Legal Medicine 16 (2014) 344–349

Contents lists available at ScienceDirect

Legal Medicine

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

Postmortem urinary catecholamine levels with regard to the cause of death

Takaki Ishikawa [a,b,e,*], Osamu Inamori-Kawamoto [a,b], Li Quan [a,c], Tomomi Michiue [a,b], Jian-Hua Chen [a,b], Qi Wang [a,b], Bao-li Zhu [a,d], Hitoshi Maeda [a,b]

[a] Department of Legal Medicine, Osaka City University Medical School, Asahi-machi 1-4-3, Abeno, 545-8585 Osaka, Japan
[b] Forensic Autopsy Section, Medico-legal Consultation and Postmortem Investigation Support Center, c/o Department of Legal Medicine, Osaka City University Medical School, Asahi-machi 1-4-3, Abeno, 545-8585 Osaka, Japan
[c] Department of Forensic Pathology, Sun Yat-Sen University Zhongshan School of Medicine, No. 74, Zhongshan II Road, Guangzhou 510080, Guangdong, PR China
[d] Department of Forensic Pathology, China Medical University School of Forensic Medicine, No. 92, Beier Road, Heping District, Shenyang 110001, Liaoning Province, PR China
[e] Division of Legal Medicine, Faculty of Medicine, Tottori University, 86 Nishi-cho, Yonago, 683-8503 Tottori, Japan

ARTICLE INFO

Article history:
Received 21 March 2014
Received in revised form 25 June 2014
Accepted 21 July 2014
Available online 8 August 2014

Keywords:
Forensic pathophysiology
Postmortem biochemistry
Urine
Catecholamine
Physical stress

ABSTRACT

Previous studies suggested that serum catecholamines are useful for investigating stress responses in the death process. The present study analyzed postmortem urinary adrenaline (Ad), noradrenaline (Nad) and dopamine (DA) in serial forensic autopsy cases ($n = 199$: 154 males and 45 females; age >9 years; survival time <0.5–168 h; within 10 days postmortem) to investigate the differences among the causes of death with special regard to hyperthermia (heatstroke; $n = 11$) and hypothermia (cold exposure; $n = 10$); other cases included fatalities from injury ($n = 47$), mechanical asphyxiation ($n = 18$), drowning ($n = 14$), intoxication ($n = 31$), fire fatality ($n = 33$) and natural death ($n = 35$). Each catecholamine level in urine was independent of the age or gender of the subjects, postmortem interval over 10 days or survival time, and did not correlate with the blood level. Urinary Adr and Nad levels were similar to those of clinical serum reference ranges, while DA was higher in all cases. Adr and Nad were higher in blunt head injury, methamphetamine abuse, hypothermia (cold exposure) and hyperthermia (heat stroke), but were low in mechanical asphyxia, drowning, fire fatality, sedative-hypnotic intoxication and acute cardiac death. DA was higher in injury, drowning, fire fatality, methamphetamine abuse and acute cardiac death, but was lower in mechanical asphyxiation and sedative-hypnotic intoxication. These profiles were quite different from those of serum levels, involving a predominant increase of DA, and may be useful for differentiating hyperthermia (heatstroke) and hypothermia (cold exposure) from drowning, sedative-hypnotic intoxication and sudden cardiac death.

1. Introduction

Catecholamines are major humoral factors and neurotransmitters that contribute to various stress responses. Previous studies that applied these markers in blood to the postmortem investigation of death mainly focused on determining fatal hypothermia (cold exposure) and evaluating the agony period, but failed to present consistent results, although their postmortem stabilities were established [1–5]. In addition, there were significant differences in catecholamine levels in blood, pericardial and cerebrospinal fluid, depending on the cause of death, with substantial interindividual variations in each cause of death [6,7], suggesting that multiple site analyses of catecholamine in blood and other body fluids are useful to investigate stress responses or toxic/hyperthermic neuronal dysfunction during the death process in individual cases, when they are used in combination with other biochemical and immunohistochemical markers [8–22]. Meanwhile, previous studies of urinary catecholamine levels mainly focused on the diagnosis of hypothermia (cold exposure), using selected control cases [3–5]; thus, there were limited data on urinary levels with regard to the cause of death that might be involved in differentiation from hypothermia (cold exposure) in routine forensic casework, and insufficient published data about hyperthermia (heatstroke) as another end of thermal disorder.

