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ARTICLE DETAILS

TITLE (PROVISIONAL)	Injury of the mammillothalamic tract in patients with subarachnoid hemorrhage: a retrospective diffusion tensor imaging study
AUTHORS	Jang, Sung Ho; Choi, Byung Yeon; Kim, Seong Ho; Chang, Chul Hoon; Jung, Young Jin; Kwon, Hyeok Gyu

VERSION 1 - REVIEW

REVIEWER	Katja Wartenberg, MD, PhD Martin-Luther University Halle-Wittenberg, Germany
REVIEW RETURNED	20-May-2014

GENERAL COMMENTS	<p>Investigating the Papez circuit with diffusion tensor imaging is a very interesting thought as many patients after SAH are suffering from cognitive impairment. This impairment cannot just be downgraded to quantifying injuries of the mamillothalamic tract, but it is a great start. This study lacks the creation of a connection between the amount of subarachnoid blood/ severity of injury, the role of the mamillothalamic tract and the impact of its injury on outcome. Why was the outcome not further studied with real quantitative tests of cognitive function (see cited paper by Kreiter et al) ? Did the authors attempt a correlation with the severity of injury ? Rather than writing a separate paragraph in the discussion about each aspect of the study the investigators should discuss all aspects together.</p> <p>What was the actual aim of the study, the incidence of injury to the mamillothalamic tract or to quantify the injury ? Why did the authors limit the age of the patient included to 30-70 years ?</p> <p>The Fisher scale is not a linear scale to assess severity of SAH, but the risk of vasospasm. The modified Fisher scale shows a more linear response in relationship with the incidence of vasospasm. The severity of injury is assessed with the Hunt Hess scale or WFNS. The amount of blood can be described with the Hijdra score. Most of the variables are probably not normally distributed, therefore their median value should be presented rather than the mean. The conclusion includes further elements of the discussion, these should be moved to the discussion part. That the assessment of the MTT utilizing DTI is necessary cannot be concluded from this study. The study limitations should also include the retrospective nature of the study and the extremely limited amount of clinical information if this cannot be improved.</p>
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REVIEWER	George Wong Chinese University of Hong Kong, China
REVIEW RETURNED	24-May-2014

GENERAL COMMENTS	<p>The authors reported a 16 SAH patient and 15 control study to investigate mamillothalamic tract DTI. While the FAs are not different, the authors found a significant difference in volume and concluded a positive findings. It would be a good opportunity to expand on previous pilot studies.</p> <p>I would suggest for the following amendments:</p> <ol style="list-style-type: none"> 1. Since this is a retrospective study, why the authors did not investigate the tracts reported in other preliminary study. In the introduction, the rationale to limit the analysis to MTT is unclear. 2. FA is considered the primary outcome in similar studies. I would argue that this is a negative study. The method to measure tract volume should be described in more detail and referenced accordingly. 3. A description and review in the literature of the type of cognitive deficit associated with MTT would be useful. Also, please propose the specific cognitive assessment for future prospective study. 4. Is the timing of 4-8 weeks appropriate? Please discuss or offer alternative suggestion. 5. Validation study of MMSE and MoCA in SAH patients should be referenced. 6. The authors implied problems with aneurysm clips and all patients had anterior circulation aneurysm. An idea of patients screened and excluded would give the readers a clearer picture. 7. Are the tract volumes or FA associated with delayed cerebral infarction or hydrocephalus? 7. Do the authors experienced problems with coils, stents, giant aneurysms, shunt etc?
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VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name Katja Wartenberg, MD, PhD

Institution and Country Martin-Luther University Halle-Wittenberg, Germany

Please state any competing interests or state 'None declared': Non declared

Investigating the Papez circuit with diffusion tensor imaging is a very interesting thought as many patients after SAH are suffering from cognitive impairment. This impairment cannot just be downgraded to quantifying injuries of the mamillothalamic tract, but it is a great start. This study lacks the creation of a connection between the amount of subarachnoid blood/ severity of injury, the role of the mamillothalamic tract and the impact of its injury on outcome.

Point 1] Why was the outcome not further studied with real quantitative tests of cognitive function (see cited paper by Kreiter et al) ?

Answer: The MMSE is the most widely used tool for screening of cognitive dysfunction. Although the MMSE is easy for clinical application and comprehensive test for cognitive impairment, it is not sufficient to reflect the detail cognitive function for the patient. As mentioned in the manuscript, due to the retrospective study, we could not obtain the real quantitative data of cognitive function of the MTT. Therefore, we already described in the discussion as follows(underline).

