Legal Medicine 15 (2013) 140–144

Contents lists available at SciVerse ScienceDirect

Legal Medicine

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

Diagnostic implications of urinary liver-type fatty acid-binding protein and 8-hydroxy-2′-deoxyguanosine in forensic autopsy cases

Masayuki Kashiwagi *, Tomoko Sugimura, Aya Matsusue, Kenji Hara, Brian Waters, Shin-ichi Kubo

Department of Forensic Medicine, Faculty of Medicine, Fukuoka University, Japan

ARTICLE INFO

Article history:
Received 6 December 2011
Received in revised form 13 November 2012
Accepted 14 November 2012
Available online 25 December 2012

Keywords:
Liver-type fatty acid-binding protein
8-Hydroxy-2′-deoxyguanosine
Urine
Antemortem reaction
Autopsy diagnosis

ABSTRACT

Background/aim: Liver-type fatty acid-binding protein (L-FABP) is a clinical biomarker of the progress of kidney disease. 8-Hydroxy-2′-deoxyguanosine (8-OHdG) is known as a biomarker of peroxidative DNA damage. We investigated both urinary L-FABP and 8-OHdG in forensic autopsy cases as biomarkers to elucidate the metabolic changes in survival periods after insults.

Methods: In 196 urinary samples from forensic autopsy cases, we measured L-FABP and 8-OHdG by enzyme-linked immunosorbent assay (ELISA) and creatinine by enzymatic assay. Urinary L-FABP/Cr and 8-OHdG/Cr were obtained.

Results: No significant correlation was observed between urinary L-FABP/Cr or 8-OHdG/Cr, and gender, age, or postmortem interval. Regarding urinary L-FABP/Cr or 8-OHdG/Cr, there were no significant differences among the causes of death. In the survival/agony period, urinary L-FABP/Cr under the cut-off value 31.3 might show that the survival/agony period was within 1 h. Under the cut-off value of urinary 8-OHdG/Cr, 17.8, might indicate that it is within 24 h.

Conclusion: Urinary L-FABP/Cr may rise within a relatively short survival/agony period, and urinary 8-OHdG/Cr may increase when the damage continues longer. Measuring the urinary L-FABP/Cr and 8-OHdG/Cr might be useful in elucidating the survival/agony period.

1. Introduction

In forensic medicine, the cause of death has a wider meaning of the causal relationship with not only the pathological cause of death, but also the passage from onset to death; however, metabolic changes in the survival period after insults are difficult to confirm in forensic autopsy cases. Comprehensive reviews of postmortem chemistry are available [1–4]. To elucidate the severity of the disease or trauma, several biomarkers in blood, such as c-reactive protein (CRP), neuron-specific enolase (NSE), and S100B for traumatic brain injury, have been reported [5,6]. Postmortem chemistry can represent one of the most important ancillary procedures for the forensic pathologist in investigating the cause and the process of death, the contributing conditions and the predisposing disorders [3]. The purpose of this study was therefore to investigate the biomarkers useful for the autopsy diagnosis, especially elucidation of the death process [7].

Liver-type fatty acid-binding protein (L-FABP) is an isoform of FABPs. In the human kidney, proximal tubule cells express mRNA of L-FABP, and a certain amount is excreted into urine [8,9]. Urinary L-FABP showed great potential for early and accurate detection of histological and functional decline in both nephrotoxin-induced and ischemia–reperfusion injury [10]. Urinary L-FABP was reported as a clinical biomarker that may be of use in monitoring and predicting the progression of chronic renal disease [11], and in the diagnosis of acute kidney injury (AKI) [12] or acute coronary syndrome [13].

8-Hydroxy-2′-deoxyguanosine (8-OHdG) is produced by the oxidization of deoxyguanosine, a composition factor of DNA, by free radicals such as active and generated oxygen. 8-OHdG is an excellent biomarker that closely reflects living cell damage caused by active oxygen, and the relationship between 8-OHdG and various stresses, including work, smoking [14–16], cancer, atherosclerosis and diabetes [17–22], has been clarified recently *in vivo*.

In postmortem chemistry, urine is not a practical alternative to blood; however, in forensic autopsy cases, blood samples are often not available. It is more stable for some markers to be analyzed in urine than in blood. For instance, in our preliminary study, it was revealed that urinary 8-OHdG was more stable than blood 8-OHdG [23]. Urine contains various biomarkers having biochemical information different from that of blood components, such as L-FABP. Previously, it was reported that increased urinary L-FABP levels represent an increase in the shedding of proximal tubule L-FABP,

* Corresponding author. Address: Department of Forensic Medicine, Faculty of Medicine, Fukuoka University, 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan. Tel.: +81 92 801 1011x3335; fax: +81 92 801 4266.
E-mail address: kasiwagi@fukuoka-u.ac.jp (M. Kashiwagi).

1344-6223/$ - see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.legalmed.2012.11.001

rather than just reflecting increased filtration of high serum L-FABP [24].

In this study, urinary L-FABP and 8-OHdG were investigated as potential supplementary biomarkers to elucidate the antemortem pathophysiological condition, especially the survival/agony period.

2. Materials and methods

2.1. Forensic autopsy cases

One hundred ninety-six urine specimens were collected from forensic autopsies performed in the Department of Forensic Medicine, Faculty of Medicine, Fukuoka University. All of the samples were collected within one-week postmortem interval (PMI) and centrifuged at 5 °C to remove sediments. The supernatant was stored at −30 °C until the assays. Obvious cases of renal disease were excluded.

