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Diatom analysis in victim's tissues as an indicator of the site of drowning

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Abstract The diagnosis of drowning is one of the most difficult in forensic pathology and previously we proposed criteria for a positive tissue analysis according to the qualitative and quantitative diatom investigations. In the positive cases, we studied the reliability of determining the site of drowning by comparing the diatom taxa found in the lung samples with those of the water samples or in the absence of these samples with the results of the water diatom monitoring programme set up in our region. In this study, we present two series of cases, the first is one of 20 corpses who died from accidental or suicidal drowning with known drowning site, and the second of 20 corpses for which the drowning site was unknown. The results showed that a concordance of the abundance of the diatom taxa in tissues compared to the site of drowning and their distribution relative to one another was 65% in the group where the site of drowning was known and 35% in the other group. The concordance of the individual distribution in the lungs of water diatom taxa may be an interesting method to guide the investigations for determining the site of drowning. The two limiting factors are the concentration of diatoms in the lungs and the development of a river monitoring programme in the district of the study.

Key words Drowning · Diatoms · Site of drowning

Introduction

The diatom analysis is one of the biological tests used to assess the diagnosis of drowning [1–3, 18, 25, 26]. This test is based on the inhalation of the diatoms in the immersion water by the victim during drowning and their entry in the circulatory system, followed by the embolization to internal organs [17, 20]. The post-mortem extraction and recovery of these algae is possible due to their silica-based extracellular coat or frustule which is resistant to enzymatic and to acid digestion. In previous studies, we have proposed criteria for a positive "diatom test" based on a qualitative and quantitative diatom identification. In our experience, the test is positive only if there are more than 20 diatom frustules per 100 µl of sediment obtained by enzymatic digestion of 10 g of lung tissue and more than 5 diatom frustules, extracted by the same technique, per 10 g of sediment from other internal organs (i.e. brain, liver, kidney, bone marrow) [10, 11].

The diatom test is still disputed because of disagreement concerning the reliability of the test and the possibility of false positives or false negatives reported by some authors [5, 6, 21]. Diatoms were found in the organs of non-drowned individuals by Pachar and Cameron [13] who found in "control" samples of lungs 5–25 diatoms/100 g and a maximum of 10 diatoms/100 g in internal organs. Polson et al. [17] showed that in 10 cases of death due to causes other than drowning, the maximum number of diatoms in 100 g of tissue was 20 in the lungs and 13 in the liver. The quantitative criteria of the diatom test are defined to exclude postmortem contamination of the tissue by the algae due to passive transfer of water by hydrostatic pressure into the lungs or into other organs after death [13, 17].

Beside the quantitative analysis, the essential criterion on which to assess the reliability of the diatom test is this qualitative comparison between the diatom microflora of the immersion water and the diatoms found in the tissues [4, 7, 8]. In our experience this comparison avoids false positive results due to contamination (exogenous or laboratory contamination).

Diatoms are present all the year round in the rivers and lakes but seasonal variations of their content are observed. As previously described, we have established a water monitoring programme of the diatom populations in the main rivers of the Strasbourg area [11]. Diatom profiles of

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each river appear to be specific if the five most frequent taxa and the month of sampling are considered. In a given river we have observed the same diatom profiles, but the relative abundance of the taxa can vary along the river. Some taxa are found to be linked to a pollution source and may thus be taken as a marker of this particular site of drowning.

In this study, we have compared the profiles of the diatom taxa extracted from the lungs with those found in water to investigate if the diatom profiles found in pulmonary tissues may be an indication of the site of drowning.

We present two series of cases, the first one investigated 20 corpses who died from accidental or suicidal drownings at known sites and the second 20 corpses for which the drowning site was unknown.

Material and methods

Cases

In this study, the cases examined were freshwater drownings in which a sample of the known or putative drowning medium was available for analysis, and was processed for the diatom test in the laboratory. The drowning medium was identified by eye witnesses of accidental or suicidal drowning and a sample of this water was collected. These drowning cases represented the first group. The putative drowning medium was defined as the water where the body was found to be immersed at the time of body recovery which may not necessarily represent the site of drowning and those cases where included in the second group.

A complete taxonomic analysis of the diatoms recovered from water samples and from organs was conducted. Diatom sampling was performed using standard techniques, ensuring that glass containers and reagents were uncontaminated.

For both series the diagnosis of drowning was corroborated by the water/lung and water/internal organs comparisons of the diatom flora [11]. When the diatom concentrations in the lungs samples were over 60 diatoms/100 µl of sediment recovered from 10 g of lung tissue, the determination of proportional abundance of each taxa is possible and can be compared to the dominant taxa of the water samples.

