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A quantitative comparison analysis of diatoms in the lung tissues and the drowning medium as an indicator of drowning

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ABSTRACT

The presence of diatoms in the lung tissues, internal organs and bone marrow is considered as the supportive evidence in the diagnosis of death by drowning. Generally, the diatoms detected in the lung tissues are regarded as insignificant since these diatoms can be detected in the lung tissues of the postmortem immersion bodies. In this study, we analyzed the relationships between the numbers of the diatoms in the lung tissues and the drowning medium. We made a comparison analysis between the diatoms in the lung tissues and the drowning medium using the ratio of diatom numbers in both samples (L/D ratio), utilizing Microwave Digestion - Vacuum Filtration - Automated Scanning Electron Microscopy method. Our data indicate that the L/D ratios in victims of the drowning group were higher than the postmortem immersion group. A higher L/D ratio provides valuable information about the cause of death in drowning victims. Quantitative diatom analysis in the lung tissues, especially combined with the diatom analysis of the drowning medium, provides supportive evidence in determining if a body recovered in water was due to drowning or not.

1. Introduction

The presence of diatoms in the lung tissues, internal organs and bone marrow is considered as the supportive evidence in the diagnosis of drowning.[1–3] Numbers and types of diatoms accurately reflect the water quality at the site where they grow. The diatoms in the medium are also influenced by the salinity, pH range, water depth, bottom conditions, and aquatic vegetation, etc.[4] Not only the cause of death but also the site of drowning may be determined basing on the qualitative and quantitative analysis of the diatoms.[5–8] Generally, the diatoms recovered in the lung tissues of the bodies cannot provide supportive evidence for the diagnosis of drowning because diatoms can be flowed into the lung with the drowning medium when the bodies are immersed after death. In order to solve this problem, some forensic experts suggested the use of quantification of the measurement of diatoms in the lung tissues. Ludes et al[9] have suggested a cut-off concentration of 20 diatoms per 10 g of the lung tissue. However, the concentration of diatoms in the drowning medium varies greatly. The fixed criteria for all drowning cases would have limited applications.

The low sensitivity of the conventional diatom test method limits the use of quantitative analysis in the diagnosis of drowning. We reported a novel method called Microwave Digestion - Vacuum Filtration - Automated Scanning Electron Microscopy method (MD-VF-Auto SEM method).[10] This high sensitive diatoms extraction and detection method provides more accurate diatom test results.[11] In this article, we made a comparison analysis of the diatom numbers in the lung tissues and the drowning medium samples with the MD-VF-Auto SEM method. Our studies indicate that there are quantitative differences of the diatoms detected in the lung tissues of drowning bodies and postmortem immersion bodies.

2. Methods

2.1. Cases

Two groups of the cadavers at our institution were included in this study. The drowning group was comprised of 56 bodies which were confirmed drowning cases according to the eyewitnesses or

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postmortem examination. The postmortem immersion group was comprised of 8 cases with the cause of death other than drowning. Among them, three cases had heart attacks; five cases died of asphyxia. These eight bodies were then submersed or dumped into the water.

2.2. Sample collection and diatom test

The lung tissue samples were collected from all the cases. Precautions were applied to avoid cross contaminations during the autopsy. In particular, the instruments for sampling were used once only and then disposed. The upper lobe of the left lung was collected for further analysis.

The superficial tissues of the lung were removed. 2 g of the inner lung tissues were taken and used for analysis. We utilized our previous described "MD-VF-Auto SEM method" for this study. The samples were digested in the microwave digestion system with 8 ml of concentrated nitric acid and 2 ml of 30% hydrogen peroxide for 20 min. Then the digestive solutions were filtrated by the membranes (the pore diameter of the filter membrane is 0.45 μm). The membranes were analyzed by SEM.[10]

10–50 ml of the putative drowning medium samples were taken from the sites of drowning or where the bodies were recovered. The water samples were tested by the same method as above.

A blank control (without lung tissues) sample and a negative control sample (with lung tissues of bodies died of other causes outside the water) were performed with each diatom analysis comparison study.

