Switching to tenofovir disoproxil fumarate in entecavir-treated chronic hepatitis B patients: A pilot randomized controlled study

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Abstract. Although hepatitis B surface antigen (HBsAg) removal is considered the goal of chronic hepatitis B treatment, it can rarely be achieved with nucleos(t)ide analogues (NAs). It has been reported that tenofovir disoproxil fumarate (TDF) is superior in reducing HBsAg compared with entecavir (ETV) in treatment-naïve patients; however, the effect of TDF in patients who have received NAs is still unclear. The aim of the present study was to evaluate the efficacy of switching from ETV to TDF in patients who were already receiving ETV. A pilot randomized controlled study for 2 years in patients who had been treated with ETV for >1 year and did not exhibit drug resistance was performed (Clinical trial registration: UMIN000021948, UMIN-CTR, May 1, 2016). A total of 20 patients were enrolled and 19 patients were randomized into 2 groups, a TDF-switching group (n=12) or an ETV-continuing group (n=7). The mean change in HBsAg levels after 2 years was greater in the TDF group compared with the ETV group, but the difference was not significant (-0.25 vs. -0.06 log IU/ml). In the TDF group, hepatitis B e antigen (HBeAg)-positive patients at baseline showed significantly greater changes in HBsAg (-0.63 vs. -0.03 log IU/ml; P=0.030). In contrast, no difference between HBeAg-positive and HBeAg-negative patients was observed in the ETV group. No significant differences of estimated glomerular filtration rate and inorganic phosphorus changes were observed among the TDF and ETV groups. In conclusion, a significant HBsAg decrease was not achieved after switching from ETV to TDF in the overall analysis, but HBeAg-positive patients showed a larger HBsAg decrease after switching treatment.

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Introduction

Hepatitis B virus (HBV) infection is a worldwide health problem, and patients who are chronically infected with HBV are at greater risk of developing liver cirrhosis and liver cancer. Worldwide, ~292 million individuals are estimated to be chronically infected with HBV (1). Once HBV infects hepatocytes, its genome translocates to the nucleus and covalently closed circular DNA (cccDNA) is formed (2). The stability of cccDNA is one of the primary reasons why it is difficult to eliminate HBV completely. The serum levels of hepatitis B surface antigen (HBsAg) are considered to be associated with cccDNA levels in the liver (3), and the removal of HBsAg is regarded as the optimal treatment endpoint, termed 'functional cure' (4,5).

Nucleos(t)ide analogues (NAs), including entecavir (ETV), tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide fumarate (TAF), as well as interferons (IFNs), are widely used for treatment of chronic HBV infection worldwide (1,2,4,5). These treatments inhibit the reverse transcription of the HBV genome and HBV DNA in the serum can be reduced rapidly. Although IFNs were reported to reduce serum HBsAg levels more efficiently when used appropriately in combination with NAs (6) or sequentially after NA discontinuation (7,8), NA monotherapies are still beneficial for most patients as they can be taken orally and have fewer side effects than IFNs (4,5). However, long-term administration of NA is required, as the discontinuation can lead to frequent hepatitis exacerbations (5). Generally, it is hypothesized that NA does not reduce cccDNA efficiently and HBsAg is released into the blood continuously in most cases during NA treatments (9). A previous report showed that high serum levels of HBsAg increases the risk

of developing hepatocellular carcinoma in patients with low levels of HBV DNA (10). Additionally, low levels of HBsAg are reported to be a surrogate marker for the safe discontinuation of NA (11,12). Therefore, NAs that can reduce HBsAg efficiently are required in clinical settings.

A phase 3 clinical trial of TDF in Japan showed a significantly greater decrease in HBsAg levels in TDF-treated individuals compared with ETV-treated patients amongst treatment-naïve patients (13). Additionally, TDF is effective in patients with ETV-resistant HBV, even if TDF is administered as a monotherapy (14). However, it is unclear whether such an effect of TDF can be obtained in NA-treated patients in whom the hepatitis is stable. In the present study, a pilot prospective randomized control study was performed to evaluate the efficacy of switching to TDF in ETV-treated patients.

