Direct-acting antiviral treatment decreases serum undercarboxylated osteocalcin in male patients with chronic hepatitis C

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Abstract. Hepatic osteodystrophy (HOD) is a common complication of chronic liver disease, including viral hepatitis. Hepatitis C virus (HCV) infection is associated with an increased risk of osteoporosis and bone mineral density (BMD) loss. Direct-acting antiviral (DAA) treatment is used to treat HCV infections; however, its effects on bone metabolism have not been reported. We compared the clinical data and bone metabolic markers at the start of DAA treatment and 1 year later in 78 patients. There were 41 female and 37 male patients. HCV was successfully treated with DAA in all patients. Bone metabolic markers included undercarboxylated osteocalcin (ucOC), 25(OH) vitamin D (VD), total type I procollagen N-propeptide (P1NP), tartrate-resistant acid phosphatase 5b (TRACP-5b), and BMD. BMD was measured in the lumbar spine (mean, L2-L4) and femoral neck using dual-energy X-ray absorptiometry. ucOC in males decreased at 1 year after treatment initiation but not in females. In males, ucOC changes were related to alterations in proteins induced by vitamin K absence-II (PIVKA-II), hemoglobin A1c, and TRACP-5b, which contributed to P1NP and lumbar BMD at the start of DAA. Changes in ucOC among women contributed to the changes in grip strength and TRACP-5b levels. DAA treatment improved ucOC, a useful bone metabolic marker, in HCV-infected male patients. Changes in ucOC contributed to changes in PIVKA-II that likely ameliorated the vitamin K deficiency. DAA treatment has been reported to improve various extrahepatic disorders and abnormal bone metabolism, especially in HOD.

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Abbreviations: DAA, direct-acting antiviral; HCV, hepatitis C virus; eGFR, estimated glomerular filtration rate; Cr, creatinine; CysC, cystatin C; CBMM, calculated body muscle mass; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HOD, hepatic osteodystrophy; TRACP-5b, tartrate-resistant acid phosphatase isoform 5b; ucOC, undercarboxylated osteocalcin; LDL, low-density lipoprotein; sdLDL, small dense low-density lipoprotein; TG, triglyceride; HbA1c, hemoglobin A1c; HOMA, homeostatic model assessment; M2BPGi, Mac-2 binding protein glycosylation isomer; FIB-4, fibrosis 4; ALBI, albumin-bilirubin grade; BMD, bone mineral density; YAM, young adult mean; P1NP, procollagen type I intact N-terminal propeptide; VD, 25(OH) vitamin D; VK, vitamin K; PIVKA-II, protein induced by vitamin K absence or antagonist II; SVR, sustained virologic response; HBV, hepatitis B virus; HBs, hepatitis B surface; HBc, hepatitis B core; SOF/RBV, sofosbuvir/ribavirin; γGTP, γ glutamyl transpeptidase

Key words: hepatitis C virus, direct-acting antivirus, undercarboxylated osteocalcin, bone metabolic marker

Introduction

Hepatitis C virus (HCV) infection may lead to cirrhosis and hepatocellular carcinoma (HCC) (1,2). However, direct-acting antiviral (DAA) treatment has been shown to cure HCV infection irrespective of the patient's sex and age (2), as well as improving survival rates (3) and HCC risk (4). Although HCV infection can lead to various extrahepatic disorders (5,6), diabetes (7), poor sleep (8), low quality of life (QoL) (9), and low muscle volume (10), these disorders were found to improve with DAA treatment. While a small clearance rate of HCV using interferon treatment was associated with a reduction in the risk of fractures (11,12), the effects of DAA on bone metabolism have not been reported.

