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Advanced liver fibrosis by transient elastography, Fibrosis 4, and alanine aminotransferase/platelet ratio index among Asian hepatitis C with and without human immunodeficiency virus infection: Role of vitamin D levels

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Key words
APRI, Fib-4, genotype 3, genotype 6, hepatitis C, HIV/hepatitis C co-infection, liver fibrosis, Thailand, vitamin D.

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Abstract

Background and Aim: Vitamin D insufficiency plays an important role in liver fibrosis in hepatitis C virus (HCV)-infected patients. We assessed liver fibrosis by transient elastography and 25 hydroxy vitamin D [25(OH)D] status in HCV-infected patients, with (HIV/HCV) or without HIV co-infection (HCV) from Thailand.

Methods: Fibrosis stage was defined as mild (< 7.1 kPa); moderate (7.2–9.4 kPa); severe (9.5–14 kPa), and cirrhosis (> 14 kPa). Hypovitaminosis D was defined as 25(OH)D < 30 ng/mL. Logistic regression analyses were used to assess predictors for significant fibrosis. Serum 25(OH) D levels, HCV genotypes (GT), interleukin-28B (IL28B) and HCV-RNA were assessed.

Results: A total of 331 HCV and 130 HIV/HCV patients were enrolled (70% male, 35% people who inject drugs [PWIDs]). HCV GT distribution was as follows: GT3 47%, GT1 34%, GT6 17%. IL-28B CC genotype (rs12979860) were found in 88% of HIV/HCV and 85% of HCV. In HCV, liver fibrosis was mild in 56.5%; moderate in 18.4%; severe in 12.4%; and cirrhosis in 12.7%. In HIV/HCV, these figures were 30.6%, 27.8%, 17.6%, and 24.1%, respectively. Patients with significant fibrosis were more often male, older, with HIV infection, hypovitaminosis D, and less likely to be infected with GT6. Factors associated with significant fibrosis by multivariate analysis were HIV infection (adjusted odd ratio [95% confidential interval]: 2.67, 1.20–5.93), $P = 0.016$, Fib-4 score > 1.45 (6.30, 2.70–14.74), $P < 0.001$, and hypovitaminosis D (2.48, 1.09–5.67), $P = 0.031$. GT 6 was less likely to have advanced liver fibrosis (0.17, 0.05–0.65), $P = 0.01$.

Conclusions: HIV infection, Fib-4 score > 1.45, and hypovitaminosis D are strong and independent predictors for the presence of advanced fibrosis in our HCV-infected patients. These data highlight the urgent need of HCV treatment and vitamin D supplement in resource-limited settings.

Introduction

Since the widespread availability of combination antiretroviral therapy (cART), there has been a dramatic decline in HIV/AIDS-related morbidity/mortality, and significant increase in the life expectancies of HIV-infected patients worldwide.[1] However, the improved survival after effective cART has been associated with higher mortality and morbidity rates contributable to chronic infection with hepatitis C virus (HCV) which is increasing.[2–4] This is especially problematic for resource-limited settings (RLS), where treatment of HCV is generally not easily accessible. HIV has a negative impact on the natural history of HCV, and compared with HCV mono-infected patients, HIV/HCV co-infected patients have a more rapid progression from chronic-active-hepatitis-to-liver-cirrhosis, end-stage liver disease, liver cancer,s and death.[5]

Factors that contribute to the rapid development of liver fibrosis/cirrhosis among HIV/HCV co-infected patients are males, acquiring HCV at an older age, heavy alcohol consumption, low CD4 cell count, HCV genotype 3 and insulin resistance.[6–12] Studies from HCV genotype 1, HIV/HCV co-infection have found that low serum levels of 25-OH-vitamin D [25(OH)D] are associated with severe liver fibrosis[13,14] and lower HCV treatment response.[14,15] However, these data were obtained mostly from resource-rich countries and may not be applicable in Asia where the financial status, behavior, culture, modes of HCV acquisition and HCV genotype distribution are different. Presently, factors associated with liver fibrosis in HIV/HCV co-infection and HCV mono-infection in RLS have not been well-characterized.

Management of HCV–related liver disease is based on staging of liver fibrosis assessed by liver biopsy. This limits the ability to assess the presence of fibrosis in RLS because liver biopsies are not easily accessible. HIV co-infection forms an additional barrier to access to liver biopsy because of widespread HIV stigmatization in many settings in Thailand. Therefore, little is currently known about the prevalence of liver fibrosis in Asia. An alternative non-invasive tool to measure the degree of liver fibrosis is transient elastography (FibroScan, TE, Echosens, Paris, France), which has been validated in both HCV mono-infected and HIV/HCV co-infected patients.[16] This is a promising tool in RLS to assess the presence of liver fibrosis among HCV-infected patients with/without HIV co-infection without the need for liver biopsy.

In the present study, we investigated the prevalence of different stages of liver fibrosis among untreated HCV-infected patients, with/without HIV co-infection. Predictors for the presence of liver fibrosis included serum 25(OH)D levels, HCV genotypes, IL28B and HCV-RNA.

