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SHORT COMMUNICATION

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Usefulness of a latex agglutination assay for FDP D-dimer to demonstrate the presence of postmortem blood

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Abstract D-dimer, a specific fragment resulting from degradation of cross-linked fibrin, is an essential marker for the diagnosis of disseminated intravascular coagulation (DIC). Rapid assay for D-dimer using monoclonal antibody coated-latex particles might be useful for discriminating between postmortem and antemortem blood in bloodstains. We tried to detect D-dimer in nine postmortem blood samples by the rapid latex agglutination assay and to quantify them automatically using the latex photometric immunoassay system. The results showed that all samples were positive and that their amounts of D-dimer were 335–2,800 μg/ml (the normal blood level, <1 μg/ml; the pathogenic blood level with DIC, 1–100 μg/ml). Next, nine stains made of postmortem blood were examined by the rapid latex agglutination assay. The result showed that only one case (D-dimer 335 μg/ml blood) showed weak positive while the others (D-dimer 600–2,800 μg/ml blood) were positive. The present study indicates that the latex agglutination assay for D-dimer can be useful to demonstrate the presence of postmortem blood.

Keywords D-dimer · FDP · Latex agglutination assay · Fibrin · Postmortem blood

Introduction

Bloodstain analysis, including species identification, blood grouping, and DNA typing, provide much useful information for the resolution of crime. Discrimination of postmortem blood from antemortem blood is also an important examination in bloodstain analysis. We have often recovered dismembered bodies buried in the ground or thrown in the sea. As a matter of fact, although it is very important for us to determine the diagnosis of vitality of wounds, if a bloodstain discovered in one place was made of antemortem blood, this would indicate that the victim might have been killed in that place, while if a bloodstain discovered in another place was made of postmortem blood, the victim might have been dismembered in that place after death.

For the discrimination of antemortem and postmortem wounds, various methods have been reported (Bonelli et al. 2003, Ortiz-Rey et al. 2003, Baroldi et al. 2001, Hernandez-Cueto et al. 2000, Ortmann et al. 2000). Bonelli et al. showed that the detection of mast cells with immunohistochemical techniques can lead to a high level of discrimination between antemortem and postmortem lesions, and Ortiz-Rey et al. analyzed the expression of fibronectin and tenascin in wounds. The other hand, some methods have been also reported for discrimination of postmortem blood from antemortem blood, including a Dot-ELISA for human myoglobin (Miyaishi et al. 1994), scanning electron microscopy (Muraoka, 1980), thin layer chromatography (Satoh et al. 1975), and quantification of nucleotides by high-performance liquid chromatography (Sugie et al. 1995). However, these techniques have their specific advantages and limitations.

Generally, it is known that D-dimer is a specific fragment resulting from cross-linked fibrin and is an essential marker for diagnosis of DIC. Low concentrations of cross-linked fibrin derivatives containing D-dimer are detected in normal blood, and high levels are found in patients with DIC and in the majority of patients having deep vein thrombosis or pulmonary embolism (Elms, 1983, Wada et al. 2003, Akman et al. 2004). To aid in the diagnosis and evaluation of DIC and other conditions, a monoclonal antibody coated-latex agglutination assay specific for D-dimer has been widely and clinically employed (Ikematsu and Kuroso, 1989, Bates et al. 2001). Compared to enzyme

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immunoassay, the latex agglutination assay is less sensitive, but the latex procedure provides a rapid and less elaborate test for elevated levels of cross-linked fibrin degradation products in patients with thrombosis (Elms et al. 1986). With change after death, high concentrations of fibrinogen and fibrin degradation products (FDP) are assumed to be produced in postmortem blood. Rutty et al. (2003) also showed to be present high concentrations of D-dimer in postmortem blood as results of the coagulation test. Therefore, the latex agglutination assay for D-dimer might be useful to distinguish whether a bloodstain left at the scene of a crime was made of antemortem or postmortem blood.

We tried to detect D-dimer in antemortem and postmortem bloods using the rapid latex agglutination assay and to quantify them automatically using a latex photometric immunoassay system. It was investigated whether or not the commercial test for the detection of D-dimer is useful to demonstrate the presence of postmortem blood in bloodstains.

