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Diatomological investigation in sphenoid sinus fluid and lung tissue from cases of suspected drowning

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ABSTRACT

We report on the presence, distribution and numbers of diatoms within specific organs as a result of drowning in fresh, treated and seawater. Specimens of sphenoid sinus fluid and lung tissue from 100 cases of suspected drowning and 20 cases where death was by natural causes, to act as a control, were examined for the presence of diatoms. In the 100 cases where the deceased was suspected to have drowned, 94 were confirmed as a death by drowning after autopsy with the other six being reported as death by another cause. No diatoms were found in cases confirmed as death by causes unrelated to drowning, with the exception of possible contamination via open wounds and through decomposition. In 94 cases, where all fatalities were confirmed as death by drowning, there were 81 cases in which diatoms were detected in samples taken from the sphenoid sinus fluid and/or lung tissue. No, or only few, diatoms were observed from the samples where the deceased drowned in treated waters such as spa or swimming pools. A significantly higher number of diatoms were detected in the sphenoid sinus fluid and lung tissue of confirmed drowning cases in fresh water compared to seawater. More diatoms were observed in sphenoid sinus fluid compared to lung tissue regardless of the water in which the deceased drowned. This study illustrates the potential use of diatom screening using both sphenoid sinus fluid and lung tissue to determine the cause of death in suspected cases of drowning. This report also highlights specific variables that need to be considered prior to such as conclusion being reached.

1. Introduction

Diatoms are one of the most common organisms used in determination of death by drowning due to their silica frustule; these are inert to acid, high temperature and enzymatic digestion and thus remain intact for a long period of time [1]. Smaller diatoms (sizes of not more than 10 μm) can enter the blood circulation from the lung vessels and migrate to the other organs [2,3]. While the presence of diatoms in lung tissues, blood, internal organs and bone marrow is key in the reporting of death by drowning, determination of the location at which the drowning occurred can be deduced by comparisons of the diatoms recovered from the organs of the deceased with those from reference water samples [4–7]. Based on qualitative and quantitative analyses of the diatoms, both the cause and place of drowning may be reported [8–11]. While diatoms can provide valuable information for forensic investigations, diatoms have been detected in cases where the deceased did not die by drowning [12]. In these cases, the presence of the diatoms was reported as potentially coming from the air, soil and seafood [13,14].

The presence or absence of diatoms in sphenoid sinus fluid was assessed to determine if this was more reliable indicator of death by drowning [15,16], however positive results for the presence of diatoms were reported in cases where death was not by drowning. To address this, we report on a comprehensive study of the presence of diatoms in sphenoid sinus fluid collected during an autopsy. As a result of this study we report on the significance of finding diatoms in suspicious deaths by drowning.

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2. Materials and methods

2.1. Sample collection

All the fluid from the sphenoid sinus and 5 g of lung tissue from the lower lobe was collected from 100 autopsy cases spanning September 2009 to September 2012. In all cases, the deceased were found near or within water leading to a suspicion of death by drowning. Samples were also collected from 20 deceased who were reported to have died by natural causes to act as a control.

2.2. Acid digestion

The sphenoid sinus fluid was centrifuged at 2900 × *g*-force for 10 min. The precipitate was treated with glacial acetic acid and concentrated H_2SO_4 (ratio 9:1), and boiled for 20 min. After this treatment, the specimen was again centrifuged (as abovementioned). The precipitate was washed with distilled water twice and then re-suspended in 10 μL distilled water in preparation for transfer to a microscope slide. The lung tissue was cut into small pieces and digested with concentrated HCl and concentrated HNO_3 (ratio 1:3) overnight [17]. The digested product was centrifuged at 2900 × *g*-force for 10 min. The precipitate was re-suspended in 200 μL distilled water for diatom examination.

Water samples acting as a positive control were collected from the river Xindian (Taipei, Taiwan) and the negative control was from distilled water.

