ORIGINAL ARTICLE

Relation of Reduction of Antibodies against Hepatitis B Virus to Hepatocellular Carcinoma Recurrence in the Patients with Resolved Hepatitis B Virus Infection Following Direct-acting Antiviral Therapy for Hepatitis C Virus Infection

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Abstract

Background: A possible interaction of hepatitis viruses at cellular and molecular levels has been suggested. Eradication of hepatitis C virus (HCV) has been reported to induce activation of hepatitis B virus (HBV)-related liver diseases.

Materials and methods: The present study examined association of HBV markers with recurrence of hepatocellular carcinoma (HCC) in patients with resolved HCV infection by direct-acting antiviral (DAA) therapy. In a patient pool of 378 patients with sustained virologic response (SVR) by DAA, the antibody to the hepatitis B surface antigen (anti-HBs), the antibody to the hepatitis B core antigen (anti-HBc), and HBV-DNA levels were estimated before and at the end of DAA therapy. These patients were HBsAg negative. Eighty-nine patients had a history of curative treatment of HCC by resection or radiofrequency ablation. A Cox proportional hazards model was used to identify risk factors for HCC recurrence, including the change ratio of the antibody against HBV proteins.

Results: Although 188 patients had resolved HBV infection, no patient showed HBV reactivation, but anti-HBs and anti-HBc levels decreased significantly. No significant difference in the HCC recurrence rate was evident between patients with and without resolved HBV infection. Changes of immune responses to HBV proteins did not affect HCC recurrence after DAA therapy for HCV infection in this cohort.

Conclusion: The mechanisms underlying diverse roles of DAA-induced SVR of HCV on HBV kinetics need to be resolved in future.

Keywords: Direct-acting antiviral, Hepatitis B reactivation, Hepatitis C, Hepatocellular carcinoma, Recurrence risk.

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Introduction

A sustained virologic response (SVR) due to therapy with interferon (IFN) has shown to decrease the incidence of hepatocellular carcinoma (HCC) in patients with HCV infection.[1–5] These patients also show low recurrence of HCC after therapy for HCC.[6–10] With the advent of DAAs, SVR can be achieved even in patients with HCV-related liver cirrhosis. Direct-acting antiviral has also shown its effect on the incidence of HCC in DAA-induced SVR patients.[11] However, there has been lack of consensus about the degree of decrease of HCC incidence in patients with advanced liver cirrhosis.[12,13] This controversial issue about an association of SVR and role of DAA on HCC recurrence is a continual debate in clinical hepatology.[13–23]

Differential types of reactivation of HBV during DAA therapy have been reported,[24–28] which may indicate possible changes of immune responses during DAA therapy.[29] Epidemiological data indicate that the fact of HBV reactivation is not common among patients with resolved HBV infection.[30–32] However, it has been shown that a significant proportion of HBsAg-negative patients show decreases in the anti-HBs, the protective antibody of HBV infection.[33–35] These findings suggest that a reduced immune response may occur with the rapid eradication of HCV during DAA therapy, and that the change in the immune response may have an influence on HCC recurrence. To examine this possibility, the present study evaluated relationships between HCC recurrence and changes in HBV-related markers before and after DAA therapy.

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Conflict of interest: None

Materials and Methods

Patients

A total of 543 HCV-infected patients who received DAA therapy at our hospital between September 2014 and February 2017 were

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Table 1: Patients' background characteristics

Age, years	68 (22–88)†
Sex, male:female	178:200 (1:1.1)
Genotype, 1:2	293:85 (3.5:1)
History of treatment for HCC, yes:no	89:289 (1:3.2)
Past history of IFN, yes:no	128:250 (1:2.0)
WBC (/μL)	4,690 (3,900–5,740)‡
Hb (g/dL)	13.4 (12.5–14.5)‡
Plt ($\times 10^4$/μL)	14.2 (10.6–19.2)‡
ALT (IU/L)	35 (25–51)‡
T. Bil (mg/dL)	0.7 (0.6–1.0)‡
PT (%)	88.5 (78.4–100.3)‡
Alb (g/dL)	4.1 (3.8–4.4)‡
HCV-RNA (logIU/mL)	6.1 (5.6–6.5)‡
AFP (ng/mL)	5.0 (3.0–9.0)‡