Materials and methods

Subjects

Normal blood samples were obtained from four healthy laboratory volunteers (26–39 years). A portion of blood taken was immediately attached to gauze and was kept for preparation of the bloodstain at room temperature. The rest of the blood was centrifuged at 3,000 rpm for 5 min and its plasma was removed. The bloodstain was allowed to dry for two weeks then cut into 2×2-mm-sized pieces, and an extraction procedure was performed using Tris-buffer (pH 8.0) 30 μl for 5 h at room temperature. After centrifugation at 3,000 rpm for 5 min, the supernatant was removed.

Nine blood samples from deceased persons (7–83 years) were obtained at postmortem examination 10 h to 7 days after death. Extracts from the postmortem bloodstain and sera were obtained in the same manner as in the normal blood samples.

Latex agglutination assay

As the latex agglutination assay, Iatro DD/E Test (Dia-Iatron, Tokyo, Japan) and Rapidia-D dimer II (Fujirebio, Tokyo, Japan) were used. Twenty-microliter aliquots of latex reagent were mixed on a reaction plate with 20 μl of antemortem plasma, postmortem serum or extracts from the bloodstains. After gentle rotation for 3 min, the test was examined for agglutination against a black background.

Quantitative analysis of D-dimer

A latex photometric immunoassay system (LPIA-200, Mishima Olympus, Shizuoka, Japan) was used. The amount of D-dimer was measured using LPIA-ACE D-D dimer (Dia-Iatron, Tokyo, Japan) as reagent, 0.5 ml plasma or serum as sample at 950-nm absorbance. The amount of FDP was also measured using LPIA FDP-P (Dia-Iatron, Tokyo, Japan) as reagent, 0.2 ml plasma or serum as sample at 800-nm absorbance.

Results and discussion

This initial study shows the feasibility of a latex agglutination technique to detect elevated levels of D-dimer in postmortem blood. The unique advantages of this latex assay are that it does not interact with fibrinogen and that it specifically detects fibrinolysis rather than fibrinogenolysis. Therefore, it can be sensitive to only some diseases such as DIC. We tried to detect the D-dimer of four normal bloods and nine postmortem bloods using Iatro DD/E Test. The results showed that all antemortem bloods were negative and all postmortem bloods were positive, and as shown in Table 1, the amount of D-dimer in postmortem bloods was 335–2,800 μg/ml (all normal bloods were less than 1 μg/ml). Furthermore, another kit, Rapidia-D dimer II, showed the same results. In relation with the reaction of these latex agglutination kits to the purified FDP fractions, some researchers have reported that these kits reacted

Table 1 D-dimer and FDP concentrations in antemortem plasma and postmortem serum measured by LPIA-200

Sample	Year	The cause of death	The time after death	D-dimer (μg/ml)	FDP(μg/ml)
AP1	39			<0.50	<2
AP2	35			0.52	<2
AP3	26			<0.50	<2
AP4	33			<0.50	<2
PS 1	60	Traumatic shock	24–36 h	916	932
PS 2	53	Stab wound	5–7 days	846	894
PS 3	81	Ligature strangulation	4–5 days	1,570	1,560
PS 4	7	Hanging	18–24 h	993	1,090
PS 5	73	Traumatic shock	8–15 h	335	375
PS 6	46	Ligature strangulation	24–36 h	600	742
PS 7	83	Subarachnoid hemorrhage	8–12 h	1,434	1,470
PS 8	79	Traumatic shock	8–12 h	1,121	1,260
PS 9	83	Traumatic shock	15–24 h	2,800	2,950

The LOQ of D-dimer and FDP were 0.50 and 2 μg/ml, respectively
AP Antemortem plasma, *PS* postmortem serum

