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Evaluation of postmortem serum calcium and magnesium levels in relation to the causes of death in forensic autopsy

Bao-Li Zhu*, Takaki Ishikawa, Li Quan, Dong-Ri Li, Dong Zhao, Tomomi Michiue, Hitoshi Maeda

Department of Legal Medicine, Osaka City University Medical School, Asahi-machi 1-4-3, Abeno, 545-8585 Osaka, Japan

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Abstract

There appears to be very poor investigation of postmortem serum calcium (Ca) and magnesium (Mg) for diagnostic evidence to determine the cause of death. The aim of the present study was a comprehensive analysis of the serum levels in relation to the causes of death in routine casework. Autopsy cases (total, $n = 360$; 5–48 h postmortem), including blunt injury ($n = 76$), sharp injury ($n = 29$), asphyxiation ($n = 42$), drownings ($n = 28$: freshwater, $n = 11$; saltwater, $n = 17$), fire fatalities ($n = 79$), methamphetamine (MA) poisoning ($n = 8$), delayed death from traumas ($n = 37$), and acute myocardial infarction/ischemia (AMI, $n = 61$), were examined. In total cases, there was no significant postmortem time-dependent rise in serum Ca and Mg. Both Ca and Mg levels in the heart and peripheral blood were significantly higher in saltwater drowning compared with those of the other groups. In addition, a significant elevation in the Ca level was observed in freshwater drowning and fire fatalities, and in the Mg level in fatal MA intoxication and asphyxiation. Topographic analyses suggested a rise in serum Ca and Mg due to aspirated saltwater in drowning, that in serum Ca in freshwater drowning and fire fatalities of peripheral skeletal muscle origin and that in serum Mg in MA fatality and asphyxiation of myocardial and/or peripheral origin. These markers may be useful especially for diagnosis and differentiation of salt- and freshwater drownings and may be also helpful to determine the causes of death involving skeletal muscle damage, including burns and MA intoxication.

Keywords: Forensic pathophysiology; Postmortem biochemistry; Serum; Calcium; Magnesium; Cause of death

1. Introduction

In forensic pathology, there have been various attempts to use biochemical markers for determining the cause of death and estimating the time after death [1–12]. In determining the cause of death, biochemical investigation may be helpful especially in cases of poor morphological evidence, e.g., drowning, asphyxiation, poisoning and acute cardiac death [1–6]. Meanwhile, electrolytes and minerals including calcium (Ca) and magnesium (Mg) have been mainly applied to estimation of the time after death [10–13]. There appears to be very poor investigation of postmortem serum Ca and Mg for diagnostic evidence to determine the cause of death. In clinical biochemistry, however, serum Ca and Mg are important markers to investigate pathophysiology, e.g. in renal, skeletal muscular and endocrine diseases, and also in traumatic skeletal muscle damage involving rhabdomyolysis and seawater near-drowning [14–16].

* Corresponding author. Tel.: +81 6 6645 3767; fax: +81 6 6634 3871.
E-mail address: baolizhu@med.osaka-cu.ac.jp (B.-L. Zhu).

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In the present study, we comprehensively examined postmortem serum Ca and Mg levels in relation to the causes of death in routine casework with special regard to their diagnostic value in seawater drowning and skeletal muscle damage.

2. Materials and methods

2.1. Materials

Blood samples of 360 autopsy cases (within 48 h postmortem) at our institute were examined: 266 males and 94 females; 2 months-94 years of age (mean, 56.2 years of age); postmortem interval, 5–48 h (mean, 18.9 h). The blood samples were collected aseptically using syringes from the left and right heart chambers, subclavian and external iliac vein. The serum was separated immediately by centrifugation and stored at -20 °C until use.