Patients and methods

Patients. A total of 20 patients were enrolled from 4 hospitals. Inclusion criteria were as follows: i) ETV had been administered for >1 year continuously without drug resistance; ii) HBsAg in the serum had been continuously positive; iii) patients ≥20 years old; and iv) and they had no history of decompensated liver cirrhosis or liver cancer. The definition of drug resistance is a 1-log (10-fold) increase in HBV DNA from the nadir in a patient who had an initial virological response (5). The exclusion criteria were as follows: i) Patients receiving immunosuppressive therapies; ii) estimated glomerular filtration rate (eGFR) <50 ml/min/1.73 m^2; iii) presence of hypophosphatemia (<2.5 mg/dl); iv) pregnant women and women suspected of being pregnant; v) breast-feeding women and vi) coinfection with hepatitis C virus or human immunodeficiency virus. The patients were randomized into 2 groups, a TDF-switching group or ETV-continuing group (Fig. 1). Randomization was performed using a random number table. Among the 20 patients enrolled in the present study, 1 patient was excluded due to a low eGFR and a total of 19 patients were randomized. The median age of the randomized patients was 62 years old (range, 32-79); 13 patients (68%) were male, and 6 patients (32%) were female. After randomization, 12 patients (median age, 63; range, 32-79; 9 males and 3 females) were assigned to the TDF switching group and 7 patients (median age, 48; range, 37-72; 4 males and 3 females) were assigned to the ETV continuing group. ETV at 0.5 mg/day was administered orally while fasting, and TDF at 300 mg/day was administered orally after a meal. The patients were observed every 3 months for 24 months and the clinical data were collected at 3, 6, 9, 12, 18 and 24 months after enrollment. The primary efficacy endpoint was the change of serum HBsAg at 24 months, and the secondary endpoints were the changes of alanine aminotransferase (ALT), eGFR and inorganic phosphorus (IP). Imaging tests including abdominal ultrasonography were performed for the screening of liver cancer. This study was registered on University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR, ID: UMIN000021948). The study enrollment was started at April 2016 and the observation was performed until March 2019. The study protocol conformed to the guidelines described in the Declaration of Helsinki (15), and was approved by the Medical Ethics Committee of Tohoku University (approval no. 2016-2-11-1). Written informed consent was obtained from each patient.

Determination of serological markers and HBV genotype. The serum levels of HBsAg were quantified using a chemiluminescent enzyme immunoassay (CLEIA) with LUMIPULSE HBsAg-HQ (Fujirebio; cat. no. 296851). Hepatitis B e antigen (HBeAg) was assessed using a CLEIA by ARCHITECT (Abbott Pharmaceutical Co. Ltd.; cat. no. G06241R03). The HBV DNA levels were quantified using reverse transcription-quantitative (RT-q)PCR assays using Cobas TaqMan HBV Auto, according to the manufacturer's protocol (Roche Diagnostics). HBcrAg was tested using a CLEIA with LUMIPULSE (Fujirebio; cat. no. 294109). HBV genotypes were determined using the IMMUNIS HBV genotype EIA kit (Institute of Immunology; cat. no. 1A65).

Statistical analysis. Statistical analysis was performed using JMP version 14.2 (SAS Institute Inc.). Statistical comparisons were performed using a χ^2 test for comparison of frequencies between the two groups or a Wilcoxon rank sum test for comparison of continuous variables between two groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Clinical characteristics of the enrolled patients. Among the 19 patients randomized in the present study, a total of 5 (26%) and 14 (74%) patients were infected with HBV genotype B and C, respectively. The clinical characteristics of the chronic hepatitis B patients in the TDF switching group (n=12) and the ETV continuing group (n=7) are shown in Table I. There were no statistically significant differences in the clinical characteristics between these groups.

Comparison of the antiviral effects between the TDF and ETV group. At randomization, the number of patients whose HBV DNA levels in the serum were lower than the detection limits were 11/12 (92%) and 5/7 (71%) in the TDF and ETV groups, respectively. At 12 months after enrollment, they were 11/12 (92%) and 6/7 (86%), and at 24 months, 12/12 (100%) and 6/7 (86%) in the TDF and ETV groups, respectively.

