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Experimental evaluation of freezing preparation for the macroscopic inspection in putrefied brain

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ABSTRACT

Purpose: To evaluate the usefulness of freezing preparation for macroscopic investigation in advanced putrefied brain.

Materials and methods: After sealing in individual plastic bags, 10 pig heads were stored at 20 °C for 5 days allow postmortem change (putrefaction) to progress. After an observation period, they were divided into 2 groups to evaluate the usefulness of the freezing effect in macroscopic investigation. The process over the postmortem period and the freezing process were examined.

Results: At day-5, the presence of air density was detected between the inner surface of the cranium and the brain parenchyma. Intra-cranial air accumulation presented on CT in all heads.

In the control group, the brain parenchyma leaked out from the hole in the meninges, and the gray-white matter difference was clear in 3/72 (4.2%), moderate in 7/72 (9.7%), ambiguous in 17/72 (23.6%), and poor in 45/72 (62.5%). In the freezing group, the brain parenchyma presented homogeneous low density after more than 14 h freezing. On opening the cranium, the entire brains were frozen, and the gray-white matter difference was clear in 33/72 (46.0%), moderate in 17/72 (24.0%), ambiguous in 15/72 (21.0%), and poor in 7/72 (10.0%). The freezing group afforded greater clarity in the gray-white matter inspection ($p < 0.05$).

Conclusion: Freezing preparation was useful for the macroscopic investigation of putrefied brain compared with the ordinary autopsy.

1. Introduction

One of the recent topics of interest in forensic medicine is postmortem CT imaging [1–3], because of its usefulness in detecting intra-cadaver condition before or without invasive investigation. For example, intra-cadaver gas distribution [4,5] and intra-visceral hypostasis [6] have been reported, which have been previously reported as difficult to detect during autopsy investigation [6,7].

The use of postmortem CT has been expanding in scope; it has been increasingly used for evaluation of bodies in an advanced state of putrefaction [8], and postmortem CT has the benefit of allowing detection of the intra-cadaver condition prior to autopsy investigation [9]. In particular, intra-cranial bleeding has been reported as an abnormal high-density region in postmortem CT images, and it can be detected even if the cadaver is in advanced putrefaction [10,11].

On the other hand, embalming of a cadaver may result in the presentation of high density in intra-body, which may lead to a mistaken interpretation as bleeding/hematoma [12]. Furthermore, the long postmortem period may decrease a lesion's density and examination solely by CT will miss a hematoma or underestimate the size of a lesion [10]. Therefore, a macroscopic investigation is required in order to diagnose an intracranial lesion (such as bleeding). But this is difficult to evaluate in an advanced putrefied body, because after opening the skull bone, the brain emerges as sludge material and at best macroscopic investigation has been limited to observing the color change in the sludge brain tissue.

In our literature review, there was no recommended procedure concerning advanced putrefied brain investigation, especially in

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macroscopic investigation. The purpose of our study was to evaluate the usefulness of freezing preparation for macroscopic investigation in advanced putrefied brain.

2. Materials and method

This study was evaluated by the institutional animal ethics committee and did not require approval because the subject animals had already been sacrificed. Within 5 h after sacrifice, the skin was removed from 10 pig heads (sex unknown, 1.92–2.34 (mean 2.09) kg). These were collected from a food supplier, and enrolled in this study. After sealing in individual plastic bags, all heads were stored at 20 °C for 5 days to allow the postmortem change (putrefaction) to progress.

2.1. Grouping

After an observation period, they were divided into 2 groups, the control group and the freezing group. CT examination was performed to evaluate the process of the postmortem period (putrefaction) in both groups.

2.2. Freezing method

In the freezing group, the bagged heads were placed in a refrigerator (Ultra Low MDF-U33V, Panasonic Healthcare Co., Tokyo). The dry ice was used in practical investigation [13], and the dry-ice sublimation temperature was at minus 79 °C. Therefore, the temperature of refrigerator was set at minus 80 °C. To evaluate the freezing process, the bagged heads were examined by CT in every 2 h.

2.3. Postmortem CT imaging

CT examination was performed by 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 collected DICOM data was transferred to an image workstation (VINCENT, FujiFilm Corp., Tokyo) and the postmortem change (appearance of putrefaction gas and brain tissue distraction) was evaluated in both groups, and the freezing effect was also evaluated in the freezing group.

2.4. Autopsy

After extracting the nose part, a sagittal sectional dissection was used. After reaching into the intra-cranial space, the brain parenchyma was extracted, taking care not to damage the dura and brain parenchyma. If the dura was adhering to the inner surface of the cranium, or the brain was difficult to extract without damage, additional cranial cutting was used.

2.5. Inspection and statistical analysis

Whole brain was inspected for maintenance of its anatomical structure (dura, surface vein, cerebral/cerebellum gyrus) macroscopically in both groups. In addition, the gray-white matter difference was inspected using coronal dissection at the level achieving the maximum cross section. The cross section was divided into 4 parts of 90 ° each, and the gray-white matter appearance was evaluated on 4 points scales: clear (3 points), moderate (2 points), ambiguous (1 point), or poor (0 point). The data was scored by 3 observers (HH, KM, AS) independently, and the total score was used for statistical investigation. The JMP (SAS Institute Inc., North California, USA, version 11.0.0) software was used with a Mann-Whitney Test. Differences with $p < 0.05$ were considered to be statistically significant.

3. Result

The head weights were 1.92–2.18 (mean 2.04) kg in the control group and 2.02–2.34 (2.13) kg in the freezing group.

At day 5 observation, the presence of air density was detected between the inner surface of the cranium and the brain parenchyma. Intra-cranial air accumulation presented on CT in all heads (Fig. 1). This was interpreted as putrefied brain.

3.1. Freezing group

After the observation period, the bagged heads were placed in the refrigerator for at least 24 h. During the freezing process, the lower density band was increasing from the outer surface to the center part of the brain parenchyma on CT images. More than 14 h needed to complete the density decrease (Fig. 2).

Fig. 1a. Pig brain CT (coronal view); pre-procedural CT. Little air is detected in the brain. The brain parenchyma continues to reach the inner-surface of the cranium.

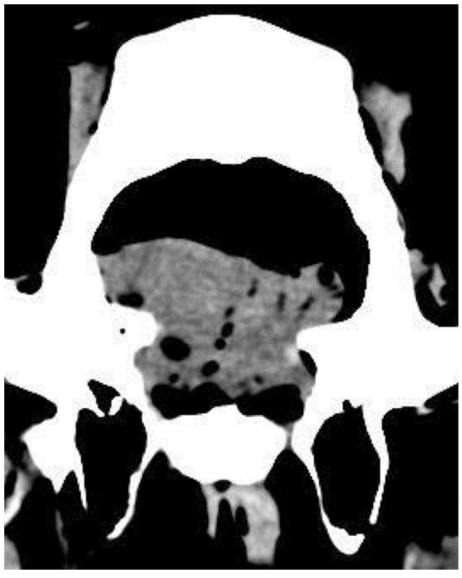

Fig. 1b. Pig brain CT; after 5 days observation. The brain parenchymal height is decreased and tends to flatten. Intra-cranial air is detected between the brain parenchyma and the inner-surface of the cranium.