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Applications for drowning identification by planktonic diatom test on rats in forensic medicine

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Abstract

We established a model of drowning, and by investigating diatoms in lung, liver, kidney, and long bone marrow of rats at different time to discuss the cause of death. The organs of 35 rats were extracted 0.5 h, 1 h, 6 h, 12 h, 24 h and 48 h after drowning and the organs of sham-drowning group killed by mechanical asphyxia were extracted 1 h after body immersed in water. The organs were digested by acid, and the diatoms were analyzed by statistics. Results shown the detection rate was 100% in lung, and the positive rate of all the extracted organs was 100% 6 hours after drowning except the sham-drowning group. No diatoms were detected in the liver, kidney and bone marrow of the sham-drowning group, just only one case was positive in the lung. So it is concluded that the detection rate of diatoms could be considered as important evidence in drowning determination.

Keywords: diatom; drowning; forensic pathology

1. Introduction

The post-mortem examination of drowning is one of the most difficult problems in forensic [1], [2], as the environment of water is more complex than the land, which makes the identification of the cause of death more difficult. Recently, forensic diatomology plays an important role in solving drowning cases [3]. The diatom test has been proposed to be useful in the diagnosis of drowning [4], [5]. It would be helpful in judging the suspected drowning cases. It is considered that the diatom is one of the important evidence

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when determining the nature of cases, and especially the diatoms were detected in more than one organ. The use of the diatom test was helpful in excluding the possibility that the body who had died by means other than drowning [6]. The present study was to investigate the value and practical significance of diatomology in determining the mode of death of water corpse.

2. Materials and Methods

2.1. Drugs and apparatus

Nitric acid, concentrated sulfuric acid and 30% hydrogen dioxide were purchased from Shanghai Chemical Reagent Company. MARS 5 Microwave Reaction System (CEM Corp., USA) and LXJ-IIB Centrifuge (Cany Precision Instruments Co., Ltd., China) were used to digest tissues and organs samples for this study.

2.2. Establishment of an animal model

Fifty-six SD rats, purchased from the Experimental Animal Center of Zhejiang University, were divided randomly into seven groups, eight rats each group. Each group was submerged in the same place of a river for one hour, deep in one meter in iron cage. The organs including lung, liver, kidney, and tibial bone marrow were extracted 0.5 h, 1 h, 6 h, 12 h, 24 h, 48 h after drowning, the sham-drowning group were killed by mechanical asphyxia, then put them into same position of river, and the organs of the sham-drowning group were extracted 1 h after bodies immersed in water. The care and handling of the animals was in accordance with the Chinese Association for Laboratory Animal Sciences.

2.3. Management of the specimen

The lung, liver, kidney and tibial bone marrow were removed by surgery. Strictly aseptic operation was executed, in order to avoid cross-contamination. Equipment and tube were cleaned by using distilled water for prevent water pollution. The autopsy specimens were frozen before cutting-up for avoiding taking organ capsule which can easily be contaminated. The specimen was taken in subcapsular 0.5 cm and weight 1-2 grams. The tissue cut were put into the digestion tank, nitric acid 5 ml was added dropwise to digested the tissue, then the specimens were digested in 0.5 MPa for 2 minutes. The digestion fluid was centrifuged 5 minutes with 5000 rpm. The supernatant was dumped, distilled water 8 ml were added dropped into digestion tank, and the tank was centrifuged 5 minutes with 5000 rpm. The supernatant was dumped once again, then leaving only a wall of water to observe diatom smear by light microscope. The marrow was from the long bone, and concentrated nitric acid was poured into the marrow cavity of long bone, 2 minutes later, the liquid was poured into flask, the next steps were the same to the other organs.

2.4. Statistical analysis

Count all the experimental data with Excel 2003 database and the SPSS 11.5 statistical package, Chi-square test was used to analyze detection rate of diatom in the organs of rats. The probability value of 0.05 was accepted as significant for differences between groups of data.

3. Results

The detection rate of diatoms was 100% in the lung in all groups except the sham-drowning group, and the positive rate of all the extracted organs was 100% 6 hours after drowning except the sham-drowning group. No diatoms were detected in the liver, kidney and bone marrow of the sham-drowning group, just only one case was positive in the lung. The detection rate of diatoms of the experimental groups was higher than the sham-drowning group (P<0.05). The results could be seen at table 1.

Table 1. The detection rate of diatoms in different groups (n=8)

Groups	0.5 h*	1 h*	6 h*	12 h*	24 h*	48 h*	Sham-drowning
Lung	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)	8 (100%)	1 (12.5%)
Liver	5 (62.5%)	6 (75%)	6 (75%)	7 (87.5%)	6 (75%)	8 (100%)	0 (0%)
Kidney	7 (87.5%)	5 (62.5%)	5 (62.5%)	4 (50%)	6 (75%)	6 (75%)	0 (0%)
Bone marrow	3 (37.5%)	5 (62.5%)	2 (25%)	3 (37.5%)	4 (50%)	6 (77%)	0 (0%)

*P<0.05 vs Sham-drowning

Species of the diatoms frequently detected in the organs in the drowning group were the Melosira granulate, Cyclotella, Actinocyclus, Navicula, Nitzschia, Achnanthidium and so on.

Fig. 1. The diatoms of organs.

4. Discussion

Diatom with many different kinds and shapes is single-celled plankton, widespread in natural water. After living falling into water, diatoms go into the lung or stomach with water, then penetrating into the body tissue through the blood circulation.

Diatom test has been used in a range of applications in forensic science for a long time [7-12]. Although the value of diatoms in drowning cases is still controversial, the supporting view is accepted by more and more people [2], [8], [13-17]. In the present study, the model of drowning was established, and the results of our study indicated that the detection rate of diatoms of the drowned alive was higher than the water corpse killed by mechanical asphyxia before put into river. Diatoms could go into the organs with the blood circulation, while sometimes the lung of the water body not drowning could also be

detected diatoms, it may be that the water was squeezed into the lungs by the water pressure, but the other organs could not be detected diatoms because blood circulation had been stopped. It also can be helpful in diagnosing the suspected drowning cases occurring in a particular area. Generally, presence of sufficient number of diatoms in vital distant body organs can establish ante-mortem drowning up to a certain extent.

The recovery of diatoms from various examined organs including lungs, liver, kidney, and long bone marrow, their qualitative and quantitative composition, can be a reliable proof of drowning. It the process of diatom examination, the anthropogenic pollution should be removed, as the diatoms are widely distributed and even it could be spread with the wind. Strict compliance with operating rules is regarded as the foundation of diatom examination; the amount of samples should be sufficient which one of the key to success is. And incubation of digested samples with hydrogen peroxide would be helpful for quantitative and qualitative analyses of diatoms [9]. Distribution of diatoms in any water body, and their correlation with the diatom species recovered from the drowned body can be a method of choice to resolve the questions related to drowning site [10, 17]. Though there are many features of drowned alive, such as the foam, goose skin, all these are not Characteristic, we should do a comprehensive analysis with the case, especially the water body was rot as soaked in water for too long, bitten by water animal, or hit by propeller. And the present study showed that diatom detection could be helpful in the diagnosis of drowning.

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