Original Article

Frequency of, and factors associated with, hepatitis B virus reactivation in hepatitis C patients treated with all-oral direct-acting antivirals: Analysis of a Japanese prospective cohort

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Aim: Several case reports have shown that hepatitis B virus (HBV) reactivation developed in hepatitis C patients with a current or previous HBV infection during direct-acting antiviral (DAA) treatment, which led to severe hepatitis or death in some cases. However, its precise frequency and risk factors are not entirely clear. We analyzed a prospective cohort.

Methods: We analyzed HBV reactivation in 461 consecutive hepatitis C patients who received 12 weeks of ledipasvir/sofosbuvir for genotype 1 or sofosbuvir plus ribavirin for genotype 2 at multiple centers.

Results: By the examination of the preserved sera at baseline, 159 patients (34%) were identified as seropositive for HBV core antibody (anti-HBc) and were included in the subsequent analysis; 4 patients were positive for HBV surface antigen (HBsAg), and the others were negative. Serum HBV DNA was undetectable or was detectable but <20 IU/mL at baseline for all patients. Serial measurement of HBV DNA at 4 weeks and 12 weeks in the preserved serum samples was available in 147 patients and identified HBV reactivation (defined as the appearance of serum HBV DNA ≥20 IU/mL) in 2 HBsAg-positive and 3 HBsAg-negative patients. No patient developed HBV-associated hepatitis. Patients who developed HBV reactivation had significantly lower anti-HBs titers and higher serum alanine transferase levels before treatment.

Conclusion: Hepatitis B virus reactivation during direct-acting antiviral therapies occurs in 3.4% (5/147) of patients who are positive for anti-HBc. A low titer of anti-HBs and a high serum alanine transferase level prior to treatment are associated with reactivation in this patient group.

Key words: DAA, HBV reactivation, IFN-free

INTRODUCTION

THE ANTIVIRAL TREATMENT of hepatitis C has advanced rapidly in recent years.1 The introduction of direct-acting antiviral agents (DAAs) has enabled highly efficient virus elimination in a short treatment period without using interferon, a non-selective antiviral agent. A combination of ledipasvir plus sofosbuvir and sofosbuvir plus ribavirin, one of the standards of care for genotype 1 and for genotype 2, respectively, achieves a sustained virologic response (SVR) of more than 95% with a treatment period of 12 weeks.2 Although DAA therapy is generally safe, several case reports have shown that hepatitis C patients who...
were seropositive for hepatitis B virus (HBV) surface antigen (HBsAg) or anti-HBV core antibody (anti-HBc) developed HBV reactivation and accompanying hepatitis followed by DAA therapies.\textsuperscript{3–7} Hepatitis B virus reactivation is commonly observed in patients receiving anticancer drugs and immunosuppressants and in transplant patients.\textsuperscript{8} It presumably occurs by suppressing the immune response to HBV. Direct-acting antivirals do not have an immunosuppressive effect, but it could be possible that the abrupt elimination of hepatitis C virus (HCV) by DAA treatment may attenuate the interference effect of HCV on HBV. The reactivation of HBV during DAA treatment may be an important problem in HCV treatment, but its frequency and risk factors are not well understood. In the present study, we analyzed a prospective cohort in which preserved serum samples were available at both baseline and during therapy to examine the frequency of, and factors associated with, HBV reactivation.

METHODS

Study cohort

A PROSPECTIVE JAPANESE study cohort was applied; 461 consecutive hepatitis C patients who started 12 weeks of treatment with ledipasvir/sofosbuvir for genotype 1 or sofosbuvir/ribavirin for genotype 2 at Osaka University Hospital, Osaka Medical Center for Cancer and Cardiovascular Disease, NTT Osaka Hospital (all Osaka, Japan), and Ikeda City Hospital (Ikeda, Japan) between November 2015 and May 2016. All patients were ≥20 years of age (Table S1) and provided written informed consent. This study is regarded as a clinical study on UMIN (ID nos. 000017703 and 000018561; protocol no. IN-US-337-2072). Four hundred and sixty-one patients were enrolled and baseline serum samples were available for all patients.