Hepatic osteodystrophy (HOD) is a common complication of chronic liver disease, including viral hepatitis (13-15). HOD comprises osteoporosis and osteomalacia, which are caused by hormonal abnormality-induced advanced liver failure

complicated with abnormal serum levels of calcium and phosphate (13-15). These complications are evident in HCV infection as it is associated with the risk of osteoporosis and loss of bone mineral density (BMD) (16). In HOD, various bone metabolic markers are altered by a hormonal abnormality (14). Tartrate-resistant acid phosphatase isoform 5b (TRACP-5b) is an osteoclast activation marker that is elevated in patients with HOD associated with chronic liver disease (14). In contrast, procollagen type I intact N-terminal propeptide (P1NP) is an osteoblast marker that is elevated in non-cirrhotic HCV-infected patients with low BMD (17,18). It is speculated that low vitamin D levels are one of the leading factors in HOD (14). Moreover, vitamin K (VK), a fat-soluble vitamin similar to vitamin D, is linked to bone metabolism (19). Cholestasis induces VK deficiency and osteoporosis (19). Consequently, VK deficiency elevates the levels of undercarboxylated osteocalcin (ucOC) (20), a marker of bone metabolism. Measurements of BMD and bone metabolic markers are useful for the diagnosis and management of osteoporosis (21,22). Therefore, changes in bone metabolic markers are expected in patients with HCV infection after DAA treatment.

We previously reported that tenofovir alafenamide for hepatitis B virus (HBV) leads to the recovery titer of the bone metabolic marker, TRACP-5b (23). In that study, we speculated that HOD appears in non-cirrhotic HBV-infected patients, and that osteoblasts were normalized by HBV treatment. Although tenofovir alafenamide cannot eliminate HBV in patients, DAA can clear HCV. In this study, we evaluated the influence of HCV clearance by DAA treatment on bone metabolism over 1 year.

Patients and methods

Patients. A total of 116 serial patients with HCV infection were treated with DAA at the Nagasaki Harbor Medical Center between January 2017 and December 2020. DAA treatment was initiated in the absence of HCC by imaging in patients without a history of HCC, and in patients with no recurrence of HCC within 6 months of the start of DAA treatment among those with a history of HCC. Patients were treated with DAAs, such as sofosbuvir/ledipasvir (SOF/LDV; Gilead Sciences), elbasvir/grazoprevir (EBR/GZR; MSD) for 1b, sofosbuvir/ribavirin (SOF/RBV) for 2a/2b (RBV; Chugai Pharmaceutical Co.), and glecaprevir/pibrentasvir (GLE/PIB; AbbVie) for the pangenotype. Combination DAA therapy was administered orally for 12 weeks in the case of SOF/LDV, SOF/RBV, EBR/GZR, and GLE/PIB and for 8 weeks in patients with chronic hepatitis who received GLE/PIB. The sustained virologic response (SVR) was determined 24 weeks after the end of treatment. SVR was achieved in all the patients; after which, all patients were followed up for HCC screening every 6 months through imaging and tumor makers. Data on concomitant medications for hypertension, hyperlipidemia, diabetes, obesity [body mass index (BMI)>25], and dialysis were obtained before treatment initiation. The diagnosis of hypertension and hyperlipidemia was based on a history and use of oral medications. All patients did not suffer from malignant diseases and thyroid disease Among the 116 patients, 87 were followed up for 1 year after the start of DAA treatment. Of the 87 patients, 78 had normal kidney function, excluding chronic kidney disease grades 4-5. We evaluated the bone metabolic markers in 78 patients, since suboptimal VK and vitamin D status in patients with advanced chronic kidney disease is not expected (24). Heavy alcohol consumption was considered as >7 drinks per week for women and >14 drinks per week for men (25). We compared the clinical data and bone metabolic markers at the start of DAA and 1 year later. All patients did not receive osteoporosis drugs including VK.

The medical records of 78 patients were retrospectively reviewed. Informed consent was obtained from each patient included in the study, and they were guaranteed the right to leave the study, if they desired. The study protocol conformed to the guidelines of the 1975 Declaration of Helsinki (26), and it was approved by the Human Research Ethics Committee of Nagasaki Harbor Medical Center (approval no. H30-031).

