipping and two patients underwent endovascular coiling (12.5%). The two remaining patients (12.5%) received conservative treatment. Average MMSE was 19.8 ± 8.8 . Average modified Fisher CT grade, WFNS, and Hijdra score were 3.4 ± 0.5 , 2.5 ± 0.9 , 2.1 ± 1.3 , and 15.7 ± 6.3 , respectively.²⁵⁻²⁸ DTI scanning was performed at 5.7 ± 1.5 weeks (range: 4~8 weeks) after SAH onset.

Table 1

A summary of the results of DTT parameters for MTT in the patient and control groups is shown in Table 2. Values for FA and tract volume were significantly lower in the patient group than in the control group, respectively ($p < .05$). The tract volume of the MTT showed strong ($r = 0.67$, $p = 0.005$) positive correlation with MMSE, however, no correlation was observed between the FA value of MTT and MMSE ($r = 0.41$, $p = 0.11$).^{32 30}

Table 2

Figure 2 shows the results for incidence of injury of the MTT with DTT parameters. Among 32 hemispheres of 16 patients, the MTT was not reconstructed in five hemispheres of four patients (right hemisphere: two, left hemisphere: one, both hemispheres: one); in contrast,

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4 the MTTs were reconstructed in all 30 hemispheres of 15 control subjects. With regard to the
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6 FA and tract volume, nine MTTs in six patients (right hemisphere: one, left hemisphere: two,
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8 both hemispheres: three) and 13 MTTs in nine patients (right hemisphere: four, left
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10 hemisphere: one, both hemispheres: four) of 32 hemispheres in 16 patients revealed a
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12 decrement of more than two standard deviations, compared with control subjects. Six MTTs
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14 in five patients (right hemisphere: three, left hemisphere: one, both hemispheres: one) showed
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16 a decrement of more than two standard deviations in both FA and tract volume. As a result,
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18 16 MTTs (right hemisphere: two, left hemisphere: two, both hemispheres: six) of 32 MTTs in
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20 16 patients showed abnormalities of the MTT. Three patients in one hemisphere and one
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22 patient in both hemispheres whose MTT was not reconstructed belonged to these 16 patients.
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24 Consequently, in the individual analysis, the prevalence of MTT abnormality was 62.5% (10
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26 of 16 patients).
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Discussion

In the current study, we investigated injury of the MTT in patients with SAH using DTT. We obtained the following results: 1) the FA value and tract volume in the patient group were significantly lower than those of the control group, 2) MMSE showed strong ($r=0.67$, $p=0.005$) positive correlation with tract volume without correlation with FA value, 3) in the individual analysis, 16 MTTs of 32 MTTs in 16 patients showed abnormalities of the MTT in terms of the FA value, tract volume, or the presence of a reconstructed MTT. As a result, ten (62.5%) of 16 patients showed abnormality of the MTT in at least one hemisphere. The FA value indicates the degree of directionality of water diffusion and has a range of zero (completely isotropic diffusion) to one (completely anisotropic diffusion). It represents the white matter organization: in detail, the degree of directionality and integrity of white matter microstructures such as axons, myelin, and microtubules.³³³⁴ In contrast, tract volume, the number of voxels in a neural tract, reflects the neural fibers contained within a neural tract.³⁴³² Therefore, a decrease in the FA or tract volume indicates an injury of the neural tract. Consequently, our results showing that the FA value and tract volume in the patient group were significantly lower than those of the control group indicate injury of the MTT in the patient group. Regarding the relation between MTT injury and cognition, MMSE showed strong positive correlation with the tract volume of the MTT without correlation with FA.³²³⁰ These results appear to indicate that the tract volume is more sensitive than the FA value for detection of injury of the MTT. The MMSE is the most widely used tool for screening cognitive dysfunction.³⁵³³ Because this study was conducted retrospectively, we could not employ detailed neuropsychological testing for evaluation of the function of the MTT. Therefore, conduct of further prospective studies, including specific neurophysiological tests

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4 for MTT function should be encouraged.
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7 The pathogenic mechanism of neural injury following SAH has not been elucidated and
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9 few DTI studies have reported on this mechanism.^{15 3634} In 2007, Liu et al. demonstrated that
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11 SAH caused global mild vasogenic edema in white matter and deep gray matter by measuring
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13 the apparent diffusion coefficient value in the subacute stage of SAH, which was
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15 undetectable on T2-weighted and diffusion-weighted MR images.³⁶³⁴ In a recent study, Yeo et
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17 al. [2012] reported injury of the CST at the midbrain in patients with SAH.¹⁵ This study
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19 suggested that frequent occurrence of SAH into perimesencephalic cisterns could be ascribed
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21 to injury of the CST, through mechanical (increased intracranial pressure or direct mass) or
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23 chemical mechanisms (a blood clot itself can cause extensive damage).^{5 23 3735} Considering
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25 that the MTT is located in close proximity to a cistern, the MTT in patients with SAH appears
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27 to be injured by mechanisms similar to those of CST injury at the midbrain.^{5 15 17 18 23}
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32 As for injury of the MTT, to the best of our knowledge, one study was reported in
33 patients with thalamic hemorrhage.³ Therefore, this is the first DTI study on injury of the
34 MTT in patients with SAH. However, several limitations of DTI should be considered.^{38 39}
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36 First, DTI is a powerful anatomic imaging tool that can demonstrate gross fiber architecture;
37 however, reflection of all fibers, particularly small fibers, can be difficult. Second, fiber
38 tracking of the MTT might be affected by artifact, such as an aneurysmal clip. Third, we
39 investigated only injury of the MTT following SAH even though other tracts such as fornix,
40 cingulum, thalamocortical tract of Papez circuit might be also injured. Therefore, conduct of
41 further studies to overcome these limitations would be necessary.
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53 In conclusion, we found injury of the MTT in the patient group, compared with the
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4 control group. In addition, in the individual analysis, 16 MTTs (50%) of 32 hemispheres
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6 showed injury of the MTT and ten (62.5%) of 16 patients showed injury of the MTT in at
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8 least in one hemisphere in terms of DTT parameters or the presence of a reconstructed MTT.
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11 ~~As for injury of the MTT, to the best of our knowledge, one study was reported in patients~~
12 ~~with thalamic hemorrhage.³ Therefore, this is the first DTT study on injury of the MTT in~~
13 ~~patients with SAH. However, several limitations of DTI should be considered.^{36,37} First, DTI~~
14 ~~is a powerful anatomic imaging tool that can demonstrate gross fiber architecture; however,~~
15 ~~reflection of all fibers, particularly small fibers, can be difficult. Second, fiber tracking of the~~
16 ~~MTT might be affected by artifact, such as an aneurysmal clip. Therefore, conduct of further~~
17 ~~studies to overcome these limitations would be necessary.~~
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Contributorship statement

Sung Ho Jang: Conceiving and designing the study, funding, data acquisition, manuscript development and manuscript writing. Byung Yeon Choi: Acquisition and analysis of data, Seong Ho Kim: Research design and data acquisition. Chul Hoon Chang: Research design and data acquisition. Young Jin Jung: Research design and technical support. Hyeok Gyu Kwon: Manuscript development, data acquisition, manuscript writing and manuscript authorization.

Competing interests

None

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Data sharing statement

No additional data are available.

