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【 ORIGINAL ARTICLE 】

Cases of Rapid Hepatitis B Surface Antigen Reduction after COVID-19 Vaccination

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Abstract:

Objective One of the therapeutic goals for chronic infection with hepatitis B virus is the clearance of hepatitis B surface antigen (HBsAg) from the blood, as a high load of HBsAg has been proposed to induce antigen-specific immunotolerance. To achieve HBsAg reduction, Pegylated interferon and nucleos(t)ide analogs are used to treat chronic hepatitis B. Following the coronavirus disease 2019 (COVID-19) outbreak, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has rapidly spread worldwide, and vaccination with mRNA COVID-19 vaccines has been conducted since 2021 in Japan. We experienced three clinical cases in which HBsAg levels rapidly decreased after injection of the COVID-19 vaccine without any incentive.

Method To examine whether the vaccine administration was involved in the HBsAg reduction, the number of patients with chronic hepatitis B showing a change in the HBsAg levels during the period before the commencement of the COVID-19 vaccination program in Japan (i.e. until the end of 2020; pre-vaccination-program period) was compared to the number of those who showed a change in HBsAg levels after the initiation of the program (i.e. 2021 onwards; post-vaccination-program period).

Results The number of patients whose HBsAg levels was reduced by >50% per year was prominent after the initiation of the vaccination program. Although the involvement of vaccination in HBsAg reduction was not statistically proven (p=0.0532), the result suggests that the administration of COVID-19 vaccines may have been involved in HBsAg reduction in patients with chronic hepatitis B.

Conclusion COVID-19 vaccines may be involved in HBsAg reduction.

Key words: HBsAg, COVID-19 vaccine, HBV, chronic hepatitis B

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Introduction

Hepatitis B virus (HBV) infection is a major public health threat worldwide, causing chronic hepatitis B [CH (B)], which can progress to liver cirrhosis and hepatocellular carcinoma. Pegylated interferon (Peg-IFN) and nucleos(t)ide analogs (NA), such as adefovir, entecavir, and tenofovir, are approved for the treatment of CH (B) and should be offered to patients (1). The median HBs-antigen (HBsAg) reduction from baseline by Peg-IFN-α-2a was reported as 0.32 and 0.44 $\log_{10}$IU/mL at 24 and 48 weeks of treatment, respectively (2). The average annual decline in HBsAg by entecavir was reported to be 0.043 and 0.044 $\log_{10}$IU/mL for HBeAg-positive and HBeAg-negative patients, respectively (3). The annual reduction in HBsAg levels by adefovir and entecavir is reported to be 0.1 $\log_{10}$IU/mL and 0.11 $\log_{10}$IU/mL, respectively (4). Thus, the rapid reduction of HBsAg is not easy to achieve. In addition to Peg-IFN and NA, immunomodulators, capsid assembly modulators, inhibitors of subviral particle release, cccDNA silencers, antisense oligonucleotides, nucleic acid polymers, and RNA interference molecules have been clinically developed for the treatment of CH (B) (5, 6).