In the overall analysis, the mean change of HBsAg was -0.20 and -0.17 log IU/ml at 12 and 24 months, respectively. When comparing the TDF and ETV groups, HBsAg changes were greater in the TDF group at 12 months (-0.25 vs. -0.13 log IU/ml) and at 24 months (-0.25 vs. -0.06 log IU/ml), but the differences were not statistically significant (Fig. 2A). The results showed that HBsAg tended to decrease more in the first 12 months in the TDF group, whereas the changes of HBsAg were lower in the ETV group during the 24 months. The changes in ALT were similar other than at 18 months when it was significantly lower in the TDF group (Fig. 2B). There were 7 patients who were positive for HBeAg at randomization, and HBeAg sero-clearance was achieved in 2/4 (50%) patients in the TDF group and 0/3 (0%) patients in the ETV group. No patients developed liver cancer during the observation period.

Table I. Clinicopathological characteristics of the patients randomized in the two groups.

Characteristics	TDF group[a], n=12	ETV group[a], n=7	P-value
Age, years	63 (49-70)	48 (40-67)	0.421
Sex, male/female	9/3	4/3	0.423
T-Bil, mg/dl	0.8 (0.6-0.8)	0.5 (0.5-0.8)	0.198
AST, U/l	20 (19-25)	17 (16-21)	0.098
ALT, U/l	17 (15-31)	16 (11-21)	0.289
g-GTP, U/l	25 (18-28)	18 (17-27)	0.928
Alb, g/dl	4.5 (3.8-5.1)	4.2 (3.9-5.2)	0.442
Cr, mg/dl	0.73 (0.70-0.77)	0.73 (0.653-0.76)	0.735
eGFR, ml/min/1.73 m^2	79.1 (74.8-90.3)	78.2 (71.7-86.3)	0.899
IP, mg/dl	3.2 (2.8-3.3)	2.8 (2.5-3.0)	0.071
PLT, x10^4/ml	19.3 (16.4-23.5)	18.7 (15.4-23.2)	0.966
FIB-4 index	1.50 (1.06-1.92)	1.24 (0.63-2.28)	0.899
AFP, ng/ml	2.3 (2.0-2.3)	2.7 (1.4-2.8)	1.000
HBV DNA, log IU/ml	BDL (BDL-BDL)	BDL (BDL-1.0)	0.258
HBsAg, IU/ml	1,006 (391-9,011)	2,500 (483-4,085)	1.000
HBeAg, +/-	4/8	3/4	0.679
HBcrAg, log U/ml	4.3 (BDL-4.9)	3.7 (3.1-4.8)	0.719
HBV genotype, B/C	4/8	1/6	0.348
ETV duration, months	62 (34-93)	40 (31-49)	0.206
NA prior to ETV, LAM/LAM+ADV/none	1/0/11	0/1/6	0.231

[a]Median (interquartile range) or number. AFP, α fetoprotein; Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BDL, below detection limit; Cr, creatinine; eGFR, estimated glomerular filtration rate; ETV, entecavir; γ-GTP, γ-glutamyltransferase; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IP, inorganic phosphorus; NA, nucleos(t)ide analogue; PLT, platelet counts; T-Bil, total bilirubin; TDF, tenofovir disoproxil fumarate.

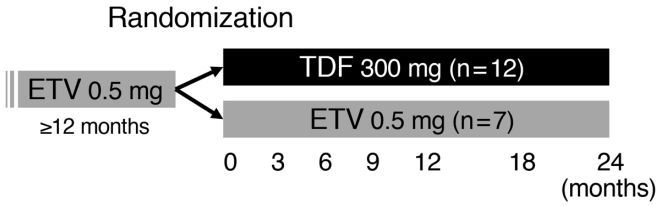

Figure 1. Schema of the design of the present study. Patients who had been treated with ETV for more than 12 months were randomly divided into 2 groups: A TDF-switching and ETV-continuing group. ETV, entecavir; TDF, tenofovir disoproxil fumarate.

Comparison of safety profiles between the TDF and ETV groups. As renal toxicity has been reported as a major adverse effect of TDF (16), the changes in the eGFR and serum IP levels were compared. However, no differences in eGFR changes were observed (Fig. 2C). The IP levels seemed to be reduced in the TDF group, but the differences were not significant at any time points (Fig. 2D). Of note, a 62-year-old male patient in the TDF group, whose serum IP levels were 2.6 mg/dl at randomization showed hypophosphatemia (2.0 mg/dl) at 12 months and TDF was switched to TAF. No data regarding this patient were included in the analysis after this time point. In this patient, switching back to ETV was not recommended as the slight signal of serum HBV DNA was detected during ETV administration. The IP levels slightly recovered to 2.2 mg/dl 6 months after switching to TAF.