†Median (range)
‡Median (interquartile range)
AFP, α-fetoprotein; Alb, albumin; ALT, alanine aminotransferase; Hb, hemoglobin; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; Plt, platelets; PT, prothrombin time; T. Bil, total bilirubin; WBC, white blood cells

initially enrolled in this retrospective study. Out of these, 378 patients met the inclusion criteria: (i) HBsAg-negative prior to DAA therapy; (ii) radiofrequency ablation or resection was employed as a curative treatment in patients with a history of HCC treatment, and there was no viable tumor prior to DAA therapy; (iii) SVR confirmed for at least 24 weeks (SVR24); (iv) observation for >1 year after termination of DAA therapy; (v) complete clinical record for the observation period; and (vi) availability of preserved blood serum samples from before and at the end of DAA therapy. The participants included 178 men and 200 women (median age, 68 years). A history of treatment for HCC was confirmed in 89 patients. The average duration of follow-up was 805 days (range: 365–1,208 days). Table 1 shows the laboratory data at the commencement of DAA therapy.

Antiviral Therapy

The following agents were used in DAA therapy: sofosbuvir/ledipasvir in 193 patients; sofosbuvir/ribavirin in 83 patients; ombitasvir/paritaprevir/ritonavir in 45 patients; asunaprevir/daclatasvir in 39 patients; ombitasvir/paritaprevir/ritonavir/ribavirin in 2 patients; and elbasvir/grazoprevir in 16 patients. Recurrence was monitored in patients with a past history of treatment for HCC by measuring des-gamma-carboxy prothrombin (DCP) and α-fetoprotein (AFP) levels and by imaging with either dynamic computed tomography or ethoxybenzyl diethylenetriamine pentaacetic acid-enhanced magnetic resonance imaging every 2–3 months.

Ethical Considerations

The present study was a retrospective analysis of patient records. All treatments were conducted in an open-label manner. While consent was obtained for preservation of blood serum samples for research purposes, the present study was a retrospective study, and an informed consent from patients was obtained on the basis of an "opt-out" option provided on our hospital website with the description of the study. Approval of the above measures was obtained from the ethics committee of the hospital (approval no. 616), and all procedures conformed to the provisions of the Declaration of Helsinki.

Assessment of HBV Reactivation

The following were measured from blood serum samples collected before and at the end of DAA therapy: levels of HBsAg and anti-HBs, hepatitis B core antibodies (anti-HBc), and HBV-DNA. Methods used for measurements were as follows: chemiluminescent immunoassay (CLIA) for HBs antigens (ARCHITECT HBsAg Reagent Kit; Abbott, Chicago, IL); CLIA for HBs antibodies (ARCHITECT Anti-HBs Reagent Kit; Abbott); CLIA for HBc antibodies (ARCHITECT Anti-HBc II Reagent Kit; Abbott), and the TaqMan probe for HBV-DNA (COBAS Ampriprep/COBAS TaqMan HBV Test, v2.0; Roche, Basel, Switzerland).

Effect of Resolved HBV Infection on the Recurrence Rate of HCV-related HCC

The duration from the end of DAA therapy to the day of diagnosis of HCC recurrence was calculated, and the cumulative recurrence rate of HCC was compared between patients with and without resolved HBV infection.

Relationship between HCC Recurrence and HBV Markers

To determine changes in anti-HBs and anti-HBc levels, rates of change in anti-HBs and anti-HBc were calculated using the formulae below. Anti-HBs change ratio = anti-HBs level at the end of DAA therapy/anti-HBs level before DAA therapy. Anti-HBc change ratio = anti-HBc level at the end of DAA therapy/anti-HBc level before DAA therapy.