Discussion

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 for MTT function should be encouraged.

Point 2] Did the authors attempt a correlation with the severity of injury ?

Answer: We already attempted a correlation between the severity of MTT injury and the MMSE in the patient group using the Pearson correlation. As a result, we found that 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 ($r=0.41$, $p=0.11$). We already described it in the part of the results as follows (underline). In addition, during this revision, we attempted to see correlation between the severity of MTT and the severity of SAH including the modified Fischer grade, WFNS, and Hijdra score. As a result, we could not observe any significant correlations between the severity of MTT and the severity of SAH (the modified Fischer grade, WFNS, and Hijdra score) ($p>.05$). So, we could not add this results. However, if reviewer want us to add this results, we will do it.

Statistical analysis

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

Results

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 ($r=0.41$, $p=0.11$).³²³⁰

Point 3] Rather than writing a separate paragraph in the discussion about each aspect of the study the investigators should discuss all aspects together.

Answer: We revised as follows.

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.³³³¹ 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 for MTT function should be encouraged.

The pathogenic mechanism of neural injury following SAH has not been elucidated and few DTI studies have reported on this mechanism.^{15 3634} In 2007, Liu et al. demonstrated that SAH caused global mild vasogenic edema in white matter and deep gray matter by measuring the apparent diffusion coefficient value in the subacute stage of SAH, which was undetectable on T2-weighted and

diffusion-weighted MR images.^{36,34} In a recent study, Yeo et al. [2012] reported injury of the CST at the midbrain in patients with SAH.¹⁵ This study suggested that frequent occurrence of SAH into perimesencephalic cisterns could be ascribed to injury of the CST, through mechanical (increased intracranial pressure or direct mass) or chemical mechanisms (a blood clot itself can cause extensive damage).^{5, 23, 37, 35} Considering that the MTT is located in close proximity to a cistern, the MTT in patients with SAH appears to be injured by mechanisms similar to those of CST injury at the midbrain.^{5, 15, 17, 18, 23}

As for injury of the MTT, to the best of our knowledge, one study was reported in patients with thalamic hemorrhage.³ Therefore, this is the first DTT study on injury of the MTT in patients with SAH. However, several limitations of DTI should be considered.^{38, 39} First, DTI is a powerful anatomic imaging tool that can demonstrate gross fiber architecture; however, reflection of all fibers, particularly small fibers, can be difficult. Second, fiber tracking of the MTT might be affected by artifact, such as an aneurysmal clip. Third, we investigated only the injury of the MTT following the SAH even though other tracts such as fornix, cingulum, thalamocortical tract of Papez circuit might be also injured. Therefore, conduct of further studies to overcome these limitations would be necessary. In conclusion, we found injury of the MTT in the patient group, compared with the control group. In addition, in the individual analysis, 16 MTTs (50%) of 32 hemispheres showed injury of the MTT and ten (62.5%) of 16 patients showed injury of the MTT in at least in one hemisphere in terms of DTT parameters or the presence of a reconstructed MTT. As for injury of the MTT, to the best of our knowledge, one study was reported in patients with thalamic hemorrhage.³ Therefore, this is the first DTT study on injury of the MTT in patients with SAH. However, several limitations of DTI should be considered.^{36, 37} First, DTI is a powerful anatomic imaging tool that can demonstrate gross fiber architecture; however, reflection of all fibers, particularly small fibers, can be difficult. Second, fiber tracking of the MTT might be affected by artifact, such as an aneurysmal clip. Therefore, conduct of further studies to overcome these limitations would be necessary.

Point 4] What was the actual aim of the study, the incidence of injury to the mamillothalamic tract or to quantify the injury ?

Answer: The aim of this study was to investigate the presence and severity of injury of the MTT in patients with SAH, using DTT.

Point 5] Why did the authors limit the age of the patient included to 30-70 years ?

Answer: Cognitive function could be affected by age particularly, the older people. So, we recruited patients younger than 70 years old to diminish the aging effects.

Point 6] The Fisher scale is not a linear scale to assess severity of SAH, but the risk of vasospasm. The modified Fisher scale shows a more linear response in relationship with the incidence of vasospasm. The severity of injury is assessed with the Hunt Hess scale or WFNS. The amount of blood can be described with the Hijdra score.

