Change in tartrate-resistant acid phosphatase isoform 5b levels, a marker of bone metabolism, in patients with chronic hepatitis B treated with tenofovir alafenamide

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Abstract. Hepatitis B virus (HBV) infection is associated with the risk of osteoporosis and bone mineral density (BMD) loss. Tenofovir alafenamide (TAF) is associated with a slightly lower degree of BMD loss compared with tenofovir disoproxil, without loss of the excellent anti-HBV effects. The aim of the present study was to verify the effect of bone metabolism in patients with HBV treated with TAF. A total of 87 patients were treated with TAF. Of these, 32 patients were treatment naïve, and 55 patients were treated with entecavir (ETV) for at least 1 year, after which ETV was switched to TAF. At the start of TAF and after 1 year, BMD in the lumbar and neck of the femur, tartrate-resistant acid phosphatase isoform 5b (TRACP-5b) levels as a marker of bone metabolism and serum inorganic phosphorus (P) were compared to estimate bone metabolism. Serum creatinine (Cr), cystatin C, urine protein and β2 microglobulin levels were evaluated to estimate kidney function. Treatment with TAF for 1 year decreased TRACP-5b levels, particularly in patients with bone disease, except for a minimal significant change (MSC; decrease of 12.4%) in TRACP-5b levels. The change in rate of TRACP-5b levels were positively associated with changes in P, Cr-estimated glomerular filtration rate and TRACP-5b levels at the start of TAF. Logistic regression analysis showed that increased BMD in the lumbar region contributed to the switch from ETV to TAF. TAF induced a decrease in TRACP-5b levels in patients with HBV. Bone disease was a contributing factor for MSC. Since TRACP-5b can be used as a marker of bone metabolism and fractures, TAF may exhibit potential in preventing fractures in patients with HBV.

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Introduction

Hepatitis B virus (HBV) infection causes cirrhosis and hepatocellular carcinoma (1,2); *de novo* HBV infection also causes acute liver failure (2). Treatment for HBV infection focuses on improving survival and quality of life by preventing disease progression (3). Long-term administration of a potent nucleoside/nucleotide analog (NA) with a high barrier to resistance is the treatment of choice regardless of the severity of liver disease, and the preferred regimens are entecavir (ETV), tenofovir disoproxil (TDF) and tenofovir alafenamide (TAF) (3). In cases of long-term administration of NA, patients are at an increased risk of renal disease and should undergo periodic renal monitoring, including at least estimated glomerular filtration rate (eGFR) and serum phosphate level (3). Chronic HBV infection is associated with kidney damage (4), and follow-up of renal function in patients with HBV is important during treatment.

Additionally, hepatic osteodystrophy (HOD) is common in chronic liver diseases, including viral hepatitis (5-7). HOD includes osteoporosis and osteomalacia, which are caused by hormonal abnormality-induced advanced liver failure complicated with abnormal serum calcium and phosphate (5-7). HBV infection is associated with the risk of osteoporosis and bone mineral density (BMD) loss (8,9). The bone metabolic marker, tartrate-resistant acid phosphatase isoform 5b (TRACP-5b), is an activation marker of osteoclasts, and is elevated in patients with HOD associated with chronic liver disease (6). TRACP-5b

levels from baseline to 3 months after treatment may predict the efficacy of bone therapy after 12 months (10). Therefore, measurements of BMD and bone metabolic markers are useful in the diagnosis and management of osteoporosis (11).

TAF is associated with a slightly lower degree of BMD loss and creatinine (Cr) elevation compared with TDF, without loss of the excellent anti-viral effects (12-14). Patients on TDF that are at a risk of development of or have already developed underlying renal or bone disease should be considered for a switch to ETV or TAF (3). However, switching from ETV to TAF is contested. When ETV was switched to TAF, serum Cr (15) or renal tubular function (16) improved, hepatitis B surface antigen (HBsAg) decreased, however no-change in BMD and renal function were observed (17). ETV is administered orally once daily under fasting conditions, and TAF is administered orally once daily. Switching from ETV to TAF can be a useful approach for improving medication adherence and satisfaction (18,19).

Based on favorable adherence, TAF is being chosen for the treatment naïve patients with HBV in our hospital, and patients have been encouraged to switch from ETV to TAF since 2017. In this study, the influence of TAF treatment on bone metabolism and kidney function for 1 year of TAF treatment was evaluated.

Patients and methods

Patients. A total of 87 patients with HBV infection were admitted to the Nagasaki Harbor Medical Center between April 2017 and February 2020. Of these, 32 patients (median age, 58.96; range, 36-86; female/male, 14/18) were naïve to treatment with TAF (Vemlidy, Gilead Sciences) (Naïve group), and 55 patients (median age, 59.89; range, 35-82; female/male, 15/40) were treated with ETV (Baraclude, Bristol-Myers Sqibb) for at least 1 year, and switched to TAF (Switch group). The reason for switching was to ensure drug compliance, adjusted for lifestyle. ETV was administered orally at a dose of 0.5 mg once daily under fasting conditions. TAF was administered orally at a dose of 25 mg once daily. No patients were treated for osteoporosis before TAF initiation. The medical records of the 87 patients were compared at the start of TAF and after 1 year. The prevention group was treated with TAF for asymptomatic HBV using immunosuppressants and/or anticancer drugs. The bone disease-positive group was defined as follows: Chronic steroid use or use of other medications that worsen bone density and/or history of fragility fracture and/or osteoporosis.

