Subependymal injuries in anterior horns of lateral ventricles and sagittal rotational acceleration applied to the head

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Abstract: The authors histopathologically surveyed 12 autopsy cases with fatal head traumata, especially regarding neural tissue injuries at the anterior horns of the lateral ventricles, compared with 32 control cases with no head traumata. Traumatic injuries, including axonal injuries as well as hemorrhages, tearing of white matter and neurofilament-containing hypertrophic astrocytes were observed in the neural tissue adjacent to the anterior horn of the lateral ventricle in 8 cases which were killed by rotational mechanism when wounded, while other 4 cases without those traumatic lesions had linear or direct injury to the head. It is suggested that traumatic changes in the subependymal zone around the anterior horn might suggest sagittal rotational acceleration to the head. The simplicity of this examination method is also emphasized.

Key words: subependymal zone, anterior horn of lateral ventricle, traumatic axonal injury, rotation of head, human autopsy

There have been few descriptions concerning neural tissue injuries in the subependymal zones (SEZ) at anterior horns (AH) of the lateral ventricles (LV) in major textbooks of neuropathology [1, 2], forensic neuropathology [3-5] and accessible English articles, apart from Makino’s report [6].

The authors of the present paper microscopically examined the neural tissue of SEZ around AH of LV in cases with or without fatal head injuries. Neural tissue injuries in these regions were seen in some of the cases with fatal head injuries, while no traumatic lesion is observed in any of the cases without fatal head injuries. It appears there is a tendency that cases with neural injuries in SEZ at AH of LV have common mode of injury; the force to the head has an element of rotational acceleration in the sagittal direction. Eight and 4 cases with and without neural tissue injuries in SEZ at AH, respectively, were analyzed in the present study, in comparison with 32 cases without fatal head injuries as control cases.

Materials and methods

The cases in the present study were those satisfying the condition criteria below, and were from the medico-legal autopsies dealt with in the Department of Forensic Medicine, Hirosaki University Graduate School of Medicine in the period between 1997 and 2009; 1) cases with a postmortem...
interval shorter of 7 days or less, 2) with a successfully fixed brain, and 3) with established cause(s) of
dearth.

The total number of cases included in this study was 44 (32 males, 12 females, age range: 0-87
year(s), 50.3 years on average, including 4 infants). All 13 cases in whom the mechanisms of injury
were able to be clearly explained both by the inspection information and the gross autopsy findings
were included. The summaries of the examined cases are shown in Table 1.

Specimens of AH of LV were prepared to allow microscopic examination of their horizontal
sections. They were derived either from fixed brains in 10% formalin solution or from unfixed brains
directly incised at autopsy. Some of the injured brains were removed from the skull by the immersion
technique in a container filled with water [7, 8] so as to prevent the fragile brains from being damaged
during the removal procedures.

A corner of the tissue block from the left hemisphere was cut off in order to differentiate the
right from the left hemispheres as shown in Figs. 1-4. All specimens were processed as usual and
stained with hematoxilin and eosin. Paraffin sections in cases with fatal head injuries were
immunohistochemically investigated using the avidin-biotin-peroxidase complex method with a
Vectastain ABC kit (Vector, Burlingame, CA, USA). Antibodies against phosphorylated
neurofilament (SMI-31; Sternberger Immunochemicals, Baltimore, MD, USA; 1:5000) and glial
fibrillary acidic protein (GFAP; DAKO, Glostrup, Denmark; 1:800) were used as primary antibodies
for definition of neurofilament and astrocytes, respectively.

Fig. 1 A coronal section of the fixed frontal lobes (sliced approximately 1.5 cm in thickness) showing anterior horns of the lateral ventricles. (Posterior view)

Fig. 2 Incisions for collecting specimens must carefully be made in each side separately so that proper portions of the anterior horns are obtained, but this is quite simple and easy.

Fig. 3 The left and right slices removed from the section of Fig. 2, showing the upper surface of horizontal sections. Arrows show the left and right anterior horns.

Fig. 4 Two central specimen (arrows) are prepared for microscopic specimens. A corner of the left specimen is transected in order to show its lateral view. The specimens are processed as usual.
Table 1. Summary of the cases included in the present study (Note: Case No. 1 to 12 are with fatal intracranial injuries. Case No. 13 to 44 are control cases.)
Table 1. (continued) Summary of the cases included in the present study (Note: Case No. 1 to 12 are with fatal intracranial injuries. Case No. 13 to 44 are control cases)

<table>
<thead>
<tr>
<th>Group</th>
<th>Case No.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Cause of death</th>
<th>Circumstances</th>
<th>Survival period (hri)</th>
<th>PMI (hrs)</th>
<th>Gross and microscopic findings of head</th>
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<td>Injuries in SEE at anterior horns of lateral ventricles</td>
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<td>Hemorrhages</td>
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</table>

Cases were analyzed by the following groups; group A) cases with fatal head injuries associated with neural tissue injuries in SEZ at AH of LV, group B) cases with fatal head injuries but without the lesions in SEZ, and group C) cases without head injuries.