Answer: As for the Fisher scale, we replaced the fisher grade with the modified Fisher scale. Average modified Fisher scale was 2.5 ± 0.9 . In addition, WFNS and Hijdra score were also assessed. Average WFNS and Hijdra score were 2.1 ± 1.3 and 15.7 ± 6.3 , respectively. We revised as follows.

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 aneurysmal 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 and 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.

Results

A summary of the demographic data for the patient and control groups is shown in Table 1. The artery distribution of aneurysmal 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.

25. Teasdale GM, Drake CG, Hunt W, et al. A universal subarachnoid hemorrhage scale: report of a committee of the World Federation of Neurosurgical Societies. *J Neurol Neurosurg Psychiatry* 1988;51:1457.

26. Hijdra A, Brouwers PJ, Vermeulen M, et al. Grading the amount of blood on computed tomograms after subarachnoid hemorrhage. *Stroke* 1990;21:1156-61.

27. Frontera JA, Claassen J, Schmidt JM, et al. Prediction of symptomatic vasospasm after subarachnoid hemorrhage: the modified fisher scale. *Neurosurgery* 2006;59:21-7; discussion -7.

Point 7] Most of the variables are probably not normally distributed, therefore their median value should be presented rather than the mean.

Answer: We totally agree with reviewer comment. So, we added the median value of DTT parameters as follows.

Point 8] The conclusion includes further elements of the discussion, these should be moved to the discussion part.

Answer: We revised as follows.

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.³³³¹ 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 for MTT function should be encouraged.

The pathogenic mechanism of neural injury following SAH has not been elucidated and few DTI studies have reported on this mechanism.^{15 3634} In 2007, Liu et al. demonstrated that SAH caused global mild vasogenic edema in white matter and deep gray matter by measuring the apparent

diffusion coefficient value in the subacute stage of SAH, which was undetectable on T2-weighted and diffusion-weighted MR images.^{36,34} In a recent study, Yeo et al. [2012] reported injury of the CST at the midbrain in patients with SAH.¹⁵ This study suggested that frequent occurrence of SAH into perimesencephalic cisterns could be ascribed to injury of the CST, through mechanical (increased intracranial pressure or direct mass) or chemical mechanisms (a blood clot itself can cause extensive damage).^{5, 23, 37, 35} Considering that the MTT is located in close proximity to a cistern, the MTT in patients with SAH appears to be injured by mechanisms similar to those of CST injury at the midbrain.^{5, 15, 17, 18, 23}

As for injury of the MTT, to the best of our knowledge, one study was reported in patients with thalamic hemorrhage.³ Therefore, this is the first DTT study on injury of the MTT in patients with SAH. However, several limitations of DTI should be considered.^{38, 39} First, DTI is a powerful anatomic imaging tool that can demonstrate gross fiber architecture; however, reflection of all fibers, particularly small fibers, can be difficult. Second, fiber tracking of the MTT might be affected by artifact, such as an aneurysmal clip. Third, we investigated only the injury of the MTT following the SAH even though other tracts such as fornix, cingulum, thalamocortical tract of Papez circuit might be also injured. Therefore, conduct of further studies to overcome these limitations would be necessary. In conclusion, we found injury of the MTT in the patient group, compared with the control group. In addition, in the individual analysis, 16 MTTs (50%) of 32 hemispheres showed injury of the MTT and ten (62.5%) of 16 patients showed injury of the MTT in at least in one hemisphere in terms of DTT parameters or the presence of a reconstructed MTT. As for injury of the MTT, to the best of our knowledge, one study was reported in patients with thalamic hemorrhage.³ Therefore, this is the first DTT study on injury of the MTT in patients with SAH. However, several limitations of DTI should be considered.^{36, 37} First, DTI is a powerful anatomic imaging tool that can demonstrate gross fiber architecture; however, reflection of all fibers, particularly small fibers, can be difficult. Second, fiber tracking of the MTT might be affected by artifact, such as an aneurysmal clip. Therefore, conduct of further studies to overcome these limitations would be necessary.

Point 9] That the assessment of the MTT utilizing DTI is necessary cannot be concluded from this study.

Answer: We revised the abstract as follows.

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.

Point 10] The study limitations should also include the retrospective nature of the study and the extremely limited amount of clinical information if this cannot be improved.

Answer: We revised as follows.

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.³³³¹ 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 for MTT function should be encouraged.