Each of the indices was used to determine relationships between changes in immunological conditions toward HBV and HCC recurrence. In addition, factors associated with HCC recurrence during the observation period were assessed in all patients. Selected factors were the anti-HBs change ratio and the anti-HBc change ratio to examine the effects of immunological change on HCC recurrence; age, sex, past history of IFN, and past history of HBV infection as patients' background characteristics; number of HCC treatments before DAA therapy (≥3 times), number of days from HCC treatment just before DAA therapy to the beginning of DAA therapy, HCC stage III at initial treatment, HCC stage III at treatment just before DAA, number of tumors (≥3) at treatment just before DAA, and maximum tumor diameter (≥2.5 cm) at treatment just before DAA; platelet count, albumin, prothrombin time, and the fibrosis-4 index at the beginning of DAA as the background characteristics of liver disease; and alanine aminotransferase (ALT), DCP, and AFP at the end of DAA treatment as the condition after DAA therapy. The diagnosis of HCC was based on the international guideline.[36] Tumor diameters and numbers were measured by two hepatologists with more than 10 years of experience, and the decisions were made by consensus.

Statistical Analysis

In order to examine changes of anti-HBs and anti-HBc levels during DAA therapy, the Wilcoxon signed-rank test was used. Hepatocellular carcinoma recurrence curves were prepared using the Kaplan–Meier method to examine the effect of past HBV infection on the recurrence rate of HCV-related HCC, while the log-rank test was used to compare groups. A Cox proportional hazards model was used to identify risk factors for HCC recurrence during the follow-up period. These statistical analyses were conducted using JMP version 13.2.0 (SAS Institute, Cary, NC) under the guidance of a statistician, and values of $p < 0.05$ were considered significant.

Results

Frequency of HBV Reactivation

Of the 378 patients with HCV infection who underwent DAA therapy, approximately half of the patients (188 patients) had resolved HBV infection. Of these, 188 patients had harbored HBV markers: 91 were positive for anti-HBs, 176 were positive for anti-HBc, and 79 were positive for both anti-HBs and anti-HBc. Of these 188 patients, 43 had histories of treatment for HCC. Out of 190 HBV marker-negative HCV patients, 46 had received treatment for HCC (Table 2). Serum HBV-DNA levels before and at the end of DAA therapy were measured in the 188 patients with a history of past HBV infection, but levels were all below the sensitivity of detection, suggesting the extremely low incidence of HBV reactivation in patients negative for HBsAg.

Table 2: Number of patients with hepatitis B virus infection and a history of treatment for hepatocellular carcinoma

	Overall number of patients	*Positive for anti-HBs*	*Positive for anti-HBc*	*History of treatment for HCC*
Resolved HBV infection (+)	188	91	176	43
History of HBV infection (–)	190	0	0	46
Total	378	91	176	89

Anti-HBc, antibody to hepatitis B core antigen; anti-HBs, antibody to hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma

