Etiologic Considerations of Fulminant Non-A, Non-B Viral Hepatitis in Japan: Analyses by Nucleic Acid Amplification Method

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The etiology of fulminant non-A, non-B hepatitis has remained unclear, even after the identification of hepatitis C and E viruses. To study the possible involvement of hepatitis B, C, D, and E virus infections, viral genomes were amplified by a sensitive polymerase chain reaction method in sera and liver tissues obtained from 20 patients serologically diagnosed with non-A, non-B fulminant hepatitis (n = 14), acute hepatitis severe type (n = 2), and ordinary acute hepatitis (n = 4). Hepatitis C or E virus RNA could not be detected in sera obtained at admission from these patients. Hepatitis B virus DNA was detected in sera from 3 patients with fulminant hepatitis and in liver from 9 patients with fulminant hepatitis or acute hepatitis severe type. These results suggest that HCV might not be involved in fulminant non-A, non-B hepatitis, and HBV might be related to some of the serologic “non-A, non-B” viral hepatitis cases in Japan.

Fulminant hepatitis (FH) is a most severe form of acute liver injury, and its outcome is often fatal. Thus far, its etiology has been examined by conventional serologic tests. The discoveries of hepatitis C virus (HCV) [1] and hepatitis E virus (HEV) [2] have enabled the development of sensitive serologic detection methods [3, 4]. It was shown that most cases of chronic non-A, non-B hepatitis were due to HCV infection [3]. Concerning acute HCV infection, we and others have reported that it is difficult to detect antibody to HCV in the early phase, and therefore nucleic acid detection methods might be required for diagnosing acute HCV infection [5, 6]. However, in regard to fulminant non-A, non-B hepatitis, the etiology has remained an enigma. For the diagnosis of such cases, conventional serologic methods may have certain limitations.

By applying molecular techniques, Yanagi et al. [7] reported that HCV was involved in 43% of fulminant non-A, non-B hepatitis in Japan. Subsequently, data suggesting the possible role of HCV in fulminant non-A, non-B hepatitis were documented [8, 9]. On the other hand, reports from the United States [10–13] and Europe [14–17] have shown that HCV might not be involved in fulminant non-A, non-B hepatitis. Thus, the role of HCV in fulminant non-A, non-B hepatitis remains controversial.

Previous studies have shown that hepatitis B virus (HBV) might have caused “atypical” hepatitis, in which patients are negative for HBV surface antigen (HBsAg) and antibody to HBV core antigen (anti-HBc) of the IgM class [18–22]. The infectious potential of such HBsAg-negative sera has also been established in chimpanzee transmission experiments [18, 20]. If such HBV infection leads to FH, it must be classified as non-B hepatitis according to classic serologic tests.

Here we report the prevalences of HBV DNA, HCV RNA, hepatitis D virus (HDV) RNA, and HEV RNA in non-A, non-B FH as well as in severe and ordinary acute hepatitis, based on the detection and analysis of the viral genomes.

Materials and Methods

Patients and materials. We treated 18 patients serologically diagnosed with non-A, non-B FH, 8 with acute hepatitis severe type (AH-ST), and 10 with ordinary acute hepatitis (AH) at Chiba University Hospital between January 1986 and June 1995. FH was diagnosed if patients developed hepatic encephalopathy of grade II or more within 8 weeks of the onset of jaundice according to published criteria [23]. Patients who showed a plasma prothrombin level of <40% without developing hepatic encephalopathy were diagnosed as AH-ST.

Among these 36 patients, we selected 20 patients (14 with FH, 2 with AH-ST, and 4 with AH) whose sera and liver samples had been stored suitably for analyzing viral nucleic acids (table 1). They were all negative for HBsAg, anti-HBc IgM, and antibody to hepatitis A virus (anti-HAV) IgM. They were also negative for IgM antibody to cytomegalovirus and to Epstein-Barr virus capsid antigen, anti-nuclear antibody, anti-smooth muscle antibody, and anti-mitochondrial antibody. None of the patients had evidence of preexisting liver disease or exposure to hepatotoxic drugs.