The pathogenic mechanism of neural injury following SAH has not been elucidated and few DTI studies have reported on this mechanism.^{15 3634} In 2007, Liu et al. demonstrated that SAH caused global mild vasogenic edema in white matter and deep gray matter by measuring the apparent diffusion coefficient value in the subacute stage of SAH, which was undetectable on T2-weighted and diffusion-weighted MR images.³⁶³⁴ In a recent study, Yeo et al. [2012] reported injury of the CST at the midbrain in patients with SAH.¹⁵ This study suggested that frequent occurrence of SAH into perimesencephalic cisterns could be ascribed to injury of the CST, through mechanical (increased intracranial pressure or direct mass) or chemical mechanisms (a blood clot itself can cause extensive damage).^{5 23 3735} Considering that the MTT is located in close proximity to a cistern, the MTT in patients with SAH appears to be injured by mechanisms similar to those of CST injury at the midbrain.^{5 15 17 18 23}

As for injury of the MTT, to the best of our knowledge, one study was reported in patients with thalamic hemorrhage.³ Therefore, this is the first DTT study on injury of the MTT in patients with SAH. However, several limitations of DTI should be considered.^{38 39} First, DTI is a powerful anatomic imaging tool that can demonstrate gross fiber architecture; however, reflection of all fibers, particularly small fibers, can be difficult. Second, fiber tracking of the MTT might be affected by artifact, such as an aneurysmal clip. Third, we investigated only the injury of the MTT following the SAH even though other tracts such as fornix, cingulum, thalamocortical tract of Papez circuit might be also injured. Therefore, conduct of further studies to overcome these limitations would be necessary. In conclusion, we found injury of the MTT in the patient group, compared with the control group. In addition, in the individual analysis, 16 MTTs (50%) of 32 hemispheres showed injury of the MTT and ten (62.5%) of 16 patients showed injury of the MTT in at least in one hemisphere in terms of DTT parameters or the presence of a reconstructed MTT. As for injury of the MTT, to the best of our knowledge, one study was reported in patients with thalamic hemorrhage.³ Therefore, this is the first DTT study on injury of the MTT in patients with SAH. However, several limitations of DTI should be considered.^{36 37} First, DTI is a powerful anatomic imaging tool that can demonstrate gross fiber architecture; however, reflection of all fibers, particularly small fibers, can be difficult. Second, fiber tracking of the MTT might be affected by artifact, such as an aneurysmal clip. Therefore, conduct of further studies to overcome these limitations would be necessary.

Reviewer: 2

Reviewer Name George Wong

Institution and Country Chinese University of Hong Kong, China

Please state any competing interests or state 'None declared': None declared

The authors reported a 16 SAH patient and 15 control study to investigate mamillothalamic tract DTI. While the FAs are not different, the authors found a significant difference in volume and concluded a positive findings. It would be a good opportunity to expand on previous pilot studies.

I would suggest for the following amendments:

Point 1] Since this is a retrospective study, why the authors did not investigate the tracts reported in other preliminary study. In the introduction, the rationale to limit the analysis to MTT is unclear.

Answer: The cognitive function is ascribed to injuries of various neural tracts including fornix, cingulum, thalamocortical tract of Papez circuit, and so on. Among these tracts, we thought that the possibility of injury of the MTT due to the SAH was higher than other tracts because of the pathway of the MTT. Although, as reviewer comment, the investigation of various tracts in SAH would be helpful and improve our study, in the current study, we focused on the injury of the MTT documented by DTT following SAH. At the time of this revision, we added this issue as a limitation in the part of the discussion. We revised as follows.

Discussion

As for injury of the MTT, to the best of our knowledge, one study was reported in patients with thalamic hemorrhage.³ Therefore, this is the first DTT study on injury of the MTT in patients with SAH. However, several limitations of DTI should be considered.^{38 39} First, DTI is a powerful anatomic imaging tool that can demonstrate gross fiber architecture; however, reflection of all fibers, particularly small fibers, can be difficult. Second, fiber tracking of the MTT might be affected by artifact, such as an aneurysmal clip. Third, we investigated only injury of the MTT following SAH even though other tracts such as fornix, cingulum, thalamocortical tract of Papez circuit might be also injured. Therefore, conduct of further studies to overcome these limitations would be necessary.

Point 2] FA is considered the primary outcome in similar studies. I would argue that this is a negative study. The method to measure tract volume should be described in more detail and referenced accordingly.