Laboratory measurements. Laboratory data and anthropometric measurements were obtained from each patient every 4-12 weeks during treatment. BMD and urinalysis were performed every 12 months. The BMI of each patient was calculated by dividing their weight in kilograms by their height^2 in meters. Laboratory examinations included platelet count, creatinine (Cr), cystatin C (CysC), albumin, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), α-fetoprotein, protein induced by VK absence-II (PIVKA-II), Mac-2 binding protein glycan isomer (M2BPGi), ucOC, 25(OH) vitamin D (VD), P1NP, and TRACP-5b. Among bone metabolic makers, TRACP-5b, ucOC, and VD were evaluated at treatment initiation and 1 year later. BMD of the lumbar spine (mean, L2-L4) and femoral neck was measured using dual-energy X-ray absorptiometry at the start of treatment and 1 year later.

Cr- and CysC-based estimated glomerular filtration rates (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 (27). The calculated body muscle mass (CBMM) was obtained as follows: [(body weight in kg x Cr)/(K x body weight in kg x CysC) + Cr], where K=0.00675 or 0.01006 for males and females, respectively (28). Small dense low-density lipoprotein (sdLDL) was assessed using a commercially available assay kit (sdLDL-EX; Denka Seiken Co.), based on a previously established method (29). The percentage of sdLDL (%sdLDL) was calculated based on sdLDL/LDL determined at the same time. Insulin resistance was evaluated by the homeostasis model assessment (HOMA-IR) method using the following formula: HOMA-IR=fasting plasma glucose (mg/dl) x 9 fasting insulin (IU/ml)/405. In contrast, insulin secretion was calculated using the homeostasis model assessment of insulin secretion (HOMA-β) formula: Insulin at time 0 (μU/ml) x 360/fasting plasma glucose (mg/dl)-63.

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 signed ranks test for differences between paired groups, a Mann-Whitney U test comparison for unpaired two groups, and a χ^2 test for comparison between discrete variables. Univariate and multivariate analyses were

Table I. Relationship among bone metabolic markers at the start of direct-acting antiviral treatment.

A, Female

	ucOC	TRACP-5b	P1NP	VD	LBMD	FBMD
ucOC	1	0.518[b]	0.7[c]	-0.04	-0.228	-0.18
TRACP-5b		1	0.709[c]	-0.03	-0.351[a]	-0.302
P1NP			1	-0.25	-0.168	-0.195
VD				1	-0.003	0.075
LBMD					1	0.65[c]
FBMD						1

B, Male

	ucOC	TRACP-5b	P1NP	VD	LBMD	FBMD
ucOC	1	0.498[a]	0.676[c]	0.352[a]	-0.441[a]	-0.178
TRACP-5b		1	0.692[c]	0.354[a]	-0.271	-0.204
P1NP			1	0.25	-0.134	-0.004
VD				1	-0.128	0.137
LBMD					1	0.632[c]
FBMD						1

[a]P≤0.05, [b]P≤0.01, [c]P≤0.001. Data are presented as correlation coefficients. ucOC, undercarboxylated osteocalcin; LBMD, lumbar BM; FBMD, femur BMD.

performed using multiple linear regression analysis. A standardized partial regression coefficient, β, was employed. Correlations were evaluated using the Pearson's correlation coefficient (R). P<0.05 was considered to indicate statistical significance.

Results

The clinical characteristics are presented by sex in Table SI. There were 41 women and 37 men. Sex differences were observed in AST, ALT, Cr, PIVKA-II, ferritin, high-density lipoprotein, CBMM, grip strength, BMD, and ucOC. In particular, bone metabolic markers significantly differed. BMD in females was lower than that in males, while the ucOC titer in females was higher than that in males. We then evaluated changes in bone metabolic markers by sex in this study. The relationships between bone metabolic markers were compared in HCV-infected patients at the start of DAA treatment (Table I). ucOC was related to TRACP-5b and P1NP in females, while in males, it was related to TRACP-5b, P1NP, VD, and LBMD. ucOC was particularly associated with P1NP in both sexes. VD was not related to other bone metabolic markers in the female sex.

We evaluated the differences among the clinical factors between the start of DAA treatment and 1 year after treatment (Table II). In both sexes, ALT, M2BPGi, α-fetoprotein, Cr, Cr-eGFR, CBMM, LDL, sdLDL, and %sdLDL were changed, while total bilirubin, FIB-4, and albumin levels were altered in men. ucOC in males decreased 1 year after the start of DAA treatment; however, TRACP-5b and lumbar BMD did not change after 1 year in either sex. Femur BMD in females decreased at 1 year after treatment, but the femur t value did not change. VD in females, but not in males, decreased. Therefore, we focused on the changes in ucOCs after DAA treatment.