Material and methods

Study design and participants. Chronic HCV (at least two occasions more than 6 months apart) with and without HIV were enrolled from Taksin Hospital, Chulalongkorn Memorial Hospital, and HIV-NAT, Thailand. Study protocol was approved by Institutional Review Board, Faculty of Medicine, Chulalongkorn University. All patients gave written consent. Basic characteristics included age, sex, risk category for HCV infection and for HIV infection if applicable, history of alcohol use, body weight (BW), body mass index (BMI), history of antiretroviral agents (ARV) use among those co-infected with HIV/HCV, calcium and vitamin D intake, and history of bone fracture. Duration of HCV infection was estimated by using the first reported exposure as the start of infection, e.g. first time the person shared used needles if PWIDs (people who inject drugs), first time of receiving blood products, first time of needle stick injury if healthcare workers and/or first time of unprotected sex.

Samples for HCV-RNA, HCV genotyping and IL28B were collected between June 2010 and April 2012. Blood was drawn after 10 h of fasting to assess CD4 count, HIV-RNA, complete blood count, glucose, lipid panels, serum creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), 25(OH)D levels, and HBV. Tests for anti-HCV antibody were repeated using chemiluminescent microparticle immunoassay (CMIA) (Abbott test generation3, Abbott Architect ci4100, Wiesbaden, Germany).

HCV viral load testing. Quantification of HCV-RNA was performed by real-time polymerase chain reaction assay (Abbott Molecular Inc. Des Plaines, IL, USA). Lower (LLD) and upper detection limits (ULD) of the assay were < 12 IU/mL and 100 000 000 IU/mL, respectively. Specimens yielding values above ULD were diluted 100-fold and retested.

HCV Genotyping and IL28B polymorphism. HCV-RNA was reversed by random primer and viral 5'UTR was amplified. Positive 5'UTR were then subjected for further amplification with primers specific for the core and NS5B regions as described previously.[17] Viral genotypes were classified based on the nucleotide sequence using BLAST search and viral genotyping tool (http://www.ncbi.nlm.nih.gov/). Human DNA was extracted from PBMCs/sera and used to detect IL28B polymorphism. PCR and direct sequencing were used to detect rs12979860. Genotype rs12979860 was analyzed directly from the chromatogram of the nucleotide sequences.

Assessment of vitamin D levels. Whole blood samples were centrifuged at 2500 r.p.m. for 20 min and plasma was stored at –80°C until use. ARCHITECT CMIA was used to quantitate the levels of 25(OH)D according to the manufacturer's instructions (Abbott, Barcelona, Spain). Hypovitaminosis D was defined as having serum levels of 25(OH)D < 30 ng/mL. Imprecision of the ARCHITECT 25-OH Vitamin D assay is < 10% but within laboratory (total) coefficient variation.

Assessment of liver fibrosis and cirrhosis. Liver stiffness was assessed by transient elastography (TE). All TE were performed by a single-blinded TE specialist to avoid interindividual variation and standard criteria with FS validation.[18] AST/platelet ratio index (APRI) score was calculated by using Wai *et al.*'s formula:[19] (AST/upper limit of normal considered as 40 IU/L)/platelet count $\times 10^9$/L. Fibrosis 4 Score (Fib-4) was calculated by using Sterling *et al.*'s formula:[20] (age × AST)/[(platelet count × ALT)$^{1/2}$].

Fibrosis stage was defined according to fibrosis score as measured by TE: mild (equivalent to Metavir F0–F1), ≤ 7.1 kPa; moderate (F2), 7.2–9.4 kPa; severe (F3), 9.5–14 kPa; and cirrhosis (F4), > 4 kPa. Furthermore, advanced liver fibrosis (F3 and F4) was defined as a fibrosis score of > 9.5 kPa. Cirrhosis was diagnosed based on definitive clinical evidence, fibrosis score > 14 kPa, and/or imaging evidence by abdominal sonogram of the liver showing cirrhosis/splenomegaly with portal hypertension.

Statistical analysis. Analysis was performed by Stata version 12.1 (Stata Corp., College Station, TX, USA). For descriptive analysis, frequencies of categorical variable were calculated. Median and interquartile range (IQR) were calculated for continuous variables. Each variable was correlated to vitamin D status and advanced liver fibrosis. Magnitude of associations was expressed as odds ratio (OR) and 95% confidence intervals (95% CI). Categorical variables were analyzed by using the Pearson's chi square test/Fisher's exact test, as appropriate. Continuous variable was analyzed using Student's *t*-test. Pearson correlation coefficiency was used to explore the association between serum vitamin D levels and advanced liver fibrosis. For multivariate analysis, mul-

tiple logistic regression analysis was used. Variables presenting $P < 0.1$ in univariate analysis were tested in the final model.