The medical records of the 87 patients were retrospectively reviewed. Informed consent was obtained from each patient included in the study, and the patients were guaranteed the right to leave the study if they desired. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki (20) and was approved by the Human Research Ethics Committee of the Nagasaki Harbor Medical Center (approval no. H30-031).

Laboratory measurements. Laboratory data and anthropometric measurements were obtained for each subject every 4-12 weeks during treatment, and BMD and urinalysis were examined every 12 months. The body mass index of each patient was calculated by dividing their weight in kg by the square of their height in meters. Laboratory examinations included platelet count, Cr, cystatin C (CysC), albumin, total bilirubin, alanine aminotransferase (ALT), calcium (Ca), inorganic phosphorus (P), α-fetoprotein, protein induced by vitamin K absence-II (PIVKA-II), Mac-2 binding protein glycan isomer (M2BPGi), total type I procollagen N-propeptide (P1NP), and tartrate-resistant acid phosphatase 5b (TRACP-5b). HBsAg, HBeAg, HBe antibody (HBeAb), HB core-related Ag (HBcrAg) and HBV-DNA were evaluated at the start of TAF administration and 1 year later. Urinalysis was performed using the β2-microglobulin-to-creatinine β2MG/Cr ratio and total protein-to-creatinine protein/Cr ratio. Radiological findings in the present study.

Osteoporosis, osteopenia and normal BMD were diagnosed according to the World Health Organization criteria (osteoporosis, T-score ≤-2.5; osteopenia, T-score between -2.5 and -1; normal BMD: T-score >-1) (21). Bone mineral density was measured at the lumbar spine (mean, L2-L4) and femoral neck using dual-energy X-ray absorptiometry (DEXA).

Cr- and CysC-based estimated GFRs (eGFRs) (ml/min/1.73 m^2) in women and men were calculated using the equations provided by the Japanese Society of Nephrology for Japanese patients (22). The sarcopenia index (SI) was calculated as follows: Cr/CysC x100 (23). The calculated body muscle mass (CBMM) was calculated as follows: (body weight in kg x Cr)/[K x body weight in kg x CysC)+ Cr], where K=0.00675 for men and 0.01006 for women (24).

Statistical analysis. Data were analyzed using StatFlex version 6.0 (Artech Co., Ltd.) and are presented as the mean ± standard deviation. Laboratory result variables were compared using a Wilcoxon test for differences between paired groups, a Mann-Whitney tests comparison for unpaired two groups, and a χ^2 test for comparison between discrete variables. A standardized partial regression coefficient β was employed. Univariate and multivariate analyses were performed using a logistic regression analysis. Correlations were evaluated using Pearson's correlation coefficient (R). $P<0.05$ was considered to indicate a statistically significant difference.

Results

The clinical characteristics of the patients at the start of TAF and after 1 year, are described in Table I. It was observed that male patients were dominant and 51 patients were ≥60 years of age. The prevention group included patients with asymptomatic HBV infection treated with TAF as part of an anticancer or immunosuppressive therapy. During the 1-year observation period, HBsAg levels were not significantly altered, but HBcrAg and HBV-DNA levels significantly decreased. Albumin levels increased and M2BPGi levels decreased. Regarding renal function, Cr levels were increased, Cr-based eGFR decreased and urine protein/Cr ratio increased. CysC, CysC eGFR, Ca, P and urine β2MG/Cr ratios were not significantly changed. In bone metabolism, lumbar BMD did not change, but the BMD of the neck of femur decreased. However, TRACP-5b levels significantly improved. P1NP was not measured after 1 year.

A focus was placed on the change in TRACP-5b associated with TAF. The change in TRACP-5b levels were compared

Table I. Clinical characteristics at start of treatment and after 1 year.