The determination of the proportional abundance of the various species of diatoms was done for the five most represented diatom species in the lung and water sample. The proportional abundance is the frequency of a given taxa (diatom) observed among the frustules present in the 100 µl of sediment of lung tissues and in 300 frustules counted in the water samples. The diatom profile of a sample is given by the different diatom taxa identified and their relative abundance.

Analysis of samples of drowning medium

Water samples were available for each case (drowning medium: n = 20, putative drowning medium: n = 20). Samples were centrifuged at 2500 rpm for 15 min and diatoms were cleaned by incubation in hydrogen peroxide (130 vol%) at 80°C for 12 h. The solutions obtained were allowed to cool at room temperature and centrifuged a second time (2500 rpm for 15 min). The supernatant was decanted and replaced with diatom-free distilled water. The process was repeated 3 times until the fluid was transparent with a final spin at 3000 rpm to produce a pellet. After removing the supernatant, the sediment was air dried and mounted in Naphrax. Examination of 300 diatoms was performed under a light microscope (Zeiss, Germany) equipped with an immersion objective (1000×), to determine the proportional abundance of each taxa. The diatoms were identified using the flora index of Krammer and Lange-Bertalot [9].

Analysis of the tissue samples

During the autopsy, 10 g of each of the following organs were taken for diatom analysis: peripheral lung tissue, kidney, liver, brain and bone marrow taken from the femurs. These samples were minced with scissors, rinsed and mixed with 500 µl of 10 mg/ml proteinase K and 100 ml of 0.01 M Tris-HCl buffer (pH 7.5) containing 2% SAC. The mixture was incubated at 50°C overnight, 500 µl proteinase K (Boehringer Mannheim, Germany) was added and the solution was diluted with 100 µl of distilled water and centrifuged at 3000 rpm for 15 min, the upper layer was then removed. The sediment (100 µl/slide) was transferred onto a coverglass, mounted in Naphrax and examined under the light microscope.

Results

In most cases, we could recover from 5 to 8 distinctive species of diatom in any given lung sample with all frustules being less than 80 µm in size . The most frequent taxa found in the lung samples were *Navicula lanceolata, N. radiosa, N. capitata, Diatoma vulgaris, Cocconeis pediculus* (Fig. 1). In the water samples, the number of distinctive types of diatoms greatly exceeded the number of species in the tissue samples.

When the site of drowning was known, the different taxa found in the lung were similar to those present in the water samples and the concordance of the diatom species recovered from both samples was 100%, whereas it was only 70% in the second group where the drowning site was unknown (Table 1). When there was no concordance between the two samples, we compared the microflora with the results of the monthly water monitoring network to find the flora which showed the closest similarity with the tissue findings to have an indication of the site of drowning.

In the known drowning site group, the relative abundance of the different taxa of diatoms in the lung samples

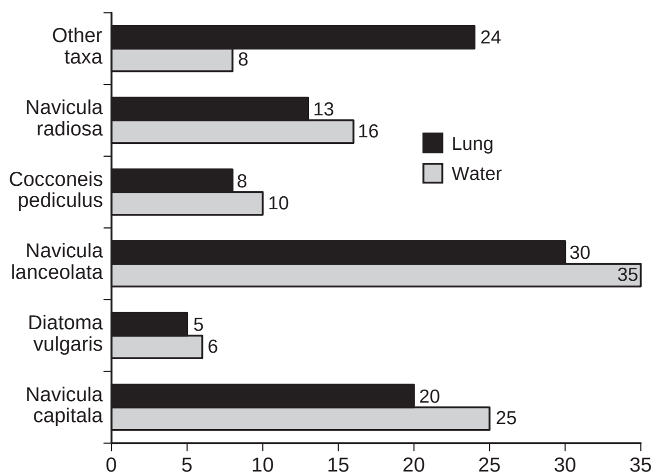

Fig. 1 Comparison of the taxa found in water samples and lung tissue in one of the cases. The results are shown as percentages

Table 1 Concordance between the diatoms recovered from lung tissues and from water samples, and their relative abundance

Results	Known drowning site	Putative drowning site
No. of cases	20	20
Concordance of diatom types from lung and water samples	20 (100%)	14 (70%)
Concordance of relative abundanceof each taxon	13 (65%)	7 (35%)

Table 2 Diagnostic indices for the comparison of the five most abundant taxa in the lung and water samples

Test	Value	Definition
Sensitivity	0.81	a/(a+c)
Specificity	0.75	d/(b+d)
False positive rate	0.25	b/(b+d)
False negative rate	0.18	c/(a+c)
Positive predictive value	0.92	a/(a+b)
Negative predictive value	0.50	d/(c+d)

a = 13: true positives and concordant results
b = 1: false positive result
c = 3: false negative results
d = 3: no concordant results
total number of cases = 20 (only those where the site of drowning was known)

was similar to those in the water in 65% of the cases. In the putative drowning medium, a concordance of the relative abundance could only be found in 35% of the cases.