Results

Characteristics of study population (Table 1). A total of 130 untreated HIV/HCV co-infected and 331 untreated HCV mono-infected patients were enrolled. None of them reported fracture nor taking calcium/vitamin D. Majority were males (70%) and former PWIDs. The HCV mono-infected group had a higher proportion of patients > 50 years (34% *vs* 18%), longer estimated duration of HCV infection (17 years *vs* 15 years), and higher proportion of alcohol consumption (77% *vs* 39%). Median 25(OH)D levels were comparable between the HCV mono-infected and HIV/HCV co-infected patients. The prevalence of vitamin D insufficiency [25(OH)D 20–30 ng/mL] and deficiency [25(OH)D < 20 ng/mL] among mono-infected patients was 53.9% and 8.9%, respectively, thus the prevalence of hypovitaminosis D was 62.8%.The prevalence of vitamin D insufficiency and deficiency among co-infected patients was 47.9% and 13.7%, respectively (hypovitaminosis D 61.6%).

HCV genotype (GT) and IL28B polymorphism. The most prevalent circulating genotype was HCV GT3 (47%), followed by GT1 (34%) and GT6 (17%). HIV/HCV co-infected patients more often had plasma HCV-RNA > 800 000 IU/mL (54% *vs* 43%).

IL28B at rs12979860 position was available for 94 HIV/HCV and 136 HCV mono-infected patients. The major allele (CC genotypes) of rs12979860 position was found in 88% of HIV/HCV and 85% HCV mono-infected patients. Only 1% of HIV/HCV and 4% of HCV mono were minor TT allele.

HIV parameters for HIV/HCV co-infected patients. The median CD4 cell count at time of TE was 494 cells/mm^3 (IQR 310–625 cells/mm^3), only 16 (12.5%) of patients had a CD4 cell count of < 200 cells/mm^3. cART was used by 91% of patients. Median duration of cART was 7 years (IQR 5–11). Protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) were used by 36.4% and 59.3%, respectively, and 78% of those on ART had HIV-RNA < 50 copies/mL. Stavudine was used in 77 (59.2 %) patients with the median duration of 33 months (IQR 15–56).

Liver fibrosis and its fibrosis markers based on the transient elastography results. Median ALT for HIV/HCV co-infected and HCV mono-infected patients were 71 IU/L (IQR 43–112) and 60 IU/L (IQR 31–97), respectively ($P = 0.004$). Figure 1 depicts the distribution of liver fibrosis staging. A greater proportion of the participants from the HIV/HCV co-infected group had advanced liver fibrosis (41.7% *vs* 25.1%). Also, 24.1% of the participants from the HIV/HCV co-infected group had cirrhosis based on the fibrosis score > 14 kPa, compared with 12.7% in the HCV mono-infected group. The proportion of patients with Fib-4 score > 3.25 and APRI score > 1.5 were significantly greater in HIV/HCV co-infection (21.6% *vs* 9.1% and 27.2% *vs* 10.8%, respectively).

Comparisons of HCV genotypes 6 to 1 and 3 (Table 2). There were no differences in the median age, sex, duration of HCV infection, duration of HIV infection, or transmission route between genotypes. However, patients with HCV GT3 had a significantly higher median ALT (77 IU/L) compared with those with GT1 (61 IU/L) and GT6 (51 IU/L), $P < 0.001$. Only 23% of the participants with HCV GT3 had normal ALT levels. HCV-RNA was significantly higher in GT6 (6.6 $\log_{10}$ IU/mL) compared with GT1 and GT3 (5.9 $\log_{10}$ IU/mL), $P = 0.01$. Furthermore, participants with HCV GT3 had higher median fibrosis scores. In contrast, fewer participants with HCV GT6 had TE > 7.1 kPa (39.7%) compared with GT3(57.9%) and GT1(47.5%).

Factors associated with advanced liver fibrosis (metavir F3-F4) (Table 3). After adjustment in the multivariate analysis, HIV co-infection (aOR 2.67 [95% CI 1.20–5.93], $P = 0.016$), Fib-4 score > 1.45 (aOR 6.30 [95% CI 2.70–14.74], $P < 0.001$), and plasma 25(OH)D level of < 30 ng/mL (aOR 2.48 [95% CI 1.09–5.67], $P = 0.031$) remained significantly associated with advanced liver fibrosis. HCV GT6 was associated with a decreased risk of liver fibrosis (aOR 0.17 [95% CI 0.05–0.65], $P = 0.01$).