The causes of death were classified as follows: acute traumatic death ($n = 262$, survival time within 24 h) from blunt injury ($n = 76$), sharp injury ($n = 29$), mechanical asphyxiation ($n = 42$: hanging, $n = 8$; strangulation, $n = 15$; aspiration, $n = 12$; others, $n = 7$), drownings ($n = 28$: freshwater, $n = 11$; saltwater, $n = 17$), fire fatalities ($n = 79$) consisting of those with blood carboxyhemoglobin (COHb) below 60% ($n = 48$) and above 60% ($n = 31$), methamphetamine (MA) poisoning ($n = 8$); delayed death from traumas (multiple organ insufficiency, $n = 37$; survival time, 1–90 days); acute myocardial infarction/ischemia (AMI, $n = 61$) (Table 1). The above-mentioned causes of death were classified on the pathological and toxicological bases, excluding cases with complications, which may have contributed to the dying process or elevated cardiac blood urea nitrogen (BUN) level (>50 mg/dl). The AMI group consisted of cases of sudden death, which showed macro- and microscopical findings of acute ischemic heart diseases without any evidence of cause of death other than a cardiac attack [17].

2.2. Biochemical analyses

Ca and Mg were measured by an orthocresolphthalein complexome method [18] and a xylidyl blue method [19], respectively. Hemoglobin contamination (<0.1 g/dl) did not interfere with the measurements. Clinical reference serum ranges were: 8.7–10.1 mg/dl for Ca and 1.8–2.6 mg/dl for Mg. BUN was measured by a urease-glutamate dehydrogenase method [20]. In cases of strong hemolysis, which may have influenced the measurements, the findings were not used in the analyses.

2.3. Toxicological analyses

Blood COHb concentration was determined using a CO-oximeter system [21,22] in all the fire fatalities. Volatile chemicals including alcohol were analyzed by head-space gas chromatography in all cases. Drug analyses were performed by gas chromatography/mass spectrometry, when preliminary screening tests were positive.

2.4. Statistical analyses

The Fisher exact test was used to compare two parameters including biochemical markers, the age of victims, survival time and postmortem interval. Comparisons between groups were performed by Student's t-test, and a nonparametric test (Mann–Whitney U-test) was used for the

Table 1
Case profiles

Cause of death	n	Age (years)		Postmortem interval (h) range	BUN (mg/dl)	
		Range	Mean		Range	Mean
Blunt injury	76	2–94	53.2	6–40	5.7–40.3	17.2
Sharp injury	29	22–90	53.5	6–46	1.3–36.7	13.3
Asphyxia	42	2–93	48.9	6–47	7.4–45.8	16.9
Drowning						
Freshwater	11	5–72	42.8	10–34	10.6–20.2	13.9
Saltwater	17	0–70	45.6	7–48	5.8–23.0	14.2
Fire fatality						
COHb < 60%	48	23–89	62.4	6–48	4.0–40.3	16.9
COHb > 60%	31	1–87	55.8	7–39	7.2–31.2	17.9
MA poisoning	8	20–52	38.3	8–34	11.1–112.0	46.5
Delayed death from traumas	37[a]	1–79	57.2	5–32	10.8–114.6	58.8
Acute myocardial infarction/ischemia	61	31–94	65.5	5–36	4.7–42.8	19.9
Total	360	0–94	52.2	5–48	1.3–114.6	22.4

COHb, carboxyhemoglobin concentration; MA, methamphetamine.

[a] Multiple organ insufficiency and secondary infection from head injury ($n = 30$), chest injury ($n = 4$), abdominal injury ($n = 3$).

comparison of Mg levels between saltwater drowning and the other groups. A p-value less than 0.05 was considered statistically significant.