First, we evaluated the relationship between changes in ucOC (ducOC) and clinical factors (dfactors) (Table SII). In women, only dTRACP-5b and dGrip strength were related to ducOC, whereas in men, dPIVKA-II, dHbA1c, and dTRACP-5b were related to ducOC. dGrip strength was not related to ducOC in men, while dPIVKA-II and dHbA1c were not related to ducOC in females. In the FIB-4 high (H) group, the FIB-4 titer exceeded 3.25 (Table SI). The high titer of FIB-4 (exceeded 3.25) is a marker of advanced fibrosis including cirrhosis (30). ducOC was not related to dFIB-4. Correlations were -0.115 (P=0.5568) in the FIB-4 high group and -0.15 (P=3115) in the normal group. Next, we evaluated ducOC using a multivariate linear regression analysis (Table III). In females, dTRACP-5b and dGrip strength were contributing factors to ducOC but dPIVKA and dHbA1c were not. Among males, dTRACp-5b, dPIVKA-II, and dHbA1c were contributing factors for ducOC but dGrip strength was not. Changes in TRACP-5b were a common factor in the ucOC changes, but the changes in grip strength contributed to changes in ucOC among females. Changes in PIVKA-II and HbA1c levels were specifically associated with the male sex. Finally, we evaluated the relationship between ducOCs and clinical factors at the start of DAA treatment (Table IV). Age is only related to ducOC in the woman (Table IV), but did not contribute to ducOC based on the regression analysis (β=-0.271, P=0.0861). We could not find any significant factors in women. Contrarily, PIVKA-II, TRACP-5b, VD, ucOC, P1NP, and LBMD were related to ducOC in men. In the multivariate linear regression analysis,

Table II. Differences in clinical factors between the start and 1 year after treatment[d].