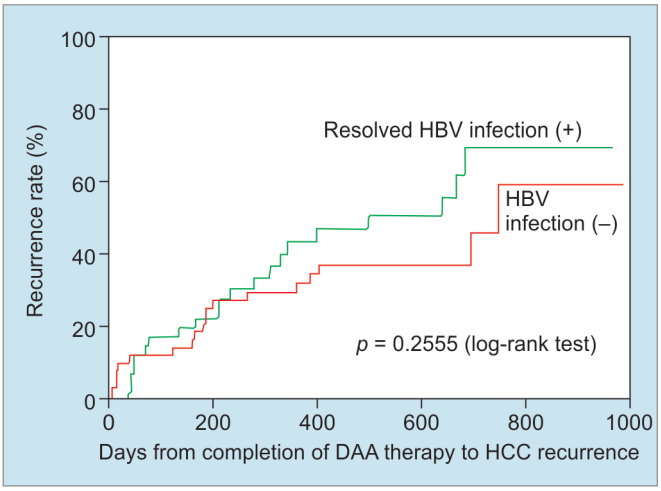


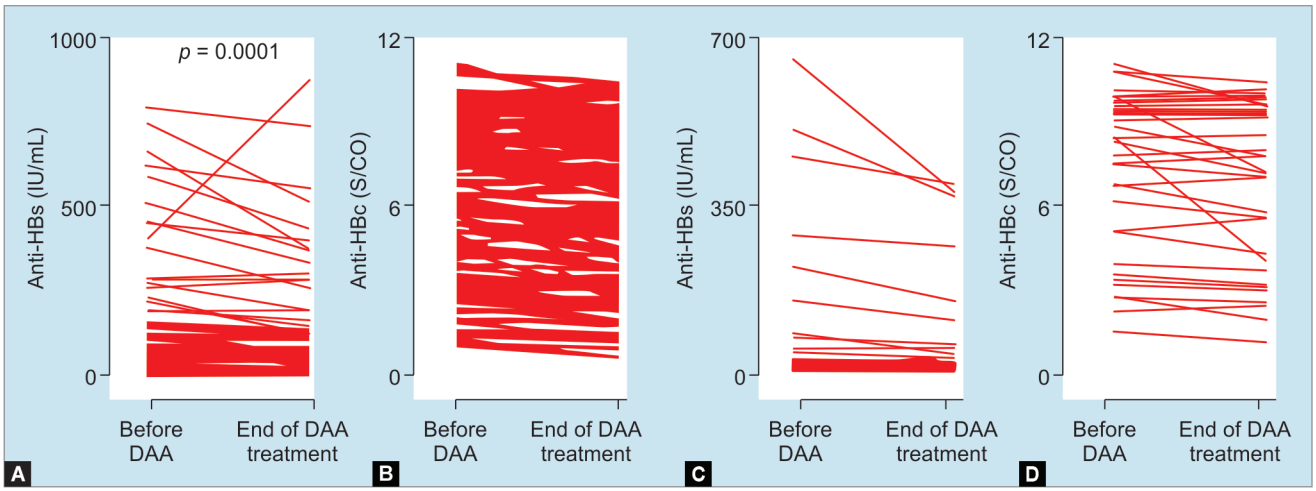
Fig. 1: Cumulative recurrence rate of HCV-related hepatocellular carcinoma in patients with and without a past history of HBV infection

Effect of Resolved HBV Infection on the Recurrence Rate of HCV-related HCC

The cumulative recurrence rate of HCC was compared between patients with and without a past history of HBV infection. No significant difference in the HCC recurrence rate was found between the presence of resolved HBV infection and the absence of HBV infection (Fig. 1).

Changes of Anti-HBs and Anti-HBc during DAA Therapy

When anti-HBs levels were compared at two points (before and at the end of DAA therapy), a significant decrease was observed after treatment (Fig. 2A). Similarly, when anti-HBc levels were compared at these points (before and at the end of DAA therapy), a significant decrease in anti-HBc levels was identified (Fig. 2B). When anti-HBs and anti-HBc levels before and at the end of DAA therapy were compared exclusively in patients with a history of treatment for HCC, significant decreases in both levels were also observed after treatment (Figs 2C and D). Forty-two of 78 patients (53.8%) had the anti-HBs change ratio and anti-HBc change ratio both under 1.0, and seven patients (9.0%) had the anti-HBs change ratio and anti-HBc change ratio both under 0.8. Twenty-five patients (32.1%) had an anti-HBs change ratio below 1.0 and an anti-HBc change ratio ≥1.0, and six patients (7.7%) had an anti-HBc change ratio below 1.0 and an anti-HBs change ratio ≥1.0. Five patients (6.4%) had both antibody change ratios ≥1.0. While reactivation resulting in positive conversion of HBV-DNA occurs at an extremely low