Preserved serum samples

Serological HBV markers including HBsAg, anti-HBs, and anti-HBc were measured for all patients at baseline. Serum HBV DNA at baseline and 4 weeks and 12 weeks after starting treatment were measured in patients who were positive for anti-HBc at baseline. Serum HBV DNA 4 weeks after the treatment was measured in the reactivation cases if there were preserved serum samples available.

Measurement of HBV markers

Hepatitis B surface antigen, anti-HBs, and anti-HBc were tested using commercial microparticle enzyme immunoassays, namely, the chemiluminescence enzyme immunoassay (CLEIA)-based HISCL HBs Ag assay (Sysmex, Kobe, Japan) (HBsAg measurement range, 0.03–2500 IU/mL; cut-off value to be positive, 0.03 IU/mL), CLEIA-based HISCL HBs Ab assay (Sysmex) (anti-HBs measurement range, 5.0–1000 mIU/mL; cut-off value to be positive, 5.0 mIU/mL), and CLEIA-based HISCL HBc Ab assay (Sysmex) anti-HBc measurement range, 0-1600 cut off index (COI); cut off value to be positive, 1). Testing for HBV DNA was carried out using the Cobas AmpliPrep/Cobas TaqMan Real-Time PCR Assay (Roche Molecular Diagnostics, Pleasanton, CA, USA; linear range, 20–200 000 000 IU/mL).

Definition of HBV reactivation

Hepatitis B virus reactivation was defined as the reappearance of serum HBV DNA ≥20 IU/mL following baseline undetectable HBV DNA or detectable but <20 IU/mL\textsuperscript{9} or a≥10-fold increase in HBV DNA compared with baseline.\textsuperscript{10}

Laboratory and liver biopsy

Laboratory testing carried out on each subject included complete blood count, serum chemistry, liver profile, prothrombin time, HCV RNA testing, and HCV genotyping. Liver biopsies were carried out initiating the combination therapy if appropriate. The histopathological interpretation of the specimens was undertaken by experienced liver pathologists. The histology, activity, and fibrosis were evaluated according to the METAVIR histological scoring system. The liver function test results including serum alanine transferase (ALT), medication, and other clinical characteristics were derived from clinical information.

Statistics

The Mann–Whitney U-test was applied for the comparison of study parameters. The significant differences in the trends among categorical data were analyzed using Fisher’s exact test or the Mantel–Haenszel χ²-test. A P-value <0.05 was regarded as statistically significant in all analyses. For statistical analyses, we used spss version 24 (IBM, Armonk, NY, USA).

RESULTS

A TOTAL OF 461 consecutive patients with hepatitis C were enrolled. The SVR rates were 98.0% for genotype 1 and 97.1% for genotype 2 (Table S1). Preserved serum samples were available for all patients at baseline and their analysis identified 159 patients seropositive for anti-HBc. Among them, 4 patients were positive for HBsAg the others were negative (Fig. 1). Serum HBV DNA was undetectable or detectable but <20 IU/mL at baseline for all 159 patients. Among 159 patients who were seropositive for anti-HBc, preserved blood samples 4 weeks and 12 weeks after starting therapy were available in 147 patients (Fig. 1). The
remaining 12 patients were negative for HBsAg at baseline and were confirmed from medical information not to develop hepatitis during DAA therapy.

The serial measurement of HBV DNA identified HBV reactivation in 5 patients, 2 of which were HBsAg seropositive. The viral load of each time point is shown in Table 1 and more detailed clinical information, including serum ALT levels, serum HCV RNA levels, and serum HBV DNA levels, is shown in Figure 2. All patients showed HCV clearance as early as 4 weeks and achieved SVR. Four patients experienced HBV reactivation at 4 weeks, and it occurred at 12 weeks in another patient. No patient developed HBV-associated hepatitis. No patient received HBV treatment, and all HBV DNA levels spontaneously declined, except for one case in which HBsAg was positive (patient no. 1).