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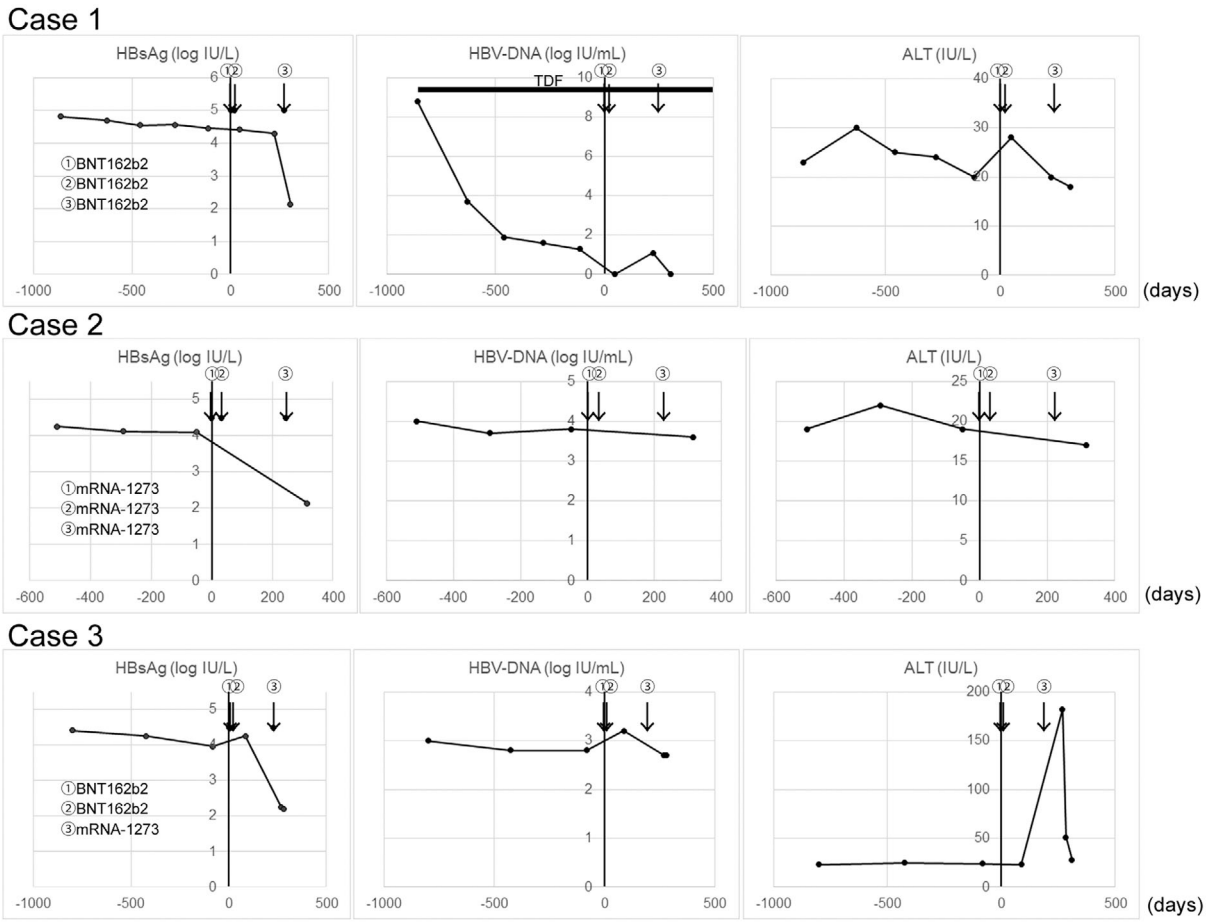

Figure 1. **Laboratory data of the three cases. Day 0 means the date of the first vaccination.**

After the coronavirus disease 2019 (COVID-19) outbreak in December 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection rapidly spread worldwide. The first case in Japan was confirmed in January 2020. Vaccination against SARS-CoV-2 with the BNT162b2 mRNA vaccine (BioNTech and Pfizer) was initiated in Japan on February 17, 2021, followed by mRNA-1273 (Moderna and Takeda) on May 24, 2021, and ChAdOXi-S adenovirus vector vaccine (Astra-Zeneca) on August 1, 2021. As of November 14, 2021, among the national population of approximately 125 million, an estimated 99.3 million (79.4%) had received at least one dose of the COVID-19 vaccine, including the 95.6 million (76.4%) who had received 2 doses of the vaccine. Of these shots, 83.6% were BNT162b2, 16.3% were mRNA-1273, and <0.1% were ChAdOx1-S (7).

However, the effects of COVID-19 vaccines on HBV infection are unknown. We found that three patients showed a rapid reduction in HBsAg after receiving COVID-19 vaccination and thus hypothesized that the administration of the COVID-19 vaccine was involved in the HBsAg reduction.

We herein report three such clinical cases and examine whether or not COVID-19 vaccination was involved in HBsAg reduction.

Case reports

Case 1

A 74-year-old Japanese woman presented at our hospital with HBeAg-positive CH (B) from 2019. Because her daughter was CH (B)-positive, she came to be examined for HBV. The HBsAg level in 2019 had been 64,521.9 IU/L, and the HBV-DNA level had been 8.8 log IU/mL. Oral tenofovir disoprixil fumarate administration was started in 2019. The HBsAg level in 2020 was 29,734.1 IU/L (Fig. 1). After 502 days, the HBsAg levels rapidly decreased to 140.2 IU/L. She was injected with the first COVID-19 vaccine 171 days after the HBsAg measurement in 2020, and the HBsAg reduction was observed after the third vaccination. All of the injected vaccines were BNT162b2. The second vaccine was injected 21 days after the first shot, and the third was injected 273 days after the first one. HBV DNA values also dropped from 1.5 to <1.0 log IU/mL. The alanine aminotransferase (ALT) levels were 24 IU/L in 2020 and 18 IU/L at the post-vaccination-program time point.

