Contribution of biventricular cardiac blood and pleural effusion strontium concentrations to the diagnosis of drowning

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Abstract: The present study evaluated cardiac blood and pleural effusion strontium (Sr) concentrations in dead bodies found in fresh water or seawater. Eighty cases that had been found in water (59 seawater, 13 fresh water, 8 unknown) were compared with 20 control cases, which had not been found in water and died due to unrelated cause of death, in terms of right ventricle cardiac blood strontium concentration (RVSr), left ventricle cardiac blood strontium concentration (LVSr) and their differences [LVSr-RVSr], as well as pleural effusion strontium concentration (PlSr). The ages of cases were between 2 and 82 years. Seventy one cases were retrieved from water within the first 24 hours. Cause of death was reported as drowning (regardless of Sr concentration) in 68 cases. Whilst 8 cases referred to the supreme board for further investigation, cause of death could not be detected in 3 cases because of advanced decomposition. Of the bodies found in seawater, LVSr and PlSr concentrations were significantly higher than the bodies found in fresh water. LVSr, RVSr and [LVSr-RVSr] concentrations were significantly higher than those of the control group. No difference was determined between the bodies found in fresh water and the control group in terms of [LVSr-RVSr] concentration.

Key Words: drowning, strontium, blood, pleural effusion, seawater, fresh water.

Up to today, different elemental analyses have been carried out to investigate the type of death in immersion deaths: blood chlorine, blood iron, blood magnesium, ANP and strontium in serum as an indicator of drowning. Necessity of using strontium (Sr) as an indicator in drowning cases was first recommended in 1932 by Icard. However, Sr was first analysed by Abdallah et al. [1-3].

Strontium is a common component in seawater. It has been reported that Sr concentration changes between 1651 and 9120 μg/L in seawater [4], sometimes reaching levels of 31000 μg/L in groundwater, and may take values between 100 and 10000 μg/L in fresh water [5]. There is a large Sr concentration difference between water medium and blood compared to other elements’ seawater/blood concentrations [2, 4]. Therefore, according to Azparren et al., in cases of drowning, no matter what the concentration would be, the difference

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of Sr concentration between blood and seawater would always be big enough to allow Sr in blood to be considered as an indicator. The mean concentration of Sr in living individuals is 11.4±0.83 µg/L, ranging between 5.7 to 15.6 µg/L in whole blood according to Piette et al., whereas it reported to range 16 to 95 µg/L in total blood according to Iyengar et al [5, 6].

The combined effect of Sr that passes into blood from seawater and hemoconcentration contribute concurrently to the increase in left ventricular blood Sr concentration (LVSr) in seawater drowning cases. This increase is not likely to be significantly affected by putrefactive processes [4]. Azparren et al. suggested that a difference between left and right cardiac blood Sr concentrations [LVSr-RVSr] higher than 75 µg/L is a typical indicator of drowning [2].

Lower Sr concentrations in the environment, may increase the difficulty in interpreting the results [7]. Since Sr concentration is low in many fresh waters, its evidential value in the diagnosis of drowning is not same for all types of water. Nevertheless, it has been reported that diagnostic Sr concentrations could have been detected in 32% of 144 fresh water drowning cases [8].

The present study is the first one carried out in human autopsy cases in Istanbul, Turkey to assess the diagnostic value of Sr concentration in drowning in this area.

**MATERIALS AND METHODS**

Eighty cases, who had been found dead in water (immersion death) and transferred to the Council of Forensic Medicine, Istanbul, between January 2009 and September 2009 for autopsy, were compared with 20 cases (14 hanging, 5 heart attack, 1 electrocution) in terms of RVSr, LVSr and their differences [LVSr-RVSr], as well as PISr concentrations, who had not been found in water and died due to reasons other than drowning.

Exclusion criteria were:
- Cases with penetrating injury, internal organ laceration and blood in body cavities;
- Cases with atrial or ventricular septal defect;
- Extremely decomposed bodies with no body fluid;
- Cases, from which cardiac blood could not be obtained.

In order to make comparison between Sr concentrations of the cases and Sr concentration of the water from which the cases were retrieved, seawater samples were obtained from Istanbul shoreline (Black Sea, Bosporus, Golden Horn, Marmara sea), whereas fresh water samples were obtained from the sources found in Istanbul hinterland (from dams and lakes); both types of water were analysed for Sr concentrations.