We also examined HBV DNA in sera and liver tissues from 20 chronic type C hepatitis patients who were negative for HBsAg but positive for IgG antibody to HBV surface antigen (anti-HBs) and anti-HBc IgG (n = 10) and also in patients who were negative for all these markers (n = 10) as controls for detection of HBV DNA.

Serum samples obtained at admission were stored at −20°C and were not thawed until analysis. Liver samples obtained by liver...
biopsy from surviving patients and from autopsy cases were frozen immediately and stored at −80°C.

**Serologic examination.** HBsAg, anti-HBs IgG, anti-HBc IgM, and anti-HBc IgG were examined by RIA (Dinabot, Tokyo), second-generation anti-HCV antibody (Abbott Laboratories, Abbott Park, IL) and IgM antibody to cytomegalovirus were examined by EIA, and antinuclear antibody, anti-smooth muscle antibody, anti-mitochondrial antibody, and IgM antibody to Epstein-Barr virus were examined by a fluorescence antibody method using serum samples at admission.

**Nucleic acid extraction from serum.** One hundred microliters of serum was diluted with 500 μL of chaos buffer (50% guanidine thiocyanate, 0.5% sodium N-lauroylsarcosine, 25 mM Tris-Cl, pH 8.0), followed by extraction with phenol-chloroform (1:1) twice and chloroform once and precipitation with ethanol. After washing with 75% ethanol and drying, nucleic acid was resuspended in 100 μL of TE buffer (10 mM Tris-Cl, 0.1 mM EDTA, pH 8.0) for DNA extraction with phenol once, with phenol-chloroform (1:1) twice, and chloroform once and then precipitation with ethanol.

**DNA extraction from the liver.** Twenty milligrams of frozen liver tissue was homogenized with TNE buffer (10 mM Tris-Cl, pH 7.4, 10 mM NaCl, 2 mM EDTA) and incubated overnight at 37°C with 2% SDS and 400 μg of protease K, followed by extraction with phenol once, with phenol-chloroform (1:1) twice, and with chloroform once and then precipitation with ethanol. After washing with 75% ethanol and drying, DNA was resuspended in 40 μL of TE buffer (pH 8.0). The DNA concentration of the solution was then determined by spectrophotometer.

**RNA extraction from the liver.** Total RNA was extracted from 20 mg of frozen liver tissue by using TRizol reagent (Gibco BRL, Rockville, MD) according to the instructions of the manufacturer. After washing with 75% ethanol and drying, RNA was resuspended in 50 μL of sterile diethyl pyrocarbonate–treated water. The RNA concentration of the solution was then determined by spectrophotometer. Between 8 and 10 μg of total RNA was obtained from each sample.

**Reverse transcription–polymerase chain reaction (RT-PCR) for detecting HCV, HDV, and HEV RNA in sera.** Fifty microliters of RNA solution was used for the RT-PCR assay. The primers for detection of each viral genome are shown in figure 1. RT was done for 30 min at 42°C in a 100-μL reaction mixture containing 100 pmol of each outer primer corresponding to each viral sequence (figure 1), 20 mM Tris-Cl, pH 8.3, 50 mM KCl, 2.5 mM MgCl₂, 0.8 mM dNTPs, 18 U of Rous-associated virus-2 reverse transcriptase (Takara Shuzo, Kyoto, Japan), and 110 U of IgM antibody to Epstein-Barr virus. After this step, nested PCR using 2.5 U of Taq polymerase (Perkin-Elmer Cetus, Norwalk, CT) and IgM antibody to Epstein-Barr virus were examined by a fluorescence antibody method using serum samples at admission.