Factor	At start	After 1 year	P-value
Female/male, n	29/58	29/58	
Age, years	59.55 (12)		-
Age bracket, n			-
≥60 years	51		
<60 years	37		
Body weight, kg	61.83 (12.1)	61.91 (12.18)	0.5899
Body mass index, kg/m^2	23.07 (3.64)		
Groups, n			
Naïve	32		
Switch	55		
Prevention	20		
HBsAg IU/ml	3,134 (9,744)	2,115 (3,697)	0.0005
HBcrAg Log U/ml	3.457 (1.612)	3.11 (1.485)	<0.0001[c]
HBcrAg positive, n	45	36	<0.001[c]
HBeAg C.O.I.	97.756 (376)	55.628 (271)	0.0352[a]
HBeAg positive, n	13	13	0.999
HBeAb % inhibition	74.41 (34.12)	72.84 (32.5)	0.2149
HBV-DNA log IU/ml	1.80 (2.522)	0.251 (0.559)	<0.0001[d]
HBV-DNA positive, n	41	17	<0.0001[d]
AST U/l	68.92 (227.13)	26.73 (14.85)	0.019[a]
ALT U/l	79.91 (310.1)	24.97 (21.05)	0.0241[a]
Platelet x10^4/μl	17.86 (7.164)	18.64 (17.49)	0.342
Albumin g/dl	4.098 (0.578)	4.226 (0.456)	0.0053[b]
Total bilirubin mg/dl	0.921 (0.985)	0.907 (0.577)	0.0198[a]
M2BPGi C.O.I	1.436 (2.05)	1.058 (1.37)	0.0014[b]
Cr mg/d	0.825 (0.248)	0.857 (0.219)	0.0007[c]
Cr-eGFR ml/min/1.73 m^2	72.945 (19.006)	68.51 (16.91)	<0.0001
Cys C mg/l	1.103 (0.345)	1.098 (0.328)	0.6944
Cys C-eGFR ml/min/1.73 m^2	70.74 (22.66)	70.36 (22.49)	0.6253
Sarcopenia index[e]	77.21 (18.4)	80.69 (817.82)	0.0424[a]
Ca mg/dl	9.074 (0.493)	9.138 (0.486)	0.2563
P mg/dl	3.127 (0.502)	3.193 (0.522)	0.4027
Urine protein/Cr g/g	0.147 (0.383)	0.199 (0.536)	0.0205[a]
Urine b2MG/Cr μg/mg	4.499 (19.301)	7.845 (32.666)	0.9357
Lumbar BMD g/cm^2	0.906 (0.206)	0.907 (0.2)	0.2041
Lumbar t-score	-1.09 (1.697)	-1.091 (1.668)	0.4505
Lumbar young adult mean	87.62 (19.614)	87.427 (18.833)	0.7057
Lumbar osteoporosis, n	19	15	0.3409
Neck of femur BMD g/cm^2	0.66 (0.142)	0.636 (0.132)	<0.0001[d]
Neck of femur t-score	-1.504 (1.111)	-1.699 (1.026)	0.0001[d]
Neck of femur young adult mean	78.785 (15.523)	75.813 (14.659)	<0.0001[d]
Neck of femur Osteoporosis, n	13	21	0.0625
TRACP-5b mU/dl	417.7 (207)	356.5 (142.3)	0.0039[b]
TRACP-5b High, n	18	13	0.1714
P1NP ng/ml	55.05 (28.25)		
P1NP High[e]	9	0	

with clinical factors (Table II). TRACP-5b at start in male patients, patients who were HBeAg-positive, HBcrAg-positive, HBV-DNA-negative, switch, treatment, high albumin (≥4 g/dl), high platelet count (≥15x10^4/μl) and low body mass index (<25) were lower than after 1 year. In conforming with the EASL guidelines (4), old age (≥60 years), bone disease [chronic steroid use or use of other medications that worsen bone density and/or history of fragility fracture and/or

Table I. Continued.

Factor	At start	After 1 year	P-value
α-fetoprotein ng/ml	10.64 (25)	4.93 (4.5)	0.6469
PIVKA-II mAU/ml	38.33 (114.3)	38.46 (106.5)	0.4463

[a]P≤0.05, [b]P≤0.01, [c]P≤0.001, [d]P≤0.0001. [e]Data are presented as the mean ± standard deviation. Co.O.I., cut off index. The sensitivity of HBsAg is 0.005 IU/ml. The sensitivity of HBcrAg is 2.9 log U/ml. The sensitivity of HBeAg is 1.0 cut off index. Positive HBeAb was <60% inhibition. The HBV-DNA detection level ranged from 1-9.1 log IU/ml. The normal range of AST is 10-40 U/l. The normal range of ALT is 5-45 U/l. The normal range of platelet counts is 14.0-37.9x10^4/μl. The normal range of albumin is 3.7-5.5 g/dl. The normal range of total bilirubin is 0.3-1.2 mg/dl. Unit of M2BPGi is C.O.I. The normal range of Cr is 0.65-1.09 (male) and 0.46-0.82 (female) mg/dl. The Cr-based eGFR is ml/min/1.73 m^2. Normal range of CysC is 0.58-0.87 (male) and 0.47-0.82 (female) mg/l. CysC-eGFR is ml/min/1.73 m^2. The sarcopenia index was calculated as follows: serum Cr/CysC x100. The normal range of calcium (Ca) is 8.5-10.2. mg/dl. The normal range of P is 2.4-4.3 mg/dl. The urine protein/Cr ratio was g/g. Urine β2MG/Cr was μg/mg. Lumbar bone mineral density is the mean of the lumbar spine 2-4 and is measured in g/cm^2. The young adult mean (lumber spine, 20-44 years of age) is 1.19 g/cm^2 in men and 1.12 g/cm^2 in women. The YAM (femur neck) was 0.95 g/cm^2 in men and 0.90 g/cm^2 in female. A T-score ≤-2.5 indicates osteoporosis. The normal range of TRACP-5b is 170-590 in males and 120-420 in females (mU/dl). The TRACP-5b High group was over the upper limits. Normal range of total P1NP is 18.1-74.1 in male, 16.8-70.1 in premenopausal female and 26.4-98.2 in postmenopausal (ng/ml). The P1NP High group was over the upper limits. The normal range of α-fetoprotein was under 10 ng/ml. The normal range of protein induced by PIVKA-II is <40 mAU/ml.

osteoporosis) and renal alteration (eGFR <60 ml/min/1.73 m^2 and/or moderate dipstick proteinuria and/or low P (<2.5 mg/dl) and/or hemodialysis] were selected for the disease group. Old age (≥60 years) and bone disease significantly decreased TRAC-5b levels. Next, whether these factors contributed to the decrease in TRCAP-5b levels with TAF use was assessed. Since bone metabolic markers have various circadian variations, MSCs were set for each marker. The MSC of TRACP-5b showed over a 12.4% of change rate [(pre-treatment-after treatment)/pretreatment x100]. Therefore, the contribution of TRACP-5b to MSCs (32 cases) was evaluated. Univariate logistic regression analysis revealed that bone disease was the only contributing factor for MSCs. The bone disease group had lower BMD in the lumbar and neck of the femur at start and after 1 year compared with the control group. A high tendency of TRACP-5b levels at the start was observed, however there was no difference in TRACP-5b levels after 1 year (Fig. 1A-C).