3. Results

3.1. Postmortem stability, topographic distribution, age- and gender-dependence

Each factor in the cardiac and peripheral blood showed no significant relation to the postmortem intervals (correlation coefficient, $k < 0.2$; $p > 0.1$). There was a good correlation in Ca level between both cardiac and peripheral blood (Fig. 1a and b). When saltwater drowning cases were excluded, the values of the left (y) and right (x) heart blood showed an equivalency: $y = 0.69x + 3.70$; $R = 0.73$, $n = 295$, $p < 0.0001$ (Fig. 1a). The subclavian (y) and external iliac (x) venous blood levels also showed an equivalency ($y = 0.76x + 2.89$; $R = 0.79$, $n = 94$, $p < 0.0001$), being mildly higher than the heart blood levels. There was an equivalency between the external iliac venous (y) and right heart (x) blood levels: $y = 0.74x + 4.10$; $R = 0.76$, $n = 152$, $p < 0.0001$ (Fig. 1b). Similar relation was observed between the external iliac venous (y) and left heart blood (x): $y = 0.72x + 4.38$; $R = 0.71$, $n = 122$, $p < 0.0001$, between the subclavian venous (y) and right heart blood (x): $y = 0.81x + 2.98$; $R = 0.86$, $n = 157$, $p < 0.0001$, and between the subclavian venous (y) and left heart blood (x): $y = 0.62x + 5.02$; $R = 0.73$, $n = 152$, $p < 0.0001$. There was no age- or gender-dependence in Ca levels. A significant inverse relation was observed between the Ca and BUN levels ($p < 0.0001$).

Mg level also showed a good correlation between both cardiac and peripheral blood (Fig. 2a and b). When saltwater drowning cases were excluded, the Mg levels were slightly lower in the left (y) than in the right (x) heart blood:

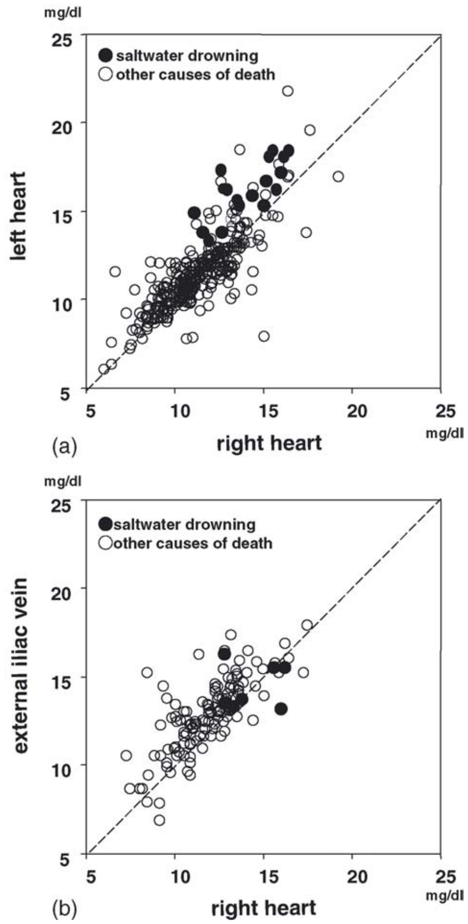

Fig. 1. Topographic comparisons of postmortem serum calcium (Ca) levels. Relation between the left and right heart blood (a), and between the external iliac venous and right heart blood (b). Details in the text.

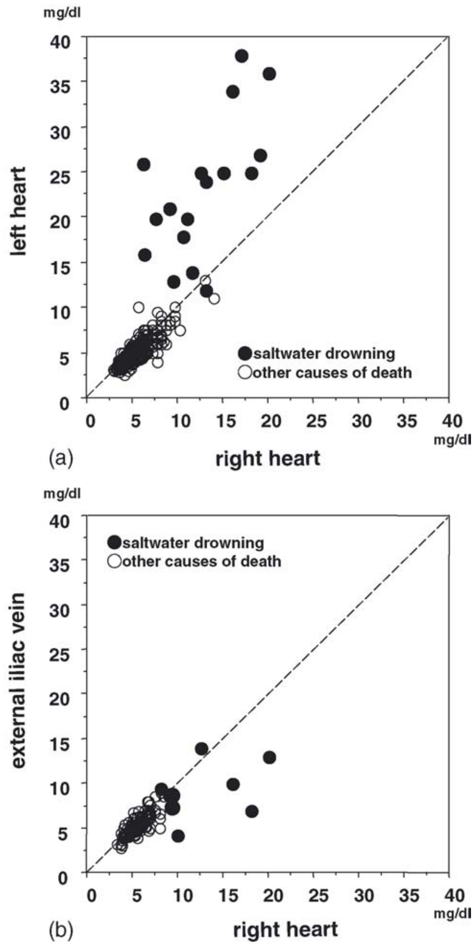

Fig. 2. Topographic comparisons of postmortem serum magnesium (Mg) levels. Relation between the left and right heart blood (a), and between the external iliac venous and right heart blood (b). Details in the text.