Samples were stored in a refrigerator at +4°C until the time of analysis. After keeping 1 ml of sample in 15% HNO₃ solution overnight, it was put into Teflon ovenware, which had been passed through distilled water and dried. It was melted in microwave oven after 6 ml of concentrated HNO₃ and 1 ml of concentrated H₂O₂ solutions were added. Melted samples were transferred to the plastic tubes. They were put into Shimadzu AAS/Electrothermal AA-6701 (ETAAS) device and analysed at 460.7 nm.

Eighty cases, which have been reported to be found dead in water, and 20 cases that formed the control group were examined in terms of age, gender, type of water (fresh or seawater), manner of death, macroscopic and microscopic findings, RVSr (80 cases and 20 controls), LVSr (79 cases and 20 controls) and PISr (52 cases and 0 controls) concentrations, as well as total lung weight/body weight ratio. Statistical analyses were performed by SPSS for Windows 11.0 version using parametric tests. Level of significance was considered to be p<0.05 for Mann-Whitney U test and to be p<0.0125 for paired comparison test, which was performed by Mann - Whitney U tests with Bonferroni Correction.

**RESULTS**

Of the cases, 72 (90%) were male and 8 (10%) were female. The mean age of the cases was 28 years (ranging between 2–82 years). The mean age of the control group was 40.6 years (ranging between 13–79 years). LVSr concentration was decreased as the age increased with statistically low correlation found between them (p=0.019, Pearson correlation; r=-0.24).

Limited number of cases was considered to be the reason for low correlation. No statistically significant change was determined in RVSr and PISr concentrations as the age increased (Mann-Whitney U test, p=0.68 and p=0.224, respectively).

Although investigational records revealed that 59 cases (73.8%) were found dead in seawater and 13 cases (16.2%) in fresh water, there was no information about 8 cases. Of the cases, 47 (58.8%) were accidents and 8 (10%) were suicides in terms of the manner of death, whereas no information could be obtained in 25 (31.2%) cases.

With regard to duration of staying in water, it was determined that 58 (72.5%) cases were retrieved from water within the first 6 hours, 6 (7.5%) cases within 7-12 hours, 7 (8.7%) cases within 13-24 hours, 4 (5%) cases within 25-48 hours, 1 case within 2-7 days, and 1 case within 8-14 days. Although decomposition was not observed in 2 cases and saponification was observed in 1 case, no information could be obtained about duration of time in water.

With regard to the distribution of cases according to the cause of death, 69 (86.2%) cases were reported as drowning. Eight cases (10%) referred to the supreme board, which is found within the body of Council of
Forensic Medicine and consisted of multidisciplinary specialists, for further examination since cause of death could not be identified. Classical pathologic findings of drowning could not be determined in these cases and cause of death could not be elucidated. Cause of death could not be determined also in three cases (3.8%) because of decomposition.

Analyses of water specimens obtained from Marmara, Black Sea and Bosporus shoreline revealed a Sr concentration changing between 2873 and 3269 μg/L. Sr concentration was between 82 and 155µg/L in the water specimens obtained from the lakes within the hinterland of Istanbul with the exception of Alibeykoy dam lake, and tap water from Council of Forensic Medicine with 437 μg/L and 45 µg/L Sr concentration, respectively.

The mean LVSr concentration was 40.7µg/L and RVsr concentration was 45.2µg/L in the control group. However, quite different from the mean values, the mean LVsr and RVsr concentrations of two cases were 121-119 µg/L and 145-61 µg/L, respectively.

Sr concentrations of the cases and the control group are shown in Table 1. Right heart blood could be obtained from all of 80 cases, whereas left heart blood could be obtained from 79 cases and pleural effusion fluid could be obtained from 52 cases. Pleural effusion fluid could not be obtained from the control group since they had none.

PLsr concentration was between 118.4 and 2486.4µg/L in 52 cases, from which pleural effusion could be obtained. The mean PLsr value of the cases was 741.8µg/L.

LVsr, RVsr and [LVsr-RVsr] concentrations were higher in the cases reported to be retrieved from seawater as compared to the control group. The difference was statistically significant (Mann-Whitney U test, p=0.001 and p=0.010 respectively).

No statistically significant difference was found between the cases pulled out of fresh water and the control group in terms of [LVsr-RVsr] concentration. (Mann-Whitney U test, p=0.11).

LVsr and RVsr concentrations were also higher in the fresh water drowning cases as compared to the control group. This increase was found statistically significant (Mann-Whitney U test, p=0.001 and p=0.010 respectively).

No statistically significant difference was found between the cases pulled out of fresh water and the control group. This increase was found statistically significant (Mann-Whitney U test, p=0.001 and p=0.001).