Ethics approval

This study protocol was approved by the Institutional Review Board of the Yeungnam university hospital.

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7 **Fig 1.** Diffusion tensor tractography (DTT) for the mammillothalamic tract (MTT). Brain CT
8 shows subarachnoid hemorrhage and the MTTs in both hemispheres of a patient (34-year old
9 male) are reconstructed between the mammillary body and the anterior thalamus. However,
10 the right MTT (red) of the patient is thinned, compared with the left MTT (green) of the
11 patients and normal control subjects.
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Fig. 2. The incidence of injury of the mammillothalamic tract

(FA: fractional anisotropy, MTT: mammillothalamic tract, TV: tract volume)

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Table 1. Demographic data for the patient and control groups.

	Patient group (n=16)	Control group (n=15)
Age (year)	51.6±13.3	48.1±15.1
Sex, male/female	9 / 7	7 / 8
Duration from onset (weeks)	5.7 ± 1.5	
<u>Modified Fisher's grading</u>	<u>3.4±0.5</u>	<u>2.5 ± 0.9</u>
<u>WFNS</u>	<u>2.1 ± 1.3</u>	
<u>Hijdra score</u>	<u>15.7 ± 6.3</u>	
Ruptured artery (ACoA:ACA:MCA:PCA)	11:1:3:1	
Operation type (clipping:coiling:non)	12:2:2	
MMSE	19.8±8.8	
<u>SAH: subarachnoid hemorrhage, WFNS: World Federation of Neurosurgical Societies,</u> ACoA: anterior communicating, ACA: anterior cerebral artery, MCA: middle cerebral artery, PCA: posterior communicating artery, MMSE: Mini-mental state examination		

Table 2. Results of diffusion tensor tractography parameters of the mammillothalamic tract in the patient and control groups.

	Hemisphere	FA	<i>p</i>	Tract volume	<i>p</i>
Patient group	Right	0.36 (0.04, <u>0.36</u>)		69.08 (30.33, <u>72.00</u>)	
	Left	0.35 (0.04, <u>0.36</u>)		78.86 (28.80, <u>80.50</u>)	
	Both	0.36 (0.04, <u>0.36</u>)	.015*	74.15 (23.39, <u>74.00</u>)	.000*
Control group	Right	0.38 (0.04, <u>0.38</u>)		96.80 (22.7, <u>96.00</u>)	
	Left	0.37 (0.03, <u>0.38</u>)		99.73 (23.8, <u>103.00</u>)	
	Both	0.38 (0.03, <u>0.38</u>)		98.27 (22.9, <u>98.50</u>)	

Values represent mean (\pm standard deviation, median value), FA: fractional anisotropy, *p*: An independent t-test for determination of variances in FA and tract volume between the patient and control groups.

* *p* < .05

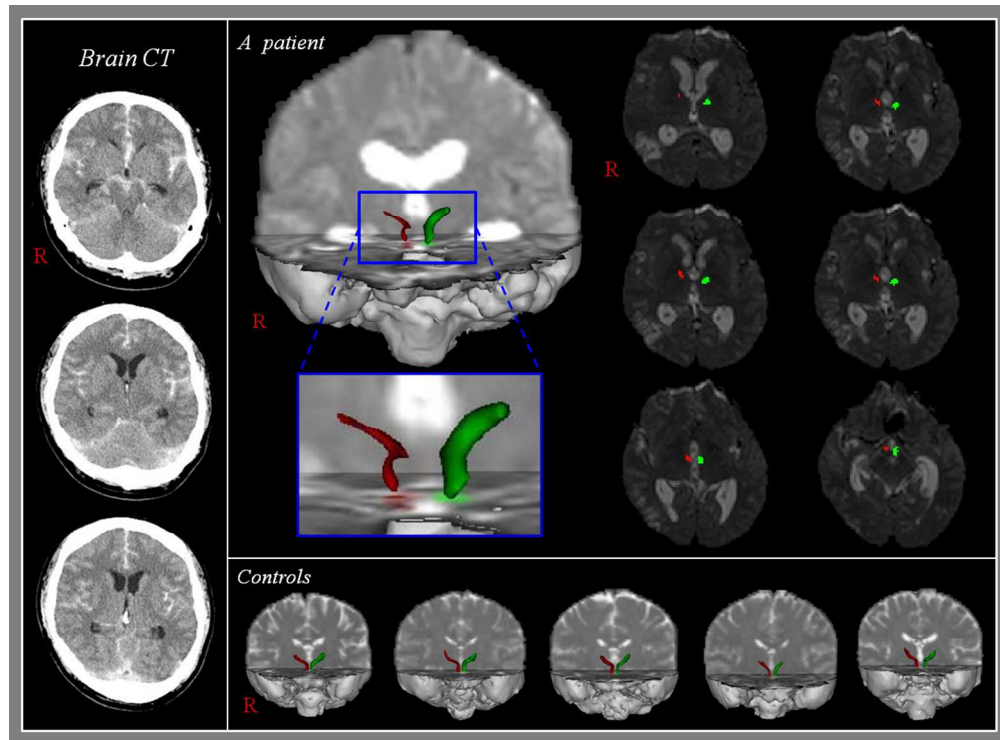


Fig 1. Diffusion tensor tractography (DTT) for the mammillothalamic tract (MTT). Brain CT shows subarachnoid hemorrhage and the MTTs in both hemispheres of a patient (34-year old male) are reconstructed between the mammillary body and the anterior thalamus. However, the right MTT (red) of the patient is thinned, compared with the left MTT (green) of the patients and normal control subjects. 148x109mm (300 x 300 DPI)

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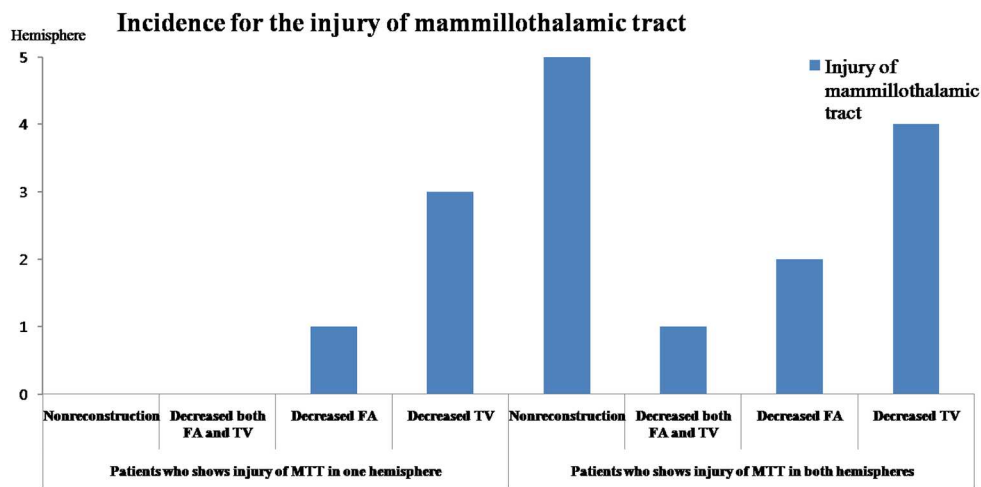


Fig. 2. The incidence of injury of the mammillothalamic tract (FA: fractional anisotropy, MTT: mammillothalamic tract, TV: tract volume)