In HIV/HCV co-infected patients (Table 4), HIV-related parameters such as CD4 cell count, HIV-RNA, cART use, and duration of ART were not associated with advanced liver fibrosis whereas d4T exposure and 25(OH)D were strongly correlated with advanced liver fibrosis. Importantly, the correlation for 25(OH)D < 30 mg/dL and liver fibrosis was seen at milder levels of liver fibrosis in HIV/HCV co-infection but not in HCV mono-infection (TE > 7.5 kPa *vs* TE > 9.5 kPa). For the multivariate analysis, d4T exposure (aOR 4.92 [95% CI 1.44–16.8; $P = 0.011$]), Fib-4 score > 1.45 (aOR5.29 [95% CI 1.52–18.35], $P = 0.009$), and 25(OH)D < 30 mg/dL (aOR 5.04 [95% CI 1.44–17.6; $P = 0.011$]) were strongly correlated with significant liver fibrosis (TE > 7.5 kPa). In addition, from the multivariate analysis, only liver fibrosis > 7.5 kPa, not ARV, was associated with 25(OH)D < 30 mg/dL (OR 3.75 [95% CI 1.22–11.55]; $P = 0.021$).

To further investigate the nature of the relationship between Fib-4 and APRI and the development of advanced liver fibrosis, we examined the correlation between Fib-4, APRI and liver stiffness which revealed positive correlation between liver stiffness and Fib-4 [Pearson correlation (r) = 0.50, $P = < 0.001$] and APRI (r = 0.49, $P = < 0.001$).

A subanalysis was performed including patients who had IL28B testing. IL28B polymorphism was not found to be a predictor for liver fibrosis [rs 12979860 CC: OR 1.27 (95% CI 0.47–3.42, $P = 0.639$)].

Discussion

In this analysis of 130 untreated HIV/HCV and 331 untreated HCV mono-infected patients using TE to measure degree of liver fibrosis, the primary finding was that people co-infected with HCV and HIV have a high prevalence of significant liver fibrosis. HIV co-infected patients were 2.67 times more likely to have advanced liver fibrosis (TE > 9.5 kPa) compared with HCV mono-infected patients. Almost 70% of our HIV/HCV co-infected patients had significant liver fibrosis (TE > 7.1 kPa) and 41.6% had advanced liver fibrosis with TE > 9.5 kPa.

Table 1 Demographic and clinical features of the study participants: 130 HIV/HCV co-infected and 331 HCV mono-infected participants

Characteristics	Total (*N* = 461)	HIV/HCV (*N* = 130)	HCV mono (*N* = 331)	*P*-value
Sex, *N* (%)				< 0.001
Male	323 (70.1)	111 (85.4)	212 (64.1)	
Age, years	43 (36–52)	42 (37–48)	44 (36–53)	0.364
Age ≥ 50 years, *N* (%)	136 (29.50)	24 (18.46)	112 (33.84)	0.001
Duration of HCV exposure, years	17 (10–24)	15 (10–21)	17.5 (10–25)	0.042
Median (IQR) body mass index (BMI), kg/m^2	23.2 (20.8–25.6)	21.6 (19.4–23.8)	23.9 (21.5–26.0)	< 0.001
BMI > 25 kg/m^2, *N* (%)	116 (29.3)	19 (15.8)	97 (35.1)	< 0.001
HCV risk factors				< 0.001
Heterosexual	25 (5.73)	25 (19.23)	5 (1.63)	
MSM	17 (3.90)	17 (13.08)	0 (0.0)	
IV drug use	154 (35.32)	81 (62.31)	73 (23.86)	
Blood transfusion	91 (20.87)	4 (3.08)	87 (28.43)	
TAS	64 (14.68)	1 (0.77)	63 (20.59)	
Unknown	80 (18.35)	2 (1.54)	78 (25.49)	
Alcohol consumption, *N* (%)	273 (66)	44 (39)	229 (77)	< 0.001
Current ALT (IU/L)	62 (35–100)	71 (43–112)	60 (31–97)	0.004
Plasma HCV-RNA, $\log_{10}$ IU/mL[†]	5.9 (5.4–6.6)	6.0 (4.9–6.6)	5.8 (5.6, 6.5)	0.829
Plasma HCV-RNA levels of > 800,000 IU/mL, *N* (%)	118 (49.2)	70 (54.3)	48 (43.2)	< 0.001
HCV genotype[‡]				
1	128 (34)	32 (31.7)	96 (34.9)	
2	2 (0.5)	0	2 (0.7)	
3	176 (46.8)	50 (49.5)	126 (45.8)	
5	1 (0.3)	0	1 (0.4)	
6	63 (16.8)	13 (12.9)	50 (18.2)	
1 + 3	5 (1.3)	5 (4.9)	0	
1 + 2	1 (0.3)	1 (0.9)	0	
FibroScan,[§] kPa	7.1 (5.2–10.5)	8.5 (6.4–13.85)	6.6 (4.9–9.5)	< 0.001
Fib-4 score, median (IQR)[¶]	1.3 (0.8–2.1)	1.7 (1.0–3.1)	1.1 (0.8–1.9)	0.001
< 1.45, *N* (%)	241 (57.2)	54 (43.2)	187 (63.2)	< 0.001
1.45–3.25, *N* (%)	126 (29.9)	44 (35.2)	82 (27.7)	
> 3.25, *N* (%)	54 (12.8)	27 (21.6)	27 (9.1)	
APRI score, median (IQR)[¶]	0.6 (0.3–1.1)	0.8 (0.4–1.6)	0.5 (0.3–1.0)	< 0.001
< 0.5, *N* (%)	188 (44.7)	38 (30.4)	150 (50.7)	< 0.001
0.5–1.5, *N* (%)	167 (39.7)	53 (42.4)	114 (38.5)	
> 1.5, *N* (%)	66 (15.7)	34 (27.2)	32 (10.8)	
25(OH)D levels,[††] ng/mL, median (IQR)	27.4 (22.9–33.6)	27 (22.4–34.3)	27.5 (23.2–33.4)	0.961
> 30 ng/mL, *N* (%)	112 (37.7)	45 (38.5)	67 (37.2)	
20–30 ng/mL, *N* (%)	153 (51.5)	56 (47.9)	97 (53.9)	
< 20 ng/mL	32 (10.8)	16 (13.7)	16 (8.9)	
Median (IQR) current CD4 cell counts, cells/mm^3		494 (310–625)		
HIV RNA < 50 copies/mL, *N* (%)		100 (78.1)		
Current antiretroviral therapy, *N* (%)		118 (90.8)		
None		12 (9.2)		
2 NRTIs and NNRTI		70 (59.3)		
2 NRTIs and boosted PI		43 (36.4)		
Other		5 (4.2)		