2.3. Calculation

With the concentration differences, we introduced the ratio of number of diatoms in the lung tissues to the drowning medium (L/D Ratio) to eliminate the influence of environment. In this research, we defined the L/D ratio of 1.0 as the baseline.

- Calculation of L/D Ratio

$$L/D\ Ratio = \frac{N_l}{N_d} \quad (1)$$

Where

L/D Ratio: Ratio of numbers of diatom in the lung tissues and the drowning medium samples
N_l: Number of diatoms in the lung tissues
N_d: Number of diatoms in the putative drowning medium

2.4. Statistical analysis

Statistical analysis was performed by *t*-test and chi-square test using SPSS 17.0 for windows. $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. The L/D ratios of each group

There were no diatoms found in the blank control samples and the negative control samples. As demonstrated in Table 1, the L/D ratios in the drowning group and the postmortem immersion group were 13.50 ± 17.47, 0.51 ± 0.43 respectively. The L/D ratios of the drowning group ranged from 0.02 to 64.67 while those of the postmortem immersion group ranged from 0.10 to 1.26. There were statistical differences between the two groups ($P < 0.05$). The proportion of the L/D ratio >1 in the drowning group is 91.07%. And that of the postmortem immersion group was only 2 out of 8 cases with the L/D ratios of 1.05 and 1.26. The proportion of the L/D ratio >1 in the drowning group was much higher than that of the postmortem immersion group. The results were statistically significant ($X^2 = 17.08 > X^2_{0.05.1} = 3.84$, $P < 0.05$).

3.2. The specificity of L/D ratios

As shown in Table 2, there were total 53 cases in both groups whose L/D ratios were >1. Among these 53 cases, only 2 of them were from the postmortem immersion group. The other 42 cases whose L/D ratios >2 were all came from the drowning group. The drowning specificity of L/D ratio >1 was 0.96 and that of L/D ratio >2 is 1.00.

4. Discussion

This article is designed to study if there are differences of the diatoms number detected in the lung tissues and the drowning medium among the drowning victims and postmortem immersion cases. The main findings of the study are that: (i) The proportion of L/D ratio >1 in the drowning group is much higher than that in the postmortem immersion group; (ii) The specificity of L/D ratio for drowning bodies >1 is 0.96 and that of L/D ratio >2 is 1.00.

In our studies, there are no diatoms detected in the blank control and negative control samples. The results of the blank control (without lung tissues) indicate that there was no cross-contamination during the sample analysis process. Few Diatoms can be found in the lung tissues of some non-drowned bodies.[12,13] The negative results in our negative control group (with lung tissues obtained from bodies unrelated to drowning) may be due to the following reasons: (i) There are no diatom in our negative control samples; (ii) There are indeed few diatoms in the lung tissues, but the total numbers of the diatoms are too small to be detected because of the total lung tissues samples (2 g) used for analysis. The negative results of both control groups come to a conclusion that the diatoms found in the lung tissues were indeed from the drowning medium.

The previous research[14] has shown that the types of diatoms in the internal organs often match those in the putative drowning medium. This concordance is important corroborating evidence for the validity of the diatom test. The victims are classified as drowning basing on the fact that the person is alive with active breathing before his/her body is submerged in the medium (water).[15] In the process of drowning, the victim's lung tissues will expand, so that the diatoms in the water are forced by the increased respiratory effort into the alveoli, penetrate into the pulmonary blood vessels through the alveoli-capillary barrier and the interstitial spaces.[16] These diatoms are then travelled to distant organs via systemic circulation. In the process of the postmortem immersion, the diatoms are passively flowed into the terminal bronchiole and the alveolar space with hydrostatic pressure difference and no "enrichment process".[17,18] The number of diatoms in the lung tissue is likely comparable to the numbers in the drowning medium. That is the reason why there are differences between the drowning group and the postmortem immersion group and why we set "L/D ratio = 1" as the baseline in this study.

In the drowning group and postmortem immersion group, we have cases with L/D ratio <1 or around 1. The L/D ratio in this range is inconclusive for drowning or postmortem immersion. We analyzed the drowning cases with L/D ratio in this range. In these cases, the victims had suffered severe physical injuries prior to their drowning. These fatal injures led to decreased respiration efforts.