DISCUSSION

The most sensitive analytic methods used to measure strontium concentration in biological samples include NAA, XRF, ICP-AES, ICP-MS, FAAS and ETAAS. Although FAAS based methods are not sensitive enough at low concentrations, AAS based methods still remain valid in many clinical and analytic laboratories. ETAAS based methods are more convenient in detecting normal concentrations of Sr at the level of µg/L. Therefore, many researchers prefer using ETAAS to detect Sr in biological samples (plasma, serum, whole blood, urine, bone and soft tissues) [9-11]. Whilst Piette et al. preferred FAAS and ICP-AES methods [5, 12], Azparren et al. [4, 8, 13], Fornes [14] and Perez-Carceles [15] preferred AAS (Zeeman) method. The present study used ETAAS method, the sensitivity of which is known to be good enough.

The average Sr concentration in a healthy individual ranges between 5.7 and 15.6 µg/L depending on nutritional habits and personal medication used, like Strontium Ranelate prescribed for the treatment of bone diseases. It was determined that LVsr, RVsr and PLsr concentrations increased as total lung weight/body weight (L/B) ratio, i.e. lung weight, of the cases increased. This increase was found to be statistically significant. (Pearson correlation; r=0.301, r=0.285 and r=0.319 respectively).

LVsr and RVsr concentrations were also higher in the fresh water drowning cases as compared to the control group. This increase was found statistically significant (Mann-Whitney U test, p=0.001 and p=0.010 respectively).

LVsr, RVsr and [LVsr-RVsr] concentrations were higher in the cases reported to be retrieved from seawater as compared to the control group. The difference was found statistically significant (Mann-Whitney U test, p=0.001 and p=0.003).

Table 1. Comparison between overall cases and the controls in terms of the left ventricle and right ventricle Sr concentrations and left ventricle-right ventricle difference

<table>
<thead>
<tr>
<th></th>
<th>LVsr (µg/L)</th>
<th>RVsr (µg/L)</th>
<th>[LVsr-RVsr] (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
<td>Mean</td>
<td>Max.</td>
</tr>
<tr>
<td>Overall cases (n:80)</td>
<td>1.4</td>
<td>366.9</td>
<td>1190.4</td>
</tr>
<tr>
<td>Retrieved from fresh water (n:13)</td>
<td>1.84</td>
<td>175.8</td>
<td>552</td>
</tr>
<tr>
<td>Retrieved from seawater (n:59)</td>
<td>13.7</td>
<td>383.7</td>
<td>1190.4</td>
</tr>
<tr>
<td>Control (n:20)</td>
<td>13.1</td>
<td>40.7</td>
<td>145.4</td>
</tr>
</tbody>
</table>

LVsr: Left ventricle cardiac blood strontium concentration, RVsr: Right ventricle cardiac blood strontium concentration, [LVsr-RVsr]: Difference between left ventricle cardiac blood strontium concentration and right ventricle cardiac blood strontium concentration.
postmenopausal osteoporosis [12, 15, 16]. In a different study, the mean Sr concentration in living individuals was 11.4±0.83µg/L ranging between 16 and 95 µg/L [5]. Sr concentration may be higher in those who regularly consume mineral water and sea foods [12, 17]. It has been experimentally demonstrated that serum strontium concentration ranges from 15 µg/L to 58.7 µg/L in the subjects receiving a solution containing 9.14 µg/L strontium for 15 days [18]. According to Piette, Sr concentration in living organisms depends on diet and drinking water [5]. Azparren stated that Sr concentration that passes from water into ventricular blood via postmortem diffusion does not exceed 125 µg/L in the left ventricle when the postmortem interval shorter than 5 days and in the absence of conditions such as exposure to severe hydrostatic pressure in deep water or serious injuries [4]. In the present study, mean LVSc concentration was 40.7 µg/L and mean RVSc concentration was 45.2µg/L in the control group. However, LVSc and RVSc concentrations were quite different from the average concentrations in two cases (121-119 µg/L and 145-61 µg/L, respectively).

Studies reported that Sr concentrations are not influenced by age and gender [15, 19]. The present study showed decrease in LVSc concentration with increased age with low statistical relevance. RVSc and PISc concentrations appeared not to be dependent on victim's age. There is no statistically significant difference between Sr concentrations (LVSc, RVSc and PISc concentrations) and gender.

Significant differences between biventricular Sr concentrations are reported in seawater drowning cases as well as animal studies [2, 3, 5, 15]. Perez-Carceles [15] and Fornes [14] reported significant increase in Sr concentrations in fresh water drowning cases, whereas Azparren [8] reported that diagnostic Sr concentrations can be detected in ventricular blood in 32% of fresh water drowning cases. It is difficult to obtain significant results due to low Sr concentrations in fresh water [5].