In the group where the site of drowning was known, the seven cases of no concordance included three cases with a false negative result,1 case false positive result and 3 cases of absence of concordance. We could calculate the sensitivity (0.81) and the specificity (0.75) of the concordance test between the lung and water profiles (Table 2).

Discussion and conclusion

The diagnosis of drowning is one of the most difficult diagnoses in forensic pathology and therefore a great number of tests have been proposed to allow a confirmation of death by drowning of a victim [19, 24]. The diatom analysis is one of these tests, but the relationship between diatoms and a diagnosis of death by drowning on the basis of the test alone is very controversial because these algae have been found at autopsy in non-drowned victims. Due to the possibility of a false positive diagnosis of drowned victims, several investigators [22, 23] are of the opinion that this test cannot be used as definitive evidence of drowning. This criticism is based on a misunderstanding of the principles of the test as described by us which must not only demonstrate the presence of diatoms in tissue in given concentrations but also the same diatoms in the drowning medium at the site of drowning. Diatom contamination of tissue from non-drowned victims can usually be distinguished from typical freshwater diatoms. The diatoms found in contamination may originate from commercial cleaning agents because many are derived from diatomaceous earth which contains distinctive types of fossilized diatoms [14]. Other authors such as Peabody [14], Neidhart and Greedyke [12], Hendey [7, 8], Auer and Möttönen[3], Pollanen [15], and Pollanen et al. [16] believe that under well-defined conditions, diatom analysis can discriminate between drowning and non-drowning cases.

As shown in a previous paper, to assess the diagnosis of drowning we have established that the analyses may be considered positive if 20 diatoms are identified per 100 µl of a pellet obtained from a 10 g lung sample [10]. For other organs, more than 5 complete diatoms, excluding fragments, are required as an indication of water inhalation. The qualitative analysis allows identification of the taxa and comparison with those extracted from water samples taken at the site where the victim was found. Pollonen et al. [16] stated that the presence of a few diatoms in the bone marrow may be a sufficient indicator of drowning. Schneider [22] has shown that diatoms can be found in the tissues of non-drowned victims. For other authors and in our experience, the false positive cases can be excluded by the quantitative analysis, the taxa identification and the comparison between species found in the water and in the organ samples. Buhtz and Burkhardt [4] have shown that a water /lung tissues comparison of the diatom species may be a reliable indicator of the site of drowning specially if the diatoms flora is determined during the whole year at the most frequent drowning sites.

This study was performed in drowning cases where the diatom test was positive and was designed to determine if the lung diatom microflora in cases of drowning can be an indicator of the site of drowning. We compared the results of the diatom recovery in two groups: a group (n = 20 cases) where the drowning site was assessed by the police investigations and an second (n = 20 bodies) where the site was unknown. All cases were freshwater drownings assessed by the diatom test previously described. The frequent concordance of diatom types in the putative drowning medium and lung samples was 70%, and 100% for the known drowning site corroborating evidence for the validity of the diatom test. However, in the putative drowning site group 30% of the cases showed a discordance between the type of diatoms recovered in the lungs and the putative drowning medium. This may be explained by a regional diatom distribution in freshwater because the site of body recovery may differ from the site of drowning.

The diatom microflora extracted from the lungs was concordant with the water taxa when the site was known. In the other cases, where the site was not found, some taxa extracted from the water samples were absent from the lung extractions. Large frustules were not found but small taxa which could easily be inhaled were found. These results can be explained by either the drowning medium being incorrectly identified or a heterogenous lung distribution of the inhaled water if the site was correct.

The correlation of relative abundance of each taxa recovered in the lung samples with those found in the water samples may thus be a reliable method to indicate the site of drowning. We found a good correlation (65%) when the site was known and in those cases the comparison of the relative abundance of the most frequent taxa identified in the lung and water samples was a relatively sensitive and specific test to indicate the site of drowning. In the other cases, the correlation was only 35% and according to the positive predictive value (0.92), we could assume that the putative site was the exact drowning site. These results show that the lung diatom microflora may be an indication of the drowning site but not conclusive evidence.

The interpretation of the results must take into account the following information:

– the same taxa are found along a given river as has been shown by a continuous water monitoring for diatoms of the area of Strasbourg and the diatom microflora profiles can be specific for the river in question [11]
– different genera can be found linked to a pollution source and may then be indicative of the site of drowning

In the group of victims where the site was unknown, the bodies were found in a given river or mostly at the confluence or junction of rivers, the diatom microflora was indicative of the river but in the absence of a particular taxa we were unable to identify the exact drowning site.

Therefore, we have shown that the diatoms extracted from the lungs may be an indicator of the site of drowning, but the results must be interpreted in the context of the post-mortem findings, the police investigations and the histological analysis.

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