216x109mm (300 x 300 DPI)

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CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	4
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	5
	2b	Specific objectives or hypotheses	5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	6-7
Participants	4a	Eligibility criteria for participants	6
	4b	Settings and locations where the data were collected	
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6-7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	6
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	6
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	6
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	

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2		assessing outcomes) and how	
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4		11b If relevant, description of the similarity of interventions	
5	Statistical methods	12a Statistical methods used to compare groups for primary and secondary outcomes	6
6		12b Methods for additional analyses, such as subgroup analyses and adjusted analyses	6
7			
8	Results		
9	Participant flow (a	13a For each group, the numbers of participants who were randomly assigned, received intended treatment, and	
10	diagram is strongly	were analysed for the primary outcome	
11	recommended)	13b For each group, losses and exclusions after randomisation, together with reasons	
12	Recruitment	14a Dates defining the periods of recruitment and follow-up	
13		14b Why the trial ended or was stopped	
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15	Baseline data	15 A table showing baseline demographic and clinical characteristics for each group	
16	Numbers analysed	16 For each group, number of participants (denominator) included in each analysis and whether the analysis was	
17		by original assigned groups	
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19	Outcomes and	17a For each primary and secondary outcome, results for each group, and the estimated effect size and its	9-10
20	estimation	precision (such as 95% confidence interval)	
21		17b For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
22	Ancillary analyses	18 Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing	9-10
23		pre-specified from exploratory	
24			
25	Harms	19 All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
26			
27	Discussion		
28	Limitations	20 Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	12-13
29	Generalisability	21 Generalisability (external validity, applicability) of the trial findings	
30	Interpretation	22 Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
31			
32	Other information		
33	Registration	23 Registration number and name of trial registry	
34	Protocol	24 Where the full trial protocol can be accessed, if available	
35	Funding	25 Sources of funding and other support (such as supply of drugs), role of funders	13
36			

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38 *We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also

39 recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials.

40 Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

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BMJ Open

Injury of the mammillothalamic tract in patients with subarachnoid hemorrhage: a retrospective diffusion tensor imaging study

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Primary Subject Heading:	Rehabilitation medicine
Secondary Subject Heading:	Neurology
Keywords:	REHABILITATION MEDICINE, Magnetic resonance imaging < RADIOLOGY & IMAGING, Neurological injury < NEUROLOGY

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4 **Title: Injury of the mammillothalamic tract in patients with subarachnoid hemorrhage:**
5 **a retrospective diffusion tensor imaging study**
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8 **Running title: Injury of the mammillothalamic tract**
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Abstract

Objective: Few studies have reported on injury of the mammillothalamic tract (MTT) in stroke patients. However, no study in patients with subarachnoid hemorrhage (SAH) has been reported. Using diffusion tensor tractography (DTT), we attempted to investigate injury of the MTT in patients with subarachnoid hemorrhage.

Methods: We recruited 16 patients with SAH and 15 control subjects. Diffusion tensor imaging (DTI) was obtained at 5.7 ± 1.5 weeks after onset and reconstruction of the MTT was performed using the probabilistic tractography method. The fractional anisotropy (FA) value and tract number of the MTT and the Mini-Mental State Examination (MMSE) score were determined. Values of FA and tract volume showing decrement of more than two standard deviations that of normal control were defined as abnormal.

Results: The FA value and tract volume in the patient group were significantly lower than those of the control group ($p < .05$). In addition, MMSE showed strong ($r = 0.67$, $p = 0.005$) positive correlation with tract volume without correlation with FA. In the individual analysis, 16 MTTs of 32 MTTs in 16 patients showed abnormalities of the MTT in terms of the FA value, the tract volume, or the presence of a reconstructed MTT. As a result, ten (62.5%) of 16 patients showed abnormality of the MTT in at least one hemisphere.

Conclusion: We found that patients with SAH showed injury of the MTT and this injury showed correlation with cognitive dysfunction.

Keywords: Mammillothalamic tract, Subarachnoid hemorrhage, Diffusion tensor tractography

Strengths and limitations of this study

- This is the first study on the injury of the mammillothalamic tract (MTT) in patients with subarachnoid hemorrhage (SAH) using diffusion tensor imaging (DTI).
- The main strength of our study is to find the relation between MTT injury and cognitive function with SAH
- Limitation of our study is that fiber tracking of the MTT might be affected by artifact, such as an aneurysmal clip. Therefore, conduct of further studies to overcome this limitation would be necessary.

Introduction

The mammillothalamic tract (MTT) connects the mammillary body and the anterior thalamus as a part of the Papez circuit.¹ Because of the anatomical characteristics of the MTT, thin, short, and located deep within the brain, accurate estimation of the MTT has been difficult in the live human brain. However, diffusion tensor tractography (DTT), which is derived from diffusion tensor imaging (DTI), allows for reconstruction of the MTT in the human brain.² Therefore, accurate estimation of the MTT in terms of DTI parameters and three-dimensional configuration is now possible.² A few studies have reported on injury of the MTT in thalamic hemorrhage and thalamic infarct, however, so far, little is known about injury of the MTT.^{3 4}

Subarachnoid hemorrhage (SAH) is extravasation of blood into the subarachnoid space accompanied by various neurological sequelae in terms of memory, executive function, language, motor function, and cranial nerve function.⁵⁻¹⁶ In particular, memory deficit is known to be one of the most common sequelae of SAH.^{7 8 10 11 14} In the past, previous studies have suggested that the neurotoxic effects of blood, vasospasm, or increased intracranial pressure are pathogenic mechanisms of neural sequelae following SAH.^{5 7 17-23} After introduction of DTI, among the neural tracts associated with memory, injuries in the fornix and cingulum were reported in patients with SAH.²⁴ However, no study on neural injury of the MTT in patients with SAH has been reported. In this study, we hypothesized that the MTT would be injured due to SAH.

In the current study, using DTT, we attempted to investigate injury of the MTT in patients with SAH.

Methods

Subjects

Among 55 patients, 39 patients were excluded due to the hydrocephalus (8 patients), intracerebral hemorrhage (14 patients), or intraventricular hemorrhage (17 patients). The remained 16 patients (male: 9, female: 7, mean age: 51.6 ± 13.3 years, range: 34~70 years) and 15 normal healthy control subjects (male: 7, female: 8, mean age: 48.1 ± 15.1 years, range: 20~67 years) with no previous history of neurological, physical, or psychiatric illness were recruited for this study. Inclusion criteria for patients were as follows: (1) first ever stroke, (2) age 30~70 years, (3) hemorrhage in the subarachnoid space due to aneurismal rupture confirmed by a neuroradiologist, (4) DTI was scanned at a chronic stage after onset, and (5) no hydrocephalus, intracerebral hemorrhage, or intraventricular hemorrhage. Severity of SAH was assessed according to the modified Fisher CT grade, World Federation of Neurosurgical Societies (WFNS) and Hijdra score.²⁵⁻²⁷ Patients who showed any lesion or artifact due to the clipping or coiling along or around the MTT pathway between the mammillary body and thalamus were excluded. This study was conducted retrospectively and the study protocol was approved by the Institutional Review Board of a university hospital.