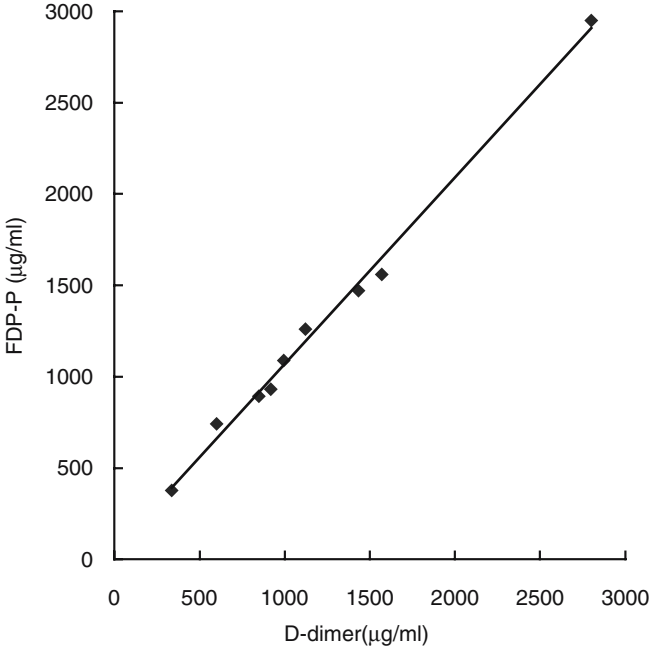

Fig. 1 Correlation between FDP and D-dimer in postmortem bloods measured by LPIA-200. The regression line represents $y=1.021x+48.481$ ($r=0.997$, $n=9$)

Table 2 Reaction of D-dimer in antemortem and postmortem bloodstains using the latex agglutination assay

Sample	D-dimer (μg/ml blood)	Iatoro DD/E Test	Rapidia-Ddimer II
AB 1	<0.50	−	−
AB 2	0.52	−	−
AB 3	<0.50	−	−
AB 4	<0.50	−	−
PB 1	916	+	+
PB 2	846	+	+
PB 3	1,570	+	+
PB 4	993	+	+
PB 5	335	±	−
PB 6	600	+	+
PB 7	1,434	+	+
PB 8	1,121	+	+
PB 9	2,800	+	+

AB Antemortem bloodstain, *PB* postmortem bloodstain, + positive, − negative, ± weak positive

strongly to DD/E complex fraction and D-dimer fraction, and slightly to high molecular weight derivative fractions and late D-dimer, but little to fibrinogen, D monomer, and E fragment (Francis et al. 1980, Mihalyi, 1983, Hunt et al. 1985, Arai 1986, Kuroso, 1986). We also tried to quantify FDP, which reflects not only the second fibrinolysis but the first one, by using anti-FDP monoclonal antibody. The result showed that the amount of FDP in postmortem bloods was 375–2,950 μg/ml (normal bloods were less than 2 μg/ml). As shown in Fig. 1, the correlation between FDP and D-dimer in postmortem bloods was significant, and it has been reported that the correlation between Iatro DD/E Test and Rapidia-D dimer II to D-dimer was close (Shinbo et al. 1997). These results indicates that high concentrations of FDP such as D-dimer are present in postmortem bloods, and the latex agglutination assay for D-dimer can be useful to determine postmortem bloods. A representative agglutination pattern of antemortem and postmortem bloods was shown in Fig. 2.

Next, nine bloodstains were measured using the rapid latex agglutination assay. The results showed that only one case (D-dimer 335 μg/ml blood) showed weak positive while the others (D-dimer 600–2,800 μg/ml blood) were positive (Table 2). As well, the limit of detection (LOD) of D-dimer in bloodstains was determined in D-dimer 0–500 μg/ml blood concentrations. The LOD was also 300–400 μg/ml blood. These results indicates that the detection of D-dimer in bloodstains may determine postmortem blood because the amount of D-dimer in antemortem blood is assumed to be less than 100 μg/ml blood.