The factors associated with HBV reactivation were analyzed (Tables 2,S2). Patients who developed HBV reactivation had significantly higher ALT levels at baseline than those who did not. Among viral markers of HBV, only the anti-HBs titer prior to treatment was significantly associated with reactivation (Table 2).

DISCUSSION

IN THE PRESENT study, we analyzed a prospective cohort and showed that 3.4% (5/147) of the patients who were seropositive for anti-HBc developed HBV reactivation during DAA therapy, even though hepatitis did not develop. The percentage of patients with current or past HBV infection in this cohort was 34%. We also showed that a low titer of anti-HBs and higher ALT levels at baseline may be a risk factor for the reactivation of those patients.

The US Food and Drug Administration warned in October 2016 about the risk of HBV reactivation in any patient with current or previous infection with HBV who were treated with DAAs for HCV. They recommended the screening of HBV infection before starting DAA treatment and appropriate monitoring followed by the treatment.11 The Pharmaceuticals and Medical Devices Agency also issued a similar warning in May 2016 in Japan. Patients in our cohort were treated with DAAs at multiple institutes before these statements were issued. All pretreatment sera of these consecutive patients were preserved, as was most sera under treatment. Because observing the unbiased natural course of HBV reactivation would be difficult in the future, this report is considered valuable.

Very recently, two observational studies on HBV reactivation during DAA treatment were published. One is a report on patients treated with pan-oral DAAs for HCV infections in areas endemic for HBV in China12 and the other is a report using a clinical trial carried out in multiple sites in Taiwan and Korea evaluating 12 weeks of a fixed-dose combination of ledipasvir/sofosbuvir.13 Wang
et al. analyzed 327 consecutive DAA-treated patients (HBsAg-positive, \( n = 10 \); occult HBV infection, \( n = 124 \)) and reported that hepatitis developed in 3 of the HBsAg-positive patients and in 3 of the patients with occult HBV infection. All patients positive for HBsAg who developed hepatitis showed HBV reactivation, but none were caused by HBV reactivation in occult HBV infection cases. Sulkowski et al. specially focused on HBV reactivation in patients with previous HBV infection. They determined anti-HBc in the preserved sera at 24 weeks after the end of treatment and identified 103 patients positive for anti-HBc of the 178 patients enrolled. They concluded that there was no evidence of reactivation in this population on the basis that all patients positive for anti-HBc showed HBV DNA <20 IU/mL at 24 weeks after the end of treatment and that none of them showed ALT flare-up during treatment or post-treatment. In the present study, we showed that 2 of 4 patients with current HBV infection and 3 of 143 patients (2.1%) with previous HBV infection showed an increase of HBV DNA >20 IU/mL during the therapy. All patients showed subsequent decreases of HBV DNA after the end of treatment without receiving nucleoside analogs (Tables 1, S3). Although data are not shown, we additionally analyzed HBV DNA after DAA therapy (post 12 and 24 weeks) in 111 patients who did not show on-treatment HBV reactivation and found one patient who was seropositive for anti-HBc but seronegative for both HBsAg and anti-HBs, developing HBV reactivation after 12 weeks (HBV DNA, 63 IU/mL) and spontaneously healing after 24 weeks (HBV DNA, <20 IU/mL). Taken together, we think that the present study was not in contradiction with previous reports because Wang et al. specifically focused on HBV reactivation and did not analyze the fluctuation of HBV DNA during therapy, but rather made the overall picture of HBV reactivation clearer.

The fact that a low HBs antibody titer before treatment poses a risk of reactivation is often reported with regard to reactivation following treatment with immunosuppressive drugs and anticancer drugs. Our observation that the HBs antibody titer before treatment was low in cases of HBV reactivation is consistent with this fact. Hepatitis B virus reactivation did not occur in patients who were negative for HBsAg but positive for both anti-HBs and anti-HBc. Because HBV replication is controlled by the host's immune system, when immunity is suppressed, HBV is reactivated. Although the HBs antibody is an antibody that neutralizes HBV, it would be closely linked to the regulation of HBV proliferation. The best known and most straightforward example is treatment with rituximab for B-cell lymphoma, in which B cells producing