Results and discussion
First of all, the diagnosis of rotational acceleration to the head was the most important condition in the present study. The authors adopted one or more of the following items for evidence of “rotational” acceleration to the brain in the sagittal direction in group A; 1) presence of acute subdural hematoma adjacent to the bridging veins, 2) presence of superficial abrasion suggesting a blunt force in the sagittal direction, 3) presence of overextension injuries in the cervical vertebrae, and 4) condition(s) of the whole body or circumstance(s) which could cause a strong whiplash action at the accident.

Two of the cases in Group A, cases No. 3 and No. 8, are described as examples. A man was strongly kicked in the face, smear the pattern of the sole of the shoe could be seen en bloc (Fig. 5, 6) and he died 8 hours in hospital in case No. 3. Case No.3 had an acute subdural hematoma (Fig. 7). In case No. 8, a helicopter in which the deceased, a copilot, had been fixed in the cabin seat by a shoulder harness, crashed into the sea water surface at a speed of at least approximately 200 km/h (124 mph).

Traumatic changes in neural tissue in SEZ at AH of LV were observed bilaterally in all cases in Group A. The microscopic traumatic changes in this portion were characterized by a) tearing of the ependymal layer (Fig. 8), b) destruction of subependymal white matter (Fig. 11), c) small round eosinophilic structures (usually several micrometers in diameter) (Fig. 9), and d) hypertrophic astrocytes containing neurofilament protein (Fig. 10, 12, 13), in addition to areas of hemorrhage in this region, as was pointed out by Makino [6].

The main contents of small round eosinophilic structures were confirmed by immunohistochemical staining with SMI-31, a neurofilament marker (Fig. 10, 13). It is presumed that this finding suggests the traumatic lesion is caused by severe neural fiber injury. Immunohistochemistry using the anti-GAFP antibody showed that the neurofilament-containing cells in SEZ were astrocytes in origin (Fig. 14). The reason why such a phagocytic reaction of astrocytes took place in a case of almost instant death such as case No. 8 (a helicopter crash) can only be explained by a localized reaction of surviving astrocytes for a short period after somatic death.

Fig. 5  A view of the bottom of the soles of the shoe used to kill the victim of Case No. 3 - they have a characteristic zigzag pattern.

Fig. 6  “Printed” bruising with zigzag patterns corresponding to those of the sole shown in Fig. 5 were clearly observed around the victim’s left orbit.

Fig. 7  A photograph taken at autopsy of Case No. 3 shows a marked acute subdural hematoma weighing approximately 100 g covering the left cerebral hemisphere.
Fig. 8  A microscopic view of the anterior horn of the left lateral ventricle of Case No. 3, hematoxylin & eosin staining, x100. No ependymal layer is to be seen in this specimen.

Fig. 9  A higher magnification view of the anterior horn of the left lateral ventricle in Case No. 3, hematoxylin & eosin staining, x400. Small round eosinophilic structures (arrows) apparently bigger than red blood cells are observed in torn neural tissue in subependymal zone.

Fig. 10  Immunohistochemical staining with SMI-31 as a primary antibody shows that the small round spherical bodies (thin arrows) and hypertrophic astrocytes (thick arrow) are neurofilament positive. x400
**Fig. 11** A microscopic view of the anterior horn of the left lateral ventricle in Case No. 8, hematoxylin & eosin staining, x100. The photo clearly shows a tear of neural tissue in the subependymal zone (arrow).

**Fig. 12** A higher magnification view of the anterior horn of the left lateral ventricle in Case No. 8, hematoxylin & eosin staining, x400. Small round eosinophilic structures (arrows) are sometimes as big as, or are smaller than, red blood cells in torn neural tissue in the subependymal zone. Thick arrows indicate hypertrophic astrocytes.

