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Application of the microwave digestion-vacuum filtration-automated scanning electron microscopy method for diatom detection in the diagnosis of drowning

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ABSTRACT

The cause of death for the decomposed corpses recovered from water is still a difficult issue in current forensic practice. In this article, we present two cases of bodies recovered from water with no positive findings to indicate the cause of their death. We apply both conventional acid digestion method as well as the microwave digestion-vacuum filtration-automated scanning electron microscopy method (MD-VF-Auto SEM) for diatom detection in different organs of both bodies. Our results indicate that MD-VF-Auto SEM method provide more accurate information and match further police investigation. This novel method would be a useful technique in assessing cause of death for body found in water.

1. Introduction

During the last few decades, many diatoms detection methods have been developed in order to isolate and enrich diatoms from different tissue and water samples, such as acid digestion method,[1] enzymatic digestion method,[2] and soluence-350.[3,4] One of the commonly used diatom detection method is acid digestion method in combination with centrifugation to enrich diatoms, followed by light microscopy examination of the samples. There are certain limitations with acid digestion method: time-consuming, diatom damage or multi-residual and limited sensitivity using light microscopy. We developed a novel diatom detection method called "Microwave Digestion-Vacuum Filtration-Automated Scanning Electron Microscopy (MD-VF-Auto SEM)".[5]

In the two cases reported here, we provide the results of diatom test as a supportive evidence for drowning or post-mortem immersion by the MD-VF-Auto SEM method.

2. Methods

2.1. Sample collection

All the instruments were single-used for each tissue and were washed with ultrapure water before sample collection. All samples must be removed in the prescribed sequence at autopsy as following: liver, kidney, bone marrow, lungs and water.

Once the abdominal cavity was opened, a piece of liver tissue weighting about 50 g was removed and saved from the left lobe of the liver using scalpels and forceps. Then, the intestinal organs were removed. The entire right kidney including the capsule were removed and saved for our analysis. For bone marrow sample, the left femur was removed and saved for analysis. For lung tissues sample collection, sternum was removed to open the thoracic cavity. Four strips of lung tissues (approx. 2–2.5 g for each piece)

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were detached by scissors from the edge of the superior lobe of left lung, at the width of less than 5 mm.

Above tissue sample were placed in separated plastic bags, 1000 ml of water samples were taken in a bottle from where the body recovered. It is preserved in the dark after adding filtered formalin to reach 5% final concentration.

2.2. Sample digestion and observation

The tissue samples of the right kidney (10 g), the left lobe of the liver (10 g), the whole bone marrow of the left femur, the lung tissues samples (2 g), and 50 ml water sampled from the discovery site were tested using the conventional acid digestion method and the MD-VF-Auto SEM method.[5] Quantitative and taxonomical analysis at species level was performed in each tissues and water sample.

2.2.1. The conventional acid digestion method

The tissues were digested with concentrated nitric acid (HNO3) and hydrogen peroxide, enriched with centrifugation at 4000 rpm for 15 min and observed with light microscopy at 400 × magnification.

2.2.2. The MD-VF-Auto SEM method

The tissues digested with microwave digestion system, enriched with vacuum filtration (pore diameter size of the filter membrane is 0.45 μm) and automatic scanned with SEM at 400 × magnification.

3. Case report

3.1. Case 1

3.1.1. Case history

A lower part of male body was found on the bank near a bridge building site during the late spring (Fig. 1). The victim was identified by means of DNA investigation. Police found that the victim was kidnapped near his home, died of asphyxia. His body was dumped in a river, and floated 80 km to the sea.

3.1.2. Autopsy findings

The injuries of the broken ends suggested that the body was dismembered by a propeller. No ante-mortem blunt and sharp force injuries were found on the body parts. The condition of the body parts did not provide any information about the cause of death.

Fig. 1. Case 1 : Cadaver discovered on the bank.

3.1.3. Laboratory analysis

3.1.3.1. Toxicological analysis. Common sedatives and hypnotics (phenobarbital, triazolam and so on), morphine, MDMA, methylamphetamine, ketamine, marijuana and tetramine were negative in the bladder flushing fluid.

3.1.3.2. Diatom test. The results of the both methods were negative. *Aulacoseira crassipunctat*, *Cyclotella meneghiniana*, *Stephanodiscus niagarae*, *Tryblionella salinarum* were found in the water samples.

3.2. Case 2

3.2.1. Case history

An unidentified, grossly decomposed male body was found floating in the sea near a mountain during the early summer (Fig. 2A). No results were obtained regarding victim identification by police investigation.

3.2.2. Autopsy findings

The body was highly decomposed and the facial soft tissues disappeared. No injuries of the hyoid and laryngeal cartilages were discovered. There were no hemorrhage found in the skins and muscles of the four limbs. The thoracic cavity was exposed and the lungs were missing (Fig. 2B). The spine was broken by a propeller force. There was a suspected sharp force injury, with a length of 1.5 cm and width of 0.5 cm in the right ventricle (Fig. 2C). It was suspected that the injury could be caused by the broken rib. The color of the both cardiac chambers was different (Fig. 2D). No defending injuries were found. The condition of the body indicated that the victim died of nonviolent factors.

3.2.3. Laboratory analysis

3.2.3.1. Toxicological analysis. Common sedatives and hypnotics (phenobarbital, triazolam and so on), morphine, MDMA, methylamphetamine, ketamine, marijuana and tetramine were negative in the liver tissues and bladder flushing fluid.

3.2.3.2. Diatom test. As demonstrated in Table 1, many diatoms were detected in the tissues digested with the MD-VF-Auto SEM method. However, the kidney tissues were the only tissues with positive results using the conventional acid digestion method.

4. Discussion

In forensic science, the cause of death for body discovered in the water can be challenging. Although there are some typical physical of drowning, it is still difficult to determine a death by drowning. The more putrefied the body is, the more difficult to find these signs. Presently, diatoms tests are widely used to provide scientific evidence for the drowning cases.[6,7] In the process of drowning, the diatoms in the water will be inhaled into the lungs. Some of the diatoms (because of their sizes) penetrate into the alveoli. If the heart is still beating, the diatoms can enter into the blood stream, travel around the body and lodge in distant organs and tissues such as liver, kidneys, bone marrows before death. The presence of diatoms in the internal organs most likely confirms the ante-mortem drowning.[8]

For decades, the conventional and classic forensic diatom test method consisted of acid digestion, centrifugation, and observation by light microscopy is widely used in forensic laboratories.[9–12] This method is lengthy; diatoms are lost during centrifugation; could be health hazardous to handle nitric acid. According to our previous study,[5] the lost ratios of centrifugation (4000 rmp, 15 min) range from 29.94 to 49.60%. Almost 50% of diatom valves in the supernatant have a maximum length of less than 15 μm, and 90% have a