Next, the relationship between TRACP-5b rate change and clinical factors at the start of TAF were evaluated (Table III). Cr-eGFR, P, P1NP and TRACP-5b were positively correlated with the TRACP-5b rate of change. Amongst these factors, Cr-eGFR and TRACP-5b were related to the TRCAP-5b rate of change in the multi-regression model. The change in these factors (at the start of TAF and after 1 year) was also evaluated in relation to the TRACP-5b rate of change (Table III). The change in P was only related to the TRACP-5b rate of change.

Changes in lumbar BMD were evaluated based on clinical factors (Table IV). The clinical factors were the same as in Table II. The Switch group exhibited increased BMD only in the lumbar region, but the control group (naïve group) did not exhibit any significant changes. Increases in BMD were significant between the start and after 1 year, [(44 cases exhibited increased BMD after 1 year compared with at the start]. Logistic univariate analysis showed that the switch was a contributing factor for the increased BMD in the lumbar spine. The Switch group did not exhibit a difference in BMD of the lumbar and neck of the femur and TRACP-5b at the start and after 1 year compared to the control (Fig. 1D-F). BMD in the lumbar region after 1 year in the switch group increased more than at the start (Fig. 1D), and TRACP-5b after 1 year after switching also decreased more than at the start (Fig. 1F).

Similarly, the change in BMD in the neck of the femur was also evaluated based on several clinical factors (Table V). Male sex, prevention group, low albumin levels and a low BMI did not decrease BMD in the neck of the femur after 1 year. Changes in BMD in the femoral neck were evaluated. There were 18 cases of increased BMD (at start-after 1 year). Logistic univariate analysis revealed that female sex was the only factor for increased BMD in the neck of the femur. The number of patients with increased BMD (18 cases) in the femoral neck was less than that in the lumbar region (44 cases, P=0.0001) and MSCs in TRACP-5b (32 cases, P=0.0289). BMD in the femoral neck was lower in females than in males at the start and after 1 year; however there was no difference between the start and after 1 year in females (Fig. 1H). BMD in the lumbar spine and TRACP-5b also showed no difference between the start and after 1 year in females (Fig. 1G and I).

Discussion

Treatment with TAF for 1 year decreased TRACP-5b levels, especially in patients with bone disease, excluding the MSC of TRACP-5b. The rate of change of TRACP-5b was associated with changes in P, Cr-eGFR and TRACP-5b levels at the start of TAF. Increased BMD in the lumbar region contributed to the switch from ETV to TAF. Increased BMD in the neck of the femur was present in female patients.

TRACP-5b at the start of TAF was related to the rate of change of TRACP-5b. HBV-infected patients exhibited hyperosteoclast function before TAF treatment. Carbon tetrachloride induced liver damage may have increased the levels of TRACP-5b (25), and HOD has been reported to increase TRACP-5b levels in patients with chronic liver

Table II. Change in TRACP-5b levels and contributing factors in the MSC of TRACP-5b.

	Comparison of TRACP-5b between at start and after 1 year			Logistical analysis for MSC of TRACP-5b		
Group (n)	At start[d]	After 1 year[d]	P-value	Odds ratio	95% Confidence interval	P-value
Female (29)	431 (216.6)	381.68 (143.3)	0.374			
Male (58)	410.1 (203.2)	343.9 (141.2)	0.0016[b]	1.082	0.422-2.773	0.8699
Age <60 years old (36)	366.73 (179.3)	313.46 (113.2)	0.0596			
Age ≥60 years old (51)	453.49 (219.3)	387.2 (153.68)	0.0311[a]	1.083	0.435-2.696	0.8646
HBeAg positive (13)	308.77 (119.1)	299.23 (141.1)	0.005[b]	0.903	0.266-3.059	0.8695
HBeAg negative (74)	438.84 (214.3)	366.99 (141)	0.3463			
HBcrAg positive (45)	412.3 (215.7)	327.5 (129.3)	0.0012[b]	1.062	0.361-2.456	0.9014
HBcrAg negative (42)	431.3 (210.2)	370.3 (128.7)	0.18			
HBV-DNA positive (41)	406.97 (208.53)	369.1 (147.86)	0.9547			
HBV-DNA negative (46)	427.4 (207.77)	346.1 (138.3)	0.0015[b]	1.232	0.5-3.039	0.6504
Bone disease negative (33)	356.57 (115)	364.5 (145.9)	0.9093			
Bone disease positive (54)	450.6 (237.2)	351.58 (141.2)	0.0007[c]	2.885	1.044-7.972	0.0411[a]
Renal alteration negative (53)	399.8 (161.63)	346.4 (137.69)	0.0043[b]			
Renal alteration positive (34)	446 (264.1)	372 (150)	0.2989	0.563	0.219-1.445	0.2322
Naïve TAF (32)	394.66 (198)	375.83 (142.8)	0.8382			
Switch ETV to TAF (55)	430.8 (212.8)	346.3 (142.3)	0.0004[c]	2.222	0.828-5.964	0.1129
Treatment (67)	403 (177.2)	339.88 (1249)	0.002[b]			
Prevention (20)	472.24 (293.28)	413.37 (185.2)	0.4347	0.755	0.248-2.304	0.6218
Albumin ≥4 g/dl (65)	407.42 (211.7)	347.75 (139.6)	0.0079[b]			
Albumin <4 g/dl (22)	453.1 (191.56)	386.4 (8,151.19)	0.2775	1.036	0.348-3.085	0.9493
Platelet ≥15x10^4/μl over (62)	405.7 (193)	352.14 (146)	0.0123[a]			
Platelet <15x10^4/μl (25)	451.38 (244.2)	366.8 (135.59)	0.1218	0.66	0.232-1.878	0.4360
BMI ≥25 (25)	380 (185)	359.5 (132.8)	0.8314			
BMI <25 (62)	432.9 (215)	355.24 (147.2)	0.001[b]	1.843	0.656-5.178	0.2458
CBMM High (57)	387 (189.9)	344.4 (134.14)	0.0387			
CBMM Low (30)	477.9 (229.1)	382.1 (157.78)	0.0573	1.117	0.431-2.899	0.8193