In this study, the amounts of D-dimer differed among the nine postmortem blood samples, ranging from 335 to 2,800 μg/ml. The differences may be attributable to differences in the cause of death, in the interval between death and examination, in the ages of the victims, or other factors. Since it was difficult to obtain blood samples shortly after death, we could not examine the amounts of D-dimer in relation to time after death. Generally, as soon as blood has stopped circulating in vessels after death, the blood starts to coagulate by the natural activation of factors along the coagulation pathways. After a while, the increased activation of factors involved in fibrinolysis

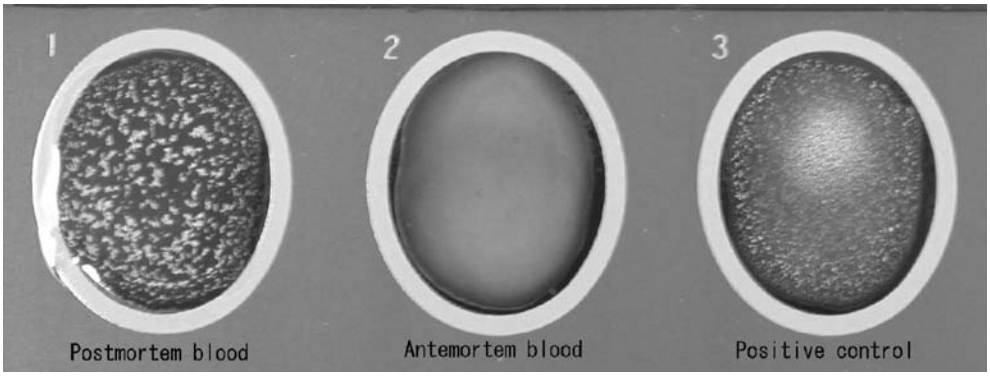

Fig. 2 A representative agglutination pattern of antemortem and postmortem bloods: *1* postmortem blood (D-dimer 600 μg/ml), *2* antemortem blood (D-dimer <0.50 μg/ml), *3* positive control

changes coagulated blood back into fluid. It is very difficult to discuss the time course of coagulation and fibrinolysis of blood shortly after death because it is impossible to collect blood samples from a human immediately after death. However, we found a very old study of blood conditions immediately after death (Sanaka, 1953). That author reported that blood in the hearts of condemned criminals showed increased coagulation until about an hour after death, after which it returned to fluidity by fibrinolysis, until all blood in the heart was fluid at 2–3 h after death. Although we do not think that these results, which were obtained using only heart blood with death by hanging (acute death), conform to every case, they do provide very important information. Therefore, we suggest that the amounts of D-dimer in bloodstains prepared a few hours after death might be sufficient for detection by latex agglutination assay.

So far, various methods have been reported for the discrimination of antemortem and postmortem bloodstains (Satoh et al. 1975, Muraoka, 1980, Miyaishi et al. 1994, Sugie et al. 1995). Miyaishi et al. exploited the fact that postmortem blood contains more myoglobin than does antemortem blood. They showed that their dot-ELISA was able to detect human myoglobin from bloodstains containing more than 10 μg/ml myoglobin. Using this method, they correctly identified all antemortem and postmortem bloodstains that they examined. Sugie et al. tried to quantitate ATP and its related compounds by high-performance liquid chromatography to identify antemortem or postmortem bloodstains. The results showed large amounts of ATP in antemortem bloodstains but not in postmortem bloodstains. The results also indicated that quantitation of ATP-related compounds may be useful for identifying whether bloodstains are antemortem or postmortem. However, these methods are more complicated and detection more time-consuming than our method.

In conclusion, the latex agglutination assay for D-dimer is a rapid and simple kit to detect postmortem blood. The detection of D-dimer in bloodstains by this assay is a definite indication of postmorten blood.

Case report

A 52-year-old male had been absent from work without notice. Two weeks later, his older sister went to his house and discovered many bloodstains on a Tatami mat although his body could not be found. Furthermore, about 3 million Japanese yen had been drawn on a bank. One week later, a policeman discovered the missing man's car on a mountain road which was some distance from the house and found some bloodstains on the carpet of the car. These bloodstains were identified as that of the missing man by DNA typing although he had not been discovered. We tried to detect D-dimer in these bloodstains by the rapid latex agglutination assay. The result showed that the former bloodstain sample was negative and the latter one was positive. Later, a man was arrested on suspicion of homicide. According to police information, it seemed that the man had killed the missing man in the house, drew the money out of the bank, and transported the corpse to the other place in the car.

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