Case 2

A 33-year-old Japanese man presented to our hospital with HBeAg-negative inactive CH (B) from 2020. A blood test performed for a health checkup in 2020 revealed him to be HBsAg-positive. Because his mother had also been HBsAg-positive, he had received postnatal HBV vaccination.

His sisters were also HBsAg-positive. The HBsAg level in 2020 was 13,234.1 IU/L (Fig. 1). After 608 days, HBsAg levels rapidly decreased to 136.1 IU/L. He was injected with the first COVID-19 vaccine 293 days after the HBsAg measurement in 2020. Because HBsAg was measured after the third vaccination, the kinetics of HBsAg during the vaccination period were unknown. All of the injected vaccines were mRNA-1273. The second vaccine was injected 31 days after the first shot, and the third was injected 243 days after the first one. HBsAg HBV DNA was 3.7 log IU/mL in 2020 and 3.6 log IU/mL at the post-vaccination-program time point. The ALT levels were 24 IU/L in 2020 and 17 IU/L at the post-vaccination-program time point. He was not administered NA.

Case 3

A 67-year-old Japanese woman presented to our hospital with HBeAg-negative inactive CH (B) from 2019. Although a blood test performed for a health checkup in 2012 had revealed her to be HBsAg-positive, she had not visited a medical institution. The HBsAg level in 2020 was 17,722.3 IU/L (Fig. 1). After 707 days, the HBsAg levels rapidly decreased to 155.2 IU/L. The patient was injected with the first COVID-19 vaccine 425 days after HBsAg measurement in 2020, and the HBsAg reduction was observed after the third vaccination. The first and second vaccines were BNT 162b2, and the third one was mRNA-1273. The second vaccine was injected 21 days after the first shot and the third was injected 226 days after the first one. HBV DNA was 2.8 log IU/mL in 2020 and 2.7 log IU/mL at the post-vaccination-program time point. The ALT levels were 25 IU/L in 2020 and increased to 182 IU/L at 42 days after the third shot before decreasing to 51 IU/L at 56 days and 28 IU/L at 84 days after the third one. She was not administered NA.

These patients showed HBsAg reduction without any incentive other than the COVID-19 vaccination. Such a rapid decline in HBsAg values is uncommon in the natural history of CH (B). We therefore investigated the relationship between HBsAg reduction and the vaccination.

Materials ane Methods

Study approval

All analyses using patient data were approved by the institutional research ethics committees of the International University of Health and Welfare (13-B-381, 21-B-53, and 21-B-51).

Study subjects

To validate the involvement of the COVID-19 vaccines in HBsAg reduction, 237 patients who were confirmed to be HBsAg-positive in 2020, before the commencement of the COVID-19 vaccination program in Japan in 2021 (Fig. 2), were included in the study. Patients whose HBsAg levels had also been measured in 2021 or 2022 were included in the study. In addition, cases lacking documentation of HBsAg levels up to 2020 were excluded from the study. A total of 88 cases were excluded due to the lack of HBsAg measurements in 2021 or 2022, 14 cases were excluded due to the lack of multiple HBsAg measurements up to 2020, 26 patients were excluded because their serum HBsAg levels were <5 IU/L in 2020, and 2 patients were excluded because the HBsAg measurement interval was <6 months. In one of those two excluded patients, the levels of HBsAg were measured toward the end of November 2020 and in early January 2021; in the other patient, the levels of HBsAg were measured toward the end of May and in early November 2021. We considered these intervals too short to analyze the kinetics of HBsAg.

A total of 107 patients were included in this analysis comparing the HBsAg changes up to the end of 2020 (pre-vaccination-program period) with those from 2021 onwards (post-vaccination-program period). The observation period in the pre-vaccination-program period (884±472 days) was longer than that in the post-vaccination-program period (484 ±142 days).

To elucidate the factors that were involved in HBsAg reduction, the 107 total patients were divided into 3 groups: 11 with an HBsAg reduction of >50% per year during the post-vaccination-program period, 50 with 10-50% reduction, and 46 patients with a <10% decrease or an increase in HBsAg levels. Among these 46 patients, 1 was excluded due to a history of starting NA during the post-vaccination-program period. The 11 patients with >50% HBsAg reduction and 45 with minimal changes were then compared. To examine whether the patients had been administered the COVID-19 vaccine at least once during the post-vaccination-program period, a questionnaire was administered by phone or letter to survey whether or not vaccination had been performed before the last HBsAg measurement during the post-vaccination-program period.