	Female			Male		
Factor	Start	1 Year	P-value	Start	1 Year	P-value
AST, U/l	35.49 (18.6)	21.8 (7.65)	<0.0001	54.27 (7.72)	23.19 (7.65)	<0.0001[c]
ALT, U/l	33.1 (27.47)	14.42 (7.7)	0.062	66.97 (71.42)	18.1 (10.659	<0.0001[c]
Platelet, x$10^4/\mu$l	19.22 (7.19)	18.84 (6.96)	0.9893	16.51 (5.91)	16.86 (5.84)	0.4735
FIB-4	2.95 (2.13)	2.52 (1.72)	0.0707	3.52 (2.69)	2.79 (2.1)	0.0007[c]
Total bilirubin, g/dl	0.7 (0.184)	0.761 (0.286)	0.1507	0.816 (0.511)	1.01 (0.745)	0.0012[b]
Albumin, g/dl	4.16 (0.327)	4.24 (0.313)	0.1378	4.18 (0.397)	4.31 (0.37)	0.0262[a]
ALBI	-2.84 (0.269)	-2.89 (0.411)	0.4292	-2.83 (0.277)	-2.87 (0.411)	0.1919
M2BPGi, COI	2.1 (1.839	1.25 (0.933)	<0.0001	2.73 (2.489	1.59 (1.94)	<0.0001[c]
α-fetoprotein, ng/ml	6.52 (7.57)	4.19 (2.27)	<0.0001	9.87 (2.27)	3.62 (1.4)	<0.0001[c]
PIVKA-II, mAU/ml	20.42 (4.82)	20.73 (6.02)	0.5659	27.32 (11.85)	26.71 (8.79)	0.432
Cr, mg/dl	0.653 (0.13)	0.701 (0.16)	<0.0001	0.863 (0.218)	0.856 (0.257)	<0.0001[c]
Cr-eGFR, ml/min/1.73 m^2	71.5 (16.6)	65.88 (16.4)	<0.0001	69.68 (18.2)	65.48 (19.1)	<0.0001[c]
CysC, mg/dl	1.08 (0.278)	1.07 (0.31)	0.5375	1.243 (0.38)	1.21 (0.44)	0.1274
CysC-eGFR, ml/min/1.73 m^2	64.76 (20.88)	68.65 (25.5)	0.5319	61.15 (23.67)	64.69 (28.1)	0.1629
CBMM	27.72 (4.37)	28.42 (4.22)	0.033	38.87 (5.71)	40.86 (5.23)	0.0003[c]
BMI	22.42 (5.92)	22.41 (5.69)	0.5313	23.13 (3.37)	23.51 (3.72)	0.0879
Grip strength	12.2 (5.9)	12.7 (7.4)	0.9851	25.2 (9.8)	25.2 (7.4)	0.5761
LDL, mg/dl	99.9 (23.62)	120.6 (29.72)	<0.0001	101.2 (25.4)	115.1 (31.2)	0.0034[b]
sdLDL	19.4 (7)	34.8 (50..8)	<0.0001	21.7 (10.8)	33.3 (30.39)	0.0001[c]
%sdLDL	0.192 (0.045)	0.268 (0.311)	0.0008	0.212 (0.08)	0.298 (0.313)	<0.0001[c]
HDL, mg/dl	66.6 (17.29	68.3 (12.3)	0.2853	56.1 (12.9)	59.7 (13.7)	0.0785
TG, mg/dl	99.1 (40.8)	101.7 (46)	0.5211	107.8 (50.4)	121.9 (171.8)	0.0726
HbA1c, %	5.78 (0.86)	5.69 (0.47)	0.6881	5.7 (0.44)	5.75 (0.61)	0.5931
HOMA-IR	2.7 (2.46)	2.56 (2.62)	0.3247	4.8 (6)	4.13 (4.85)	0.5397
HOMA-β	95.3 (81.7)	82 (46.2)	0.2221	107.9 (85.5)	82 (46.2)	0.4321
TRACP-5b, mU/dl	463.7 (202)	458.7 (265.3)	0.8017	400.8 (151.8)	381.9 (141.5)	0.9561
VD, ng/ml	15.9 (5.16)	13.6 (5.17)	0.0003	17.9 (6.3)	16.9 (5.17)	0.3898
ucOC, ng/ml	6.89 (6.39)	7.15 (7.1)	0.5818	4.22 (6.87)	4.15 (2.44)	0.0168[b]
Lumbar BMD, g/cm^2	0.724 (0.17)	0.733 (0.17)	0.9537	0.969 (0.2)	0.97 (0.197)	0.3897
Lumbar t-value	-2.34 (1.46)	-2.12 (1.48)	0.2366	-0.64 (1.63)	-0.646 (1.63)	0.7998
Femur BMD, g/cm^2,	0.533 (0.11)	0.521 (0.11)	0.0074	0.689 (0.14)	0.683 (0.14)	0.1494
Femur t-value	-2.3 (1)	-2.37 (0.99)	0.0572	-1.34 (1.1)	-1.42 (1.1)	0.1421

[a]P<0.05, [b]P<0.01, [c]P<0.001. [d]Data are presented as the mean ± standard deviation. BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; FIB-4, fibrosis-4; M2BPGi, Mac-2 binding protein glycosylation isomer; COI, cut off index; ALBI, albumin-bilirubin grade; PIVKA-II, protein induced by vitamin K absence or antagonist II; Cr, creatinine; eGFR, estimated glomerular filtration rate; CysC, cystatin C; CBMM, calculated body muscle mass; LDL, low-density lipoprotein; sdLDL, small dense low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; HbA1c, hemoglobin A1c; HOMA-IR, Homeostatic model assessment- insulin resistance; TRACP-5b, tartrate-resistant acid phosphatase 5b; VD, vitamin D; ucOC, undercarboxylated osteocalcin; BMD, bone mineral density.

P1NP and lumbar BMD at the start of DAA associated with changes in ucOC.

Discussion

In patients treated with DAA for HCV, ucOC in males decreased 1 year after the start of treatment in contrast with females. In males, changes in ucOC were related to changes in PIVKA-II, HbA1c, and TRACP-5b, which contributed to P1NP and lumbar BMD at the start of DAA treatment. In contrast, among females, a change in ucOC contributed to changes in grip strength and TRACP-5b levels.