Figure 1

Clinical evaluation

Cognitive function was evaluated at the time of DTI scanning. The mini-mental state examination (MMSE) was used for assessment of cognitive impairment. The reliability and

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4 validity of the MMSE have been well established.²⁸
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10 **Diffusion tensor tractography**

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13 A 6-channel head coil on a 1.5 T Philips Gyroscan Intera (Philips, Ltd, Best, The
14 Netherlands) with single-shot echo-planar imaging was used for acquisition of DTI data. For
15 each of the 32 non-collinear diffusion sensitizing gradients, we acquired 67 contiguous slices
16 parallel to the anterior commissure-posterior commissure line. Imaging parameters were as
17 follows: acquisition matrix = 96×96 ; reconstructed to matrix = 128×128 ; field of view =
18 $221 \times 221 \text{ mm}^2$; TR = 10,726 ms; TE = 76 ms; parallel imaging reduction factor (SENSE
19 factor) = 2; EPI factor = 49; $b = 1000 \text{ s/mm}^2$; NEX = 1; and a slice thickness of 2.3 mm
20 (acquired voxel size $1.73 \times 1.73 \times 2.3 \text{ mm}^3$). Affine multi-scale two-dimensional registration
21 using the Oxford Centre for Functional Magnetic Resonance Imaging of Brain (FMRIB)
22 Software Library (FSL; www.fmrib.ox.ac.uk/fsl) was used for removal of eddy current-
23 induced image distortions.²⁹ Fiber tracking was performed using a probabilistic tractography
24 method based on a multi-fiber model, and applied in the current study utilizing tractography
25 routines implemented in FMRIB Diffusion (5000 streamline samples, 0.5 mm step lengths,
26 curvature thresholds = 0.2).²⁹⁻³¹ MTTs were determined by selection of fibers passing through
27 three regions of interest (ROIs). Seed ROIs were placed on the mammillary body on the axial
28 image.² Two target ROIs were drawn at the portion of the MTT area (between the portion of
29 the fornix and the red nucleus in the anteroposterior direction) at about the bicommissural
30 level and the portion of the anterior thalamus on the axial image.² Of 5000 samples generated
31 from each seed voxel, results for each contact were the visualized threshold point at 5
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4 streamline through each voxel for analysis. Values of fractional anisotropy (FA) and tract
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6 volume which was determined by counting the voxels of MTT were measured using
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8 MATLAB™ (Matlab R2007b, The Mathworks, Natick, MA, USA). Values of FA and tract
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10 volume showing decrement of more than two standard deviations that of normal control were
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12 defined as abnormal.
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14 15 16 17 18 19 **Statistical analysis**

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22 We used SPSS software (v.15.0; SPSS, Chicago, IL) for data analysis. Demographic
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24 data in duration from onset and age was tested for normality. Data on MTTs that were not
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26 reconstructed were excluded in statistical analysis. An independent t-test was used for
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28 determination of variances in the value of FA and tract volume between the patient and
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30 control group. Subsequently, using Pearson correlation, DTT parameters for FA and tract
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32 volume of the patient group were used in determination of correlation with MMSE.³² The
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34 significant level of the *p* value was set at 0.05.
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Results

A summary of the demographic data for the patient and control groups is shown in Table 1. The artery distribution of aneurismal rupture for SAH in 16 patients was as follows: the anterior communicating artery: 11 patients (68.8%), the middle cerebral artery: three patients (18.8%), the posterior communicating artery: one patient (6.2%), and the anterior cerebral artery: one patient (6.2%). In addition, 12 patients (75%) patients underwent aneurysmal clipping and two patients underwent endovascular coiling (12.5%). The two remaining patients (12.5%) received conservative treatment. Average MMSE was 19.8 ± 8.8 . Average modified Fisher CT grade, WFNS, and Hijdra score were 2.5 ± 0.9 , 2.1 ± 1.3 , and 15.7 ± 6.3 , respectively.²⁵⁻²⁸ DTI scanning was performed at 5.7 ± 1.5 weeks (range: 4~8 weeks) after SAH onset. Demographic data of the patient (duration from onset and age) and control (age) group were met the normality ($p > .05$).

Table 1

A summary of the results of DTT parameters for MTT in the patient and control groups is shown in Table 2. Values for FA and tract volume were significantly lower in the patient group than in the control group, respectively ($p < .05$). The tract volume of the MTT showed strong ($r = 0.67$, $p = 0.005$) positive correlation with MMSE, however, no correlation was observed between the FA value of MTT and MMSE ($r = 0.41$, $p = 0.11$).³²

Table 2

Figure 2 shows the results for incidence of injury of the MTT with DTT parameters. Among 32 hemispheres of 16 patients, the MTT was not reconstructed in five hemispheres of

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4 four patients (right hemisphere: two, left hemisphere: one, both hemispheres: one); in contrast,
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6 the MTTs were reconstructed in all 30 hemispheres of 15 control subjects. With regard to the
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8 FA and tract volume, nine MTTs in six patients (right hemisphere: one, left hemisphere: two,
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10 both hemispheres: three) and 13 MTTs in nine patients (right hemisphere: four, left
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12 hemisphere: one, both hemispheres: four) of 32 hemispheres in 16 patients revealed a
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14 decrement of more than two standard deviations, compared with control subjects. Six MTTs
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16 in five patients (right hemisphere: three, left hemisphere: one, both hemispheres: one) showed
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18 a decrement of more than two standard deviations in both FA and tract volume. As a result,
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20 16 MTTs (right hemisphere: two, left hemisphere: two, both hemispheres: six) of 32 MTTs in
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22 16 patients showed abnormalities of the MTT. Three patients in one hemisphere and one
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24 patient in both hemispheres whose MTT was not reconstructed belonged to these 16 patients.
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26 Consequently, in the individual analysis, the prevalence of MTT abnormality was 62.5% (10
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28 of 16 patients).
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Discussion

In the current study, we investigated injury of the MTT in patients with SAH using DTT. We obtained the following results: 1) the FA value and tract volume in the patient group were significantly lower than those of the control group, 2) MMSE showed strong ($r=0.67$, $p=0.005$) positive correlation with tract volume without correlation with FA value, 3) in the individual analysis, 16 MTTs of 32 MTTs in 16 patients showed abnormalities of the MTT in terms of the FA value, tract volume, or the presence of a reconstructed MTT. As a result, ten (62.5%) of 16 patients showed abnormality of the MTT in at least one hemisphere. The FA value indicates the degree of directionality of water diffusion and has a range of zero (completely isotropic diffusion) to one (completely anisotropic diffusion). It represents the white matter organization: in detail, the degree of directionality and integrity of white matter microstructures such as axons, myelin, and microtubules.³³ In contrast, tract volume, the number of voxels in a neural tract, reflects the neural fibers contained within a neural tract.³⁴ Therefore, a decrease in the FA or tract volume indicates an injury of the neural tract. Consequently, our results showing that the FA value and tract volume in the patient group were significantly lower than those of the control group indicate injury of the MTT in the patient group. Regarding the relation between MTT injury and cognition, MMSE showed strong positive correlation with the tract volume of the MTT without correlation with FA.³² These results appear to indicate that the tract volume is more sensitive than the FA value for detection of injury of the MTT. The MMSE is the most widely used tool for screening cognitive dysfunction.³⁵ Because this study was conducted retrospectively, we could not employ detailed neuropsychological testing for evaluation of the function of the MTT. Therefore, conduct of further prospective studies, including specific neurophysiological tests