HBsAg measurement

HBsAg levels were measured by the Architect HBsAg assay (Abbott, Abbott Park, USA).

Statistical analyses

The chi-square test and univariable logistic regression analyses were performed using the GraphPad Prism software program, version 5 (GraphPad Software, San Diego, USA) and Easy R (EZR) (8). $p<0.05$ was considered statistically significant.

Results

Distribution of patients with a >50% decrease in HBsAg levels by vaccination-program period

We first compared the number of patients with rapid HBsAg reduction before and after the initiation of the vaccine program in Japan (n=107). An HBsAg reduction of

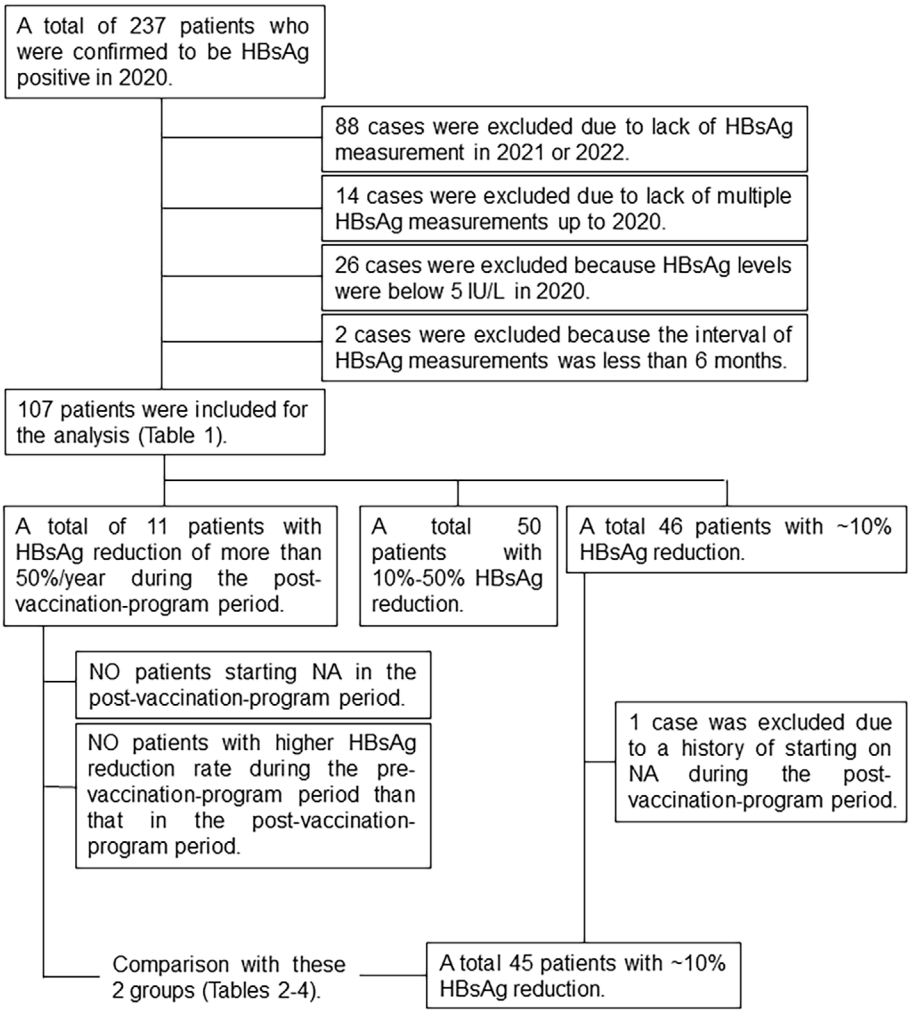

Figure 2. **Flowchart of the HBsAg-positive patient selection process.**

Table 1. **Contingency Table of the Cases.**

	HBsAg reduction of more than 50%		Total
	(+)	(-)	
Pre-vaccination-program period	3	104	107
Post-vaccination-program period	11	96	107

The Number of Cases in Whose HBsAg was Reduced by More than 50% during Pre-vaccination-program and Post-vaccination-program Periods in the 107 Cases. p=0.027, Based on the Two-tailed Chi-square Test.

>50% per year during the pre-vaccination-program period was observed in 3 patients (2.8%). In contrast, 11 patients (10.3%) showed HBsAg reduction in the post-vaccination-program period. This difference was statistically significant (Table 1; p=0.027). No patient showed a reduction in both the pre- and post-vaccination-program periods. Rapid HBsAg induction (two-fold increase per year) was observed in two patients during the pre-vaccination-program period and in three patients during the post-vaccination-program period.