[a]P≤0.05, [b]P≤0.01, [c]P≤0.001. [d]Data are presented as the mean ± standard deviation. TRACP-5b was compared between the two groups at the start and after 1 year. The MSC of TRACP-5b was 12.4%. The group that contributed to MSCs (32 cases) was analyzed by logistical analysis. The bone disease-positive group was defined as follows: Chronic steroid use or use of other medications that worsen bone density and/or history of fragility fracture and/or osteoporosis. Renal alteration was defined as follows: eGFR < ml/min/1.73 m^2 and/or moderate dipstick proteinuria and/or low P (<2.5 mg/dl) and/or hemodialysis. The treatment group was treated with TAF for chronic HBV infection. The prevention group was treated with TAF for asymptomatic HBV using immunosuppressants and/or anticancer drugs. The CBMM High group was defined as follows: >27.903 in women and >39.731 in men.

disease (6). HOD is based on cirrhosis and is caused by insufficient liver-related factors, vitamin K, vitamin D, parathyroid hormone (PTH) and fibroblast growth factor (FGF)23 (5-7). However, in the present study, low albumin and low platelet counts were not contributing factors for the MSC of TRACP-5b. Previous population-based studies have described the relationship between HBV infection and osteoporosis (8,9). It is speculated that HOD appears in pre-cirrhosis related to HBV.

TRACP-5b levels are reflected in osteoclast function, number and volume (6,26), and is a bone turnover marker and predictor of fracture-independent BMD (27,28). High serum P was related to changes in TRACP-5b levels, and a decrease in P for 1 year was positively related to a decrease in TRACP-5b levels. More osteoblasts were normalized by TAF, and bone reabsorption was recovered. As a result, P was resorbed to the bone, and serum P decreased (6). The results showed that TAF was effective for the amelioration of osteoclasts. Since changes in TRACP-5b are related to fracture (11,27,28), BMD in the lumbar and neck of the femur was not improved by TAF; thus whether TAF could prevent fractures will be the focus of future studies.

BMD in the neck of the femur decreased during the observation period. A previous study described mean hip BMD at 1 year after TAF treatment was lower than that at the start of treatment, but less than 1 year after TDF treatment (12-14).

