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Short report

Haematic silicon in drowning

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ABSTRACT

The aim of this paper was to evaluate silicon (Si) concentration in human whole ventricular blood as a further potential chemical marker in the diagnosis of drowning. We employed an acidic digestion for the extraction of soluble Si, and an alkaline digestion for the determination of total Si, including *particulate matter*, both arising from drowning medium. 29 suspected drowning situations, 24 in fresh water (*Fw*) and 5 in seawater (*Sw*), were examined. The difference in Si concentration between the left and right ventricular blood (Si Δ_{L-R}) was measured and alkaline Si Δ_{L-R} seems, indeed, a potentially significant complementary tool in the diagnosis of *Fw* drowning, because insoluble silicon fraction does not undergo hemo-dilution or hemo-concentration, and the Δ_{L-R} is not affected by exogenous factors. In spite of the limited number of cases investigated, a good correlation was observed between the analytical results and the macro-microscopic autoptic findings.

1. Introduction

The search for new methods and signs for the correct post-mortem diagnosis of drowning continues to be a priority in forensic pathology.[1] To date, in fact, there is no real "test of drowning" and the medical-legal diagnosis of death by drowning is based on the convergence of many signs.

The assessment in the drowning victims of target chemical substances is a complementary useful tool in forensic science.[2] In this regard, strontium has been shown to be most useful in the diagnosis of seawater (*Sw*) drowning.[1,3–6]

Being silicon present in natural waters usually at higher levels than strontium, it could be of great usefulness as a freshwater (*Fw*) drowning marker. In such matrices, it is usually present in the form of both soluble and insoluble species, with the latter arising mainly from *plankton*, including suspended crystalline particulate and some forms of *phytoplankton*, particularly diatoms. These silicon fractions can pass from the alveoli into the pulmonary capillaries and enter the blood circulation, arriving at the left ventricle, as specifically demonstrated more than a century ago by Stockis[7] for crystalline elements suspended in drowning media, and later by several authors both in autoptic and experimental studies.[8–12]

In this regard, it is worth to remember the value we attributed to the morphological and micro-analytical detection of silicon in the lungs, as better specified later. That is, among drowning parameters, we also considered the presence in the distal respiratory tract of exogenous material as small composite *boli*, including biotic matter (*phytoplankton*, especially *diatoms*), and amorphous and microcrystalline *plankton* portions.[13,14] This finding prompted us to investigate the concentration of the element in the blood of victims suspected of having drowned.[15]

Here we present the overall results of a double-blind study (chemical and forensic analyses) carried out on 29 suspected drowned subjects (24 in *Fw*, 5 in *Sw*) and on 15 control cases (including cases for the set-up of the analytical procedure[15]). For each case, human whole blood, whose sampling requires skill and care, from both left (L) and right (R) heart cavities was analysed by acidic (Ac) and alkaline (Alk) digestions to determine the soluble silicon fraction and the siliceous suspended crystalline particulate, respectively.[15] The differences in silicon concentration between the left and right ventricles (Δ_{L-R}) were calculated and compared to medico-legal investigations in order to evaluate their correlation with drowning cases.

2. Materials and methods

2.1. Forensic autopsy cases

We studied a total of 44 cadavers selected from medico-legal autopsies performed in the Department of Forensic Medicine, University of Pavia, and in associated Institutes, including 29

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drowning situations and 15 control autopsies, over the period 2010–2013. As drowning situations were investigated cases of bodies retrieved out of water; of these, 16 were males and 13 were females. The mean age of the subjects was 49.9 years (range: 3–85 years). The mean post-mortem interval (PMI) was 3.7 days (1–7), while the submersion interval varied from few minutes to 27 h (mean 9.1 h).

In 24 cases, drowning situations occurred in *Fw*: 20 in natural water (rivers, ditches, lakes), 2 in swimming pools, 1 in tap water, 1 in rice-field, and 5 cases occurred in *Sw*. The 15 control autopsies concerned persons who died from natural or traumatic causes, or by hanging. The mean age was 49.9 years and the PMI was 2.9 days.

Two subjects retrieved from swimming pools underwent cardiopulmonary resuscitation and survived each about 2.5 h (cases n. 23 and n. 24), therefore they are considered aside from the overall casuistry of drowned subjects.

The parameters considered suggestive of "drowning situation" and selected in our investigation were: corpses recovered from the water, morphological parameters and chemical tests.

Blood sampling during autoptic examination was performed with great care, similar to that required for the collection of specimens for the diatom test: the anterior chest wall of the cadaver was accurately dried; new gloves were worn; cleaned, dried and sterilized instruments for every single procedure up to pericardium sectioning were employed; disposable plastic syringes with attached needles, one for every cardiac cavity, were used (glass syringes should be avoided). In order to prevent the occurrence of obstruction and to facilitate blood drawing, a needle of appropriate gauge (21 gauge, 0.8 mm, from Sigma Aldrich) was chosen. For this purpose, another needle without syringe, chosen on the basis of the aforementioned criteria, may be inserted through the cardiac wall into the cavity. The puncture of the cardiac walls should be performed preferably in the posterior side lifting up the extremity of the heart; the application of a sterile dressing or the use of a toothed forceps may be of help. When the blood is clotted, it is advisable to perform an incision of the cardiac wall and collect the blood with a spoon. During this manoeuvre, special attention must be paid to avoid crossing the cavity (atrium or ventricle) or perforating the opposite heart wall; if this happens, in fact, the needle tip would reach the pericardial sac or (even worse) the cardiac site opposite to that from which the specimen must be obtained. It is prefer to perform punctures and drawings starting from the left cardiac cavities, which usually contain smaller amounts of blood.