2.3. Slide preparation

The re-suspended precipitate (10 μL) was smeared onto a microscope slide and dried in a 50 °C oven. Naphrax (RI 1.73, Brunel Microscopes Ltd., Chippenham, UK) was used to fix the slide cover.

2.4. Diatom examination

The morphological characteristics of the diatoms were examined by phase-contrast/differential interference-contrast microscopy (ZEISS Axio Imager. A2, Jena, Germany) using the 40× or 100× object lens. Species of diatom were identified by following the instructions found in reference [18] and the following websites [19,20].

3. Results and discussion

3.1. Diatom examination

In our comprehensive study of 120 samples from both sphenoid sinus fluid and lung tissue, diatoms were recorded as being present in 83 cases. Almost all the diatoms were identified to species level, with only a few identified to genus level due to the ambiguous minor microscopic features. Results of diatom counting for the sphenoid sinus fluid and lung tissue samples from the 100 cases where death by drowning was a possibility are listed in Table 1. The total number of diatoms in both the sphenoid sinus fluid and

Table 1
Results of distribution of diatoms screened from sphenoid sinus fluid (SSF) and lung tissue (LT) based on 100 suspected drowning cases.

Case	SSF	LT	Total[a]	Case	SSF	LT	Total	Case	SSF	LT	Total
1	>10,000	>10,000	>10,000	35[c]	1	430	431	69[c]	8	1	9
2	100	>10,000	>10,000	36	239	71	310	70[c]	NA	9	9
3	>10,000	2960	>10,000	37	93	200	293	71[c]	4	3	7
4	>10,000	540	>10,000	38[c]	230	10	240	72[c]	NA	6	6
5	464	>10,000	>10,000	39	160	63	223	73[c]	5	0	5
6[b]	2190	3480	5670	40	130	80	210	74[c]	3	0	3
7	403	5100	5503	41[c]	5	200	205	75[d]	3	0	3
8	4193	1060	5253	42[c]	202	0	202	76	2	1	3
9	3	4830	4833	43	0	183	183	77[d]	2	0	2
10	369	3600	3969	44[c]	71	100	171	78[c]	2	0	2
11	3120	20	3140	45	150	10	160	79[c]	2	0	2
12[c]	2778	1	2779	46	140	14	154	80[c]	1	1	2
13	174	2600	2774	47[c]	148	0	148	81	1	0	1
14	2224	220	2444	48[c]	143	1	144	82[d]	1	0	1
15	0	2100	2100	49	12	100	112	83[d]	1	0	1
16	1764	120	1884	50	103	6	109	84[c]	0	0	0
17[c]	NA	1840	1840	51	7	98	105	85[c]	0	0	0
18	13	1520	1533	52	79	22	101	86[c]	0	0	0
19[c]	1365	80	1445	53	78	1	79	87[c]	0	0	0
20	1240	17	1257	54[c]	72	2	74	88[c]	NA	0	0
21	1252	1	1253	55	13	60	73	89[c]	NA	0	0
22	1136	0	1136	56	17	40	57	90[c]	NA	0	0
23[b]	605	420	1025	57	45	8	53	91[d]	0	0	0
24	782	100	882	58	39	14	53	92[d]	0	0	0
25	477	400	877	59[c]	44	0	44	93[d]	0	0	0
26[c]	859	0	859	60[c]	40	0	40	94[d]	0	0	0
27	311	420	731	61[c]	40	0	40	95[d]	0	0	0
28[c]	303	420	723	62[c]	1	36	37	96[d]	0	0	0
29	380	340	720	63	11	13	24	97[b]	0	0	0
30	560	5	565	64[c]	20	0	20	98[b]	0	0	0
31	408	120	528	65	20	0	20	99[b]	0	0	0
32[c]	211	288	499	66	16	0	16	100[b]	0	0	0
33	448	0	448	67[c]	10	0	10				
34	288	150	438	68[c]	9	0	9				

NA: not applicable, no sphenoid sinus fluids found.
[a] Total: summation of SSF and LT.
[b] Non-drowning case.
[c] Body found on shore or sea.
[d] Body found in treated water.