Figs 2A to D: Changes in antibody to the hepatitis B surface antigen (anti-HBs) (A) and antibody to the hepatitis B core antigen (anti-HBc) levels (B) before and at the end of DAA therapy in all patients with a past history of HBV infection. Changes in anti-HBs (C) and anti-HBc levels (D) before and at the end of DAA therapy in patients with both resolved HBV infection and a history of treatment for hepatocellular carcinoma

Table 3: Factors associated with recurrence of hepatocellular carcinoma after completing antiviral therapy in patients who achieved SVR

	Univariate analysis		*Multivariate analysis*	
Factor	*Odds ratio (95% CI)*	*p*	*Odds ratio (95% CI)*	*p*
Age	0.991 (0.957–1.027)	0.6024		
Sex (male)	0.628 (0.319–1.186)	0.1537		
Past history of IFN (yes)	0.786 (0.421–1.477)	0.4495		
Resolved HBV infection (yes)	1.432 (0.767–2.691)	0.2581		
Number of HCC treatments before DAA (≥3 times)	2.709 (1.366–5.235)	0.0051	1.7163 (0.8045–3.5598)	0.1589
Number of days from HCC treatment to DAA	0.998 (0.997–0.999)	0.0009	0.9998 (0.9975–0.9997)	0.0064
HCC stage III at initial treatment	1.799 (0.824–3.629)	0.1336		
HCC stage III at treatment just before DAA	2.193 (0.694–5.954)	0.1671		
Number of tumors (≥3) at treatment just before DAA	3.374 (1.699–6.544)	0.0008	2.0633 (0.9623–4.3238)	0.0624
Maximum tumor diameter (≥2.5 cm) at treatment just before DAA	1.231 (0.489–4.129)	0.6874		
Platelet count (at start of DAA)	0.959 (0.890–1.029)	0.2537		
Albumin (at the beginning DAA)	0.973 (0.479–1.954)	0.9400		
Prothrombin time (at start of DAA)	0.996 (0.978–1.016)	0.7018		
Fibrosis-4 index (at start of DAA)	0.996 (0.913–1.059)	0.9050		
AFP (at end of DAA treatment) <8 vs ≥8 (≥8)	1.031 (1.009–1.047)	0.0091	2.6695 (1.2462–5.5706)	0.0123
	2.507 (1.285–4.782)	0.0078		
DCP (at end of DAA treatment)	1.004 (0.999–1.008)	0.0681		
ALT (at end of DAA treatment)	0.999 (0.964–1.031)	0.9674		
Anti-HBs change ratio	0.058 (0.001–2.090)	0.1384		
Anti-HBc change ratio	0.163 (0.014–3.476)	0.2212		

AFP, α-fetoprotein; ALT, alanine aminotransferase; DAA, direct-acting antiviral; DCP, des-gamma-carboxy prothrombin; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; IFN, interferon; SVR, sustained virologic response
Anti-HBs change ratio = anti-HBs level at the end of DAA therapy/anti-HBs level before DAA
Anti-HBc change ratio = anti-HBc level at the end of DAA therapy/anti-HBc level before DAA

frequency, reductions of antibody production against HBV were observed in most patients.

HCC Recurrence-related Factors Following Completion of Antiviral Therapy in Patients Who Achieved SVR with DAA

The factors that were associated with recurrence during the period of observation were analyzed. On univariate analysis, the number of past HCC treatments prior to DAA therapy (≥3), length of time from last HCC treatment to the start of antiviral therapy, number of HCC nodules, and AFP at completion of antiviral therapy were found to be significant. However, immunological change resulting from DAA therapy (anti-HBs change ratio and anti-HBc change ratio) was not a significant factor. The multivariate analysis showed that the duration from last HCC treatment to starting antiviral therapy and AFP at completion of antiviral therapy were independent factors (Table 3).

Discussion

When a comparison was made between SVR and non-SVR patients with past histories of treatment for HCC who had undergone IFN treatment, there were significant improvements in both the survival and recurrence rates in SVR patients compared to non-SVR patients, but the recurrence rate remained relatively high.[10] Subsequent studies comparing IFN and DAA therapies after treatment for HCC showed no significant difference in the recurrence rate.[22,23] In this context, although DAA is supposed to inhibit HCC recurrence, the recurrence rate seems to nevertheless remain high.