Bilateral cardiac blood samples were collected into polypropylene tubes, carefully labelled, and stored at −20 °C. No anticoagulation reagents were added to the samples so as to avoid any possible source of contamination. Before use, all samples were defrosted and homogenized by vortex.

2.2. Chemical investigation

Ventricular whole blood from L and R side of the heart was analysed ($n = 3$ or 4, depending on sample availability) following the below reported procedures for acidic and alkaline microwave digestions, respectively. The (L–R) difference in silicon concentration was calculated for control cases, *Fw* and *Sw* suspected drownings. In the last cases, (Δ_{L-R}) in strontium concentration was also considered because of the recognized role of this element as marker of *Sw* drowning situations.[1,3–6] Si and Sr levels in drowning medium were determined too.

2.3. Reagents

Tetramethylammonium hydroxide (TMAH, 25% w/w) TraceSELECT® Ultra and ultrapure HNO_3 (65% w/w) were supplied by *Sigma Aldrich*, H_2O_2 (30% w/w) from *Carlo Erba Reagents*. Ultrapure water (resistivity 18.2 MΩ cm^{-1} at 25 °C) was produced in laboratory by a *Millipore Milli-Q system*. Silicon and strontium standard solutions were daily prepared from a 1.0 mg mL^{-1} stock solutions.

2.4. Apparatus

For the microwave digestion of the samples, a CEM Mars microwave oven (CEM s.r.l., Cologno al Serio, Italy) equipped with temperature and pressure regulation through a sensor vessel, providing 1600 W output power at 100% power setting was used. PFA PTFE (PerFluoroAlkoxy PolyTetraFluoroEthylene) (Xpress, 55 mL) and TFM™ PTFE (second-generation modified PolyTetraFluoroEthylene) vessels (EasyPrep™, 100 mL) were used for acid and alkaline digestions, respectively. Evaporation of the digested acid solutions was carried out by XpressVapTM accessory.

Measurements were performed by an inductively coupled plasma quadrupole mass spectrometer (ICP-MS) (*Elan DRC-e, Perkin–Elmer, Shelton, CT, USA*) equipped with a standard ICP torch, cross flow nebulizer, nickel sampler and skimmer cones and dynamic reaction cell™ (DRC) and by an ICP-OES PerkinElmer Optima 3300 DV.

PTFE vessels, micropipette tips and PP tubes were cleaned in 10% HNO_3 overnight and rinsed thoroughly with ultrapure water.

2.5. Procedure

2.5.1. Microwave-assisted acid digestion

A sample of 2 g of whole blood was accurately weighed into the PFA PTFE vessels of the microwave digestion system, and 5 mL of HNO_3 plus 2 mL of H_2O_2 were added. Microwave heating was then performed at 1600 W for 15 min, 200 °C. After cooling, the contents were evaporated to small volume (about 0.5 mL), diluted to 10 mL in calibrated polypropylene tubes and analysed by DRC-ICP-MS for silicon determination. Four points calibration curves were generated in the range 50–500 μg L^{-1}. Standard solutions were prepared in 0.5% HNO_3. The instrumental detection and quantification limits (IDL = 12 μg L^{-1}, IQL = 35 μg L^{-1}) were calculated from linear regression parameters as three and ten times the standard deviation of the calibration blank signal ($n = 12$), respectively. The accuracy of the method was tested by analysis of Seronorm™, Trace Elements Whole Blood with a silicon content of 1.6(1) mg L^{-1}. Strontium determination was carried out by ICP-OES following the operating conditions and the instrumental parameters suggested by the manufacturers (IDL = 3 μg L^{-1}, IQL = 10 μg L^{-1}).

2.5.2. Microwave-assisted alkaline digestion

The determination of total silicon in whole blood was obtained by a sensitive and accurate analytical method validated in a previous study.[15] Briefly, 1 g of whole blood was exactly weighed into 100 mL TFM PTFE tubes; 6 mL of TMAH, two drops of 1-octanol as antifoaming agent, 1 mL of H_2O_2 and 3 mL of ultrapure water were subsequently added. Microwave vessels were sonicated (15 min, 60 °C) and irradiated for 15 min at 180 °C, after a temperature ramp of 20 min. The clear solutions obtained from digestion were left to cool, diluted to 40 mL in calibrated PP tubes and analysed by DRC-ICP-MS.

Method detection and quantification limits (MDLs and MQLs, respectively) were calculated for both acidic and alkaline digestions on the basis of the instrumental detection and quantification limits (IDLs and IQLs, respectively) evaluated from linear regression parameters and are reported in Table 1.