**DNA extraction from the liver.** Twenty milligrams of frozen liver tissue was homogenized with TNE buffer (10 mM Tris-Cl, pH 7.4, 10 mM NaCl, 2 mM EDTA) and incubated overnight at 37°C with 2% SDS and 400 μg of protease K, followed by extraction with phenol once, with phenol-chloroform (1:1) twice, and with chloroform once and then precipitation with ethanol. After washing with 75% ethanol and drying, DNA was resuspended in 40 μL of TE buffer (pH 8.0). The DNA concentration of the solution was then determined by spectrophotometer.
bromide. To avoid contamination of samples, the extraction and amplification steps were done in separate laboratories, and negative and positive controls were included in each step. The amplified regions of each viral genome are schematically shown in figure 2.

The sensitivity of RT-PCR for detecting HCV RNA was examined by using the HCV RNA fragment generated from the plasmid containing the HCV genome sequence by in vitro transcription [24]. The HCV RNA fragment diluted to an equivalent of 1–1000 copies was added to the control serum, and nucleic acids extracted from those sera were used in the RT-PCR assay. The HCV RNA fragment equivalent to 10 copies was constantly detected, so the sensitivity of our PCR assay for detection of HCV RNA was considered to be $\geq 10$ copies per specimen.

The sensitivity of RT-PCR for detecting HBV DNA and HEV RNA was also examined by the same procedure as described above. The HDV DNA or HEV RNA fragment was generated from the plasmid containing the HDV genome sequence [25] or HEV genome sequence (gift of G. Reyes [2]), respectively. Both RNA fragments equivalent to 10 copies were constantly detected, so the sensitivity of our PCR assay for the detection of HDV RNA and HEV RNA was considered to be $\geq 10$ copies per specimen.

**PCR for detecting HBV DNA in sera.** Fifty microliters of DNA solution was used for the nested PCR assay for detection of HBV DNA. The first round of PCR was done using each outer primer set for the surface or core gene (figure 1). First PCR product (2 $\mu$L) was applied to the second round of amplification with each inner primer set (either surface or core gene) (figure 1); 10 $\mu$L of the second PCR products was analyzed by 8% PAGE and staining with ethidium bromide. The HBV DNA was semiquantitatively determined by diluting the original serum from 1 to 1000 and performing PCR. Reciprocal values for the dilution at the point of becoming positive were used to quantify the amount of HBV DNA in serum.

**Southern blot analysis for PCR products for HBV DNA sequence in the liver.** Five micrometers of DNA was used for PCR amplification as described above. Multiple primers covering different regions of the HBV genome (pre-S, S, core, X; figure 1) of subtype adr [27] were used in the PCR assay for detection of HBV DNA. The locations of these primers are shown in figure 2. Second PCR product (10 $\mu$L) was electrophoresed on a 3% NuSieve 3:1 agarose gel (FMC, Rockland, ME), stained with ethidium bromide, and photographed under UV light. The separated PCR products were denatured by soaking the gel twice in 0.5 M sodium hydroxide–1.5 M sodium chloride for 15 min each and neutralized by soaking twice in 0.5 M Tris-Cl, pH 7.5, and 1.5 M sodium chloride for 15 min each. DNA was transferred to Hybond-N+/nylon transaboves and hybridized with 32P-labeled whole HBV DNA clone (subtype adr). The filters were washed twice at room temperature in a buffer containing 0.1% SDS–2 1 1 SSC for 30 min, and twice in 0.5% SDS–0.1 SSC for 30 min, and twice at 60°C in the same buffer for 30 min each. The filters were then exposed to autoradiographic film (Kodak, Tokyo) at $-70$ °C with intensifying screens and developed.

**Sequence analysis.** For sequence analysis, nested PCR for detection of pre-S, surface, and X regions of HBV DNA was done using other sets of primers: P50, S2B (5'-ATTGAGAGAGTCGAGAATT-TCCACACACCAG3'; nt 147–128), PS1 (5'-GGGTCCTACATATGGATCT-CGAGCAGGCC3'; nt 1468–2807), and S1B for the pre-S region; S1B, S7B, and S5 for the surface region; and X2, X2B, X7, and X2B for the X region. From 15 $\mu$L of the second PCR products, amplified DNA was recovered from 1.5% NuSieve GTG agarose gels (FMC) after electrophoresis on a Magic PCR Preps DNA purification column (Promega, Madison, WI) according to the instructions of the manufacturer. The purified DNAs were then sequenced.