### Table 1: Clinical characteristics of patients with hepatitis B virus (HBV) reactivation

| No. | Age | Sex | Liver disease | HCV genotype | Liver disease History | HCV therapy | HBsAg | anti-HBs, mIU/mL | anti-HBc, COI | HBV DNA, IU/mL | Baseline | Post 4 weeks | Post 12 weeks | Post 24 weeks |
|-----|-----|-----|---------------|--------------|----------------------|-------------|-------|----------------|--------------|----------------|----------|--------------|--------------|--------------|
| 1   | 75  | F   | LC            | CH           | Chronic hepatitis     | IFN → NR   | NA    | 4.04           | 0.1          | >20            | 454.0    | <20          | ND          | <20          |
| 2   | 72  | M   | LC            | CH           | Chronic hepatitis     | IFN → NR   | NA    | 4.04           | 0.1          | >20            | 454.0    | <20          | ND          | <20          |
| 3   | 69  | M   | LC            | CH           | Chronic hepatitis     | IFN → NR   | NA    | 4.04           | 0.1          | >20            | 454.0    | <20          | ND          | <20          |
| 4   | 75  | M   | LC            | CH           | Chronic hepatitis     | IFN → NR   | NA    | 4.04           | 0.1          | >20            | 454.0    | <20          | ND          | <20          |
| 5   | 54  | M   | LC            | CH           | Chronic hepatitis     | IFN → NR   | NA    | 4.04           | 0.1          | >20            | 454.0    | <20          | ND          | <20          |

In patient no. 3, treatment with rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone (R-CHOP) was given for diffuse large B-cell lymphoma (DLBCL) and achieved complete remission 2 years before sofosbuvir/ledipasvir (SOF/LDV) treatment. In patient no. 1, reactivation of HBV during and after R-CHOP: HCC, hepatocellular carcinoma; IFN, interferon; LC, liver cirrhosis; NA, not available; ND, not detected; NR, non-responder; R-CHOP, rituximab, cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone; w, weeks.
anti-HBs are directly depleted and HBV is frequently reactivated. Many other immunosuppressive agents, such as corticosteroids, cyclosporine, and tacrolimus, are thought to suppress acquired immunity. In the case of DAA treatment, it is hypothesized that HBV may reactivate as the innate immunity of hepatocytes induced by the proliferation of HCV rapidly attenuates. Even in such cases, the anti-HBs antibody may be significant in controlling the growth of HBV.

Patients who developed HBV reactivation had significantly higher ALT levels at baseline than those who did not. Alanine transferase levels are generally known as sensitive indicators of liver injury. Considering that liver injury develops with an immune response, a sharp drop...
in the immune response as well as liver injury accompanying the disappearance of HCV may be involved in the reactivation of HBV. In this analysis, there was no association between HBV reactivation and the presence or absence of liver cirrhosis. However, because DAA treatment is more often undertaken in hepatitis C patients with advanced liver disease compared to treatment with anticancer drugs, it may be necessary to give more consideration to the severity when HBV-related hepatitis develops.

In conclusion, HBV reactivation during DAA therapy occurs in 3.4% (5/147) of patients who are positive for anti-HBc. A low baseline titer of anti-HBs and higher ALT levels are associated with the reactivation. The present study provides a non-biased basic understanding of the frequency and natural course of HBV reactivation during DAA therapy.

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SUPPORTING INFORMATION

ADDITIONAL SUPPORTING INFORMATION may be found online in the supporting information tab for this article.

Table S1 Baseline characteristics of study patients with hepatitis C treated with all-oral direct-acting antivirals.
Table S2 Baseline characteristics of hepatitis B surface antigen (HBs) negative, anti-HBs negative, and anti-hepatitis core antibody-positive patients with and without hepatitis B virus reactivation.
Table S3 Hepatitis B serological markers of hepatitis C patients with hepatitis B virus reactivation.
Table S4 Baseline Characteristics of Study Patients (lower ALT level group vs higher ALT level group)