HBsAg dependent changes on HBeAg positivity. As HBeAg affects the efficacies of antiviral treatments (17), the HBsAg decrease after 24 months between the HBeAg-positive and HBeAg-negative patients were compared. The HBsAg levels at baseline and at 24 months in each patient are shown in Fig. 3A. Notably, an HBeAg-positive patient in the TDF-switching group lost their HBsAg signal. In the TDF-switching group, a significantly greater decrease in HBsAg was observed in HBeAg-positive patients than in the HBeAg-negative patients (-0.63 vs. -0.03 log IU/ml; P=0.030; Fig. 3B). In contrast, no differences between HBeAg-positive and HBeAg-negative patients was observed in the ETV-continuing group.

Case presentations. A patient who showed ALT flare-ups after switching from ETV to TDF exhibited loss of HBsAg subsequently. The patient was a 77-year-old male and a liver biopsy 8 years before the enrollment showed METAVIR scores (18) of F2 and A2. He had a history of diabetes mellitus and underwent an operation for esophageal cancer. He started administration of ETV 8 years prior to inclusion, and it was stopped once 4.5 years later. After that, a hepatitis relapse with HBV DNA of 7.6 log IU/ml and HBsAg of 2.98 log IU/ml was observed and ETV was restarted 2.5 years ago, and has been continued for 30 months before switching to TDF. The clinical course after the treatment switch to TDF is shown in Fig. 4. The HBV genotype was C and HBcrAg levels evaluated 9 months

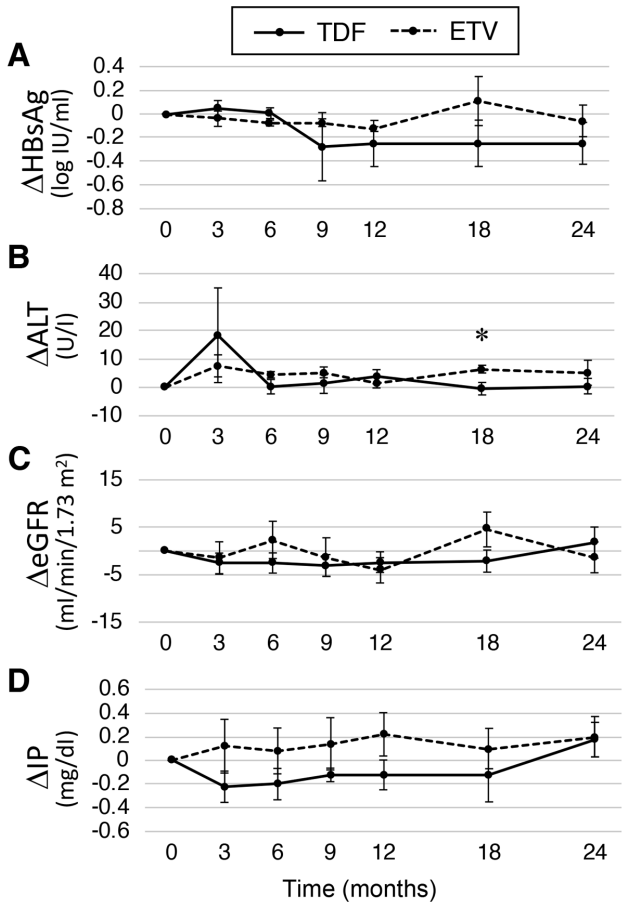

Figure 2. Comparison of the changes in the assessed parameters from baseline amongst the TDF-switching group and ETV-continuing group. (A) HBsAg, (B) ALT, (C) eGFR and (D) IP. There were no significant differences other than the ALT changes at 18 months. Error bars indicate standard errors. *P<0.05. HBsAg, hepatitis B surface antigen; ALT, alanine aminotransferase; eGFR, estimated glomerular filtration rate; IP, inorganic phosphorus; ETV, entecavir; TDF, tenofovir disoproxil fumarate.