<table>
<thead>
<tr>
<th>HCV</th>
<th>Inner primer</th>
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<tbody>
<tr>
<td>Pre-S</td>
<td>S7B: 5'-ACACGCTCACTACCACTGAG-3' (2853–2872)</td>
</tr>
<tr>
<td></td>
<td>S1B: 5'-GTCTGCGCTGATCTGTA-3' (2993–2974)</td>
</tr>
<tr>
<td>Surface(1)</td>
<td>S8B: 5'-GATATGAGAAGACAGCAATA-3' (242–261)</td>
</tr>
<tr>
<td>core</td>
<td>S8B: 5'-GATATGAGAAGACAGCAATA-3' (242–261)</td>
</tr>
<tr>
<td>X</td>
<td>S8B: 5'-GATATGAGAAGACAGCAATA-3' (242–261)</td>
</tr>
<tr>
<td>Surface(2)</td>
<td>S8B: 5'-GATATGAGAAGACAGCAATA-3' (242–261)</td>
</tr>
<tr>
<td>X</td>
<td>S8B: 5'-GATATGAGAAGACAGCAATA-3' (242–261)</td>
</tr>
<tr>
<td></td>
<td>S8B: 5'-GATATGAGAAGACAGCAATA-3' (242–261)</td>
</tr>
<tr>
<td>HCV</td>
<td>X2: 5'-GCTGACCTTTCACATCTCGCC3' (1426–1445)</td>
</tr>
<tr>
<td></td>
<td>X2: 5'-GCTGACCTTTCACATCTCGCC3' (1426–1445)</td>
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<td></td>
<td>X2: 5'-GCTGACCTTTCACATCTCGCC3' (1426–1445)</td>
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<tr>
<td>HEV</td>
<td>X2: 5'-GCTGACCTTTCACATCTCGCC3' (1426–1445)</td>
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<td>X2: 5'-GCTGACCTTTCACATCTCGCC3' (1426–1445)</td>
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<td>X2: 5'-GCTGACCTTTCACATCTCGCC3' (1426–1445)</td>
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**Figure 1.** Sequences of primers used for polymerase chain reaction to detect hepatitis B, C, D, and E virus (HBV, HCV, HDV, HEV) genome.
was added to the solution, and they were used in the RT-PCR assay. The HBV RNA fragment equivalent to 10 copies was constantly detected, so the sensitivity of our PCR assay for detection of HBV RNA was considered to be $\geq 10$ copies per specimen.

**Immunohistostaining of HBV core antigen (HBcAg) in liver tissue.** After deparaffinizing and rehydrating, the liver sections were incubated in 3% H$_2$O$_2$ and 10% egg albumin before incubation with primary antibody (rabbit anti-HBc) followed by the second antibody (biotinized anti-rabbit IgG). Vectastain ABC kits (Vector Laboratories, Burlingame, CA) were used for the linking and labeling steps of the immunoperoxidase procedure. The slides were developed in diaminobenzidine, dehydrated, and coverslipped. All washes were done with PBS, and all incubations were done at room temperature.

**Results**

**Serologic examination.** The results of serologic examination for each patient are shown in table 1. All were negative for HBsAg, anti-HBc IgM, anti-HAV IgM, and anti-HCV antibody. The patients positive for anti-HBc IgG were also positive for anti-HBs IgG, and 7 of 14 FH, 2 of 2 AH-ST, and 0 of 4 AH patients were positive for these antibodies (table 1).

**Detection of HCV RNA and HEV RNA in sera.** Sera from patients with serologically acute non-A, non-B hepatitis were analyzed for the prevalence of circulating HCV RNA or HEV RNA by RT-nested PCR. Neither HCV RNA nor HEV RNA was detected in any of the 14 pretreatment serum samples from FH, any of 2 from AH-ST, or any of 4 from AH patients (table 2), in sharp contrast to the positive results obtained with each corresponding control specimen (data not shown). HCV RNA in the sera obtained after treatment with blood products was also examined, and positive results were obtained for 4 of 10 patients with FH or AH-ST (nos. 5, 6, 15, 16).