In men, ucOC was improved by DAA treatment; thus, lowering of the fracture risk is expected. We did not evaluate the level of vitamin K in serum, but the cause of ucOC changes is speculated to be the amelioration of VK metabolism, as elevation of PIVKA-II is related to VK deficiency (31). Improvement of cholestasis and microbiota causes VK deficiency in patients (19). Bile acid metabolism is associated with changes in cholestasis and the microbiota (32). In this study,

Table III. Factors contributing to ducOC.

	Female				Male			
	Univariate		Multivariate		Univariate		Multivariate	
Factor	β	P-value	β	P-value	β	P-value	β	P-value
dTRACP-5b	0.454	0.0037[b]	0.429	0.0036[b]	0.657	<0.0001[c]	0.472	0.0006[c]
dGrip Strength	-0.569	0.0006[c]	-0.484	0.001[c]	0.03	0.869	0.075	0.5146
dPIVKA-II	-0.176	0.271	-0.395	0.0047[b]	0.563	0.0003[c]	0.358	0.0063[b]
dHbA1c	0.051	0.7504	-0.004	0.975	0.541	0.0008[c]	0.276	0.0302[a]

[a]P≤0.05, [b]P≤0.01, [c]P≤0.001. The relationship between the factors and ducOC was evaluated using multiple regression models. β is the standardized partial regression coefficient. ducOC, changes in undercarboxylated osteocalcin; PIVKA-II, protein induced by vitamin K absence or antagonist II; TRACP-5b, tartrate-resistant acid phosphatase 5b; HbA1c, hemoglobin A1c.

Table IV. Relationship between the ducOC and factors at the start of direct-acting antiviral treatment.

	Female (Correlation analysis)		Male (Correlation analysis)		Male (Regression analysis)	
Factor	R	P-value	R	P-value	β	P-value
Age	0.436	0.0104	0.169	0.3594		
α-fetoprotein	-0.159	0.3734	-0.125	0.492		
PIVKA-II	0.101	0.5903	0.401	0.0201	0.223	0.1465
LDL	0.163	0.3678	0.041	0.823		
HDL	0.109	0.5477	0.103	0.5702		
TG	0.127	0.484	-0.119	0.5262		
HbA1c	-0.054	0.7664	-0.167	0.3561		
HOMA-IR	-0.075	0.6822	-0.164	0.3654		
HOMA-β	-0.022	0.9055	-0.2	0.2685		
TRACP-5b	-0.242	0.1758	0.376	0.0304	-0.134	0.5072
VD	-0.002	0.929	0.447	0.0084	0.259	0.1013
ucOC	0.059	0.7472	0.947	<0.0001		
P1NP	-0.051	0.7782	0.51	0.0031	0.492	0.0319
M2BPGi	-0.291	0.1009	-0.164	0.3657		
FIB-4	-0.217	0.2269	-0.089	0.6235		
ALBI	0.103	0.5729	0.04	0.8272		
CysC	-0.104	0.5675	-0.159	0.3791		
Cr	-0.164	0.3637	-0.223	0.2151		
CBMM	-0.042	0.7961	-0.170	0.3174		
Grip Strength	0.115	0.9375	0.025	0.8925		
BMI	0.011	0.294	-0.24	0.1805		
Lumbar BMD	-0.173	0.3018	-0.391	0.0194	-0.349	0.0175
Lumbar t-value	-0.151	0.367	-0.395	0.0182		
Femur BMD	-0.119	0.4811	-0.076	0.6678		
Femur t-value	-0.112	0.5056	-0.076	0.6635		