Possible involvement of COVID-19 vaccine administration in HBsAg reduction

To elucidate whether the COVID-19 vaccine was involved in HBsAg reduction, the 11 patients with >50% HBsAg reduction/year (HBsAg reduction group) were compared with the 45 patients with a <10% decrease or an increase in the HBsAg level (minimal change group). The answers to the vaccine questionnaire were collected from 10 patients in the HBsAg reduction group and 37 in the minimal change group. Ten patients (100%) in the HBsAg reduction group

Table 2. Relationship between HBsAg Reduction and COVID-19 Vaccination.

	HBsAg reduction		Total
	More than 50%	Less than 10%	
COVID-19 vaccinated	10	29	39
Unvaccinated	0	8	8
Total	10	37	47

The relationship was analyzed by the one tailed chi-square test.
p=0.0532

Table 3. Relationship between HBsAg Reduction and NA Medication.

	HBsAg reduction		Total
	More than 50%	Less than 10%	
NA medication	3	11	14
(-)	8	34	42
Total	11	45	56

The relationship was analyzed by the one-tailed chi-square test.
p=0.4230

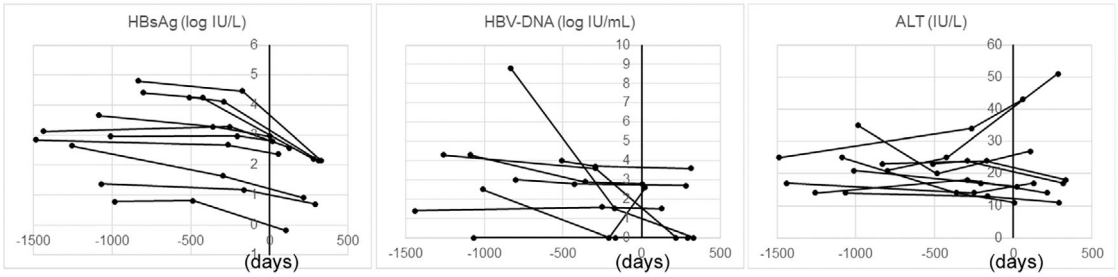

Figure 3. Kinetics of HBsAg, HBV DNA, and ALT in the HBsAg reduction group. Day 0 is the date of the first vaccination.

had been injected with the COVID-19 vaccine before the last HBsAg measurement during the post-vaccination-program period. In contrast, 29 patients (78.4%) had received the vaccine while 8 (21.6%) had not received the vaccine before the last HBsAg measurement during the post-vaccination-program period. Although the difference was not statistically significant (Table 2, p=0.0532), the results suggest that COVID-19 vaccines may be involved in HBsAg reduction.

The NA medication rate in the HBsAg reduction group was comparable to that in the minimal change group (Table 3). In the HBsAg reduction group, HBV DNA seemed to decrease without changes in HBsAg and ALT levels during the pre-vaccination-program period (Fig. 3). A univariate logistic regression analysis was performed to elucidate the factors involved in HBsAg reduction (Table 4). The rate of change in HBV DNA levels during the pre-vaccination-program period in the HBsAg reduction group (-57.7%/year) showed a greater reduction than that in the minimal change group (5.9% increase per year). HBV DNA reduction in the pre-vaccination-program period was involved in HBsAg reduction in the post-vaccination-program period. In addition, the HBsAg level in the HBsAg reduction group [929.9 IU/L (median)] was higher than that in the minimal change group [215.1 IU/L (median)]. The presence of high HBsAg levels during the pre-vaccination-program period was also found to be associated with an increased reduction in its levels in the post-vaccination-program period.

Discussion

mRNA vaccines induce the elaboration of type 1 interferon (IFN-1), including IFN-α and IFN-β (9, 10). The COVID-19 mRNA vaccines are also thought to cause the production of IFN-1 (11, 12). In addition, mRNA vaccines induce inflammatory responses in mice, such as cytokine production and immune cell activation (13). mRNA is recognized as a pathogen-associated molecular pattern by Toll-like receptors (TLR)7, TLR8, TLR9, retinoic acid-inducible gene-1 (RIG-1), and melanoma differentiation-associated protein 5 (MDA5) in macrophages and dendritic cells. The binding of these factors to RNA activates nuclear factor-κB and IFN regulatory factor 3/7, leading to the production of IFN-1 and pro-inflammatory cytokines (11, 12).