$y = 0.70x + 1.13$; $R = 0.97$, $n = 295$, $p < 0.0001$ (Fig. 2a). External iliac venous (y) blood level showed an equivalency to the right cardiac (x) level: $y = 0.80x + 0.89$; $R = 0.80$, $n = 123$, $p < 0.0001$ (Fig. 2b). There was an equivalency between the subclavian (y) and external iliac (x) venous blood: $y = 1.00x + 0.17$; $R = 0.86$, $n = 94$, $p < 0.0001$. Similar relation was observed between the external iliac venous (y) and left heart blood (x) ($y = 0.807x + 1.418$; $R = 0.667$, $n = 122$, $p < 0.0001$), between the subclavian venous (y) and right heart blood (x) ($y = 0.80x + 0.91$; $R = 0.93$, $n = 157$, $p < 0.0001$), and between the subclavian venous (y) and left heart blood (x) ($y = 0.85x + 1.16$; $R = 0.849$, $n = 152$, $p < 0.0001$). In saltwater drowning, the left cardiac level was markedly higher than the right, as described below. There was an age-dependent decrease in cardiac and peripheral Mg levels ($p < 0.0001$). Gender-difference or relation to BUN was not significant.

3.2. Difference in relation to the causes of death

3.2.1. Calcium

Both cardiac blood Ca levels in saltwater drowning were markedly higher than in blunt injury, sharp injury, asphyxia and AMI ($p < 0.0001$) (Fig. 3a). Fire fatalities showed a moderate elevation, when compared with blunt injury, sharp injury, AMI ($p < 0.0001$) and asphyxia ($p < 0.05$). In freshwater drowning cases, a mild elevation was observed in comparison with blunt injury ($p < 0.05$). In those groups, peripheral blood levels also showed a significant elevation (saltwater drowning versus blunt injury, sharp injury, asphyxia and AMI, $p < 0.05$; fire fatalities versus blunt injury and asphyxia, $p < 0.05$; freshwater drowning versus blunt injury, $p < 0.05$) (Fig. 3b). In MA fatality and delayed traumatic death cases, cardiac and peripheral Ca levels were lower than the other groups ($p < 0.05$–0.0001).

The left cardiac level was higher than the right cardiac and peripheral levels in saltwater drowning ($p < 0.05$–0.0001), whereas the external iliac venous level was higher than both cardiac levels in fire fatalities ($p < 0.001$) and freshwater drowning cases ($p < 0.0001$). In addition, in asphyxiation and MA fatality, the external iliac venous levels were higher than the right cardiac level ($p < 0.05$). There was no significant difference between fire fatalities with a high (>60%) and a low (<60%) COHb.

When salt- and freshwater drownings and fire fatalities were excluded, postmortem cardiac serum Ca level (mean ± S.D.) showed a mild rise in comparison with the clinical reference values (8.7–10.1 mg/dl): left, 11.0 ± 1.6 mg/dl; right, 10.8 ± 1.8 mg/dl.

