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Short Communication

Freezing preparation for macroscopic forensic investigation in putrefied brain

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ABSTRACT

Purpose: To evaluate the usefulness of the applied freezing technique in putrefied brain for macroscopic investigation.
Materials and methods: From October 2015 to September 2016, first the brains of 10 cadavers (control group: male 6, female 4, age 20–80 (mean 61.5), postmortem intervals (PMI) 14–75 (mean 29.7) days) were inspected following the standard practice (without freezing preparation), and then with 10 cadavers (freezing group: male 7, female 3, age 41–88 (mean 60.4), PMI 7–75 (mean 29.2) days) the freezing technique was used before the autopsy. The cut brain was investigated, and the gray-white matter difference was evaluated macroscopically.
Results: In the control group, the brain parenchyma leaked out like sludge in 5, and there was difficulty maintaining its structure in 7. The gray-white matter difference was well visible in 3, but hard to distinguish in 3, and the total scores ranged from 0 to 9 (mean 4.4) points. In the freezing group, the entire putrefied brain was extracted as a solid organ, the gray-white matter differences were well visible, and the total scores were 6.7–9 (8.3) points. The gray-white matter difference was preserved in the freezing group ($p < 0.05$).
Conclusion: The freezing procedures to evaluate the putrefied brain have been successfully applied, and it could be statistically more useful in putrefied brain investigation than the ordinary procedure. Postmortem CT can be useful to evaluate not only the degree of brain putrefaction, but also the degree of brain parenchyma freezing.

1. Background

One of the recent topics of interest in forensic medicine is postmortem CT [1,2], and it has been used for investigation of causes of death in many situations [3–6]. Some causes of death could be evaluated mainly by postmortem CT, and its usefulness has been recognized in supporting autopsy investigation [7,8]. On the other hand, postmortem CT has inherent limitations in the cases of specific causes of death [9]. Therefore it has been requested to compare its findings with those from toxicology and histopathological investigation [10,11]. In specific instances, such as putrefaction (long postmortem term), the cadaver affords a limited sample for toxicological investigation and the histopathological examination also suffers limitations because of the organ deterioration. In putrefaction of the brain, the organ was destructed after extraction from the cranium, so that it had severe limitations, not only in macroscopic but also in histopathological investigation [12].

In our recent report about an animal experiment, using freezing procedure prior to opening the cranium, the putrefied brain could be extracted and undergo cut surface investigation macroscopically [13]. Therefore, it is anticipated to be of use in cadaver autopsies.

In this report, we applied the freezing technique in autopsy scene for macroscopic investigation, and we also evaluated its usefulness comparing with the standard practice.

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2. Materials and method

This study was approved by our institutional ethics committee (No. 16-015). From October 2015 to September 2016, 20 putrefied cadavers (male 13, female 7, 20–88 (mean 61.0) y.o., 7–75 (mean 29.5) day postmortem interval (PMI)) were enrolled in this study. First the brains of 10 cadavers (designated as the control group) were inspected using standard practice (without freezing preparation), and the next 10 cadavers (designated as the freezing group) were used freezing technique before the autopsy. Two putrefied cadavers were excluded in this study, because of severe head trauma (n = 1), and severe encroaching (n = 1).

Before the autopsy examination, all cases were examined by CT using a 16-slice multi-detector CT scanner (Supria, Hitachi Corp., Tokyo). The scan parameters were as follows: 120 kV, 215 mA, 0.75 s/rotation, beam pitch 1.3125, collimation 1.25 × 16, slice thickness 5.0 mm. The intra-cranial air density volume, intra-cranial volume (estimated as maximum brain volume), and percentage air density volume (% air) were evaluated using an image workstation (VINCENT, FujiFilm Tokyo). The equation for% air was:

% air = 100* (intra-cranial air density volume)/(intra-cranial volume). The degree of putrefaction was also estimated as softened or liquefied [14], equivalent as stage II or stage III in Watanabe's staging [12], according to the CT image appearance.

2.1. Freezing procedure

In the freezing group, at least 14 h prior to the scheduled autopsy, dry-ice blocks (1 kg each) were placed at both sides and the dorsal part of the cadaver's head. Before the autopsy, postmortem CT imaging was repeated to confirm the brain freezing based on decreasing brain parenchymal CT density [15,16].

2.2. Sawing cranium and brain extraction

The standard skull opening procedure was used in the control group: having dissected the scalp, the skull was opened with an oscillating saw. If the brain parenchyma spilled out from the cranium, the sludge brain parenchyma was collected using a vat to measure its weight and to investigate it macroscopically.