It seems clear that SVR with DAA therapy decreases the risk of the development of HCC in HCV-infected patients. A large-scale retrospective cohort study reported a significantly lower incidence of HCC in SVR patients when comparing SVR and non-SVR patients who had undergone DAA therapy. Although a significant proportion of non-SVR patients have cirrhosis, the importance of SVR remains high.[11] On the other hand, HCC is known to occur at a high frequency in patients with liver cirrhosis even after achieving SVR with DAA. The importance of HCC surveillance is thus widely recognized.[5,37–43] This highlights the fact that, while SVR reduces the incidence of HCC, it is not fully suppressed in patients with liver cirrhosis. An additional high risk of developing HCC doubtlessly exists in patients with a history of treatment for HCC. Other studies have suggested that DAA therapy may promote HCC recurrence.[13,14] Because tumor cells could be present in the liver parenchyma of patients with a history of treatment for HCC, any decline in the immune function due to DAA therapy may contribute to the proliferation of tumor cells resulting in the development of clinically overt recurrent HCC.

The present study focused on the relationship between changes of the immune response to HBV proteins and recurrence of HCC. Rapid eradication of HCV by DAA therapy brings about a dramatic reduction in lymphocytes infiltrating the hepatic tissue, which reduces serum levels of natural killer (NK) cell-stimulating cytokines and normalizes the NK cell phenotype.[44,45] This may simultaneously affect the immune response toward the other antigens such as HBV proteins and tumor cells present in the liver. In the present study, HBV-DNA-positive conversion was not seen in patients with resolved HBV infection. However, 53.8% of patients showed reductions in both anti-HBs and anti-HBc levels, and 93.6% of patients showed reductions of either anti-HBs or anti-HBc levels. There was a high rate of a decreased immune response toward HBV proteins, though none of the patients showed positive HBV-DNA conversion.

Elucidating the extent to which changes in immune function affect HCC recurrence is difficult, but in the present study, both a resolved HBV infection and a change of the immune responses to HBV proteins did not affect HCC recurrence after DAA therapy for HCV infection. Changes in the immune function may not affect the HCC recurrence rate according to the report that HCV eradication by DAA does not impact rates of delisting for HCC progression or rates of HCC recurrence post-liver transplantation.[46]

We previously reported that the altered immune response after DAA therapy might affect the severity of HCC recurrence, not the recurrence rate,[47] but the conclusion was withheld because there was a problem with the statistical analysis. Considering that many clinicians have thought that an unexpected recurrence has occurred,[14–17] the promotion of recurrence by DAA might be a phenomenon that is extremely rare but possible in practice so that one cannot show a statistically significant difference. Since the rate of HCC recurrence is naturally high, statistical evidence of the promotion of recurrence by DAA may be difficult to obtain if it is a phenomenon that very rarely occurs. It is still necessary to conduct a study of the effect of immunological change during DAA therapy and its effect on the severity of HCC recurrence. After all, careful monitoring using diagnostic imaging and tumor markers is required in patients with a history of HCC treatment even after eradication of HCV.

There were some limitations in this study. This is a retrospective, single-center study, and a prospective multicenter study is needed to confirm the present results. Since the number of patients in the present study was limited, an investigation with a larger cohort is needed to confirm the results. The rate of past HBV infection in the Japanese population is relatively high, and it might be difficult to conduct the same study in an area with a low incidence of HBV infection. However, the findings of this study could be useful even for investigators in areas with a low incidence of HBV infection.

Conclusion

Although anti-HBs and anti-HBc levels decreased significantly in patients with resolved HBV infection, HBV reactivation is rare, and changes of immune responses to HBV proteins did not affect HCC recurrence after DAA therapy for HCV infection.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

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