3.2.2. Magnesium

Cardiac blood Mg levels in saltwater drowning were markedly higher than in blunt injury, sharp injury, asphyxia, freshwater drowning, fire fatalities, delayed death from traumas and AMI ($p < 0.0001$) (Fig. 4a). A mild elevation

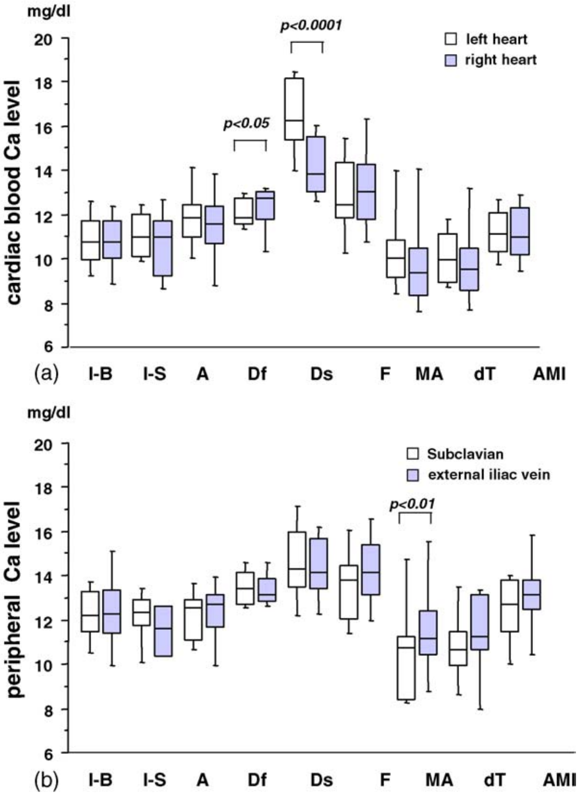

Fig. 3. Postmortem serum calcium (Ca) levels in the heart blood (a) and peripheral blood (b). I–B, blunt injury; I–S, sharp injury; A, mechanical asphyxiation; Df, freshwater drowning; Ds, saltwater drowning; F, fire fatalities; MA, fatal methamphetamine poisoning; dT, delayed traumatic death; AMI, acute myocardial infarction/ischemia. Details in the text.

was observed in asphyxiation and fatal MA intoxication, when compared with blunt injury, sharp injury, freshwater drowning, fire fatalities, delayed death from traumas and AMI groups ($p < 0.01$–0.001). In saltwater drowning and MA fatality, peripheral levels also showed a significant elevation (saltwater drowning versus blunt injury, sharp injury, asphyxia, freshwater drowning, delayed death from traumas and AMI, $p < 0.05$–0.01; MA fatality versus fire fatalities and AMI, $p < 0.05$) (Fig. 4b).

The left cardiac level was markedly higher than the levels in the other sites in saltwater drowning ($p < 0.0001$), whereas the right cardiac level was significantly high compared with the left cardiac level in asphyxiation ($p < 0.01$), freshwater drowning ($p < 0.05$) and fire fatalities ($p < 0.0001$). In fire fatalities, the external iliac venous levels were higher than the left cardiac level ($p = 0.0014$). There was no significant difference between fire fatalities with a high (>60%) and a low (<60%) COHb.

When saltwater drowning, asphyxiation and MA fatality were excluded, Mg levels (mean ± S.D.) in the left and right heart blood, respectively, ranged as follows, showing an age-