Note: Median and interquartile range (IQR) is presented, unless otherwise indicated. APRI = [(AST/ULN) × 100]/Platelets count 10^9/L (ULN = the upper limit of normal); Fib-4 = [age (years) × AST (IU/L)]/[platelet count (10^9/L) × ALT(IU/L)$^{1/2}$].

[†]HCV-RNA was available for 129 HCV/HIV co-infected and 111 HCV mono-infected participants.

[‡]HCV genotype was available for 101 HCV/HIV co-infected and 275 HCV mono-infected participants.

[§]FibroScan was available for 108 HIV/HCV co-infected and 331 HCV mono-infected participants.

[¶]Fib-4 and APRI were available for 125HIV/HCV co-infected and 296 HCV mono-infected participants.

[††]Vitamin D levels were available for 117HIV/HCV co-infected and 180 HCV mono-infected participants.

ALT, alanine aminotransferase; APRI, AST/platelet ratio index; IV, intravenous; HCV, hepatitis C virus; HIV, human immunodeficiency virus; MSM, men having sex with men; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TAS, tattoo, acupuncture, sharing of contaminated needles.

To our knowledge, this study is the first of its kind to report the prevalence of liver fibrosis among HCV-infected Asians with/without HIV co-infection. Even though the participants from the HCV mono-infected group had a longer duration of expected HCV infection (17.5 years *vs* 15 years) and had a higher proportion of patients over 50 years old (34% *vs* 18%). The prevalence of advanced liver fibrosis was higher among the HIV/HCV co-infected group (41.7% *vs* 25.1%). Only 12.7% of the participants from the HCV mono-infected group had cirrhosis, whereas in the HIV/HCV co-infected group, 24.1% had cirrhosis. These findings are consistent with previous studies that the prevalence of liver fibrosis is much higher in individuals co-infected with HCV and HIV.[6,21,22] The prevalence of cirrhosis in our HIV/HCV co-infected population (24.1%) was higher than Europe (13%)[21] and Brazil (8.5%).[23]Given the significant risk for hepatocellular carcinoma (HCC) development in this population, our findings highlight the urgent need for HCV treatment and HCC screening for patients in RLS. Given that 47% of the HCV patients in our patients have HCV GT3 and 90% of them have IL28B rs12979860 CC allele, they are good candidates for HCV treatment with pegylated interferon alfa (PegIFN) and ribavirin.

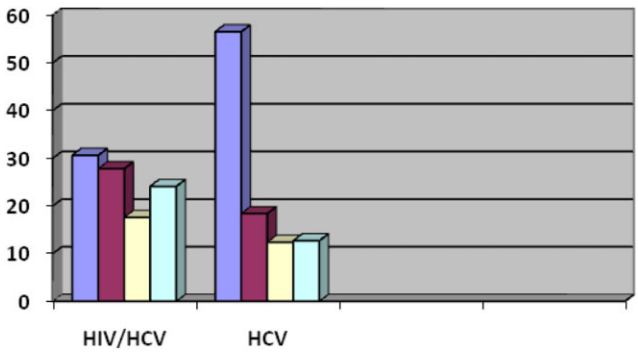

Figure 1 Distribution of liver fibrosis staging among HIV/HCV and HCV mono-infected patients. ■, F1 (< 7.1 kPa); ■, F2 (7.2–9.4 kPa); ■, F3 (9.5–14 kPa); ■, F4 (> 14 kPa).

