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Blood creatinine level in postmortem cases

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ABSTRACT

Blood chemical analysis for the diagnosis of diseases in forensic cases should be conducted in the same way as for clinical cases. However, it is sometimes difficult to obtain serum samples in forensic cases because of postmortem changes such as hemolysis and putrefaction. This study aimed to evaluate renal function in postmortem cases by blood creatinine analysis. The blood creatinine level was measured by high performance liquid chromatography (HPLC) using whole blood samples taken from 77 postmortem cases, and the relationships between blood creatinine level, postmortem interval, and cause of death were examined. The median blood creatinine level was found to be 1.15 mg/dL, with no significant differences between blood samples taken from different parts of the body. The blood creatinine level was stable for 3 days after death and gradually increased after that period, in line with a previous study using enzymatic analysis that found the serum creatinine level was stable in the early postmortem period. The blood creatinine level was high in the cases of blunt injury, intoxication, and in deaths caused by fire. This was considered to reflect acute renal dysfunction. However, the postmortem blood creatinine level remained higher than the clinical normal value despite omitting cases with renal dysfunction from the analysis. Therefore, we next investigated the change in postmortem creatinine levels in mice and found that the blood creatinine level increased with the emergence of rigor mortis. Our findings indicate that HPLC is useful in the postmortem evaluation of renal function even in the cases where serum cannot be obtained. However, the presence of rigor mortis should be considered in the evaluation of blood creatinine values.

1. Introduction

Creatinine is a metabolite of creatine phosphate, which is produced from creatine and adenosine triphosphate (ATP) by creatine kinase. Creatine phosphate is stored in muscle tissue as an energy source, and after giving its phosphate to adenosine diphosphate, it becomes creatinine through the breakdown of creatine. Creatinine is carried to the kidneys by blood and is excreted in the urine. Accordingly, creatinine is widely used as a biomarker of renal function and muscle disorders [1,2] in clinical practice.

Previous studies in forensic medicine have reported that creatinine is stable in cadaveric blood and body fluids [3–8]. Recently, Uemura et al. [8] reported that postmortem serum creatinine level was stable for 3 days after death and that its value was not affected by the cause of death except in the case of renal dysfunction. They concluded, therefore, that creatinine might be a valuable biomarker in postmortem cases. However, the measured value was 3.29 ± 2.35 mg/dL in their study, which was much higher than normal clinical values of around 0.6–1.2 mg/dL in men and 0.4–0.9 mg/dL in women. Postmortem creatinine values have also been reported to be high compared to clinical values [5–7].

Measurement of postmortem creatinine is usually performed by enzymatic analysis, as it is for fresh serum samples taken from living individuals. However, measurement of creatinine level in postmortem cases may be affected by postmortem hemolysis, bilirubin, and chyle. These factors have been investigated in the clinical setting [9–12], but changes are more marked in postmortem samples than those in clinical samples. Moreover, in many cases, serum cannot be obtained because of postmortem changes.

In efforts to develop a new method for postmortem evaluation of renal function, in this study we measured blood creatinine levels in postmortem cases using high performance liquid chromatography (HPLC), and compared the results with those obtained by the enzymatic method. We also investigated creatinine levels in samples taken from the right and left atria and from peripheral blood. Finally, we investigated the change in creatinine levels during the early postmortem period in murine blood samples.

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2. Materials and methods

2.1. Blood samples

This study was approved by the institutional review board (No. E-222). Human whole blood samples were collected from 77 autopsy cases (52 males, 25 females; mean age, 55.1 ± 19.3 years, median, 59.0 years) from 2008 to 2009. All samples were stored at −70 °C until use. The postmortem intervals were 0.5 days in 4 cases, 0.5–1 day in 31 cases, 1–2 days in 16 cases, 2–3 days in 10 cases, 4–7 days in 7 cases, 8 days–1 month in 6 cases, and more than 1 month, in 3 cases. Cause of death determined by autopsy in cases where postmortem time was within 3 days was as follows: penetrating trauma in 4, blunt trauma in 9 cases, thermal injury in 10, carbon monoxide (CO) poisoning in 3, asphyxiation in 6, drowning in 7, drug intoxication in 4, internal cause of death in 13, and unknown cause (i.e., functional death, such as cardiac arrhythmia) in 5.

Among the 77 cases, 11 cases (1 penetrating trauma case, 2 blunt trauma cases, 1 thermal injury case, 2 asphyxiation cases, and 4 internal cause of death cases) were found to have renal dysfunction, as indicated by medical history and confirmed by pathological changes in renal histological sections. This renal dysfunction group was compared to the remaining cases without renal dysfunction. No relationship between renal dysfunction and cause of death was observed.

In addition to whole heart blood, right and left atrial blood and peripheral blood from the left femoral vein were also collected for comparison in 10 cases.

2.2. Animal samples

A total of 16 male ICR mice (4 weeks old, CLEA Japan Inc., Tokyo) were used. After the mice were killed by a lethal dose of anesthesia, blood was obtained from 4 mice each at baseline, 30 min, 1 h, and 2 h after death for measurement of serum creatinine.