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4 for MTT function should be encouraged.
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7 The pathogenic mechanism of neural injury following SAH has not been elucidated and
8 few DTI studies have reported on this mechanism.^{15 36} In 2007, Liu et al. demonstrated that
9 SAH caused global mild vasogenic edema in white matter and deep gray matter by measuring
10 the apparent diffusion coefficient value in the subacute stage of SAH, which was
11 undetectable on T2-weighted and diffusion-weighted MR images.³⁶ In a recent study, Yeo et
12 al. [2012] reported injury of the CST at the midbrain in patients with SAH.¹⁵ This study
13 suggested that frequent occurrence of SAH into perimesencephalic cisterns could be ascribed
14 to injury of the CST, through mechanical (increased intracranial pressure or direct mass) or
15 chemical mechanisms (a blood clot itself can cause extensive damage).^{5 23 37} Considering that
16 the MTT is located in close proximity to a cistern, the MTT in patients with SAH appears to
17 be injured by mechanisms similar to those of CST injury at the midbrain.^{5 15 17 18 23}
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32 As for injury of the MTT, to the best of our knowledge, one study was reported in
33 patients with thalamic hemorrhage.³ Therefore, this is the first DTI study on injury of the
34 MTT in patients with SAH. However, several limitations of DTI should be considered.^{38 39}
35 First, DTI is a powerful anatomic imaging tool that can demonstrate gross fiber architecture;
36 however, reflection of all fibers, particularly small fibers, can be difficult. Second, fiber
37 tracking of the MTT might be affected by artifact, such as an aneurysmal clip. Third, we
38 investigated only injury of the MTT following SAH even though other tracts such as fornix,
39 cingulum, thalamocortical tract of Papez circuit might be also injured. Therefore, conduct of
40 further studies to overcome these limitations would be necessary.
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53 In conclusion, we found injury of the MTT in the patient group, compared with the
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4 control group. In addition, in the individual analysis, 16 MTTs (50%) of 32 hemispheres
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6 showed injury of the MTT and ten (62.5%) of 16 patients showed injury of the MTT in at
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8 least in one hemisphere in terms of DTT parameters or the presence of a reconstructed MTT.
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Contributorship statement

Sung Ho Jang: Conceiving and designing the study, funding, data acquisition, manuscript development and manuscript writing. Byung Yeon Choi: Acquisition and analysis of data, Seong Ho Kim: Research design and data acquisition. Chul Hoon Chang: Research design and data acquisition. Young Jin Jung: Research design and technical support. Hyeok Gyu Kwon: Manuscript development, data acquisition, manuscript writing and manuscript authorization.

Competing interests

None

Funding

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Data sharing statement

No additional data are available.

Ethics approval

This study protocol was approved by the Institutional Review Board of the Yeungnam university hospital.

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4 **Figure legend**
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7 **Fig 1.** Diffusion tensor tractography (DTT) for the mammillothalamic tract (MTT). Brain CT
8 shows subarachnoid hemorrhage and the MTTs in both hemispheres of a patient (34-year old
9 male) are reconstructed between the mammillary body and the anterior thalamus. However,
10 the right MTT (red) of the patient is thinned, compared with the left MTT (green) of the
11 patients and normal control subjects.
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19 **Fig. 2.** The incidence of injury of the mammillothalamic tract
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22 (FA: fractional anisotropy, MTT: mammillothalamic tract, TV: tract volume)
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Table 1. Demographic data for the patient and control groups.

	Patient group (n=16)	Control group (n=15)
Age (year)	51.6±13.3	48.1±15.1
Sex, male/female	9 / 7	7 / 8
Duration from onset (weeks)	5.7 ± 1.5	
Modified Fisher grade	2.5 ± 0.9	
WFNS	2.1 ± 1.3	
Hijdra score	15.7 ± 6.3	
Ruptured artery (ACoA:ACA:MCA:PCA)	11:1:3:1	
Operation type (clipping:coiling:non)	12:2:2	
MMSE	19.8±8.8	

WFNS: World Federation of Neurosurgical Societies, ACoA: anterior communicating, ACA: anterior cerebral artery, MCA: middle cerebral artery, PCA: posterior communicating artery, MMSE: Mini-mental state examination

Table 2. Results of diffusion tensor tractography parameters of the mammillothalamic tract in the patient and control groups.

	Hemisphere	FA	<i>p</i>	Tract volume	<i>p</i>
Patient group	Right	0.36 (0.04, 0.36)		69.08 (30.33, 72.00)	
	Left	0.35 (0.04, 0.36)		78.86 (28.80, 80.50)	
	Both	0.36 (0.04, 0.36)	.015*	74.15 (23.39, 74.00)	.000*
Control group	Right	0.38 (0.04, 0.38)		96.80 (22.7, 96.00)	
	Left	0.37 (0.03, 0.38)		99.73 (23.8, 103.00)	
	Both	0.38 (0.03, 0.38)		98.27 (22.9, 98.50)	

Values represent mean (\pm standard deviation, median value), FA: fractional anisotropy, *p*: An independent t-test for determination of variances in FA and tract volume between the patient and control groups.

* *p* < .05

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4 **Title: Injury of the mammillothalamic tract in patients with subarachnoid hemorrhage:**
5 **a retrospective diffusion tensor imaging study**
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Abstract

Objective: Few studies have reported on injury of the mammillothalamic tract (MTT) in stroke patients. However, no study in patients with subarachnoid hemorrhage (SAH) has been reported. Using diffusion tensor tractography (DTT), we attempted to investigate injury of the MTT in patients with subarachnoid hemorrhage.

Methods: We recruited 16 patients with SAH and 15 control subjects. Diffusion tensor imaging (DTI) was obtained at 5.7 ± 1.5 weeks after onset and reconstruction of the MTT was performed using the probabilistic tractography method. The fractional anisotropy (FA) value and tract number of the MTT and the Mini-Mental State Examination (MMSE) score were determined. Values of FA and tract volume showing decrement of more than two standard deviations that of normal control were defined as abnormal.

Results: The FA value and tract volume in the patient group were significantly lower than those of the control group ($p < .05$). In addition, MMSE showed strong ($r = 0.67$, $p = 0.005$) positive correlation with tract volume without correlation with FA. In the individual analysis, 16 MTTs of 32 MTTs in 16 patients showed abnormalities of the MTT in terms of the FA value, the tract volume, or the presence of a reconstructed MTT. As a result, ten (62.5%) of 16 patients showed abnormality of the MTT in at least one hemisphere.

Conclusion: We found that patients with SAH showed injury of the MTT and this injury showed correlation with cognitive dysfunction.

Keywords: Mammillothalamic tract, Subarachnoid hemorrhage, Diffusion tensor tractography

Strengths and limitations of this study

- This is the first study on the injury of the mammillothalamic tract (MTT) in patients with subarachnoid hemorrhage (SAH) using diffusion tensor imaging (DTI).
- The main strength of our study is to find the relation between MTT injury and cognitive function with SAH
- Limitation of our study is that fiber tracking of the MTT might be affected by artifact, such as an aneurysmal clip. Therefore, conduct of further studies to overcome this limitation would be necessary.

