Are pollen findings one more tool for drowning investigation?

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Abstract

A medicolegal and botanical collaboration has to lead to an interdisciplinary procedure when studying dead bodies found in water with no clear circumstances. Hereby we present a report or a preliminary attempt as a basis for encouraging further work in the field. An unidentified 25-35 year old body was found immersed in a fresh-water ditch in state of decomposition. Structural alterations avoid any *a priori* identification of asphyxia or traumatic cervical lesions and a drowning investigation was proposed. Taxonomic species identification was carried out to relate the pollen aspiration with the water medium in lung, spleen and liver. We presented pollen grains in spleen, as a closed organ, as a clue for death investigation in the absence of diatom findings. Where and when pollen grains occur may ascertain that a body or an object was in some place at a certain time. Since the consequences of aspiration depend on many factors, such as the quantity, size and solidity of the material aspirated and whether the aspiration is chronically recurrent or acute, the pollen investigation must be done with precaution. We conclude that in the absence of other microscopic structures, biochemical blood analyses, or histological findings, forensic palynology may be of help to relate to the death scenario.

Introduction

The forensic botanic analysis consists of species identification and an estimation of the percentage that each plant species represents in an evidentiary sample. It refers mainly to the study of pollen and spores, together with microscopic bodies such as dinoflagellates, diatoms and other microfossils. Its evidence can reveal not only the particular season, but also the geographical origin. Information about where and when pollen grains occur may ascertain that a body or an object was in a certain place at a certain time. Spores will refer to the reproductive bodies of ferns, mosses and fungi. An obvious use has been in examination of soil caught up in the commission but there are also significant implications for revealing the timing of the generation of documents, etc. There are other not so usual items that have been little investigated and forensic palynology has been an underutilized form of trace evidence. Drowning, as water aspiration to death, has extensively been discussed in medicolegal cases, and immersions are commonly solved by diatoms (microalgae), strontium (Sr) and other trace elements analyses. Some drownings are sometimes difficult to resolve and a joint determination of Sr and other biochemical markers like Fe is recommended. However, haemodilution is not so evident in some samples and other form elements might be relevant apart from diatoms. Besides, significant differences in the amount of strontium absorbed into the bloodstream from fresh water are much lower than in seawater and that is an inconvenience for diagnosis resolving. Freshwater drownings (FWD) are even more difficult to research if no blood can be analysed because of the long post-mortem interval (PMI). Hereby the pollen study recovers importance when diatoms and microalgae are absent in the analysed organs. To this respect, there is very scarce mention or no mention of pollen with relation to drowning or the diagnosis of death in literature. We present a FWD case of an unidentified 25-35 year-old woman who was found immersed in a water ditch in state of decomposition whose microscopic findings could suggest the pollen analysis as one optional tool for forensic diagnosis.

Materials and Methods

Water-medium (500 mL) and fresh organs (left-lung, spleen and liver) were shipped to the National Institute of Toxicology & Forensic Science (INTCF, Madrid). Tissue samples (10 g) were cleaned with ultrapure type I water for 5 min and prepared for diatom (silica-structures) analysis with nitric acid (65˚) and K-proteinase in 0.01 M Tris-ClH buffer, following centrifugation (2500 r.p.m., 7 min). One mL sediments were placed in dissolution with 4 mL distilled water following microfiltration with cellulose membrane (1.2 µm pore diameter) and set over glass-slides for drying at 56˚C; once dried 2 - 3 immersion oil drops were added on top of the slide for transparency to allow light microscopic visualization (10×, 40×, 100×). Counts of diatom valves at high magnification were yielded from the strewn slides. A comparison, when possible, was carried between organs findings and water-medium. Chirurgic material was treated with chlorine so the possibility of posterior sample contamination at the lab manipulation was discharged. The identification of pollen grains and nomenclature were performed using the classical methodology and identification keys following Valdés *et al.* (1987) and Moore *et al.* (1991). The main purpose of the investigation presented here was oriented to find diatoms, and even though, pollen grains were seen and identified, so no special technique for pollen collection was applied. Similarly, no information about pollen concentrations in the air could be compared *a posteriori* with those found in the water medium and samples analysed. It is possible that for future analysis results accuracy may be improved if the classical methods from the palinology investigation are applied.

Results and Discussion

Pollen grains were encountered in spleen (9 specimens found) and lung (N = 12) (*closed* and *open* organs, respectively), as well as in the water-medium (N = 7), and lacked in the liver. The identification included the types (t) of plants with similar pollens and species as follows: Cytisus t., Trifolium t., Pinus t., Rosaceae (possibly Rubus t.) and *Quercus caducifolium*. We could differentiate Trifolium t. pollen grain trizonocolporate with three apertures (**Figure 1A**) and Pinus t. with the pollen body (b) and two air sacs (**Figure 1B**). Similar types of pollen grains found in water medium (negative control) were also present in the lung and spleen. The spleen is as a closed organ, and the pollen presence may be indicative of aspiration, at least as a proof of an income. Such fact acquires significance since entry of structures, as diatoms, from the lungs and through the digestive tract into the systemic circulation had been proved to lead to positive results. By contrast, microalgae and other foreign bodies present in open organs (gastric and pulmonary contains) may have penetrated post-mortem. In the present case, the finding of pollen in the closed organs may be indicative of a lively aspiration either by air or through the water-medium but there is no literature to this respect. Somehow, immersion artefacts may occur in any corpse immersed in water, irrespective of whether death was from drowning or the person was dead on entering the water.

In our present case, the aspiration, either by air or by water is not ascertain. The victim might have been in contact with the found plant species elsewhere, but like it occurred in other medicolegal cases, detailed investigations indicated that this was unlikely because of the moderate percentage of pollen presence encountered. Similar findings of pollen grains were described in water medium and analysed organs in the present case. The consequences of aspiration depend on many factors, such as the quantity, size and solidity of the material aspirated and whether the aspiration is chronically recurrent or acute. The only similar representative description with pollen grains related in a death explanation was a posterior granulomatous lung inflammation after finding affected tissular lung areas or pneumonitis due to foreign bodies reaction from a water aspiration of microalgae and pollen grains (near-drowning event). In our present study, however, due to the long PMI, no histopathologic information could be considered to address a conclusion of asphystic death.

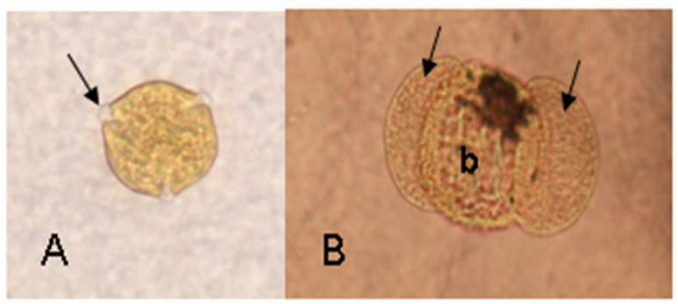

Figure 1. Light micrographs (40X magnification) of the plant groups (*type*, t) (A): Trifolium t. pollen grain trizonocolporate with three apertures (arrow) in subtriangular polar view and (B): Pinus t. grain with the pollen body (b) and two air sacs (arrows).

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