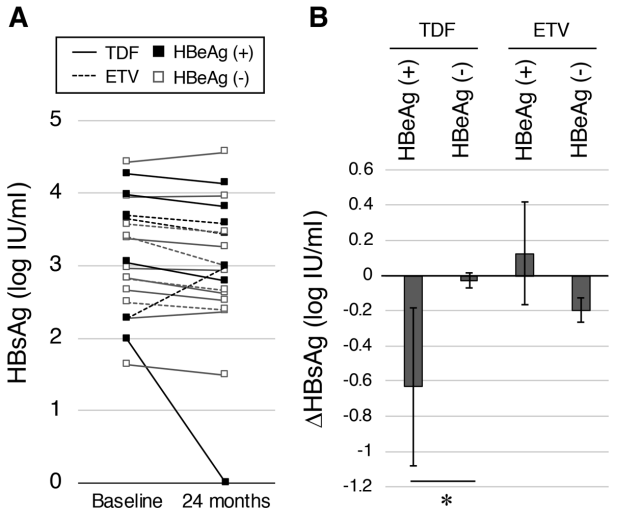

Figure 3. Changes in HBsAg between baseline and 24 months later. (A) HBsAg levels at baseline and at 24 months later in each patient. Black boxes with black lines and white boxes with gray lines indicate HBeAg positive and HBeAg negative at baseline, respectively. (B) Comparison of HBsAg changes from baseline to 24 months later amongst the four groups depending on HBeAg positivity at baseline as well as treatment groups. Error bars indicate standard errors. *P<0.05. HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; ETV, entecavir; TDF, tenofovir disoproxil fumarate.

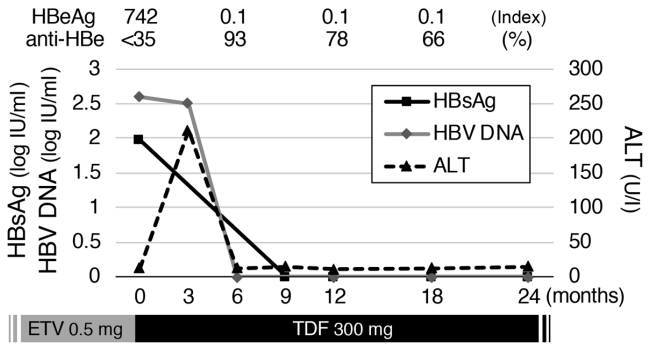

Figure 4. Clinical course of a patient with an HBV genotype C in the TDF-switching group in which HBsAg signal disappeared after 9 months. A transient increase of ALT was observed after 3 months, and subsequently, HBV DNA and HBeAg levels became undetectable. HBsAg, hepatitis B surface antigen; ALT, alanine aminotransferase; HBV, hepatitis B virus.

after the treatment switch was 4.6 log U/ml. No other patients showed such ALT elevations.

Discussion

In the present pilot study, the effects of switching to TDF in ETV-treated patients without drug resistance was assessed. The primary aim was to evaluate differences in the decrease in HBsAg signal after the treatment switch, but they were not significant in the overall analysis. A similar result for changes in HBsAg was reported recently in a 48-week randomized trial targeting patients who had been treated with ETV for >5 years (19). However, when analyzed in the TDF-switching group in the present study, a greater decrease in HBsAg signal in HBeAg-positive patients compared with HBeAg-negative patients was observed. Of note, even when a patient whose HBsAg disappeared rapidly (Fig. 4) was removed from the analysis, there was still a similar tendency (P=0.068). As most patients (85%) were HBeAg-negative in the previous study (19), it is possible that switching to TDF in ETV-treated patients may have had an additional effect on the HBsAg decrease only in HBeAg-positive patients. Consistent with this, in treatment-naïve patients, TDF was reported to result in a greater decrease in HBsAg signal in HBeAg-positive patients compared with HBeAg-negative patients (-0.37 vs. 0.07 log IU/ml) (13). Additionally, a randomized controlled study in South Korea in patients whose responses to ETV were partial showed that switching from ETV to TDF was superior than continuing ETV for the suppression of HBV DNA (20). In this previous study, all patients in the TDF-switching group were HBeAg-positive (n=22). Based on the aforementioned previous study and the present study, switching ETV to TDF may be considered in ETV-treated patients whose HBeAg is still positive. The mechanisms underlying the more prominent decrease in HBsAg in the HBeAg-positive patients are still unclear, but they may be related to the fact that HBeAg positivity during ETV administration indicates limited suppression of HBV mRNA transcription from cccDNA, even if HBV DNA is undetectable in the serum. In such patients, TDF may exert additional effects. Another possible mechanism is that HBeAg may affect the anti-viral effects of TDF. HBeAg is known to modulate immune responses (21). Furthermore, it was previously shown

that HBeAg may modulate intracellular vesicle trafficking (22). Such potential effects of HBeAg may alter the effect of TDF, but further investigation is required.