**Detection of HBV DNA in sera.** The same pretreatment sera were analyzed for HBV DNA by nested PCR. HBV DNA was detected in 3 (21%) of 14 patients with FH (nos. 4, 7, 10) but in none of 6 with AH-ST or ordinary AH (table 2). The quantitative assessment for HBV DNA revealed that the amount of HBV DNA was $\sim 10$ copies in all 3 patients.

HBV DNA in sera from 20 patients with chronic type C hepatitis with or without HBV seromarkers such as anti-HBs IgG and anti-HBc IgG was also examined. HBV DNA could not be detected in any sera from 10 patients without HBV seromarkers nor from 10 patients positive for anti-HBs IgG and anti-HBc IgG.
HDV RNA was also examined by RT-PCR in serum samples from patients who were positive for HBV DNA, but it was not detected in any of them.

**Detection of HBV DNA in the liver.** Next, we examined HBV DNA by nested PCR in liver tissue to clarify the possible correlation with serum HBV DNA. Four different regions of the HBV genome (pre-S, S, core, X) were analyzed (table 3). HBV DNA was detected in 7 of the 14 patients with FH (nos. 1, 2, 4, 5, 6, 9, 10), in both patients AH-ST (nos. 15, 16), and in no patients with AH. In total, 9 (45%) of 20 patients were positive for HBV DNA in the liver. Figure 4 shows the results of Southern blot hybridization after nested PCR for detection of various regions of the HBV genome in patient 1 (FH). All four regions examined were positive for HBV DNA in this patient, but in the other 8 patients, only parts of the HBV genome were detected (table 3).

HBV DNA in the liver from 20 patients with chronic type C hepatitis with or without HBV seromarkers was also examined. HBV DNA could not be detected in any liver tissues from 10 patients without HBV seromarkers. Of 10 patients positive for anti-HBs IgG and anti-HBc IgG, HBV DNA was detected in 2 (20%) at the core region and in 4 (40%) at the surface region (table 3).

**Analysis of HBV DNA sequence.** To verify that these amplified products were really HBV-derived, we attempted to obtain viral genome sequences of HBV from the liver of the 9 patients who were positive for HBV DNA. We were able to obtain partial sequences of pre-S1, pre-S2, and/or S regions for 3 patients (nos. 1, 2, and 5). Of 1098 nt sequenced, 12 missense mutations were found (figure 5). However, no mutation could be detected in the common antigenic epitope, the “a” determinant [28]. Sequencing of the pre-S gene revealed a 51-nt deletion between nt 2906 and 2956 in a patient with FH (patient 2). On the basis of Southern blot analysis of PCR products, this mutant was also revealed to coexist with the virus without the deletion (data not shown). We were also able to obtain the partial sequence of the X region (nt 1538–1646) by direct sequencing in 1 (patient 1) of the 4 FH patients who were positive for HBV X with Southern blot analysis. The sequence was the same as that of the wild type of the X gene of subtype adr [27] (data not shown).

**Detection of HBcAg in the liver.** To analyze the expression of HBcAg in liver tissue, we performed immunohistostaining for HBcAg. HBcAg was immunostained in the liver from FH patients, but we could not detect HBcAg in any liver tissues from FH and AH-ST patients who were positive for HBV DNA as determined by nested PCR (data not shown).

**Discussion**

FH has a poor prognosis despite the fact that patients undergo intensive treatment. It is well-known that HAV, HBV, and HDV are associated with FH. The etiologies of acute liver...
transfusion hepatitis. In that study, a retrospective survey, 2566 transfused patients were followed at our hospital from 1982 to 1990, but none of the 231 patients with posttransfusion hepatitis developed FH. On the basis of these results, we consider it unlikely for HCV to be the major cause of fulminant non-A, non-B hepatitis.

