Legal Medicine 17 (2015) 299–303

Contents lists available at ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

Detection of chlorine and bromine in free liquid from the sphenoid sinus as an indicator of seawater drowning

Naoko Tanaka [a,*], Hiroshi Kinoshita [a], Mostofa Jamal [a], Ayaka Takakura [a], Mitsuru Kumihashi [a], Nobuyuki Miyatake [b], Kunihiko Tsutsui [c], Kiyoshi Ameno [a]

[a] Department of Forensic Medicine, Faculty of Medicine, Kagawa University, 1750-1 Miki, Kagawa 761-0793, Japan
[b] Department of Hygiene, Faculty of Medicine, Kagawa University, 1750-1 Miki, Kagawa 761-0793, Japan
[c] Department of Health Science, Faculty of Medicine, Kagawa University, 1750-1 Miki, Kagawa 761-0793, Japan

ARTICLE INFO

Article history:
Received 15 May 2015
Received in revised form 11 August 2015
Accepted 27 August 2015
Available online 28 August 2015

Keywords:
Seawater drowning
Bromine
Chlorine
Liquid in sphenoid sinus
Energy-dispersive X-ray spectroscopy (EDX)

ABSTRACT

We have investigated the usefulness of elemental analysis by energy-dispersive X-ray spectroscopy (EDX) in the examination of free liquid from the sphenoid sinus of drowning victims. We detected both chlorine and bromine in liquid taken from the sphenoid sinus of seawater drowning victims. Because these elements were below the quantification limit in freshwater cases, we could easily distinguish seawater from freshwater drowning cases. Detection of these elements from the liquid in the sphenoid sinuses of drowning victims may be useful as a supportive measure for seawater drowning.

1. Introduction

The diagnosis of drowning presents a major problem in forensic medicine [1]. Diagnosis of wet drowning is greatly assisted not only by macroscopic autopsy findings such as froth around the mouth and nostrils and in the airways, emphysema aquosum, Paltauf's spots and pleural effusion, but also by the detection of diatoms and chemical substances in the victims [2–7].

It is important for forensic pathologists not only to identify the cause of death, but also to determine the site of drowning. It is well known that seawater differs from freshwater in its mineral composition [8,9]. Seawater contains large amount of chlorine, sodium, strontium and bromine, as compared with freshwater [8–10]. Electrolyte analysis of pleural effusion samples [11–13], hemodilution [14], diatom analysis [4,5] and identification of strontium [6] have all been used as indicators of the specific type of drowning medium. It has been reported that it is easy to distinguish seawater from freshwater by its bromine concentration using energy dispersive X-ray spectroscopy (EDX) [10]. EDX is a simple and convenient method to detect various elements simultaneously [15]. It uses small desktop equipment, and special sample preparation is not necessary [16]. The detection of bromine, for the supportive chemical marker of seawater drowning by EDX has been applied for blood sample alone [15]. However, other autopsy samples such as aqueous liquid in sphenoid sinus have not been applied yet. We have focused on the difference of mineral composition to distinguish seawater drowning from freshwater drowning.

The presence of aqueous liquid in the paranasal sinuses (Sveshnikov's sign) is an additional diagnostic finding in wet drowning [17], and it is recognized as a sign of wet drowning in conjunction with other findings. The sphenoid sinus is particularly accessible at autopsy, and liquid can be easily aspirated with a cannula [18]. It has been reported that the average volume of liquid in the sphenoid sinus of drowning victims is larger than that of non-drowning victims [18,19]. In recent postmortem analyses, CT imaging studies have indicated that the accumulation of liquid in the paranasal sinuses, such as the maxillary and sphenoid sinuses, is one of the most notable findings in drowning cases. However, the specificity of this finding is not high [20,21]. In the present study, we investigated the application of elemental analysis of the liquid in the sphenoid sinus in order to diagnose seawater drowning.

Specifically, the aim of this study is to test the diagnostic value of the concentrations of bromine and chlorine in the liquid from the sphenoid sinus in drowning cases by EDX. Detection of chlorine and bromine provides a supportive measure of seawater drowning.

* Corresponding author.
E-mail address: ntanaka@med.kagawa-u.ac.jp (N. Tanaka).

http://dx.doi.org/10.1016/j.legalmed.2015.08.005
1344-6223/

2. Materials and methods

2.1. Quantification of chlorine and bromine

An EDX apparatus, the Rayny EDX-720 (Shimadzu, Kyoto, Japan) was used for the detection and quantification of chlorine and bromine. The experimental design, including both the configuration of the EDX and sample preparation, was in accordance with previous reports [22,23]. In brief, a 100 μl sample was carefully pipetted onto ST-30 filter paper with a paraffin circle (Shimadzu, Kyoto, Japan). Following drying of the filter paper, we performed EDX analysis. Sodium chloride and bromine ion standard solutions (Wako Pure Chemical Industries, Osaka, Japan) were diluted with distilled water. Confirmation of the characteristic X-ray results was performed using the Kα lines of chlorine and bromine, respectively. The X-ray intensity was determined to be the average of three.