Answer: As reviewer comment, FA value is considered the primary outcome. In addition, the tract volume is also important outcome in the field of DTT study. Therefore, FA value and tract volume are the most popular parameter in the field of DTT study to demonstrate the abnormality of the neural tracts. 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 axon, myelin, and microtubule. In contrast, tract volume, the number of voxels in a neural tract, reflects the neural fibers contained within a neural tract. Therefore, a decrease in the FA value or tract volume indicates an injury of the neural tract. In the current study, our results that FA values or tract volume in patient group were significantly lower than those of control group indicated the injury of the MTT. In addition, injured MTT could contribute to the cognitive function following SAH. However, FA values of the MTT did not show the correlation with the MMSE. We thought that decreased FA of the MTT might not be sufficient to reflect the MMSE because the MMSE is affected by various tracts such as the fornix, cingulum, thalamocortical tract of Papez circuit, and so on. Nevertheless, decreased tract volume of the MTT showed the strong positive correlation with the MMSE. So, we thought that tract volume is more sensitive than the FA value for detection of injury of the MTT. We already described this issue. Regarding the methods to measure tract volume, we described the methods in detail as follows.

Diffusion tensor tractography

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 MATLABM (Matlab R2007b, The Mathworks, Natick, MA, USA). Values of FA and tract volume showing decrement of more than two standard deviations that of normal control were defined as abnormal.

Discussion

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.³³³¹ 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.

Point 3] A description and review in the literature of the type of cognitive deficit associated with MTT would be useful. Also, please propose the specific cognitive assessment for future prospective study.

Answer: We revised as follows.

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.³³³¹ 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 for MTT function should be encouraged.

The pathogenic mechanism of neural injury following SAH has not been elucidated and few DTI studies have reported on this mechanism.^{15 3634} In 2007, Liu et al. demonstrated that SAH caused global mild vasogenic edema in white matter and deep gray matter by measuring the apparent diffusion coefficient value in the subacute stage of SAH, which was undetectable on T2-weighted and diffusion-weighted MR images.³⁶³⁴ In a recent study, Yeo et al. [2012] reported injury of the CST at the midbrain in patients with SAH.¹⁵ This study suggested that frequent occurrence of SAH into perimesencephalic cisterns could be ascribed to injury of the CST, through mechanical (increased intracranial pressure or direct mass) or chemical mechanisms (a blood clot itself can cause extensive damage).^{5 23 3735} Considering that the MTT is located in close proximity to a cistern, the MTT in patients with SAH appears to be injured by mechanisms similar to those of CST injury at the midbrain.^{5 15 17 18 23}

As for injury of the MTT, to the best of our knowledge, one study was reported in patients with thalamic hemorrhage.³ Therefore, this is the first DTT study on injury of the MTT in patients with SAH. However, several limitations of DTI should be considered.^{38 39} First, DTI is a powerful anatomic imaging tool that can demonstrate gross fiber architecture; however, reflection of all fibers, particularly small fibers, can be difficult. Second, fiber tracking of the MTT might be affected by artifact, such as an aneurysmal clip. Third, we investigated only injury of the MTT following SAH even though other tracts such as fornix, cingulum, thalamocortical tract of Papez circuit might be also injured. Therefore, conduct of further studies to overcome these limitations would be necessary. In conclusion, we found injury of the MTT in the patient group, compared with the control group. In

addition, in the individual analysis, 16 MTTs (50%) of 32 hemispheres showed injury of the MTT and ten (62.5%) of 16 patients showed injury of the MTT in at least in one hemisphere in terms of DTT parameters or the presence of a reconstructed MTT. As for injury of the MTT, to the best of our knowledge, one study was reported in patients with thalamic hemorrhage.³ Therefore, this is the first DTT study on injury of the MTT in patients with SAH. However, several limitations of DTI should be considered.^{36 37} First, DTI is a powerful anatomic imaging tool that can demonstrate gross fiber architecture; however, reflection of all fibers, particularly small fibers, can be difficult. Second, fiber tracking of the MTT might be affected by artifact, such as an aneurysmal clip. Therefore, conduct of further studies to overcome these limitations would be necessary.

Point 4] Is the timing of 4-8 weeks appropriate? Please discuss or offer alternative suggestion.

Answer: We revised as follows.

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 aneurysmal 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

Point 5] Validation study of MMSE and MoCA in SAH patients should be referenced.