Introduction

The mammillothalamic tract (MTT) connects the mammillary body and the anterior thalamus as a part of the Papez circuit.¹ Because of the anatomical characteristics of the MTT, thin, short, and located deep within the brain, accurate estimation of the MTT has been difficult in the live human brain. However, diffusion tensor tractography (DTT), which is derived from diffusion tensor imaging (DTI), allows for reconstruction of the MTT in the human brain.² Therefore, accurate estimation of the MTT in terms of DTI parameters and three-dimensional configuration is now possible.² A few studies have reported on injury of the MTT in thalamic hemorrhage and thalamic infarct, however, so far, little is known about injury of the MTT.^{3 4}

Subarachnoid hemorrhage (SAH) is extravasation of blood into the subarachnoid space accompanied by various neurological sequelae in terms of memory, executive function, language, motor function, and cranial nerve function.⁵⁻¹⁶ In particular, memory deficit is known to be one of the most common sequelae of SAH.^{7 8 10 11 14} In the past, previous studies have suggested that the neurotoxic effects of blood, vasospasm, or increased intracranial pressure are pathogenic mechanisms of neural sequelae following SAH.^{5 7 17-23} After introduction of DTI, among the neural tracts associated with memory, injuries in the fornix and cingulum were reported in patients with SAH.²⁴ However, no study on neural injury of the MTT in patients with SAH has been reported. In this study, we hypothesized that the MTT would be injured due to SAH.

In the current study, using DTT, we attempted to investigate injury of the MTT in patients with SAH.

Methods

Subjects

Among 55 patients, 39 patients were excluded due to the hydrocephalus (8 patients), intracerebral hemorrhage (14 patients), or intraventricular hemorrhage (17 patients). The remained ~~We recruited~~ 16 patients (male: 9, female: 7, mean age: 51.6±13.3 years, range: 34~70 years) and 15 normal healthy control subjects (male: 7, female: 8, mean age: 48.1±15.1 years, range: 20~67 years) with no previous history of neurological, physical, or psychiatric illness were recruited for this study. Inclusion criteria for patients were as follows: (1) first ever stroke, (2) age 30~70 years, (3) hemorrhage in the subarachnoid space due to aneurismal rupture confirmed by a neuroradiologist, (4) DTI was scanned at a chronic stage after onset, and (5) no hydrocephalus, intracerebral hemorrhage, or intraventricular hemorrhage. Severity of SAH was assessed according to the modified Fisher CT grade, World Federation of Neurosurgical Societies (WFNS) and Hijdra score.²⁵⁻²⁷ Patients who showed any lesion or artifact due to the clipping or coiling along or around the MTT pathway between the mammillary body and thalamus were excluded. This study was conducted retrospectively and the study protocol was approved by the Institutional Review Board of a university hospital.

Figure 1

Clinical evaluation

Cognitive function was evaluated at the time of DTI scanning. The mini-mental state

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4 examination (MMSE) was used for assessment of cognitive impairment. The reliability and
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6 validity of the MMSE have been well established.²⁸
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9 **Diffusion tensor tractography**

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12 A 6-channel head coil on a 1.5 T Philips Gyroscan Intera (Philips, Ltd, Best, The
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14 Netherlands) with single-shot echo-planar imaging was used for acquisition of DTI data. For
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16 each of the 32 non-collinear diffusion sensitizing gradients, we acquired 67 contiguous slices
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18 parallel to the anterior commissure-posterior commissure line. Imaging parameters were as
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20 follows: acquisition matrix = 96×96 ; reconstructed to matrix = 128×128 ; field of view =
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22 $221 \times 221 \text{ mm}^2$; TR = 10,726 ms; TE = 76 ms; parallel imaging reduction factor (SENSE
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24 factor) = 2; EPI factor = 49; $b = 1000 \text{ s/mm}^2$; NEX = 1; and a slice thickness of 2.3 mm
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26 (acquired voxel size $1.73 \times 1.73 \times 2.3 \text{ mm}^3$). Affine multi-scale two-dimensional registration
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28 using the Oxford Centre for Functional Magnetic Resonance Imaging of Brain (FMRIB)
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30 Software Library (FSL; www.fmrib.ox.ac.uk/fsl) was used for removal of eddy current-
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32 induced image distortions.²⁹ Fiber tracking was performed using a probabilistic tractography
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34 method based on a multi-fiber model, and applied in the current study utilizing tractography
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36 routines implemented in FMRIB Diffusion (5000 streamline samples, 0.5 mm step lengths,
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38 curvature thresholds = 0.2).²⁹⁻³¹ MTTs were determined by selection of fibers passing through
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40 three regions of interest (ROIs). Seed ROIs were placed on the mammillary body on the axial
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42 image.² Two target ROIs were drawn at the portion of the MTT area (between the portion of
43
44 the fornix and the red nucleus in the anteroposterior direction) at about the bicommissural
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46 level and the portion of the anterior thalamus on the axial image.² Of 5000 samples generated
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48 from each seed voxel, results for each contact were the visualized threshold point at 5
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50 streamline through each voxel for analysis. Values of fractional anisotropy (FA) and tract
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4 volume which was determined by counting the voxels of MTT were measured using
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6 MATLAB™ (Matlab R2007b, The Mathworks, Natick, MA, USA). Values of FA and tract
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8 volume showing decrement of more than two standard deviations that of normal control were
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10 defined as abnormal.
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12 13 14 **Statistical analysis**

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17 We used SPSS software (v.15.0; SPSS, Chicago, IL) for data analysis. Demographic
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19 data in duration from onset and age was tested for normality. Data on MTTs that were not
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21 reconstructed were excluded in statistical analysis. An independent t-test was used for
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23 determination of variances in the value of FA and tract volume between the patient and
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25 control group. Subsequently, using Pearson correlation, DTT parameters for FA and tract
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27 volume of the patient group were used in determination of correlation with MMSE.³² The
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29 significant level of the *p* value was set at 0.05.
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Results

A summary of the demographic data for the patient and control groups is shown in Table 1. The artery distribution of aneurismal rupture for SAH in 16 patients was as follows: the anterior communicating artery: 11 patients (68.8%), the middle cerebral artery: three patients (18.8%), the posterior communicating artery: one patient (6.2%), and the anterior cerebral artery: one patient (6.2%). In addition, 12 patients (75%) patients underwent aneurysmal clipping and two patients underwent endovascular coiling (12.5%). The two remaining patients (12.5%) received conservative treatment. Average MMSE was 19.8 ± 8.8 . Average modified Fisher CT grade, WFNS, and Hijdra score were 2.5 ± 0.9 , 2.1 ± 1.3 , and 15.7 ± 6.3 , respectively.²⁵⁻²⁸ DTI scanning was performed at 5.7 ± 1.5 weeks (range: 4~8 weeks) after SAH onset. Demographic data of the patient (duration from onset and age) and control (age) group were met the normality ($p > .05$).