In contrast to the results from pretreatment serum, 4 of 10 patients with FH or AH-ST were seropositive for HCV RNA after treatment with blood products. However, this does not necessarily mean that HCV is the cause of fulminant non-A, non-B hepatitis. In Japan, as a common practice, most patients with FH are given plasma exchange treatment using large amounts of blood products, sometimes up to 100 L. Thus, anti-HCV antibody and HCV RNA could easily have contaminated these products before the Japan Red Cross introduced screening with second-generation anti-HCV antibody in 1992. Therefore, we think such positivity for HCV RNA must have been the consequence of the treatment. We have recently demonstrated that GB virus C, which is considered to be genetically related to HCV and had been considered a causative agent of FH, was not detected in a single patient with FH in

injury have thus far been examined on the basis of serologic tests. Regarding the etiology of fulminant viral hepatitis in Japan, it was reported that HAV was involved in 8% and HBV in 47%, but the etiology of the remaining 45%, based on immunologic and serologic analyses, remained unknown [29]. The genome sequences of hepatitis viruses have now been disclosed, and delicate molecular biologic detection methods have become available. As the serologic status might be influenced by the severe biologic reactions in FH cases, we examined the etiology of the so-called non-A, non-B FH in our patient population by nucleic acid amplification methods.

As for HCV involvement in FH, evidence is conflicting; reports from the United States [10–13] and Europe [14–17] have shown that HCV might not be involved in sporadic fulminant non-A, non-B hepatitis, while reports from Japan and Taiwan have shown nearly half of the fulminant non-A, non-B hepatitis patients to be positive for HCV RNA [7–9]. In the current study, we could not detect HCV RNA in any sera taken at admission from patients with FH. Before the identification of HCV, Dienstag [30] reported that most posttransfusion acute non-A, non-B hepatitis did not develop to FH. Takano et al. [31] also reported the prospective assessment of donor blood screening for antibody to HCV as a means of preventing post-

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Results of Southern blot hybridization after nested polymerase chain reaction amplification to detect different regions of hepatitis B virus DNA in liver tissue obtained from patient 1 (fulminant hepatitis). Regions shown: lane 1, surface; lane 2, surface; lane 3, pre-S; lane 4, core; lane 5, X.

![Figure 5](https://example.com/figure5.png)

**Figure 5.** Deduced amino acid residues of pre-S1 (A), pre-S2 (B), and surface region (C) in 3 patients with fulminant hepatitis (patients 1, 2, and 5). Consensus amino acid sequences (top rows) and numbers above sequences are according to subtype adr [27]. ///, deletion; --, identity with consensus sequence.
pretreatment sera but was detected after the administration of large amounts of blood products, similar to the results of HCV RNA in this study [32].

It is known that HEV causes AH in areas in which it is endemic [33], but in the present study, HEV RNA was detected in none of the patients. In Japan, HEV is not endemic, and none of our patients had a history of visiting such areas in which it is endemic, and thus our non-A, non-B hepatitis patients had little chance of being infected with HEV. Accordingly, it is not surprising that none of our patients showed any evidence of infection with HEV. It seems that HEV is also unlikely to be a major etiologic agent for fulminating non-A, non-B hepatitis in Japanese patients.

Previous studies have demonstrated the presence of HBV infections in serologically diagnosed non-A, non-B chronic hepatitis [34–38] and FH [10, 14, 39–42]. In the current study, 3 (21%) of 14 patients were seropositive for HBV DNA and 7 (50%) of 14 patients with fulminating “non-A, non-B” hepatitis were positive for HBV DNA in the liver. Thus, it was revealed that HBV DNA was frequently detectable in so-called non-A, non-B FH.

The detection of HBV DNA in fulminant non-A, non-B hepatitis might suggest that HBV is involved in its pathogenesis. However, this evidence may be insufficient to confirm a direct correlation between HBV and fulminant non-A, non-B hepatitis. It has been reported that HBV DNA is detectable by a sensitive PCR method in patients with previous HBV infection [34], so we also examined chronic type C hepatitis patients with comparable HBV seromarkers. In the control study, we could not detect HBV DNA in the serum or liver from patients without HBV seromarkers, but we could detect HBV DNA in the liver from some patients possessing HBV seromarkers.