The cause of death, the survival period, and the PMI had been diagnosed based on the autopsy, pathological, toxicological and other examination findings. Further, the clinical records and the police investigated records were referenced for these diagnoses.

A summary of the cases, including age, gender, PMI, and survival/agony period in each cause of death, is shown in Table 1. The possible error in estimating PMI was within 1 h in witnessed death cases ($n = 138$), ranged from several hours to about 12 h ($n = 48$) and within 24 h ($n = 10$) in other cases, in accordance with circumstantial evidence and postmortem findings of their cases.

The examined cases, for which the survival period was known, were divided into three groups according to the suspected survival/agony period: within 1 h (group A), within 24 h (group B), and over 24 h (group C). One hundred eighteen cases were included in group A, 29 in group B and 23 in group C. Another 26 cases had an unknown survival duration.

2.2. Quantitative analysis of L-FABP, 8-OHdG, creatinine, and urea

L-FABP was measured using an L-FABP ELISA kit (Human L-FABP ELISA Kit; CMIC Co. Ltd., Tokyo, Japan) according to the manufacturer's instructions [11]. Using an 8-OHdG-ELISA kit (New 8-OHdG Check; NIKKEN SEIL Co. Ltd., Shizuoka, Japan), 8-OHdG levels were measured according to the manufacturer's instructions [25,26]. Creatinine (Cr) was measured by enzymatic assay involving a peroxidase-coupled reaction [27]. Urea was measured by urease and leucine dehydrogenase methods [28].

This study was approved by the Fukuoka University School of Medicine Ethics Review Board (No. 389).

2.3. Statistical analyses

Histograms of the overall distributions of urinary L-FABP/Cr and 8-OHdG/Cr were significantly right-skewed and indicated the need for nonparametric testing. We used the Mann–Whitney U test to analyze differences in measurements between male and female cases, and between natural and traumatic causes of death. Correlation analysis was performed by the Spearman rank correlation. The Kruskal–Wallis test was used to test for overall group differences and the Steel–Dwass test, a post hoc nonparametric multiple comparisons procedure, was used to test for between-group differences in the causes of death and the survival/agony period. These statistical analyses were performed with the JMP 9 software program (SAS Institute, Inc., Cary, NC). $P < 0.01$ was considered significant.

2.4. Cut-off point of urinary L-FABP/Cr and 8-OHdG/Cr on the ROC curve

In order to clarify the standard value of urinary L-FABP/Cr and 8-OHdG/Cr for diagnostic implications, we examined the cut-off point of the value using the receiver operating characteristic (ROC) curve [29–31].

3. Results

Urinary L-FABP and 8-OHdG levels were corrected by urinary Cr levels, yielding urinary L-FABP/Cr and 8-OHdG/Cr. The median of urinary L-FABP/Cr (μg/g Cr) was 11.8 (interquartile range (IQR): 0–161.1). The median of urinary 8-OHdG/Cr (μg/g Cr) was 14.9 (IQR: 10.9–23.6).

Both urinary L-FABP/Cr ($P = 0.1258$) and 8-OHdG/Cr ($P = 0.1177$) showed no significant difference between males and females. Furthermore, there were no statistically significant correlations using the Spearman rank correlation between urinary L-FABP/Cr and age or PMI, or urinary 8-OHdG/Cr and age or PMI.

There was also no significant correlation between urinary L-FABP/Cr and 8-OHdG/Cr. In cases in which alcohol was detected from blood or urine (>0.01 mg/ml), no significant differences were

Table 1
Summary of cases.

Cause of death	n	Age Median (IQR)	Gender	PMI Median (IQR)	Survival time[b] Group A/B/C
Natural diseases	41	61 (37.25–72)	32/9	24 (14.75–56.5)	5/2/15
Cardiac diseases	19	66.5 (46–75.5)	15/4	27 (20–60)	1/0/4
Cerebrovascular stroke	6	55.5 (44–64.25)	5/1	18 (11.5–114)	2/2/0
Infections	5	53 (0.42–67)[a]	5/0	20 (4.5–26)[a]	0/0/5
Others	11	44 (28–76)	7/4	19 (12–48)	2/0/6
Trauma	155	52 (36–64)	117/38	20 (12–36)	113/27/8
Injury	63	51.5 (34.75–61)	52/11	15.5 (11–27)	41/11/7
Drowning	28	53 (37–66)	22/6	60 (36–72)	26/2/0
Fire fatality	20	62 (50.5–67.5)	12/8	15 (10–23.5)	20/0/0
Mechanical asphyxiation					
Strangulation	15	48 (27–67)	11/4	30 (15–34)	14/1/0
Choking	6	43 (21.25–54.25)	4/2	12 (9.375–33.75)	5/1/0
Intoxication	9	41 (31.5–54)	5/4	30 (14.5–54)	2/5/1
Hypothermia	8	74 (51.25–80.5)	5/3	20.5 (11–60)	1/6/0
Others	6	43 (25.25–55.75)	6/0	25 (15.75–49.5)	4/1/0

IQR: interquartile range; gender: male/female.
[a] For causes of death that number five or less, the number in parentheses expresses the range.
[b] The number of the cases does not include cases with an unknown survival/agony period.