Detection of hepatitis C virus and antibodies in postmortem blood and bloodstains

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Abstract
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 leaving the body for several days anti-HCV antibodies and HCV RNA may persist in blood.

Text
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 from 12 patients (8 men and 4 men, mean age was 68.5 ± 10.7, range was 44-84 years) at the University Hospital, Kyoto Prefectural University of Medicine, and Aiseikai Yamashina Hospital with informed consent. 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.

Bloodstained samples were prepared by soaking cotton buds in 0.1mL of HCV-infected whole blood samples (n=8) for one minute and then drying 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 bloodstained 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 33-79 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 bloodstained samples and blood samples were detected using immunochromatography with Ortho Quick Chaser HCV Ab (Ortho Clinical Diagnostics, Tokyo, Japan). Before testing, the bloodstained samples were soaked in 200µL saline; 100µL of extracted solution was analyzed by immunochromatography.

HCV RNA was extracted from 100µL of undiluted whole blood and 100µL of solution extracted from bloodstained materials with a QIAamp Viral RNA kit (QIAGEN, Hilden, Germany). The RNA was eluted in 50µL of ribonuclease-free water and was used for the genome amplification of the partial core region using reverse transcriptase-polymerase chain reaction (RT-PCR) with a One Step RT-PCR Kit (QIAGEN) in 50µL aliquots containing 1µL RNA, 2µL QIAGEN One Step RT-PCR Enzyme Mix, 400µM dNTP and 0.6µM of No. 256 (5’-CGCGCGACTAGGAAGACTTC-3’, sense) and NO.186 (5’-ATGTACCCCATGAGGTCGGC-3’, antisense) primers, and 5 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 sec, 55°C for 30 sec, 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 electrophoresed onto a 1.5% agarose gel at 100V for 30 min. The electrophoresed agarose gel was stained with ethidium bromide (0.5 µg/mL). The image from 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 five infected serum samples taken from a single serum sample (5.4 LogIU/mL using TaqMan method) that was diluted to concentrations between X100 and X1600. The genotype of the HCV strain was determined using the putative C gene of the HC-J4 isolate as described (16).

The analysis of anti-HCV antibodies and HCV-RNA from 8 bloodstained 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 bloodstained samples and in all 4 blood samples. HCV-RNA was detected in all samples. On day 60, anti-HCV antibodies were detected in 5 of 8 bloodstained samples and in all 4-blood samples. HCV-RNA was detected in 7 of 8 bloodstained samples and all 4 blood samples.

Among the 10 anti-HCV antibody-positive autopsy blood samples, HCV-RNA was detected in 5 samples (lanes A-C, F, and G in Table 2). The genotype of the HCV isolated in case C was 2a and the others were 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 has 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 cadaver’s geographic origin (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.

Acknowledgements

We are grateful to the donors of blood samples. We thank Dr. Jiro Yasuda of the National Research Institute of Police Science for helpful discussions. This work was supported by a Grant-in-Aid for Scientific Research C (22590641).

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Table 1. Results of anti-HCV antibody and HCV RNA detection in blood and blood stained samples.

<table>
<thead>
<tr>
<th>Test sample type</th>
<th>Number of positive results after the following days of storage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Anti-HCV Antibody</td>
<td></td>
</tr>
<tr>
<td>Blood stained</td>
<td>8</td>
</tr>
<tr>
<td>Blood</td>
<td>4</td>
</tr>
<tr>
<td>HCV RNA</td>
<td></td>
</tr>
<tr>
<td>Blood stained</td>
<td>8</td>
</tr>
<tr>
<td>Blood</td>
<td>4</td>
</tr>
</tbody>
</table>
Table 2. Profiles of anti-HCV antibody positive autopsy cases and HCV RNA detection results.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/Sex</th>
<th>Postmortem time (day)</th>
<th>Cause of death</th>
<th>HCV RNA</th>
<th>genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>52/F</td>
<td>1</td>
<td>Drowning</td>
<td>+</td>
<td>1b</td>
</tr>
<tr>
<td>B</td>
<td>42/F</td>
<td>1</td>
<td>Unknown</td>
<td>+</td>
<td>1b</td>
</tr>
<tr>
<td>C</td>
<td>33/M</td>
<td>1</td>
<td>Hypothermia</td>
<td>+</td>
<td>2a</td>
</tr>
<tr>
<td>D</td>
<td>52/M</td>
<td>1</td>
<td>Drug intoxication</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>46/M</td>
<td>1</td>
<td>Asphyxia</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>45/M</td>
<td>2</td>
<td>Hemorrhage</td>
<td>+</td>
<td>1b</td>
</tr>
<tr>
<td>G</td>
<td>48/M</td>
<td>2</td>
<td>Drug intoxication</td>
<td>+</td>
<td>1b</td>
</tr>
<tr>
<td>H</td>
<td>62/M</td>
<td>2</td>
<td>Burn</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>65/M</td>
<td>2</td>
<td>Strangulation</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>J</td>
<td>79/M</td>
<td>14</td>
<td>Unknown</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

M: male, F: female