Our findings disagree with prior studies that patients infected with HCV GT1 tend to have higher plasma HCV-RNA levels than those infected with HCV GT3.[24,25] In fact, we found that patients infected with HCV GT6 had higher HCV-RNA than those infected with GT1 and GT3. Although patients infected with HCV GT6 had higher median plasma HCV-RNA and a greater proportion of plasma HCV-RNA > 800,000 IU/mL, they had lower median fibrosis score by TE and a lower proportion of patients with advanced liver fibrosis compared with GT3 and 1. By multivariate analysis, HCV GT3 was only marginally significantly associated with advanced fibrosis. In a cross-sectional study from France and Spain, of 314 liver biopsy specimens from HCV mono-infected patients[26] and 283 HIV/HCV co-infected patients by TE,[12] the association between HCV GT3 and advanced liver fibrosis was

Table 2 Hepatitis C (HCV) genotypes 1, 3 and 6 are compared with each other as well as to the study participants' characteristics

Characteristics	Total (*n* = 367)	HCV genotypes, *N* (%)			*P*-value
		Genotype 1 (*n* = 128)	Genotype 3 (*n* = 176)	Genotype 6 (*n* = 63)	
Age, years Median (IQR)	44 (37–52)	44 (37–52)	44 (37–52)	46 (36–53)	0.987
Male	261 (71.12)	87 (67.97)	130 (73.86)	44 (69.84)	0.518
MSM	14 (4.08)	6 (4.96)	7 (4.19)	1 (1.82)	0.618
History of intravenous drug use	120 (34.99)	36 (29.75)	66 (39.52)	18 (32.73)	0.213
Duration of HCV infection, years	18 (12–25)	16 (10–24)	19 (13–25)	20 (10–25)	0.262
HIV co-infection	95 (25.89)	32 (25.00)	50 (28.41)	13 (20.63)	0.463
HCV-RNA, $\log_{10}$ IU/mL Median (IQR)	6.0 (5.7–6.6)	5.9 (5.7–6.4)	5.9 (5.5–6.6)	6.6 (5.9–6.8)	0.010
ALT, IU/L Median (IQR)	66 (42–108)	61 (37–100)	77 (53–114)	51 (32–83)	< 0.001
Normal	109 (32.15)	43 (36.13)	37 (22.84)	29 (50.00)	< 0.001
Abnormal	230 (67.85)	76 (63.87)	125 (77.16)	29 (50.00)	
Platelet count < 100,000/mm^3	22 (6.57)	6 (5.13)	14 (8.75)	2 (3.45)	0.278
Fib-4 score Median (IQR)	1.36 (0.88–2.24)	1.35 (0.8–2.16)	1.49 (0.97–2.49)	1.11 (0.86–1.68)	0.034
< 1.45	179 (54.08)	64 (55.17)	77 (48.73)	38 (66.67)	0.064
> 1.45	152 (45.92)	52 (44.83)	81 (51.27)	19 (33.33)	
APRI score					
Median (IQR)	0.63 (0.38–1.18)	0.59 (0.31–1.12)	0.75 (0.5–1.4)	0.42 (0.33–0.85)	< 0.001
< 0.5	128 (38.67)	52 (44.83)	44 (27.85)	32 (56.14)	< 0.001
> 0.5	203 (61.33)	64 (55.17)	114 (72.15)	25 (43.86)	
Fibroscan, kPa					
Median (IQR)	7.6 (5.4–10.95)	7.2 (5.4–11.4)	8.1 (5.6–11.6)	6.5 (5.2–8.4)	0.008
< 7.1	174 (48.88)	64 (52.46)	72 (42.11)	38 (60.32)	0.029
≥ 7.1	182 (51.12)	58 (47.54)	99 (57.89)	25 (39.68)	

ALT, alanine aminotransferase; APRI, AST/platelet ratio index; IQR, interquartile range; MSM, men having sex with men.

Table 3 Univariate and multivariate logistic regression analyses of the factors associated with advanced liver fibrosis (TE > 9.5 kPa)

Variables	Univariate			Multivariate		
	OR	95% CI	*P*	aOR	95% CI	*P*
Male gender	1.69	(1.05,2.72)	0.032	1.01	(0.40–2.52)	0.988
Current age of ≥ 50 years	2.96	(1.91,4.6)	< 0.001	—	—	—
Age by 10 years	1.83	(1.46–2.29)	< 0.001	1.51	(0.88–2.58)	0.133
BMI > 25 kg/m^2	1.75	(1.09–2.80)	0.020	2.25	(0.89–5.72)	0.087
HCV-RNA of > 100,000 IU/mL	2.03	(0.94,4.38)	0.072	—	—	—
HCV-RNA of > 800,000 IU/mL	1.12	(0.64–1.95)	0.685	—	—	—
Current abnormal ALT	4.58	(2.68–7.80)	< 0.001	—	—	—
HIV co-infection	2.12	(1.34, 3.36)	0.001	2.67	(1.20–5.93)	0.016
HCV genotype	—	—	< 0.001	—	—	0.010
Genotype 3	1.32	(0.81, 2.16)	—	0.96	(0.44–2.10)	—
Genotype 6	0.32	(0.14, 0.74)	—	0.17	(0.05–0.65)	—
Fib-4 score of > 1.45	8.51	(5.15–14.07)	< 0.001	6.30	(2.70–14.74)	< 0.001
APRI score of > 0.5	10.31	(5.62–18.89)	< 0.001	—	—	—
Estimated duration of HCV infection of > 20 years	1.93	(1.21–3.07)	0.006	1.10	(0.47–2.60)	0.828
Current alcohol consumption	0.86	(0.54–1.35)	0.510	—	—	—
Hypovitaminosis D [25(OH)D < 30 (ng/mL)]	1.78	(1.04–3.06)	0.037	2.48	(1.09–5.67)	0.031
IL28 B						
rs12979860 CC allele	1.4	(0.58–3.37)	0.045	—	—	—