The present study comprehensively analyzed postmortem adrenaline (Ad), noradrenaline (Nad) and dopamine (DA) in the

* Corresponding author at: Division of Legal Medicine, Faculty of Medicine, Tottori University, 86 Nishi-cho, Yonago, 683-8503 Tottori, Japan. Tel.: +81 859 38 6123; fax: +81 859 38 6120.
E-mail address: takaki@med.tottori-u.ac.jp (T. Ishikawa).

http://dx.doi.org/10.1016/j.legalmed.2014.07.006
1344-6223/

urine in routine forensic casework, as compared with blood levels, with special regard to fatal thermal disorders including hyperthermia (heatstroke) and hypothermia (cold exposure), as well as the causes of death that may be involved in differentiation, including drowning, intoxication and acute cardiac death.

2. Materials and methods

2.1. Autopsy materials

Serial forensic autopsy cases ($n = 199$: 154 males and 45 females; age >9 years; survival time <0.5–168 h; within 10 days postmortem) were examined at our institute over a period of 9 years (2005–2013). Urine samples were collected by puncturing the bladder using sterile syringes after opening the abdominal cavity at autopsy. Blood samples from the right cardiac chamber were also collected from cases within 48 h after death ($n = 147$), and the serum was immediately separated by centrifugation. These samples were stored at −20 °C until use.

The causes of death were classified into injury ($n = 47$), mechanical asphyxiation ($n = 18$), drowning ($n = 14$), intoxication ($n = 31$), fire fatalities ($n = 33$), hypothermia (cold exposure, $n = 10$), hyperthermia (heat stroke, $n = 11$), and natural death ($n = 35$), based on routine macromorphological, micropathological and toxicological bases, as shown in Table 1. Intoxication cases ($n = 31$) included fatal methamphetamine (MA) abuse ($n = 12$), sedative-hypnotic intoxication ($n = 11$), and others ($n = 8$: carbon monoxide, $n = 4$; alcohol, $n = 3$; detergent, $n = 1$); all fatal MA abusers had physical and/or circumstantial evidence of chronic abuse, including injection scars and criminal records, despite hair analysis was not included in routine examination. Fire fatalities comprised those with blood carboxyhemoglobin (COHb) of <30% (low COHb cases, $n = 13$), 30–60% (intermediate COHb cases, $n = 12$) and >60% (high COHb cases, $n = 8$). Case history and pathological and toxicological data were collected from autopsy documents. Well-documented, clearly accountable cases were collected for all groups to establish the cause of death, postmortem interval and survival time. The postmortem interval was defined as the time from the estimated time of death to autopsy. The survival time was the period from the onset of fatal insult to death.

2.2. Analytical procedure

Catecholamines (Adr, Nad and DA) in urine and serum were measured using high performance liquid chromatography [23–25]. The ranges of measurements were 2.0–10,500 pg/ml for Adr, 1.8–19,500 pg/ml for Nad, and 2.1–18,750 pg/ml for DA. Serum samples were diluted with saline before measurement (10- to 1000-fold). The accuracy and reliability were checked for reproducibility following 10 serial dilutions, and nonreproducible data were not used. Clinical serum reference ranges for peripheral blood were <100 pg/ml for Adr, 100–450 pg/ml for Nad, and <20 pg/ml for DA.

Blood %COHb saturation was analyzed on a CO-oximeter system (Ciba Corning 270, New York or Radiometer, Copenhagen) [26,27]. Blood cyanide and alcohol levels were determined by head-space gas chromatography/mass spectrometry [28]. Drug analyses were performed by gas chromatography/mass spectrometry.