The different HBV genotypes are known to have varying effects on the clinical course in chronic infections. In our previous study, it was shown that the HBsAg decrease was greater in patients with HBV genotype B than in those with HBV genotype C (17). A higher frequency of HBV genotype B in individuals from northeast Japan, where our institutions are located, than overall in Japan, has been established previously (23). In the present study including HBV genotype B at 26%, differences between genotypes could not be found (data not shown), possibly due to the small number of patients.

It has been reported that ETV does not affect renal function, even in patients with severe renal dysfunction (24). In contrast, TDF is known to have a potential side effect on renal toxicity (25). Proximal tubular dysfunction causes a decrease in phosphorus reabsorption leading to decreased bone mineral density. The present study showed no statistical differences in eGFR and IP levels, but there was a tendency to reduction of IP levels in the TDF group and a patient in the TDF group presented with hypophosphatemia. Therefore, the treatment switch from ETV to TDF should be considered carefully in older patients, who are more likely to also suffer from chronic kidney diseases or osteoporosis. As TAF was reported to have fewer side effects on the kidneys and bones (26), it may be an option to switch NAs to TAF in such patients. Switching from TDF to TAF was reported to contribute to recovery of renal dysfunction in patients with HBV (27) and in those infected with human immunodeficiency virus (28). A previous study assessing switching from ETV to TAF showed a greater decrease in HBsAg levels (29), and another study showed that such a decrease was observed in patients with a low baseline HBsAg (30). Additionally, it has been shown that HBsAg is decreased more prominently in patients with non-liver cirrhosis, HBV genotype B, HBeAg negative patients or patients with low hepatitis B core-related antigen (HBcrAg) (31). Although no similar tendency was observed in the present study, further studies with larger cohorts are required to clarify the differences between switching from ETV to TDF with that to TAF.

Recent studies have shown that only acyclic nucleoside phosphonates (ANPs) such as adefovir dipivoxil and TDF, increased IFN-λ3 levels in the gastrointestinal tract (32), inhibited lipopolysaccharide-mediated interleukin (IL)-10 production and induced IL-12p70 in peripheral blood mononuclear cells towards HBV elimination (33). Such additional immunomodulatory effects with ANPs, which were not observed with ETV, may have a favorable effect on the HBsAg decrease, particularly in HBeAg-positive patients. In our HBeAg-positive case with HBsAg disappearance after switching from ETV to TDF, such an immunomodulatory effect may have played a role. Clarification of the HBeAg effects on TDF is needed.

There are some limitations to the present study. First, this study was a small pilot study, and the number of HBeAg-positive patients were limited; therefore, the results should be verified in larger studies. The allocation of groups was intended to be equal in the study design, but the difference in patient numbers was made unintentionally due the small study size. Second, although HBcrAg has been reported to be associated with cccDNA in the liver (34), the changes in HBcrAg could not be evaluated due to a lack of relevant data. Third, TDF may affect bone mineral density (26), but this could not be evaluated. These parameters should be analyzed sequentially in future studies.

In conclusion, the present pilot randomized control study showed that switching to TDF did not have additional effects on the decrease in HBsAg in previously ETV-treated patients. However, when analyzing only the TDF-switching group, the HBeAg-positive patients showed a greater decrease in HBsAg than the HBeAg-negative patients. Further studies are required to confirm the effects and to determine the underlying mechanisms.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JI designed the study, analyzed the data and wrote the manuscript. TA and TK designed the study, collected the data and critically reviewed the manuscript. NO, TU, EK, MN, TI, AS, MT and KS collected the data. AM designed the study and critically reviewed the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study protocol used in the present study conformed to the guidelines described in the Declaration of Helsinki, and has been approved by the Medical Ethics Committee of Tohoku University (approval no. 2016-2-11-1). Written informed consent was obtained from each patient.

Patient consent for publication

Not applicable.

Competing interests

JI received research funding from Gilead Sciences and AbbVie. The other authors declare that they have no conflict of interests.

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