Answer: The MMSE is easy for clinical application and comprehensive test for cognitive dysfunction. However, it is not sufficient to reflect the detail cognitive function. Compared to the MoCA, MoCA is more sensitive to cognitive function than MMSE [1]. Nevertheless, the MMSE is the most widely used tool for screening cognitive dysfunction. In addition, the reliability and validity of the MMSE have been well established [2]. As mentioned in the manuscript, due to the retrospective study, we could not obtain the real quantitative data of cognitive function of the MTT. Therefore, we suggested further studies need to investigate the direct specific neurophysiological tests regarding the function of MTT in the part of discussion and already described this issue as limitation.

[1] Schweizer TA1, Al-Khindi T, Macdonald RL Mini-Mental State Examination versus Montreal Cognitive Assessment: rapid assessment tools for cognitive and functional outcome after aneurysmal subarachnoid hemorrhage. *J Neurol Sci* 2012;15:316(1-2):137-40.

[2] Dick JP, Guiloff RJ, Stewart A, et al. Mini-mental state examination in neurological patients. *J Neurol Neurosurg Psychiatry* 1984;47:496-9.

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

Discussion

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 for MTT function should be encouraged.

Point 6] The authors implied problems with aneurysm clips and all patients had anterior circulation aneurysm. An idea of patients screened and excluded would give the readers a clearer picture.

Answer: We totally agree with reviewer comment. We excluded patients who showed the artifact due to the clipping and coiling along or around the MTT pathway between the mammillary body and thalamus. We revised as follows.

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.

Point 7] Are the tract volumes or FA associated with delayed cerebral infarction or hydrocephalus?

Answer: We already excluded the patients with the hydrocephalus, intracerebral hemorrhage, or intraventricular hemorrhage. Therefore, tract volumes or FA were no associated with delayed cerebral infarction or hydrocephalus.

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.

Point 8] Do the authors experienced problems with coils, stents, giant aneurysms, shunt etc?

Answer: We have experienced problems with artifacts due to the coils and clips. Therefore, in the current study, we already excluded the patients who showed the artifacts due to the clipping and coiling along or around the MTT pathway between the mammillary body and thalamus. We revised as follows.

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.

VERSION 2 – REVIEW

REVIEWER	Katja E. Wartenberg Martin-Luther-University Halle-Wittenberg, Germany
REVIEW RETURNED	17-Jun-2014

GENERAL COMMENTS	<p>Point 4] What was the actual aim of the study, the incidence of injury to the mamillothalamic tract or to quantify the injury ? Answer: The aim of this study was to investigate the presence and severity of injury of the MTT in patients with SAH, using DTT. -> Then this should be formulated in the hypothesis in the introduction accordingly</p> <p>Point 7] Most of the variables are probably not normally distributed, therefore their median value should be presented rather than the mean. Answer: We totally agree with reviewer comment. So, we added the median value of DTT parameters as follows. -> Were all clinical variables checked for normal distribution ? Most of the time, age is not normally distributed in small patient samples. Please check table 1.</p>
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REVIEWER	George Wong The Chinese University of Hong Kong, Hong Kong, China.
REVIEW RETURNED	04-Jul-2014

GENERAL COMMENTS	Thank you for the revision. The number of SAH patients screened to arrive at the current cohort should be included in the method/result section.
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VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name Katja E. Wartenberg

Institution and Country Martin-Luther-University Halle-Wittenberg, Germany

Please state any competing interests or state 'None declared': None declared

What was the actual aim of the study, the incidence of injury to the mamillothalamic tract or to quantify the injury ?

Answer: The aim of this study was to investigate the presence and severity of injury of the MTT in patients with SAH, using DTT.

Point 1] Then this should be formulated in the hypothesis in the introduction accordingly

Answer: We added the hypothesis in the part of the introduction as follows.

Introduction

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.

Most of the variables are probably not normally distributed, therefore their median value should be presented rather than the mean.

Answer: We totally agree with reviewer comment. So, we added the median value of DTT parameters as follows.

Point 2] Were all clinical variables checked for normal distribution ? Most of the time, age is not normally distributed in small patient samples. Please check table 1.

Answer: We checked the normal distribution and we found that demographic data of patient (duration from onset and age) and control (age) group were met the normality. We revised as follows.

Statistical analysis

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

Results

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 ($p > .05$).

Reviewer: 2

Reviewer Name George Wong

Institution and Country The Chinese University of Hong Kong, Hong Kong, China.

Please state any competing interests or state 'None declared': None declared.

Point 1] Thank you for the revision. The number of SAH patients screened to arrive at the current cohort should be included in the method/result section.

Answer: We revised as follows.

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.