2.3. Statistical analysis

Fischer's exact test was used to compare two parameters, including catecholamine levels, gender and age of subjects, survival time and postmortem interval. A Scheffe or Games-Howell test was used for multiple comparisons among groups, and comparisons between groups were performed by the non-parametric Mann–Whitney *U* test. These analyses were performed using Microsoft Excel and Statview (version 5.0; SAS Institute Inc., Cary). $p < 0.05$ was considered significant. In Figs. 1–3, the results of data analysis are shown as box-plots, for which 50% of the data are summarized in the box. The line in each box represents the median, and the lines outside of each box represent the 90% confidence interval. The sensitivity and specificity in distinguishing two groups using cut-off Adr, Nad and DA values were estimated by receiver-operating characteristics (ROC) analysis [29]. The areas under the curves were calculated and analyzed by the one-tailed test. The optimal compromise between sensitivity and specificity was determined graphically.

Table 1
Case profiles of subjects ($n = 199$, over 9 years of age).

Cause of death	Case number (male/female)	With/without CPR	Age (y) (median)	Urine volume (ml) (median)	Survival time (h) (median)	Postmortem time (h) (median)
Blunt injury	40 (36/4)	7/33	20–88 (56)	1–550 (55)	<0.5–48 (1.0)	12–192 (25)
Head injury	10 (7/3)	3/7	35–88 (60)	1–550 (60)	<0.5–48 (16)	12–96 (30)
Others	30 (29/1)	4/26	20–79 (56)	4–400 (50)	<0.5–12 (0.5)	12–192 (25)
Sharp instrument injury	7 (5/2)	0/7	27–75 (53)	2–200 (30)	<0.5–3 (0.7)	12–96 (34)
Asphyxia	18 (14/4)	1/17	9–75 (59)	4–650 (40)	<0.5–3(0.5)	12–168 (30)
Hanging	6 (5/1)	0/6	26–75 (49)	4–650 (35)	<0.5 (0.5)	12–48 (30)
Strangulation	8 (6/2)	0/8	9–75 (54)	5–400 (30)	<0.5 (0.5)	12–168 (30)
Others	4 (3/1)	1/3	40–72 (66)	15–300 (100)	<0.5–3(2.0)	12–60 (24)
Drowning	14 (11/3)	0/14	30–79 (62)	10–350 (50)	<0.5 (0.5)	12–120 (37)
Salt water	4 (3/1)	0/4	36–79 (72)	10–300 (45)	<0.5 (0.5)	24–60 (30)
Fresh water	7(6/1)	0/7	30–71 (59)	15–350 (50)	<0.5 (0.5)	12–96 (38)
Bath water	3 (2/1)	0/3	33–72 (64)	20–50 (30)	<0.5 (0.5)	24–120 (40)
Fire fatality	33 (25/8)	2/31	44–95 (70)	4–750 (50)	<0.5–2 (0.5)	12–96 (20)
Intoxication	31 (16/15)	1/30	20–76 (45)	7–1000 (200)	0.5–48 (3.0)	6–240 (37)
Methamphetamine	12 (7/5)	0/12	26–55 (46)	7–450 (210)	0.5–6 (3.0)	12–240 (55)
Sedative-hypnotic intoxication	11 (5/6)	1/10	20–57 (37)	50–1000 (350)	3–12 (6)	6–60 (37)
Others	8 (4/4)	0/8	32–76 (53)	15–750 (35)	3–48 (3)	24–86 (35)
Hyperthermia	11 (4/7)	0/11	24–83 (70)	3–300 (100)	6–168 (6)	24–120 (31)
Hypothermia	10 (9/1)	1/9	45–84 (66)	2–450 (135)	6–12 (6)	24–240 (84)
Natural death	35 (31/4)	4/31	14–85 (65)	2–600 (70)	<0.5–48 (0.5)	5–44 (20)
Acute cardiac death	13 (13/0)	2/11	43–82 (63)	3–600 (125)	<0.5–48 (0.5)	12–84 (26)
Others	22 (18/4)	2/20	14–85 (66)	2–400 (30)	<0.5–168 (48)	12–96 (33)
Total	199 (154/45)	16/183	9–95 (60)	1–1000 (60)	<0.5–168 (0.5)	6–240 (30)

CPR, cardiopulmonary resuscitation.