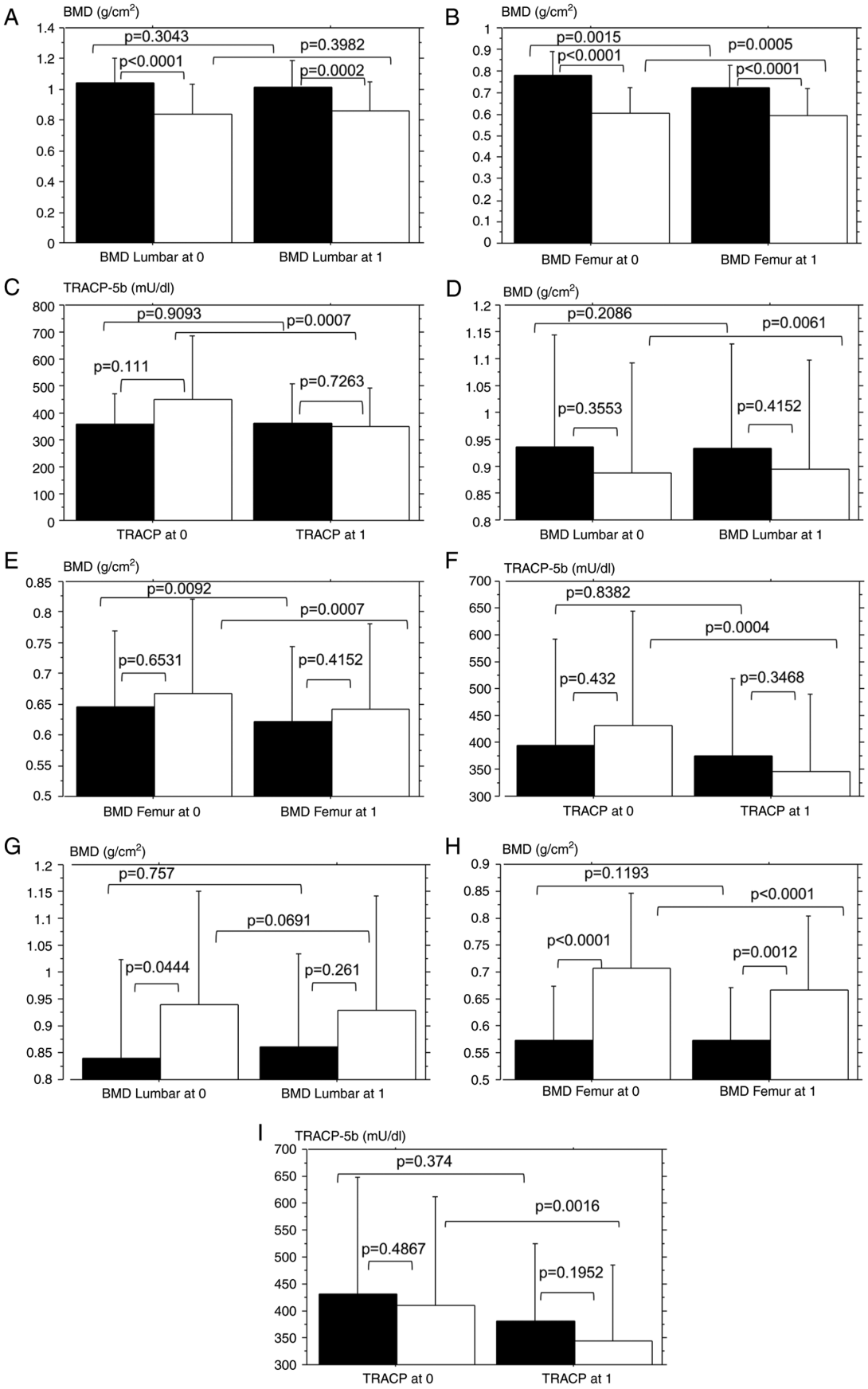

Figure 1. Change in lumbar BMD, neck of femur BMD and TRACP-5b. Differences in BMD in the lumbar and neck of the femur and TRACP-5b levels between the start of TAF (0) and 1 year after (1). (A) Lumbar BMD, (B) neck of femur BMD and (C) TRACP-5b levels were compared between bone disease-positive (white bar) and negative (black bar) conditions. (D) Lumbar BMD, (E) neck of femur BMD and (F) TRACP-5b levels were compared between naïve (black bars) and patients who switched (white bars). (G) Lumbar BMD, (H) neck of femur BMD and (I) TRACP-5b levels were compared between females (black bars) and males (white bars). In each graph, the x-axis at the start of tenofovir alafenamide administration (0) and after 1 year (1). BMD, bone mineral density; TRACP-5b, tartrate-resistant acid phosphatase isoform 5b.

Table III. Rate of change in TRACP-5b levels and clinical factors.

	Factors at start and TRACP-5b rate of change				Change in factor and TRACP-5b change rate			
Factor	R	P-value	β	P-value	R	P-value	β	P-value
Age	-0.007	0.9547						
Body weight	-0.05	0.6609			-0.148	0.1961		
Body mass index	-0.132	0.2481						
HBsAg	-0.004	0.9711			0.014	0.9022		
HBcrAg	0.09	0.4589			0.138	0.2679		
HBeAg	-0.129	0.2581			0.094	0.4359		
HBV-DNA	-0.081	0.4813			-0.038	0.7431		
AST	0.064	0.5755			0.067	0.5561		
ALT	0.067	0.5567			0.079	0.4925		
Platelet	0.06	0.5982			-0.044	0.7019		
Albumin	0.02	0.8607			-0.001	0.9905		
Total bilirubin	0.152	0.1803			0.158	0.1645		
M2BPGi	0.186	0.1384			0.14	0.2837		
Cr	-0.178	0.1168			-0.022	0.848		
Cr-eGFR	0.221	0.0498[a]	0.267	0.003[b]	0.025	0.8288		
Cys C	-0.145	0.2042			0.006	0.9618		
Cys C-eGFR	0.122	0.2868			0.004	0.9743		
Sarcopenia index	-0.035	0.7608			-0.037	0.7626		
Ca	0.069	0.552			0.191	0.1026		
P	0.254	0.0244[a]	0.097	0.3656	0.312	0.0055[b]	0.312	0.0057[b]
Urine protein/Cr	-0.125	0.3111			0.057	0.6601		
Urine b2MG/Cr	0.167	0.1906			-0.081	0.5436		
Lumbar BMD	-0.205	0.0772			-0.184	0.1304		
Lumbar t-score	-0.213	0.066						
Lumbar young adult mean	-0.208	0.0737						
Neck of Femur BMD	-0.127	0.279			0.036	0.7697		
Neck of Femur t-score	-0.15	0.2003						
Neck of Femur young adult mean	-0.145	0.2163						
TRACP-5b	0.532	<0.0001[c]	0.533	0.0003[c]				
P1NP	0.393	0.0008	-0.005	0.9702				
α-fetoprotein	0.097	0.4062			0.043	0.7195		
PIVKA-II	-0.081	0.4898			0.073	0.5418		

[a]P≤0.05, [b]P≤0.01, [c]P≤0.001. The relationship between factors and changes in TRACP-5b was evaluated using correlation and multiple regression models. R is the correlation coefficient. β is the standardized partial regression coefficient. Only factors with a significant R value were analyzed by multi-regression analysis.

