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Detection of Hepatitis C Virus and Antibodies in Postmortem Blood and Bloodstains▽

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To evaluate the risk of accidental hepatitis C virus (HCV) infection, we examined whether anti-HCV antibodies and HCV RNA were detectable in HCV-infected blood samples from living donors, cadavers, and bloodstains. We showed that even after blood has left the body for several days, anti-HCV antibodies and HCV RNA may persist in it.

At the scenes of crimes or accidents, bloody materials are often handled without adequate precautions against infection because most first responders are not medical specialists. This study's objective was to test whether or not such samples are no longer potentially infectious. We used hepatitis C virus (HCV) as the representative infectious agent. To simulate the types of exposures first responders might encounter, we tested whether HCV RNA and antibodies are detected in blood and bloodstains kept at room temperature for up to 60 days and from blood of actual postmortem cases up to 14 days after death.

HCV-infected blood samples were obtained with informed consent from 12 patients (8 men and 4 women; mean age, 68.5 ± 10.7 years; range, 44 to 84 years) at the University Hospital, Kyoto Prefectural University of Medicine, and at Aiseikai Yamashina Hospital. Prior to our experiments, the serum titers of HCV RNA of all samples were determined, using the COBAS TaqMan HCV assay (Roche Molecular Systems, Pleasanton, CA), to range from 5.4 to 7.0 log IU/ml (average, 6.363 ± 0.42 log IU/ml). All samples were stored at −80°C until use.

Bloodstain samples were prepared by soaking cotton buds in 0.1 ml of HCV-infected whole-blood samples ($n = 8$) for 1 min and then drying them at room temperature for up to 60 days. Samples of HCV-infected whole blood ($n = 4$) were placed in sealed 2-ml test tubes and kept at room temperature for up to 60 days. The prepared blood and bloodstain samples were analyzed at 1, 3, 9, 27, and 60 days after preparation.

The postmortem whole-blood samples were obtained between December 2008 and April 2010 from 10 forensic autopsies performed on individuals (7 men and 3 women; mean age, 52 ± 13.15 years; range, 33 to 79 years) who had tested positive for anti-HCV antibodies. These blood samples were stored at −80°C for a week before use.

Anti-HCV antibodies from the bloodstain and whole-blood samples were detected using immunochromatography with Ortho Quick Chaser HCV antibody (Ortho Clinical Diagnostics, Tokyo, Japan). Before testing, the bloodstain samples were soaked in 200 μl saline; 100 μl of extracted solution was analyzed using immunochromatography.

HCV RNA was extracted from 100 μl of undiluted whole blood and 100 μl of solution extracted from blood-stained materials with a QIAamp viral RNA kit (Qiagen, Hilden, Germany). The RNA was eluted in 50 μl of RNase-free water and used for genome amplification of the partial core region using reverse transcriptase PCR (RT-PCR) with a One Step RT-PCR kit (Qiagen) in 50-μl aliquots containing 1 μl RNA, 2 μl Qiagen One Step RT-PCR enzyme mix, 400 μM deoxynucleoside triphosphate (dNTP), 0.6 μM concentrations of primers 256 (5′-CGCGCGACTAGGAAGACTTC-3′; sense) and 186 (5′-ATGTACCCCATGAGGTCGGC-3′; antisense), and Qiagen One Step RT-PCR buffer supplied by the manufacturer. The amplification was performed as described by Okamoto et al. (16). Reverse transcription was performed at 50°C for 30 min. DNA polymerase was initially activated at 95°C for 15 min for PCR. PCR amplification was performed for 35 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, followed by a final step at 72°C for 10 min. Amplification was carried out in a PC-320 thermal cycler (ASTEC, Fukuoka, Japan). The PCR product was mixed with a 6× loading buffer double dye and subjected to electrophoresis on a 1.5% agarose gel at 100 V for 30 min. The electrophoresed agarose gel was stained with ethidium bromide (0.5 μg/ml). The image from

TABLE 1. Results of anti-HCV antibody and HCV RNA detection in blood and bloodstain samples

Test sample type	No. of positive results after the following days of storage:				
	0	3	9	27	60
Anti-HCV antibody					
Bloodstain	8	8	7	7	5
Blood	4	4	4	4	4
HCV RNA					
Bloodstain	8	8	8	8	7
Blood	4	4	4	4	4

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TABLE 2. Profiles of anti-HCV antibody-positive autopsy cases and HCV RNA detection results

Case	Age (yr)/ sex[a]	Postmortem time (day)	Cause of death	HCV RNA	Genotype
A	52/F	1	Drowning	+	1b
B	42/F	1	Unknown	+	1b
C	33/M	1	Hypothermia	+	2a
D	52/M	1	Drug intoxication	−	
E	46/M	1	Asphyxia	−	
F	45/M	2	Hemorrhage	+	1b
G	48/M	2	Drug intoxication	+	1b
H	62/M	2	Burn	−	
I	65/F	2	Strangulation	−	
J	79/M	14	Unknown	−	

[a] M, male; F, female.

the agarose gel was captured under UV transillumination on a LAS 4000 mini camera system (Fujifilm, Tokyo, Japan).

The limit of HCV detection of the RT-PCR method was 2.06 log IU/ml. This value was extrapolated from the results for five infected serum samples taken from a single serum sample (5.4 log IU/ml using the TaqMan method) that had been diluted to concentrations between ×100 and ×1,600. The genotype of the HCV strain was determined using the putative C gene of the HC-J4 isolate as described previously (16).

The analysis of anti-HCV antibodies and HCV RNA from 8 bloodstain samples and 4 whole-blood samples kept up to 60 days at room temperature is summarized in Table 1. On day 27, anti-HCV antibodies were detected in 7 of 8 bloodstain samples and in all 4 whole-blood samples. HCV RNA was detected in all samples. On day 60, anti-HCV antibodies were detected in 5 of 8 bloodstain samples and in all 4 whole-blood samples. HCV RNA was detected in 7 of 8 bloodstain samples and all 4 whole-blood samples.

Among the 10 anti-HCV antibody-positive autopsy blood samples, HCV RNA was detected in 5 samples (Table 2, cases A, B, C, F, and G). The genotype of the HCV isolated in case C was 2a, and that of the others was 1b.

The detection of HCV RNA and anti-HCV antibodies in these specimens does not prove that HCV could be transmitted to humans. Previous studies have demonstrated that the RNA of the entire HCV genome synthesized *in vitro* can infect chimpanzees and produce the progeny virus (10). However, although some models and tissue culture systems have been developed (e.g., replicon systems [11], JFH-1 cells [18], and immune-deficient mice [15]), infection and cultivation of wild-type HCV have not yet been successful in model systems. Therefore, we are unable to directly test whether the samples used in this study could infect human cells. Although these results do not prove that the samples were infectious, they highlight the need for first responders and law enforcement personnel to exercise caution when handling bloody materials, even if not fresh.

The results of our study may have an additional application. Recently, the number of unidentified cadavers has increased worldwide (3). The geographic distribution of various viruses has been used to determine the geographic origins cadavers (5–9). The worldwide distribution of HCV genotypes has also been reported (1, 2, 4, 12–14, 17, 19). In the present study, all of the samples were taken from Japanese individuals, and the viral genotypes were 1b and 2a, which are commonly detected in Japan. Therefore, it may be possible to estimate the geographic origin of a cadaver or bloodstain from the HCV viral genotype, if present. Further studies are necessary to confirm this hypothesis.

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