Data are presented as correlation coefficients (R). β is the standardized partial regression coefficient. BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; FIB-4, fibrosis-4; M2BPGi, Mac-2 binding protein glycosylation isomer; COI, cut off index; ALBI, Albumin-bilirubin grade; PIVKA-II, Protein induced by vitamin K absence or antagonist II; Cr, Creatinine; eGFR, estimated glomerular filtration rate; CysC, cystatin C; CBMM, calculated body muscle mass; LDL, Low-density lipoprotein; sdLDL, small dense Low-density lipoprotein; HDL, High-density lipoprotein; TG, Triglyceride; HbA1c, hemoglobin A1c; HOMA-IR, Homeostatic model assessment- insulin resistance; TRACP-5b, tartrate-resistant acid phosphatase 5b; VD, vitamin D; ucOC, undercarboxylated osteocalcin; BMD, bone mineral density.

these changes were not evaluated despite research on the ability of HCV treatment to change the composition of bile acids and gut microbiota (33,34). Moreover, it might be related to the VK-bile acid-microbiota axis that emphasizes how changes in ucOC are related to those in PIVKA-II. VK deficiency should also be considered a part of HOD in HCV infection. Changes in ucOC were related to changes in HbA1c levels in men, but changes in HOMA-IR were not. Dietary intake of VK has been linked to a lower incidence of diabetes (35), and the beneficial effect of VK on glycemic control has also been reviewed (36). Since DAA improves glycemic control in diabetes (7), future studies on the relationship between diabetes and bone metabolism are needed. γGTP, AST, and ALT in males was higher than in females but heavy alcohol consumption rates did not differ between females and males. However, we speculate that sex differences in AST, ALT, and PLT may be related to alcohol consumption, and further analysis of the quantity of alcohol consumption in this difference should be evaluated. Future studies on the relationship between alcohol consumption and bone metabolism are also needed.

VD was not related to changes in ucOC in either sex or other bone metabolic markers in women in the present study. VD was previously reported to be a critical hormone in the development of HOD (14). We evaluated 25(OH)D in this study; however, the active form of VD is 1,25(OH)D (14). We could not determine the significance of 25(OH)D; therefore, further evaluation is needed to clarify the relationship between VD and bone metabolism in patients with HCV.

Among women, ucOC did not change after DAA treatment as changes in ucOC were related to differences in grip strength. Muscles and bones are affected by chronic liver disease (37). DAA treatment ameliorates muscle volume in patients with HCV (10). Hand-grip strength predicts the risk of osteoporosis (38). CBMM, an absorption marker of muscle volume and grip strength (9), was increased in this study. Since changes in ucOC are related to alterations in TRACP-5b in women, ducOC reflected an improvement in bone metabolism. Contrary to that in males, bone metabolism was more affected by grip strength than VK in females treated with DAA. In mice models, it has been reported that sex-specific differences in gene expression of VK-related genes indicate that male and female mice respond differently to dietary VK manipulation (39). However, sex differences of VK-related genes in humans have not been reported. Thus, further investigations are required to observe sex differences in bone metabolism.

The relationship between QoL and bone metabolism has been investigated (40,41). DAA treatment effectively ameliorates muscle volume (10) and sleep quality (8) in patients. In this study, bone metabolism was also improved by DAA in patients with poor bone metabolic conditions, including low lumbar BMD and high P1NP. Therefore, we speculate that improvement in several poor conditions, such as bone metabolism, muscle function (9,10), and sleep (8), can improve QoL. We believe that DAA treatment for HCV infection is significant for improving the QoL of all patients.

The present study had some limitations. This was a single-center, small, retrospective study with a 1-year observational analysis. Since all cases achieved SVR, we could not compare cases that achieved SVR with cases that did not achieve SVR. However, it may be possible to obtain additional information on the relationship between HCV infection and bone metabolism.

In conclusion, ucOC, a useful bone metabolic marker, was improved by DAA treatment in male HCV-infected patients. Changes in ucOC contributed to alterations in PIVKA-II, which likely ameliorated the VK deficiency. Changes in ucOCs may prevent fractures in patients with HCV clearance. DAA treatment improved extrahepatic HCV-related disorders and abnormal bone metabolism. SVR by DAA was also effective for the prevention of osteoporosis or occurrence of fractures, similar to interferon treatment (11); therefore, it may improve the QoL of affected patients in the future.

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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 upon reasonable request.

Authors' contributions

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

Ethics approval and consent to participate

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

Patient consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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