Thus, as for the FH cases without any HBV seromarkers, the detection of HBV DNA might mean the possible involvement of HBV as an etiologic agent. Actually, Lugassy et al. [39] and Chazouilleres et al. [41] have already demonstrated the existence of “occult” HBV DNA in the liver or sera from patients with FH who had been negative for serologic HBV markers.

Our control study indicated that the detection of HBV DNA in the liver with HBV seromarkers might reflect the evidence of past HBV infection. However, in HBV-induced FH, it was previously described that an unusually strong and rapid immune clearance of HBsAg and enhanced production of anti-HBs might be involved in its pathogenesis [43–45]. Such mechanism might contribute to the results that anti-HBs was detected in patients with FH or severe AH caused by HBV. Thus, there still remains a possibility that HBV caused the FH in patients who were positive for both HBV DNA and anti-HBs or anti-HBc IgG.

To further assess the HBV status in the liver, we used RT-PCR assay to detect HBV RNA in liver samples from 12 FH patients. However, we could not detect HBV RNA in any liver samples examined. The lack of detection of HBV RNA suggests that active replication of HBV was not occurring at the time of collection of these samples. However, the liver tissues examined for HBV RNA were all autopsy samples, and the majority of the hepatocytes in these samples had fallen into severe necrosis or apoptosis due to the original disease, so we have to be careful in interpreting the results on HBV RNA obtained from these samples.

We do not know the reason why HBV DNA could be detected in FH patients negative for HBsAg and anti-HBc IgM. One possible explanation for the seronegativity of HBsAg is the low level of HBV replication. In fact, we hardly detected HBV DNA in serum and liver with the one-step PCR method, consistent with other reports [10, 14, 42]. In addition, we could not detect HBeAg in the liver by immunohistologic examination, a less sensitive method than the molecular biologic detection systems. Another explanation could be found in the mutations in the coding regions of the HBV genome. It was reported that a single mutation at the common “a” determinant of HBsAg caused a change in the immunologic epitope [28, 46], and mutations in the pre-S region led to the inhibition of secretion of the envelope protein [47, 48]. In addition, mutations in the precore region have also been suggested to induce AH in patients negative for anti-HBc IgM [18]. Recently, it was reported that an 8-nt deletion in the X region (corresponding to nt 1642–1649) might suppress replication and expression of HBV DNA, and it seems to be responsible for the absence of serologic markers despite the presence of HBV infection [22].

In view of these observations, we tried to identify the region of the viral genome that might be responsible for the lack of production of HBsAg. As shown in figure 5, we could obtain nucleotide sequences responsible for HBsAg in 3 patients in this analysis. However, we could not find any mutation in the common “a” determinant in these 3 patients. In addition, we could obtain the partial sequence of X region for 1 patient, and the sequence revealed the wild type of the HBV X gene of subtype adr [27]. The specific deletion of 8 nt in the X region of HBV reported to be present in HBsAg-seronegative AH patients by Uchida et al. [22] was not found in this case. The specific mutations in the core promoter region observed in 72% of type B FH patients in Japan [49], in 10% in the United States [50], and in 38% in Europe [51] were not found in this case. To further clarify this, we will need to obtain the full nucleic acid sequences of these viruses.

We also examined the possible HDV involvement in those patients who were positive for HBV DNA, but HDV RNA could not be detected in any of them.

The etiologic agent of sporadic acute non-A, non-B viral hepatitis has remained obscure. Serologic assay systems have limitations because they are based on the immunologic response by the host. Therefore, sensitive molecular diagnostic systems, such as the PCR method, are required to examine the presence of such an infectious agent. By applying the sensitive PCR method, we have shown that HCV had little involvement in the etiology of fatal FH, at least in our patient population in the Chiba area, as well as the existence of HBV DNA in some of the patients in whom we could not diagnose HBV infection by conventional serologic assay.
References


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