There was no control after 1 year; however, BMD in the lumbar spine and TRACP-5b did not worsen after 1 year. There were 18 cases of increased BMD in the femur, less than the number of cases of increased BMD in the lumbar (44 cases) and MSC (32 cases) groups. Females exhibited increased BMD in the femoral neck, and TRACP-5b did not decrease after 1 year. Differences between the femur and lumbar vertebrae in patients with CHB treated with TAF will continue to be observed in the future.

Increased Cr and decreased Cr-eGFR were observed after 1 year of TAF treatment. However, CysC and CysC eGFR were not altered significantly during the treatment period. The SI indicates muscle volume and prognosis in patients in the intensive care unit (23). SI elevation reflects an increase in Cr, whilst CysC remains unchanged, and this is indicative of muscle volume gain. Changes in muscle mass should also be evaluated in future studies. Urine protein/creatinine ratio was elevated after 1 year, but the β2MG/creatinine ratio did not differ during the observational period. Previous reports did not identify the adverse effects of TAF on the kidney (14-17). In contrast to previous reports, the prevention group in the present study (20 cases) were treated with anticancer agents and/or immunosuppressants at the start of TAF administration, and this was continued after 1 year. It is hypothesized that there

Table IV. Change of BMD in the lumbar region and factors contributing to the increased BMD in the lumbar region.

	Comparison with BMD in Lumbar			Factors contributing to the increase in BMD in the lumbar region		
Group (n)	At start	After 1 year	P-value	Odds ratio	95% confidence interval	P-value
Female (29)	0.839 (0.184)	0.861 (0.171)	0.757			
Male (58)	0.94 (0.184)	0.93 (0.211)	0.0691			
Age <60 years old (36)	0.906 (0.155)	0.908 (0.146)	0.896			
Age ≥60 years old (51)	0.906 (0.239)	0.906 (0.234)	0.1428			
HBeAg positive (13)	0.858 (0.157)	0.888 (0.143)	0.2791			
HBeAg negative (74)	0.913 (0.213)	0.91 (0.208)	0.4752			
HBcrAg positive (45)	0.925 (0.181)	0.936 (0.177)	0.7915			
HBcrAg negative (42)	0.875 (0.243)	0.866 (0.229)	0.0926			
HBV-DNA positive (41)	0.923 (0.202)	0.942 (0.169)	0.7531			
HBV-DNA negative (46)	0.89 (0.211)	0.881 (0.219)	0.147			
Bone disease negative (33)	1.046 (0.155)	1.013 (0.173)	0.3043			
Bone disease positive (54)	0.841 (0.195)	0.857 (0.193)	0.3982			
Renal alteration negative (53)	0.888 (0.205)	0.876 (0.208)	0.8126			
Renal alteration positive (34)	0.935 (0.208)	0.959 (0.178)	0.0875			
Naïve TAF (32)	0.934 (0.21)	0.933 (0.193)	0.2086			
Switch ETV to TAF (55)	0.888 (0.204)	0.894 (0.204)	0.0061[a]	3.923	1.409-10.925	0.0089[a]
Treatment (67)	0.898 (0.2)	0.891 (0.194)	0.1459			
Prevention (20)	0.936 (0.235)	0.972 (0.251)	0.9999			
Albumin ≥4 g/dl (65)	0.898 (0.208)	0.889 (0.199)	0.3172			
Albumin <4 g/dl (22)	0.936 (0.202)	0.913 (0.21)	0.3061			
Platelet count ≥15x10^4/μl (62)	0.902 (0.216)	0.913 (0.21)	0.089			
Platelet count <15x10^4/μl (25)	0.916 (0.181)	0.89 (0.173)	0.6231			
Body mass index ≥25 (25)	1.051 (0.214)	1.049 (0.203)	0.8562			
Body mass index <25 (62)	0.846 (0.172)	0.852 (0.171)	0.1029			
CBMM High (57)	0.955 (0.213)	0.953 (0.211)	0.1972			
CBMM Low (30)	0.81 (0.155)	0.816 (0.139)	0.511			

[a]P<0.01. BMD in the lumbar spine was compared at the start and after 1 year. Data are presented as the mean ± standard deviation. Increased BMD was defined as: BMD at the start < BMD after 1 year. An increase in BMD was observed in 44 patients. The contributing factors were analyzed using logistic regression analysis.

is a relationship between concomitant drug use with TAF and proteinuria.