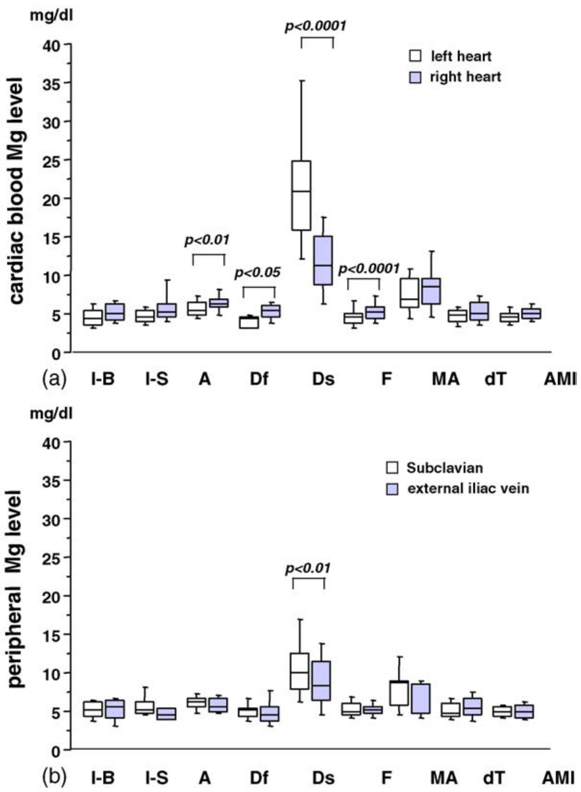

Fig. 4. Postmortem serum magnesium (Mg) levels in the heart blood (a) and peripheral blood (b). Abbreviations are shown in Fig. 3. Details in the text.

dependence ($p < 0.0001$): subjects below 60 years of age, 5.21 ± 1.07 mg/dl (n = 145) and 5.84 ± 1.40 mg/dl (n = 139); those above 60 years of age, 4.62 ± 1.02 mg/dl (n = 156) and 5.30 ± 1.41 mg/dl (n = 144).

4. Discussion

Previous studies suggested an early and progressive rise in serum Ca and Mg levels depending on the time after death [13,23,24]. In the present study, however, such a postmortem time-dependent rise was not evident during 5–48 h after death, although the cadaveric blood levels showed an increase in comparison with the clinical reference values. There was a greater increase in Mg, suggesting an influence of agonal and/or postmortem breakdown of tissues, where the Mg concentration is markedly high compared with the plasma level [1,11,14,16]. Such interference may become stationary within several hours after death. Under those conditions, a significant difference in postmortem serum Ca and Mg levels was observed between the causes of death.

Both Ca and Mg levels in the cardiac and peripheral blood were significantly higher in saltwater drowning than in the other groups. This was similar to clinical findings in saltwater near-drowning cases [25,26]. A significant elevation in the left cardiac levels of both markers compared with those in the other sites suggested the influence of saltwater aspiration [27–30]. Although an elevated serum Ca level was also observed in fire fatalities and freshwater drowning, these groups showed a higher level in the peripheral blood, suggesting an increase of skeletal muscle origin. As to fire fatalities, this finding is consistent with that in clinical cases of deep burns, which show a significant increase in serum Ca level in the early phase [16].

A lower serum Ca level was observed in MA fatality and delayed traumatic death cases, which were usually accompanied by skeletal muscle damage and renal failure. These findings as well as an inverse relation of serum Ca to BUN level suggested a contribution of massive skeletal muscle damage and/or renal failure to a reduced postmortem serum Ca level [31].

In addition, an elevated serum Mg level was observed in asphyxiation and fatal MA intoxication. These groups showed varied topographic differences in Mg level: a higher level was observed in the right heart blood in asphyxiation, whereas there was no significant topographic difference in MA fatality. Furthermore, the peripheral level was higher than the cardiac level in fire fatalities. Such topographic distributions suggested, with respect to the larger Mg contents in the muscle than the other tissues [32], that the origin of increased serum Mg may be skeletal muscle and/or myocardium, possibly being varied depending on the causes of death [14]. For an age-dependent decrease in the postmortem serum Mg level, a possible contributory factor may be reduced nutrition [33]. In respect to these hypotheses, further investigations are necessary in combination with the other markers of skeletal muscle and myocardial injury. Single site sampling may be not valid in postmortem biochemistry. Multiple site sampling including cardiac and peripheral blood is useful.

In conclusion, the findings of the present study suggested no significant postmortem time-dependent rise in serum Ca and Mg during the early postmortem period. Although there was an increase in cadaveric blood levels, a significant difference in postmortem serum Ca and Mg levels was observed between the causes of death. These markers may be useful especially for diagnosis and differentiation of salt- and freshwater drownings and may be also helpful to determine the causes of death involving skeletal muscle damage, including burns and MA intoxication. For this purpose, topographic analyses are necessary.

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