In the freezing group, the sawing level was placed relatively close to orbit to make a wide operating space to extract the brain parenchyma. If the skin had become leathery due to a long postmortem interval, or the skin was hard to remove from the skull, the dissection of the skin was performed after sawing the skull. An additional saw was considered in particular situations, such as the brain tissue was attached to the inner surface of the cranium, or the brain tissue was intentionally dissected at the level of the tentorium cerebelli to extract the cerebellum separately from the cerebrum.

2.3. Macroscopic investigation and fixation

Extracted brains were investigated macroscopically. If the putrefied brain was destructed before formaldehyde fixation, a cut macroscopic inspection was employed. After the brain surface macroscopic investigation, the whole brain parenchyma was placed in a 7.4% diluted buffered formaldehyde fixative solution for 2 weeks at room temperature (20 degree Celsius). Because the softened or liquefied brain cannot be fixed only using 7.4% diluted buffered formaldehyde fixation, 24 h prior to the cut inspection, the whole brain was placed in a minus 5 Celsius freezer with the diluted formaldehyde fixative. Using this additional freezing technique, the putrefied brain can be handled like a solid organ, and at the same time the formaldehyde kept its liquid state because pure formaldehyde freezing point was minus 92 degree Celsius. After confirming the freezing of the brain parenchyma, the ideal cut could be employed for macroscopic investigation.

2.4. Statistical analysis

The male-to-female ratio, age, postmortem interval, brain volume, and% air were compared between two groups, statistically. To evaluate the cross gray-white matter appearance, a 4-point scale was employed: 3 points as clear, 2 as moderate, 1 as ambiguous, or 0 as poor. The data was scored by 3 observers (HH, KM, AS) independently, and the total score was used for statistical investigation. Variables were compared using the Chi-squared test, and a Mann-Whitney *U* Test using JMP (SAS Institute Inc., North California, USA, version 11.0.0) software. A P value of less than 0.05 was considered to indicate a statistically significant difference.

3. Results

The backgrounds of the groups were shown in the Table 1. In the control group, the causes of death were as follows; drowning (n = 4), cardiac infarction (n = 2), hemo-pericardium (n = 1), and unknown (n = 3). In the freezing group, the causes of death were as follows: drowning (n = 2), brain hemorrhage (n = 2), multiple organ failure (n = 1), drug poisoning (n = 1), hypo-nutrition (n = 1), cardiac infarction (n = 1), and unknown (n = 2). In statistical evaluation, there were no significant differences between two groups in male-to-female ratio, age, and PMI.

3.1. Postmortem CT

In the control group, intra-cranial air presented in all cases, and its volume range was 55.2–485.4 (mean 255.2) ml. The% air compared with intra-cranial volume ranged from 3.8 to 39.5 (mean 19.0)%. The brain parenchyma decreased in volume and those were estimated as softened, 7, and liquefied, 3. In the freezing group, all cases presented intra-cranial air, and the volume range was 153.1–681.6 (mean 423.3) ml, and the% air was 11.0–47.3 (mean 29.4)% (Fig. 1a). The softened type was 3, and liquefied type was 7. Brain parenchymal density was 32.2–51.4 (mean 43.4) HU, and −6.6 to 23.1 (mean 3.6) HU after freezing preparation (Fig. 1b). In statistical evaluation, there were no significant differences between two groups in the brain volume and% air.

3.2. Autopsy

In the control group, all brain parenchyma were separated from the inner-surface of the cranium. After opening the cranium, the brain parenchyma leaked out like sludge in 5, and there was difficulty maintaining its structure in 7. After cutting the parenchyma,

Table 1
Summary of data.

	Control group (=10)	Freezing group (n = 10)	P value
Male-to-female ratio	6: 4	7: 3	0.6388
Age (year)	20–80 (61.5)	41–88 (60.4)	0.3843
PMI (day)	14–75 (29.7)	7–75 (29.2)	0.6728
Brain volume (ml)	1049–1620 (1294)	1200–1630 (1433)	0.1038
% air (%)	3.8–39.5 (19.0)	11.0–47.3 (29.4)	0.0588
Gray-white matter difference inspection score	0.0–9.0 (4.4)	6.7–9.0 (8.3)	0.0424

PMI: postmortem interval.
% air = 100* (intra-cranial air density volume)/(intra-cranial volume).