Calculations for Fib-4 and APRI scores are described in the Materials and Methods section.
95% CI, 95% confidence interval; aOR, adjusted odds ratio; APRI, AST/platelet ratio index; HCV, hepatitis C virus; HIV, human immunodeficiency virus; OR, odds ratio.

Table 4 Univariate and multivariate logistic regression analysis of the factors associated with advanced liver fibrosis (TE ≥ 7.5 kPa) only HIV/HCV co-infected patients

Variables	Univariate			Multivariate		
	OR	95%CI	*P*	aOR	95% CI	*P*
Male gender	1.24	(0.41–3.73)	0.702	—	—	—
Age increased by 10 years	1.48	(0.81–2.71)	0.208	—	—	—
BMI > 25 kg/m^2	3.88	(0.82–18.23)	0.086	1.34	(0.17–10.84)	0.782
HCV-RNA of > 800,000 IU/mL	0.95	(0.42–2.11)	0.892	—	—	—
Current abnormal ALT	3.50	(1.48–8.25)	0.004	—	—	—
HCV genotype	1.37	(0.47–4.03)	0.335	1.59	(0.42–6.03)	0.419
Genotype 3						
Genotype6	0.52	(0.13–2.04)	—	0.53	(0.09–2.99)	—
Fib-4 score of > 1.45	4.55	(1.92–10.76)	0.001	5.29	(1.52–18.35)	0.009
APRI score of > 0.5	5.18	(2.15–12.47)	< 0.001	—	—	—
Estimated duration of HCV infection of > 20 years	1.30	(0.54–3.13)	0.558	—	—	—
Current alcohol consumption	1.11	(0.48–2.58)	0.800	—	—	—
Hypovitaminosis D [25(OH)D < 30 (ng/mL)]	2.00	(0.88–4.53)	0.096	5.04	(1.44–17.62)	0.011
rs12979860 CC allele	1.38	(0.57–3.33)	0.468	—	—	—
ART Regimen	—	—	0.494	—	—	—
Efavirenz or nevirapine	Ref	1	—	—	—	—
Protease inhibitor	0.96	(0.4–2.28)	—	—	—	—
Stavudine exposure	2.66	(1.17–6.07)	0.020	4.92	(1.44–16.79)	0.011
Didanosine exposure	0.87	(0.35–2.14)	0.756	—	—	—
Indinavir exposure	0.54	(0.17–1.74)	0.301	—	—	—
CD4 ≤ 500 cells/mm^3	0.66	(0.29–1.47)	0.306	—	—	—
HIV-RNA < 50 copies/mL	2.61	(0.95–7.16)	0.063	2.01	(0.45–9.03)	0.362

95% CI, 95% confidence interval; aOR, adjusted odds ratio; APRI, AST/platelet ratio index; ART, antiretroviral therapy; BMI, body mass index; HCV, hepatitis C virus; HIV, human immunodeficiency virus; OR, odds ratio.

highly significant. Surprisingly, we found that patients with HCV GT6 were less likely to have liver fibrosis compared with infection with HCV GT1 and 3. To our knowledge, we are the first to compare HCV-RNA and liver fibrosis between patients infected with HCV GT6 to those infected with GT1 or 3. Data from Caucasians indicate that the progression rate for fibrosis is much faster among patients infected with HCV GT3 compared with those with HCV GT1.[12,26] However, data from our cohort indicate that HCV GT3 was not significantly correlated with liver fibrosis compared with GT1. Our finding is similar to another HCV study from India where HCV GT3 is the most circulating genotype (75%).[27] Hissar *et al*. reported that GT3 was not correlated with liver fibrosis.[27] Therefore, it is highly likely that host genetic factors may play an important role in liver fibrosis. Host genetic factors among HCV GT3 Asians and Caucasians should be further explored.[27]

Although ALT elevations are not a good marker of liver fibrosis especially in HIV co-infected patients, in this study we found that Fib-4 score was strongly correlated with advanced liver fibrosis. Participants with Fib-4 score of > 1.45 had a sevenfold increased risk of having advanced liver fibrosis compared with those with Fib-4 score of < 1.45. Fib-4 is cheap and very easy to perform, requiring only levels of ALT, AST, platelet and age. No additional tests, machines, or skills are required besides laboratory tests routinely given twice a year. From this observation, Fib-4 can be used as a surrogate marker to detect advanced liver fibrosis, especially in RLS where liver biopsy and FibroScan machine are not widely accessible as well as a tool for monitoring liver disease progression.[28,29]