Table 1

A summary of the results of DTT parameters for MTT in the patient and control groups is shown in Table 2. Values for FA and tract volume were significantly lower in the patient group than in the control group, respectively ($p < .05$). The tract volume of the MTT showed strong ($r = 0.67$, $p = 0.005$) positive correlation with MMSE, however, no correlation was observed between the FA value of MTT and MMSE ($r = 0.41$, $p = 0.11$).³²

Table 2

Figure 2 shows the results for incidence of injury of the MTT with DTT parameters. Among 32 hemispheres of 16 patients, the MTT was not reconstructed in five hemispheres of

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4 four patients (right hemisphere: two, left hemisphere: one, both hemispheres: one); in contrast,
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6 the MTTs were reconstructed in all 30 hemispheres of 15 control subjects. With regard to the
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8 FA and tract volume, nine MTTs in six patients (right hemisphere: one, left hemisphere: two,
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10 both hemispheres: three) and 13 MTTs in nine patients (right hemisphere: four, left
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12 hemisphere: one, both hemispheres: four) of 32 hemispheres in 16 patients revealed a
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14 decrement of more than two standard deviations, compared with control subjects. Six MTTs
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16 in five patients (right hemisphere: three, left hemisphere: one, both hemispheres: one) showed
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18 a decrement of more than two standard deviations in both FA and tract volume. As a result,
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20 16 MTTs (right hemisphere: two, left hemisphere: two, both hemispheres: six) of 32 MTTs in
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22 16 patients showed abnormalities of the MTT. Three patients in one hemisphere and one
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24 patient in both hemispheres whose MTT was not reconstructed belonged to these 16 patients.
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26 Consequently, in the individual analysis, the prevalence of MTT abnormality was 62.5% (10
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28 of 16 patients).
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Discussion

In the current study, we investigated injury of the MTT in patients with SAH using DTT. We obtained the following results: 1) the FA value and tract volume in the patient group were significantly lower than those of the control group, 2) MMSE showed strong ($r=0.67$, $p=0.005$) positive correlation with tract volume without correlation with FA value, 3) in the individual analysis, 16 MTTs of 32 MTTs in 16 patients showed abnormalities of the MTT in terms of the FA value, tract volume, or the presence of a reconstructed MTT. As a result, ten (62.5%) of 16 patients showed abnormality of the MTT in at least one hemisphere. The FA value indicates the degree of directionality of water diffusion and has a range of zero (completely isotropic diffusion) to one (completely anisotropic diffusion). It represents the white matter organization: in detail, the degree of directionality and integrity of white matter microstructures such as axons, myelin, and microtubules.³³ In contrast, tract volume, the number of voxels in a neural tract, reflects the neural fibers contained within a neural tract.³⁴ Therefore, a decrease in the FA or tract volume indicates an injury of the neural tract. Consequently, our results showing that the FA value and tract volume in the patient group were significantly lower than those of the control group indicate injury of the MTT in the patient group. Regarding the relation between MTT injury and cognition, MMSE showed strong positive correlation with the tract volume of the MTT without correlation with FA.³² These results appear to indicate that the tract volume is more sensitive than the FA value for detection of injury of the MTT. The MMSE is the most widely used tool for screening cognitive dysfunction.³⁵ Because this study was conducted retrospectively, we could not employ detailed neuropsychological testing for evaluation of the function of the MTT. Therefore, conduct of further prospective studies, including specific neurophysiological tests

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4 for MTT function should be encouraged.
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7 The pathogenic mechanism of neural injury following SAH has not been elucidated and
8 few DTI studies have reported on this mechanism.^{15 36} In 2007, Liu et al. demonstrated that
9 SAH caused global mild vasogenic edema in white matter and deep gray matter by measuring
10 the apparent diffusion coefficient value in the subacute stage of SAH, which was
11 undetectable on T2-weighted and diffusion-weighted MR images.³⁶ In a recent study, Yeo et
12 al. [2012] reported injury of the CST at the midbrain in patients with SAH.¹⁵ This study
13 suggested that frequent occurrence of SAH into perimesencephalic cisterns could be ascribed
14 to injury of the CST, through mechanical (increased intracranial pressure or direct mass) or
15 chemical mechanisms (a blood clot itself can cause extensive damage).^{5 23 37} Considering that
16 the MTT is located in close proximity to a cistern, the MTT in patients with SAH appears to
17 be injured by mechanisms similar to those of CST injury at the midbrain.^{5 15 17 18 23}
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32 As for injury of the MTT, to the best of our knowledge, one study was reported in
33 patients with thalamic hemorrhage.³ Therefore, this is the first DTI study on injury of the
34 MTT in patients with SAH. However, several limitations of DTI should be considered.^{38 39}
35 First, DTI is a powerful anatomic imaging tool that can demonstrate gross fiber architecture;
36 however, reflection of all fibers, particularly small fibers, can be difficult. Second, fiber
37 tracking of the MTT might be affected by artifact, such as an aneurysmal clip. Third, we
38 investigated only injury of the MTT following SAH even though other tracts such as fornix,
39 cingulum, thalamocortical tract of Papez circuit might be also injured. Therefore, conduct of
40 further studies to overcome these limitations would be necessary.
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53 In conclusion, we found injury of the MTT in the patient group, compared with the
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4 control group. In addition, in the individual analysis, 16 MTTs (50%) of 32 hemispheres
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6 showed injury of the MTT and ten (62.5%) of 16 patients showed injury of the MTT in at
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8 least in one hemisphere in terms of DTT parameters or the presence of a reconstructed MTT.
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Contributorship statement

Sung Ho Jang: Conceiving and designing the study, funding, data acquisition, manuscript development and manuscript writing. Byung Yeon Choi: Acquisition and analysis of data, Seong Ho Kim: Research design and data acquisition. Chul Hoon Chang: Research design and data acquisition. Young Jin Jung: Research design and technical support. Hyeok Gyu Kwon: Manuscript development, data acquisition, manuscript writing and manuscript authorization.

Competing interests

None

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Data sharing statement

No additional data are available.

Ethics approval

This study protocol was approved by the Institutional Review Board of the Yeungnam university hospital.

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Figure legend

Fig 1. Diffusion tensor tractography (DTT) for the mammillothalamic tract (MTT). Brain CT shows subarachnoid hemorrhage and the MTTs in both hemispheres of a patient (34-year old male) are reconstructed between the mammillary body and the anterior thalamus. However, the right MTT (red) of the patient is thinned, compared with the left MTT (green) of the patients and normal control subjects.

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4 **Fig. 2.** The incidence of injury of the mammillothalamic tract
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7 (FA: fractional anisotropy, MTT: mammillothalamic tract, TV: tract volume)
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Table 1. Demographic data for the patient and control groups.