Switching ETV to TAF was a contributing factor in the increased BMD in the lumbar spine. The Switch group showed decreased TRACP-5b levels. TAF treatment resulted in less BMD loss than TDF treatment (12-14), but BMD gain was not observed. In the switch from ETV to TAF, BMD was not changed after 48 weeks in a previous report (17), and there was no significant increase in the incidence of osteoporosis/osteopenia in patients with CHB treated with TDF or ETV compared to those without treatment (29). It may seem that reduced BMD may be partly due to underlying chronic liver disease, and several patients with CHB may already have pre-existing low BMD prior to commencing antiviral therapy (8,9,30). Long-term observations are required to explore the anti-HBV effects of NAs and BMD.

The present study has some limitations. This was a single-center, small retrospective study, including prevention and a 1 year observational analysis. Thus, it was not possible to evaluate HOD-related bone related hormones, such as FGF23, PTH, vitamin D and vitamin K. The protective effects of TAF on renal function has been now widely established (3,12). However, it may be possible to ascertain additional useful information regarding the relationship between HBV infection and bone metabolism.

In conclusion, patients with HBV infection complicated by bone disease exhibited decreased TRACP-5b levels after treatment with TAF. Switching ETV to TAF increased BMD in the lumbar spine and decreased the TRAC-5b levels. TAF is acceptable for improving/maintaining bone metabolism in patients with HBV infection, and TRACP-5b was shown to be a useful bone metabolic marker, especially when attempting to prevent fractures in patients with HBV.

Table V. Change of BMD in the neck of femur and factors contributing to the increased BMD in the neck of femur.

	Comparison with BMD in the neck of femur			Factors contributing to the in BMD in the neck of femur		
Group (n)	At start[e]	After 1 year[e]	P-value	Odds ratio	95% confidence interval	P-value
Female (29)	0.572 (0.101)	0.574 (0.098)	0.1193			
Male (58)	0.705 (0.14)	0.667 (0.137)	<0.0001[d]	0.308	0.102-0.928	0.0364[a]
Age <60 years old (36)	0.702 (0.128)	0.671 (0.11)	0.0011[b]			
Age ≥60 years old (51)	0.629 (0.146)	0.61 (0.142)	0.0008[c]			
HBeAg positive (13)	0.603 (0.079)	0.594 (0.078)	<0.0001[d]			
HBeAg negative (74)	0.669 (0.148)	0.642 (0.138)	0.0467[a]			
HBcrAg positive (45)	0.693 (0.136)	0.672 (0.117)	0.0003[c]			
HBcrAg negative (42)	0.622 (0.156)	0.595 (0.152)	0.0104[a]			
HBV-DNA positive (41)	0.644 (0.121)	0.637 (0.113)	0.0402[a]			
HBV-DNA negative (46)	0.674 (0.159)	0.635 (0.147)	<0.0001[d]			
Bone disease negative (33)	0.78 (0.108)	0.726 (0.102)	0.0015[b]			
Bone disease positive (54)	0.604 (0.12)	0.594 (0.124)	0.0005[c]			
Renal alteration negative (53)	0.663 (0.1439	0.634 (0.134)	0.0001[d]			
Renal alteration positive (34)	0.655 (0.142)	0.638 (0.131)	0.0092[c]			
Naïve TAF (32)	0.647 (0.124)	0.623 (0.122)	0.0007[c]			
Switch ETV to TAF (55)	0.668 (0.153)	0.643 (0.138)	0.0012[c]			
Treatment (67)	0.665 (0.134)	0.643 (0.118)	<0.0001[d]			
Prevention (20)	0.641 (0.175)	0.608 (0.181)	0.0621	0.782	0.192-3.188	0.7314
Albumin ≥4 g/dl (65)	0.651 (0.149)	0.635 (0.141)	<0.0001[d]			
Albumin <4 g/dl (22)	0.685 (0.118)	0.639 (0.106)	0.2238	2.582	0.703-9.493	0.1531
Platelet count ≥15x10^4/μl (62)	0.729 (0.141)	0.714 (0.119	<0.0001[d]			
Platelet <15x10^4/μl (25)	0.632 (0.134)	0.606 (0.125)	0.0457[a]			
Body mass index ≥25 (25)	0.729 (0.141)	0.714 (0.119)	0.1043			
Body mass index <25 (62)	0.632 (0.134)	0.606 (0.125)	<0.0001[d]	0.357	0.115-1.105	0.074
CBMM High (57)	0.7 (0.129)	0.678 (0.125)	<0.0001[d]			
CBMM Low (30)	0.583 (0.136)	0.551 (0.105)	0.0114[a]			

[a]P≤0.05, [b]P≤0.01, [c]P≤0.001, [d]P≤0.0001. BMD in the neck of femur was compared at the start and after 1 year. [e]Data are presented as the mean ± standard deviation. Increased BMD was defined as: BMD at the start < BMD after 1 year. An increase in BMD was observed in 18 patients. The contributing factors for increased BMD after 1 year were analyzed using logistic regression analysis.

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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

TO and TIc wrote the manuscript, analyzed the data and designed the study. HM, SM, YM, MY, SY, MK, TH, HY, TIk, OM, YK, YN, NT and KN collected the data. All authors have read and approved the final manuscript. KN and NT confirm the authenticity of all the raw. data.

Ethics approval and consent to participate

The present study was approved by the Human Research Ethics Committee of the Nagasaki Harbor Medical Center (approval no. H30-031). Informed consent was obtained from each patient included in the study, and the patients were guaranteed the right to leave the study if they desired.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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