Sr concentration difference more than 75µg/L between ventricles [LVSc-RVSc] accepted as a strong criterion for drowning in seawater. In waters Sr concentration is higher than 800 µg/L (seawater), classical drowning cases’ [LVSc-RVSc] concentration difference is significantly higher than in atypical drowning cases [3]. In the analyses of water obtained from Istanbul shoreline, Sr concentrations ranged between 2873 and 3269 µg/L. In the present study, mean [LVSc-RVSc] difference was 91.3µg/L in the sea drowning cases and was consistent with the concentrations defined as strong criteria for drowning in seawater.

Despite low number of cases reported to be retrieved from fresh water (n:13), LVSc and RVSc concentrations were found significantly higher than those of the control group. However, in the present study, there are 3 cases reported to be pulled out of Alibeykoy dam reservoir, which is found in Istanbul hinterland as fresh water source. LVSc and RVSc concentrations of these cases were found to be high as was also observed in the seawater cases. Sr concentrations measured in Alibeykoy reservoir is 437µg/L. This concentration has been obtained only from a single region. There are many residential areas around Alibeykoy dam reservoir; thus it might be possible to detect different concentrations of Sr in different regions of the reservoir due to pollution with drain water. Therefore, LVSc and RVSc concentrations of Alibeykoy cases (n=3) were high and discordant with other fresh water case concentrations. Hence, we determined that LVSc and RVSc concentrations of fresh water cases were consistent with the literature when Alibeykoy cases were excluded.

Although, Azparren et al. determined significant differences in "lung weight/body weight" (L/B) ratio in the seawater drowning cases (LVSc>172 µg/l), no correlation was demonstrated between Sr concentration and L/B ratio when compared with the subjects found in water but not considered to be drowned (LVSc<172 µg/l) [13]. In the present study, statistically significant increase was determined in LVSc, RVSc and PISc concentrations with increasing L/B ratio, and total lung weight.

It has been also reported that Sr concentrations might differ greatly not only between drowning cases and non-drowning cases, but also between the samples obtained from different parts of the body in drowning cases [2, 5, 12, 20, 21]. Sr concentrations in body fluids are reported not to be considerably influenced by hemolysis (due to osmotic alterations), early decomposition, and resuscitation [1]. However, blood Sr concentration may increase due to mixture of blood Sr with water Sr resulting from disintegration of skin due to decomposition and probable traumatic postmortem injuries [21]. It has been reported that heart blood Sr concentration may increase due to decomposition in water and both ventricles are influenced similarly in such cases [18]. In such a case (retrieved from seawater), 660 and 677.6 µg/L concentrations of LVSc and RVSc respectively were obtained in a saponified case with undetermined cause of death, consistent with literature.

Uncertainty may appear in the interpretation of the findings as the number of variables (such as the difference between LVSc and RVSc concentrations [LVSc-RVSc]) used for interpretation increased [8]. Hence, among the cases of present study, there were cases in which both LVSc and RVSc concentrations were quite high or cases with higher concentrations of LVSc in favor of RVSc. It was thought that such concentration differences might be due to resuscitation, aspiration before drowning, long agonal period, breathing during the struggle before death, or more than one of these factors together.
CONCLUSION

1. LVSr, RVSr and [LVSr-RV Sr] concentrations were found higher in the cases reported to be retrieved from water (regardless of sea or fresh water) as compared to the control group.

2. The mean LV Sr was 366.9 µg/L and mean RV Sr was 300.5 µg/L in all cases retrieved from water; mean LV Sr was 383.7 µg/L and mean RV Sr was 292.5 µg/L in the cases retrieved from seawater; mean LV Sr was 175.8 µg/L and mean RV Sr was 244.7 µg/L in the cases retrieved from fresh water.

3. LV Sr and PlSr concentrations were higher in the cases retrieved from seawater as compared to the fresh water cases.

4. No statistically significant difference was found between the seawater cases and fresh water cases in terms of RV Sr concentrations.

5. It was observed that LV Sr, RV Sr and PlSr concentrations increased as lung weight/body weight (L/B) ratio increased.

6. No statistically significant difference was found between fresh water cases and the control group in terms of [LV Sr-RV Sr] concentrations.

7. LV Sr, RV Sr and [LV Sr-RV Sr] concentrations were higher in the seawater cases as compared to the control group.

References