	Patient group (<i>n</i> =16)	Control group (<i>n</i> =15)
Age (year)	51.6±13.3	48.1±15.1
Sex, male/female	9 / 7	7 / 8
Duration from onset (weeks)	5.7 ± 1.5	
Modified Fisher grade	2.5 ± 0.9	
WFNS	2.1 ± 1.3	
Hijdra score	15.7 ± 6.3	
Ruptured artery (ACoA:ACA:MCA:PCA)	11:1:3:1	
Operation type (clipping:coiling:non)	12:2:2	
MMSE	19.8±8.8	

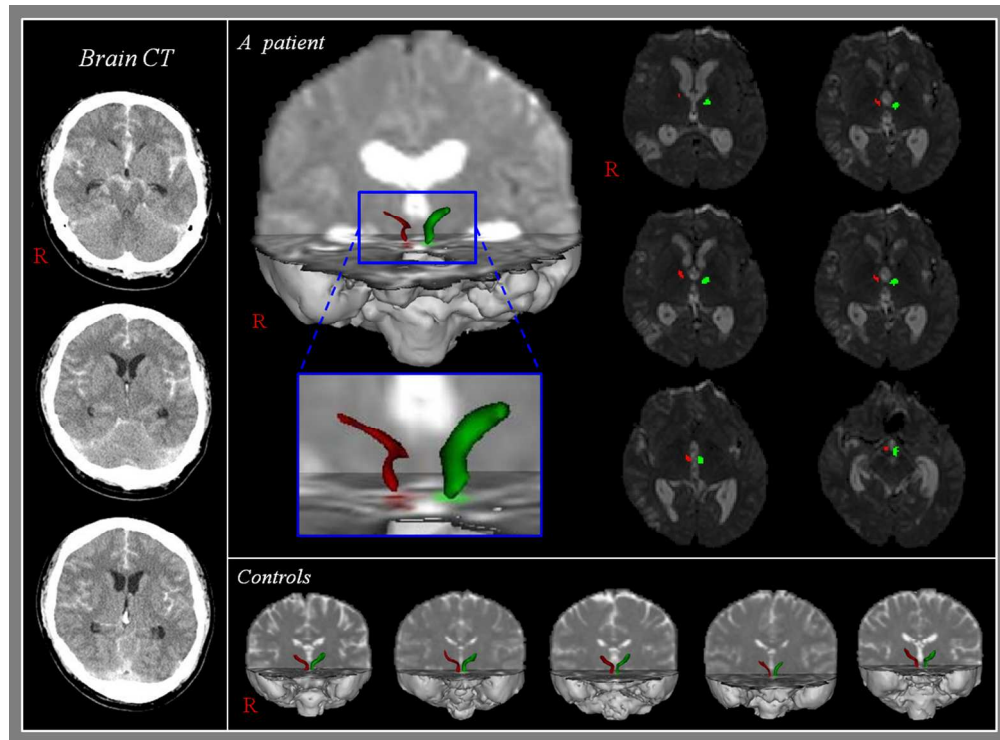
WFNS: World Federation of Neurosurgical Societies, ACoA: anterior communicating, ACA: anterior cerebral artery, MCA: middle cerebral artery, PCA: posterior communicating artery, MMSE: Mini-mental state examination

Table 2. Results of diffusion tensor tractography parameters of the mammillothalamic tract in the patient and control groups.

	Hemisphere	FA	<i>p</i>	Tract volume	<i>p</i>
Patient group	Right	0.36 (0.04, 0.36)		69.08 (30.33, 72.00)	
	Left	0.35 (0.04, 0.36)		78.86 (28.80, 80.50)	
	Both	0.36 (0.04, 0.36)	.015*	74.15 (23.39, 74.00)	.000*
Control group	Right	0.38 (0.04, 0.38)		96.80 (22.7, 96.00)	
	Left	0.37 (0.03, 0.38)		99.73 (23.8, 103.00)	
	Both	0.38 (0.03, 0.38)		98.27 (22.9, 98.50)	

Values represent mean (\pm standard deviation, median value), FA: fractional anisotropy, *p*: An independent t-test for determination of variances in FA and tract volume between the patient and control groups.

* *p* < .05



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Fig 1. Diffusion tensor tractography (DTT) for the mammillothalamic tract (MTT). Brain CT shows subarachnoid hemorrhage and the MTTs in both hemispheres of a patient (34-year old male) are reconstructed between the mammillary body and the anterior thalamus. However, the right MTT (red) of the patient is thinned, compared with the left MTT (green) of the patients and normal control subjects.
148x109mm (300 x 300 DPI)

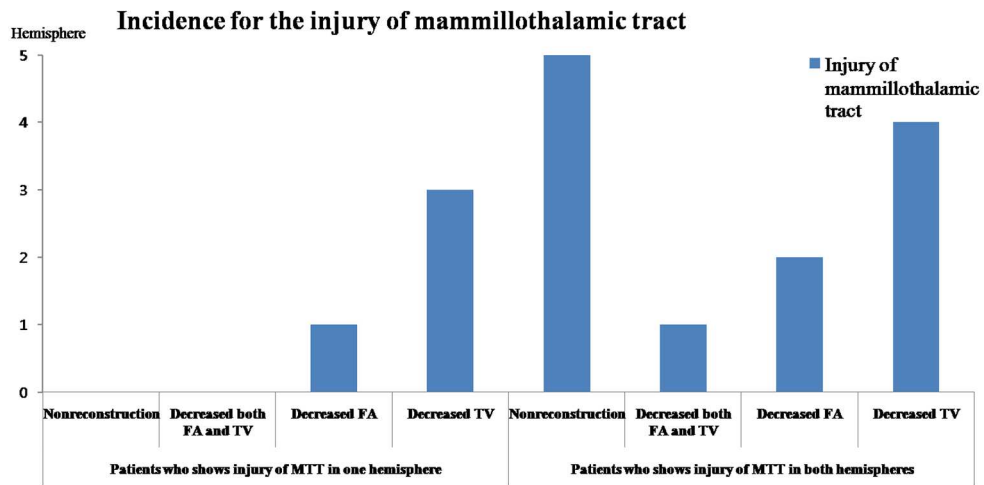


Fig. 2. The incidence of injury of the mammillothalamic tract (FA: fractional anisotropy, MTT: mammillothalamic tract, TV: tract volume)

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CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	4
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	5
	2b	Specific objectives or hypotheses	5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	6-7
Participants	4a	Eligibility criteria for participants	6
	4b	Settings and locations where the data were collected	
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6-7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	6
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	6
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	6
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	

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2		assessing outcomes) and how	
3			
4		11b If relevant, description of the similarity of interventions	
5	Statistical methods	12a Statistical methods used to compare groups for primary and secondary outcomes	6
6		12b Methods for additional analyses, such as subgroup analyses and adjusted analyses	6
7			
8	Results		
9	Participant flow (a	13a For each group, the numbers of participants who were randomly assigned, received intended treatment, and	
10	diagram is strongly	were analysed for the primary outcome	
11	recommended)	13b For each group, losses and exclusions after randomisation, together with reasons	
12	Recruitment	14a Dates defining the periods of recruitment and follow-up	
13		14b Why the trial ended or was stopped	
14			
15	Baseline data	15 A table showing baseline demographic and clinical characteristics for each group	
16	Numbers analysed	16 For each group, number of participants (denominator) included in each analysis and whether the analysis was	
17		by original assigned groups	
18			
19	Outcomes and	17a For each primary and secondary outcome, results for each group, and the estimated effect size and its	9-10
20	estimation	precision (such as 95% confidence interval)	
21		17b For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
22	Ancillary analyses	18 Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing	9-10
23		pre-specified from exploratory	
24			
25	Harms	19 All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
26			
27	Discussion		
28	Limitations	20 Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	12-13
29	Generalisability	21 Generalisability (external validity, applicability) of the trial findings	
30	Interpretation	22 Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	
31			
32	Other information		
33	Registration	23 Registration number and name of trial registry	
34	Protocol	24 Where the full trial protocol can be accessed, if available	
35	Funding	25 Sources of funding and other support (such as supply of drugs), role of funders	13
36			

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38 *We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also

39 recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials.

40 Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

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