

Original Communication

Biochemical blood markers and sampling sites in forensic autopsy

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Abstract

Forensic pathologists often hesitate to use biochemical blood markers due to the risk of large postmortem changes and deviations from healthy subjects. Biochemical analyses of postmortem blood, if possible, may help to evaluate pathological status and determining the cause of death in forensic diagnosis, for example, in sudden unexpected death without obvious cause, or young adults with no apparent cause of death or antemortem information. Even commercially available biochemical markers were re-evaluated in the blood samples of 164 forensic autopsy cases. Biochemical markers examined were HbA1c, fructosamine, blood nitrogen urea (BUN), creatinine, total protein, total bilirubin, γ -glutamyl transpeptidase (γ -GTP), triglyceride, total cholesterol, C-reactive protein (CRP) and pseudocholeline esterase (pChE). We collected cardiac blood (left cardiac blood and right cardiac blood) and peripheral blood (femoral vein blood) to clarify the differences in measured values by sampling site. The measured values were analyzed in relation to postmortem interval, etiology of death and sampling sites. Of all eleven markers, HbA1c is the most useful and reliable because of its negligible postmortem changes and small deviation from healthy subjects. Total bilirubin, BUN, CRP and total cholesterol can be useful if we set appropriate limit ranges and pay attention to the interpretation. For the evaluation of changes due to postmortem intervals, none of the markers except for triglyceride showed significant changes up to three days postmortem. As for sampling sites, femoral vein blood is generally recommended considering postmortem changes, but left cardiac blood was suitable for creatinine, pChE, and total cholesterol. For clinical forensic diagnosis of biochemical blood markers, we must determine the “forensic abnormal value” after collecting more cases by known causes with more information about the population.

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1. Introduction

In forensic autopsies, antemortem information such as present and past illnesses is rarely available, and the forensic

pathologist must make a decision on the basis of autopsy findings. In clinical medicine, a lot of information, including biochemical markers in the blood, is available and contributes to the diagnosis of disease, in addition to physical findings. For forensic pathologists, biochemical analysis of the postmortem blood, if possible, may help in evaluating pathological status and determining cause of death in such cases. However, to date, forensic pathologists have often hesitated to use biochemical markers in the blood for forensic diagnosis due to concern about large postmortem changes and large deviations from healthy subjects.

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There is a complete review by Coe on autopsy samples, covering a lot of markers¹ and a report by Tsuji et al. on an animal study,² with respect to postmortem changes in the markers. Additionally, there are several reports on the usefulness of the individual markers in autopsy diagnosis.^{3–15} However, there is insufficient information on commercially available blood markers and differences in sampling sites in forensic autopsy cases. We chose to measure eleven clinically available biochemical markers in the blood from three sampling sites. The cost for measuring these eleven markers by one sample was very low because these markers are routinely measured in clinical medicine. The cost/benefit factor is very important for low-budget forensic facilities.

Our aim was to re-examine and evaluate commercially available blood markers and their usefulness in forensic diagnosis. We investigated how biochemical markers in the blood suffer from postmortem changes and showed differences due to the etiology of death, while determining suitable sampling sites. These will be useful for taking post-mortem changes into consideration when selecting markers and interpreting results.

2. Materials and methods

2.1. Blood samples

With the permission of the Ethics Committee of Graduate School of Medicine, The University of Tokyo (No. 690), blood was obtained from 164 consecutive autopsy cases in our department from April 2003 to March 2006 (age 0–98, average age 54.9 ± 21.8 , median age 57.0, male 112, female 52). The postmortem interval of the sampling of specimens are as following: 0–12 h (25 cases), 13–69 h (69 cases), 25–48 h (54 cases), 49–72 h (16 cases). Causes of death were as follows: blunt injury (52 cases), sharp injury (seven cases), asphyxiation (18 cases), drowning (four cases), fire death (five cases), intoxication (nine cases), internal death (39 cases) and others (30 cases). Care was paid so that the deceased would not be identified from the data. The bodies were preserved refrigerated and forensic autopsies were performed within a day after they were found. The blood was sampled from the right and left heart cavities and femoral vein within 72 h postmortem, as far as possible. As soon as whole blood was obtained, the sera was separated by centrifugation at 1000g, 30 min, and stored at -20°C , while the whole blood was stored at 4°C as long as a day, until shipping to the laboratory of SRL, Co. Ltd. (Tokyo, Japan), where the samples were analyzed within a day.

2.2. Biochemical analyses

We selected the biochemical markers on the basis of post-mortem stability reported in previous reports.^{1–4} The 11 markers included HbA1c (latex aggregation method, standard range: 4.3–5.8%) and the fructosamine (calorimetry method: 205–285 mM) for chronic hyperglycemia, blood

nitrogen urea (BUN) (urease UV method: 6–20 mg/dL) and creatinine (enzyme method: male 0.61–1.04 mg/dL, female 0.47–0.79 mg/dL) for renal failure, total protein (Biu-ret method: 6.7–8.3 g/dL) for malnutrition, total bilirubin (vanadinate oxidation method: 0.2–1.0 mg/dL) and γ -glutamyl transpeptidase (γ -GTP) (JSCC standardization method: male < 70 IU/L, 37°C , female < 30 IU/L, 37°C) for liver function, triglyceride (enzyme method: 50–149 mg/dL) and total cholesterol (enzyme method: 150–219 mg/dL) for hyperlipidemia, C-reactive protein (CRP) (latex aggregation method, < 0.3 mg/dL) for inflammation, pseudocholine esterase (pChE) (rate assay, male 242–495 IU/L, 37°C , female 200–495 IU/L, 37°C) for liver function and organic phosphate poisoning. The sera volume of required to measure the 11 markers was 2 mL.

The laboratory rejected 2.4% of samples for bilirubin measurement, but not those for other markers. However, we could not measure all the markers in substantial numbers of cases because of a lack of sample volume.

2.3. Statistical analyses

Data are expressed as the mean \pm SD. Statistical significance was determined as follows: for postmortem interval, Spearman's rank correlation was carried out (Table 2). For etiology of death, one-way ANOVA was carried out. When there was a difference among groups, Scheffe's posthoc test between multiple groups was performed (Table 3). For regional differences, one-way repeated measures ANOVA was carried out. When there was a difference among groups, a paired *t*-test for pair-wise comparisons was performed (Table 4). The software for the above-mentioned statistical analysis was Statview Ver. 4.11 (Abacus Concepts Inc., Berkeley, CA). Significant level was 0.05 (5%).

3. Results

We summarized the data for right cardiac blood, which were obtained in almost all cases (Table 1). First, HbA1c showed almost the same mean value as healthy subjects, and a very low ratio of abnormal values (24.8%), as compared with fructosamine (77.7%), another marker for chronic hyperglycemia. The next group of markers showed much higher mean values than the healthy subjects, and a higher ratio of abnormal values (37.3–95.1%). This group included total bilirubin, triglyceride, BUN, CRP, γ -GTP, fructosamine and creatinine. In the third group, pseudocholine esterase (pChE) and total cholesterol showed lower mean values than the healthy subjects and a higher ratio of abnormal values (64.1%, 72.9%). In the last group, total protein showed almost the same mean value as healthy subjects, but a wide variability, and therefore, a high ratio of abnormal values (75.3%).

To evaluate changes due to postmortem intervals, we classified postmortem intervals into four groups (0–12 h, 13–24 h, 25–48 h, 49–72 h) and carried out correlation analysis between the results obtained for each of the

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