**Fig. 13** Hypertrophic astrocytes (arrows) stained by immunohistochemical staining with anti-neurofilament (SMI-31), x400
On the other hand, some linear or direct force toward the head was more remarkable in the cases in group B than those in group A, even though the devastation of the intracranial structure was extremely severe. There was no neural tissue injury in SEZ at AH of LV, despite severe brain injuries, in cases of No. 9 (Fig. 15) and No. 10 (Fig. 18), for example. The victim of No. 9 killed himself by shooting himself in the head. The tears of the ependymal layer, which were seen in the right AH of LV (Fig. 17), were presumed to be artifacts, because they were not accompanied with any vital reaction. There were severe fractures injuring the left occipital lobe and the left cerebellar hemisphere (Fig. 18), but there was no traumatic tear of the ependymal layer except for the partial tissue defects (Fig. 19, 20).

No neural tissue injury was observed in the SEZ in any of the cases in group C, the control cases which were unrelated to head injuries, although the tissue around the AH of LV is as fragile as in other areas of the brain. It is presumed that the neural tissue injury in this portion was not formed as an artifact in routine manipulation when the brain was removed from the skull at autopsy.
Fig. 16 A view of the anterior horn of the left lateral ventricle in Case No. 9, hematoxylin & eosin staining, x100. The absence of ependymal layer, indicated by an arrow, is not an abnormal finding.

Fig. 17 A view of the anterior horn of the right lateral ventricle in Case No. 9, hematoxylin & eosin staining, x100. The spaces indicated by asterisks are presumed to be due to artifacts, because no vital reactions were observed around the loci.

Fig. 18 Brain of Case No. 10 with severe lacerations in the left occipital lobe and in the left cerebellar hemisphere (arrows), which were made by deep depressed fractures of the occipital bone. Note massive traumatic subarachnoidal hemorrhage.
An interesting finding around the attachment portion of the choroid plexus was shown in the figure of the article reported by Berry and Rice [9] among several articles dealing with brain injuries with intraventricular hemorrhages in LV that have been published in the field of forensic pathology. Surprisingly enough, small round eosinophilic structures discussed in the present study are apparently different from red blood cells shown in “Fig. 6” in their article, although there was no comment concerning those substances in their text.

It has been reported by Makino [6] that the attachment loci of choroid plexus in LV are the second most fragile part regarding traumatic hemorrhage around the cerebral ventricles, next to the posterior horns of LV. Taking the results of these studies together, it is possible that the hemorrhagic lesion shown in “Fig. 6” in the paper by Berry and Rice could be associated with injuries of neural fibers.
The present study arise another question; how and why do the injuries including hemorrhages and tear of neural tissue associated with neural fiber damage occur in SEZ at AH of LV as “special sites” in the cases of group A?

The authors propose three possible hypotheses to explain the mechanism of the localization:

1) increase of inner pressure in the ventricles creates greater tension around a wall with a smaller curvature than a larger one
2) a strong force concentrates at an AH, because the LV forms stratified structure around entire cerebral hemispheres and because the AH is located at the edge of the structure
3) the region around the AH is essentially more fragile than other parts of the ventricles.

The authors consider hypothesis 1) is the most likely among the above mentioned, because areas of traumatic hemorrhage in SEZ are seen not only in AH but also in lateral corners of the LV such as around the hippocampus, as well as posterior horns of the LV in our daily experience.

Further systematic surveys will be necessary to clarify whether hypothesis 2) is tenable or not. The authors consider that the hypothesis 3), although slightly unlikely, should be considered, because we often encounter some unexplainable gross lesion near the AH of infant brains in daily postmortem examinations, although there is no reliable data yet on this issue.

The authors presume that the above mentioned hypotheses require discussion based on further studies including pretty more cases. Forensic pathologists examining cases with fatal head injuries must be aware of the need to investigate evidence of diffuse axonal injuries (DAI) [1-5] which may be caused by a strong acceleration to the head with a sudden rotational motion. However, it takes more than 6 hours of survival to be able to diagnose DAI by the development of axonal retraction balls. This fact makes it difficult to diagnose DAI in instant death or fatal cases with a very short survival period. The mechanism of how the head of a victim was injured will more clearly be explained even in instant or sudden death cases, if it is certified that the neural injuries in SEZ at AH of LV reflect evidence of rotational acceleration of the head.

Finally, the authors emphasize that it is quite easy for forensic pathologists to prepare the specimens of AH of LV as mentioned above. They will easily be able to re-examine whether our hypotheses are correct or only hypotheses by adding 2 pieces of brain tissue in routine postmortem examinations, which is another central significance of the present study.

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Ethical statement

This study is an epidemiological study with anonymous data, which is categorized in subjects, and therefore does not need to be discussed by the Ethics Committee of the authors’ institutes according to the regulation of the Ethical Guideline for Epidemiological Study advocated the Ministry of Health, Labour and Welfare, Japan (the latest revision: 1st Dec. 2008).
References