IFN has been used for CH (B) treatment (5, 6). In addition, the role of TLRs in the elimination of HBV infection has been reported in previous studies. TLR7 plays an important role in HBV (14). CL097 (TLR7/8 agonist)-conjugated HBV protein has been reported to reverse immune tolerance in HBV-transgenic mice (15). A TLR7 agonist (T7-EA) and HBsAg administration were shown to interrupt the immune tolerance in an HBV mouse model using adeno-associated virus serotype 8 encoding the HBV genome (16). TLR7 (R-848) and TLR9 (CpGODN) ligands have been reported to be useful as adjuvants in prophylactic and therapeutic HBV vaccines (17). Thus, it is reasonable to conclude that COVID-19 mRNA vaccines have antiviral activity against HBV. It was reported that a single dose of mRNA vaccine was only around 30% effective against SARS-CoV-2, with two doses being more effective (18). Thus, booster injection may provide a strong boost to the immune response. Indeed, the reduction of HBsAg was observed after the third vaccination in Cases 1 and 3.

In the HBsAg reduction group, changes in HBV DNA levels were able to be assessed in 8 patients, and only 2 showed an HBV DNA reduction of >1 $\log_{10}$ IU/mL in the

Table 4. Univariate Analysis of Factors: Influence on HBsAg Reduction More than 50%.

	A total of 11 cases whose HBsAg was reduced by more than 50% (HBsAg reduction group)	A total of 45 cases whose HBsAg was reduced to less than 10% (minimal change group)	p value
Age in 2020	67 (median)	62	0.465
Sex	5/6 (male/female)	25/20	0.549
HBsAg change rate during the pre-vaccination-program period	-16.4%/year (median)	-13.6%/year	0.977
HBsAg in 2020	929.9 IU/L (median)	215.1 IU/L	0.035
HBV DNA change rate during the pre-vaccination-program period	-57.7%/year (median)	5.6%/year	0.032
HBV DNA in 2020	2.2 log IU/mL (median)	1.7 log IU/mL	0.318
HBV DNA change rate during the post-vaccination-program period	-16.1%/year (median)	0%/year	0.723
ALT change rate during the pre-vaccination-program period	-2.9%/year (median)	0%/year	0.744
ALT in 2020	18 IU/L (median)	19 IU/L	0.595
ALT change rate during the post-vaccination-program period	-9.5%/year (median)	0%/year	0.220
PLT in 2020	181,000 /μL (median)	180,500 /μL	0.321
HBeAg	1/8 (positive/negative)	1/35	0.315

Univariable logistic regression analysis was performed.

post-vaccination-program period. Thus, HBsAg reduction can occur without a reduction in HBV DNA levels. IFN-α inhibits HBV replication by reducing RNA transcription from covalently closed circular DNA (19), suggesting that IFN-α reduces both HBV DNA and HBsAg levels. However, HBsAg does not reflect the viral load. It is likely that the surveillance period after vaccine administration was too short to observe a reduction in HBV DNA levels, or the IFN production by the vaccine might have been too low to induce HBV DNA reduction. Alternatively, mechanisms other than IFN production may also exist.

Notably, the involvement of the COVID-19 vaccine in HBsAg reduction was not proven by the statistical analysis (p=0.0532). Many reports using IFN in patients with CH (B) have shown that IFN-non-responders exist. HBsAg reduction is observed in limited patients, even if patients received PEG-IFN therapy for 48 weeks. These patients may not show HBsAg reduction after vaccine administration. Although this study surveyed all cases of HBsAg-positive patients encountered in 2020 in our hospital, the number of analyzable patients was small. The strength and duration of IFN induction by vaccination has not been investigated. In addition, cases of HBV reactivation after COVID-19 vaccination were reported (20).

It is unclear why some people had reduced HBsAg levels and others did not, despite receiving the same vaccination. The relationship between HBsAg reduction and the number or interval of vaccination is also unknown. In addition, an analysis is required using vaccinated and unvaccinated groups to examine the relationship between vaccination and HBsAg reduction. Therefore, further studies will be required to verify these results and clarify the mechanisms underlying the HBsAg reduction, potentially leading to the development of new CH (B) treatments.

In conclusion, the number of patients whose HBsAg level was reduced by >50% per year during the post-vaccination-program period was larger than that during the pre-vaccination-program period. Thus, COVID-19 vaccines may be involved in HBsAg reduction. However, large-scale studies will be required to address this issue.

The authors state that they have no Conflict of Interest (COI).

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