2.2. Water samples

Samples of seawater ($n = 25$) and freshwater ($n = 25$) were used. The seawater samples were collected from various places in the Inland Sea of Japan region, around Kagawa Prefecture. Freshwater samples were collected from ponds ($n = 15$), rivers ($n = 5$), irrigation canals ($n = 3$), a paddy ($n = 1$) and a reservoir ($n = 1$).

2.3. Liquid samples from sphenoid sinuses

Twenty-two drowning cases and 11 non-drowning cases that underwent forensic autopsy between January 2009 and December 2013 were selected for the study. This autopsy study was approved by the Ethics Committee of Kagawa University and was conducted according to the Declaration of Helsinki Principle.

A summary of the cases in each group is shown in Table 1. The diagnosis of drowning was based on the presence of autopsy findings such as froth in the airways, emphysema aquosum, pleural effusion and a positive diatom test [2–5]. The exclusion criteria included a long postmortem interval (more than 4 days). Non-drowning victims without putrefactive changes were used as controls. Liquid in the sphenoid sinus was aspirated through the use of a syringe after piercing through the fossa hypophysialis during autopsy. A liquid sample of 100 μl from the sphenoid sinus was used for EDX analysis. Of these drowning victims, 11 had drowned in the sea, seven in ponds, two in rivers, one in an irrigation canal and one in a paddy.

2.4. Statistical analysis

Data were analyzed using JMP® 11.0 (SAS Institute Inc., Cary, NC, USA) software. The Kruskal–Wallis test and the Steel–Dwass test were used to determine significance. A p value of less than 0.05 was considered statistically significant.

Table 1
Summary of the cases in each group.

Group	Seawater drowning	Freshwater drowning	Non-drowning
Number of case	11	11	11
Age (years old)	63.4 ± 10.9 (48–84)[a]	75.7 ± 15.2 (43–97)	67.6 ± 16.1 (36–85)
Sex (male/female)	8/3	5/6	9/2
Postmortem interval (h)	40.2 ± 18.9 (15–72)	38.9 ± 19.1 (15–72)	29.3 ± 22.0 (12–72)
Volume of liquid in sphenoid sinus (ml)	2.05 ± 1.43 (0.1–5.0)	0.81 ± 0.81 (0.1–2.0)	0.12 ± 0.15 (0–0.5)

[a] Mean ± SD (range).

3. Results

3.1. Volume of liquid samples in the sphenoid sinus

The volumes of each of the liquid samples from the sphenoid sinuses of seawater drowning victims, freshwater drowning victims and non-drowning victims were 2.05 ± 1.43 ml, 0.81 ± 0.81 ml, and 0.12 ± 0.15 ml (mean ± SD), respectively. Five non-drowning victims had no liquid in the sphenoid sinus. The volumes of liquid samples from the sphenoid sinuses were significantly different between seawater drowning victims and non-drowning victims ($p < 0.001$) and also between freshwater drowning victims and non-drowning victims ($p < 0.05$). There was no significant difference in the volumes of liquid samples from the sphenoid sinuses between seawater drowning victims and freshwater drowning victims ($p = 0.076$).

3.2. Quantification of chlorine and bromine using EDX

For a calibration curve for chlorine prepared using EDX, a regression line of $y = 0.991x - 1.282$ was obtained between the measured intensity (cps/uA) and concentration (mg/ml). The correlation coefficient was $r = 0.999$ (Fig. 1). The linear range for this calibration curve was confirmed up to 35.5 mg/ml. The quantification limit of chlorine was 5.33 mg/ml when the signal-to-noise ratio was taken as 10. From three separate measurements, the recovery rate at a concentration of 7.10 mg/ml in distilled water was 103 ± 2.60% (mean ± SD).

For a calibration curve for bromine prepared using EDX, a regression line of $y = 289.1x + 11.49$ was obtained for bromine ($r = 0.997$, Fig. 2). The linear range was confirmed up to 500 μg/ml, and the quantification limit was 20.0 μg/ml in this experimental condition. From three separate measurements, the recovery rate at a concentration of 100 μg/ml in distilled water was 93.2 ± 5.37% (mean ± SD).

3.3. Concentrations of chlorine and bromine in water samples

The concentrations of chlorine and bromine in seawater samples were 15.6 ± 4.0 mg/ml (mean ± SD; range, 8.4–23.6 mg/ml) and 79.5 ± 18.9 μg/ml (mean ± SD; range, 47.7–118 μg/ml), respectively. In all freshwater samples, both chlorine and bromine were below the quantification limit (Fig. 3).

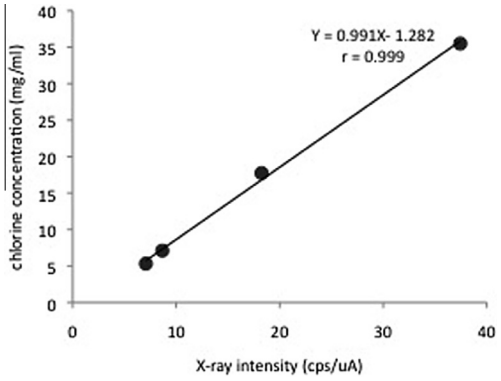

Fig. 1. Calibration curve of chlorine.