In this study, patients with hypovitaminosis D(25(OH)D levels < 30 ng/mL) were almost three times more likely to have advanced liver fibrosis. Recently, low serum 25(OH)D levels have been reported in patients with chronic HCV GT1 and were found to be associated with severe liver fibrosis.[13,14] Vitamin D plays a major role for calcium homeostasis and bone health, but vitamin D is now also widely recognized as a critical factor involved in the immune system, inflammatory response, and fibrogenesis.[30] Recent studies have found that low serum levels of 25(OH)D are also associated with low sustained virological response (SVR) to Peg-IFN/ribavirin therapy.[14,15] Furthermore, vitamin D supplementation improves early virological response (EVR) (94% *vs* 48%) and SVR (86% *vs* 42%) in HCV GT1 treated with Peg-IFN/ribavirin.[31] Therefore, hypovitaminosis D in our HCV population has clinical implication both for liver fibrosis progression and for treatment efficacy. Ensuring adequate vitamin D levels in this population is important.

Almost of our HIV/HCV co-infected patients were on cART, with a median duration of 7 years. Furthermore, only 13% had current CD4 cell counts of 200 cells/mm^3. We did not find any association between several HIV-related factors, such as current or nadir CD4 count, HIV-RNA or duration of cART, and the presence of liver fibrosis. This finding is inconsistent with previous studies that showed advanced liver disease progression among HIV/HCV co-infected patients with current CD4 < 500 cells/mm^3, and nadir CD4 of < 250 cells/mm^3.[12,32] We found only d4T exposure was positively correlated (sixfold increased) with advanced liver fibrosis, which might be explained by mitochondrial toxicity and/or insulin resistance. The data on the effect of cART on liver fibrosis is conflicting. Macias *et al*. showed that the use of PIs was able to slow down the progression of the liver disease.[33] Moreover, a recent report on the use of didanosine has been shown to be associated with liver fibrosis,[34] whereas another recent publication from the EuroSida group failed to support whether ART could ameliorate the progression of liver disease.[21]

Our study had some limitations. First, we did not perform liver biopsy for the assessment of the presence of liver fibrosis. However, TE has already been validated for both HIV/HCV and HCV mono-infected Caucasian patients in several studies. The positive predictive value of TE for diagnosis of advanced liver fibrosis has been as high as 95%.[28] In addition, when we combined the APRI, Fib-4 and TE together, the result of advanced fibrosis was the same. Therefore the combined use of TE and Fib-4 or APRI to evaluate advanced liver fibrosis could avoid a liver biopsy. Second, this was a cross-sectional study that precluded analysis of the progression of liver fibrosis. In order to see the trend of liver fibrosis using the TE, longitudinal studies are recommended. Third, data on bone mass density, parathyroid hormone, calcium and phosphate were not available. Therefore it is unclear whether low levels of vitamin D had any effect on the bones or any calcium homeostasis in this population or not. Lastly, because insulin resistance has not been investigated in our study population, we were not able to confirm the positive association between insulin resistance and advanced liver fibrosis, especially in HIV/HCV co-infected patients with the median of 8 years of cART.s

Regardless of these limitations, the findings from this study did have several important clinical implications. Our results underline the need for HCV treatment in chronically infected patients, especially for those co-infected with HIV. In this study, most of our HIV/HCV co-infected participants were not on HCV therapy and 70% had significant liver fibrosis at a median age of 40 years. Without the availability of HCV treatment, these patients will develop end-stage liver disease within the next decade. In addition, the majority of our patients had HCV non-GT1, along with favorable IL28B (89% CC genotype), and would therefore be good candidates for the treatment with Peg IFN/ribavirin.

In conclusion, advanced liver fibrosis is seen in 41.7% and 25.1% of HIV/HCV co-infected and HCV mono-infected patients from Thailand, respectively. HCV-RNA tends to be higher among patients infected with HCV GT6, but advanced liver fibrosis is seen less often in this group, compared with those infected with GT1 or GT3. Strong and independent predictors for the presence of advanced fibrosis were HIV infection, Fib-4 score > 1.45, d4T use and hypovitaminosis D. These data highlight the urgent need of HCV treatment and HCC screening for HCV patients in RLS. In addition, the role of vitamin D supplementation should be further explored as a relatively cheap therapeutic option to reduce liver fibrosis and improve SVR.

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Authors' contribution

All authors contributed to the study design. AA drafted the manuscript and conducted the study. TA performed all statistical analyses. SA performed all laboratory experiments for genotyping and polymorphisms. RAR, PT, SJ, OP, and ST conducted the study at their perspective sites, helped collect data and necessary specimens. YP, GVM, JMAL and KR provided feedback and finalized the manuscript.

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