2.3. Blood creatinine analysis by HPLC

Measurement of blood creatinine concentration was done by HPLC on an Inertsil ODS-SP column (3-μm and 5-μm particle sizes, 150 × 3.0 mm i.d., GL Sciences Inc., Tokyo). The HPLC system consisted of a TOSOH DP-8020 HPLC pump (flow rate of 0.3 mL/min), TOSOH UV-8020 UV detector (set at 234 nm), and CO-8020 column heater (set at 40 °C). The HPLC mobile phase consisted of 60% of 10 mM KH_2PO_4 (pH 3.5) and 40% of CH_3CN with 4 mM SDS. A total of 100 μL of acetonitrile was added to an equivalent volume of whole blood sample. After vortex mixing, samples were centrifuged at 10,000 *g* for 10 min. A total of 100 μL of supernatant was diluted 10-fold and analyzed by HPLC.

Stock solutions of creatinine at 1, 2.5, 5, 7.5, and 10 mg/dL were prepared by dissolving solid creatinine (Nacalai Tesque, Kyoto, Japan) in deionized water. A volume of 5 μL of stock solution was analyzed to establish a calibration curve. Stock solutions were made and analyzed 3 times. The calibration curve was determined by plotting the peak areas of the samples.

In 26 cases (two sets of 13 cases), enzymatic analysis was performed in addition to HPLC analysis for comparison.

2.4. Statistical analyses

Comparisons between groups were performed using the Mann–Whitney U test. Analyses were performed using Microsoft Excel, and a p-value less than 0.05 was considered statistically significant. In Figs. 2–4, the results of data analysis are shown as box plots, in 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 maximum and minimum values.

3. Results

3.1. Accuracy of creatinine measurement by HPLC

Fig. 1(a) is a chromatogram of a representative postmortem blood sample, and Fig. 1(b) is the chromatogram of the 10 mg/dL standard creatinine. The R^2 values of the calibration curves were more than 0.995. Using this regression line, five standard samples were analyzed three times for 3 days, and the data showed a high level of reproducibility (SD = ±0.1) (Table 1). The range of values for standard samples STD1, STD2, STD3, STD4, and STD5 were 0.9–1.2 mg/dL, 2.4–2.6 mg/dL, 4.8–5.1 mg/dL, 7.4–7.6 mg/dL, and 9.8–10.1 mg/dL, respectively.

3.2. Comparison between the conventional enzymatic method and HPLC method

We compared the levels of creatinine of 13 postmortem blood samples that were analyzed using both the conventional enzymatic method and HPLC method. The creatinine value was significantly different between the two methods: 0.82 ± 0.32 mg/dL using the HPLC method and 1.84 ± 0.63 mg/dL using the enzymatic method. There was no correlation between these methods ($R^2 = 0.048$). However, the data obtained with the enzymatic method showed variation in samples in which hemolysis and chyle were observed. Therefore, we selected another 13 samples without hemolysis and chyle and measured the creatinine level. A high correlation was observed ($R^2 = 0.94$).

3.3. Creatinine level in postmortem blood samples

Table 2 shows the blood creatinine levels measured by HPLC. The median value of blood creatinine for all 72 cases was 1.15 mg/dL (males 1.15 mg/dL, females 1.18 mg/dL). There was no statistical significant difference between blood creatinine level and age or sex.

3.4. Relationship between blood creatinine level and postmortem interval

Fig. 2 shows the relationship between blood creatinine level measured by HPLC and postmortem interval. The median values of blood creatinine were 0.73 mg/dL (0.5 days), 1.04 mg/dL (1 day), 1.13 mg/dL (2 days), 0.89 mg/dL (3 days), 2.17 mg/dL (4–7 days), 2.18 mg/dL (8 day–1 month), and 4.63 mg/dL (more than 1 month). Blood creatinine level was stable in the early stage but increased markedly after 1 month, with a significant difference seen between the results at 0.5 day, 1 day, 2 days, or 3 days and 1 month.

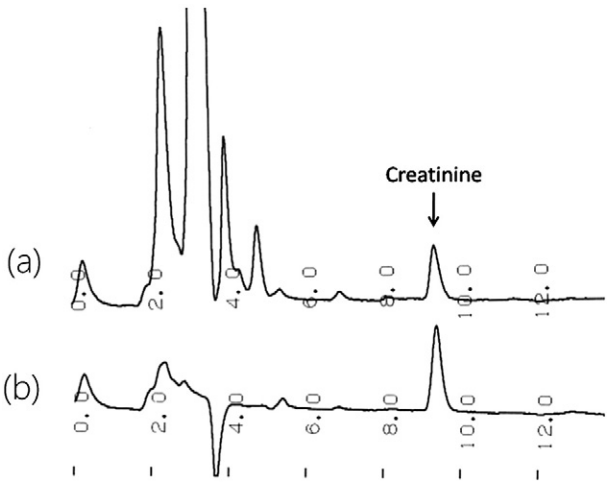

Fig. 1. Chromatogram of creatinine obtained by HPLC. (a) A representative chromatogram of a sample and (b) a chromatogram using a creatinine standard at 10 mg/dL. The X-axis shows retention time (min) and the Y-axis shows the optical density at 234 nm in HPLC.