Original article

Decline in serum 25 hydroxyvitamin D levels in HIV–HBV-coinfected patients after long-term antiretroviral therapy

Anchalee Avihingsanon[1,2], Tanakorn Apornpong[1], Reshmie A Ramautarsing[1,3], Sasiwimol Ubolyam[1], Pisit Tangkijvanich[4], Jintanat Ananworanich[1,2,5], Joep MA Lange[3], Gail Matthews[6], Sharon R Lewin[7,8], Kiat Ruxrungtham[1,2], HIV-NAT 105 study team*

[1]HIV Netherlands Australia Thailand (HIV-NAT) Research Collaboration, The Thai Red Cross AIDS Research Centre, Bangkok, Thailand
[2]Division of Allergy and Immunology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
[3]Department of Global Health, Academic Medical Center, University of Amsterdam, Amsterdam Institute for Global Health and Development (AIGHD), Amsterdam, the Netherlands
[4]Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
[5]SEARCH, The Thai Red Cross AIDS Research Centre, Bangkok, Thailand
[6]Kirby Institute, University of New South Wales, Sydney, Australia
[7]Department of Infectious Diseases, Alfred Hospital and Monash University, Melbourne, Australia
[8]Centre for Biomedicine, Burnet Institute, Melbourne, Australia

*Corresponding author email: anchaleea2009@gmail.com

Background: Vitamin D insufficiency plays an important role in the development of fibrosis in chronic liver disease.
Methods: This was a cross-sectional study from Thailand. Liver fibrosis was assessed by transient elastography. Serum 25 hydroxyvitamin D (25[OH]D)<30 ng/ml was defined as hypovitaminosis D. 25(OH)D was assessed prior to and following tenofovir disoproxil fumarate (TDF). Factors related to 25(OH)D levels were determined by logistic regression analysis.
Results: A total of 158 HIV–HBV-coinfected patients (32% female, median age 43 years) were included. Overall, liver disease was mild with 13.4% having a fibrosis score (FS) of 7.1–14 kPa and 2% with a FS>14 kPa. Median (IQR) duration on TDF was 5 years (4–7). The median estimated glomerular filtration rate was 96.9 ml/min/1.73 m². The median (IQR) serum 25(OH)D levels prior to and following TDF were 24.8 ng/ml (21.3–30.6) and 22.8 ng/ml (18.0–27.7), respectively; $P \leq 0.001$). The proportion of patients with hypovitaminosis D significantly increased from 72.2% (95% CI 64.7, 78.6) prior to TDF to 84.2% (95% CI 77.7, 89.0) after taking TDF ($P=0.01$). Factors associated with hypovitaminosis D by multivariate analysis were female sex (adjusted OR 3.8, 95% CI 1.1, 13.7; $P=0.038$) and duration of antiretroviral therapy (ART)>5 years (OR 3.3, 95% CI 1.2, 8.8; $P=0.017$). Vitamin D levels were not associated with significant liver fibrosis.
Conclusions: Although our HIV–HBV-coinfected patients live in the tropics, there was a high prevalence of hypovitaminosis D, especially in female patients and those receiving prolonged ART. Since HIV–HBV-coinfection requires long-term use of the HBV-active drug, TDF, which can also contribute to bone loss, routine vitamin D assessment and supplementation as necessary should be considered.

Introduction

Chronic hepatitis B (CHB) remains a major global public health problem. Despite the availability of a highly-effective vaccine since 1982, it is estimated that >350–400 million people around the world have been infected with HBV and nearly 75% of them are from the Asia Pacific region [1]. In addition, 15% to 40% of those patients are at risk of developing cirrhosis and/or liver cancer if treatment is not administered. HIV–HBV coinfection accelerates the progression of HBV disease resulting in a higher rate of chronic hepatitis, end-stage liver disease and hepatocellular carcinoma [2,3]. The prevalence of CHB in HIV-infected patients varies

between 4–10% in Europe and the US [4,5] and 9–10% in Thailand [6–9].

Vitamin D plays a major role in calcium homeostasis and bone health. In addition to its effect on bone metabolism, vitamin D is now widely recognized as a crucial factor involved in the immune system, inflammatory response and fibrogenesis [10]. It is thought that optimal calcium absorption is correlated with a serum 25-hydroxyvitamin D (25[OH]D) level of ≥30 ng/ml. Lower levels result in an increased production of parathyroid hormone [10,11]. However, the optimal vitamin D level for non-skeletal functions is not fully understood.

Vitamin D from the skin and diet is first hydroxylated in the liver to 25(OH)D, which is transported to the kidney, then undergoes a second hydroxylation to be converted into 1,25(OH)D in the proximal renal tubule [10]. Therefore, any disease in the liver and proximal renal tubule would interfere with production of the active metabolites of vitamin D, resulting in hypovitaminosis D (serum 25[OH]D of <30 ng/ml) and abnormal calcium and bone metabolism. Hypovitaminosis D has been reported in up to 80% of adult HIV-infected individuals from countries at high latitude [12–15] and tropical countries [16,17]. Approximately 68–92% of patients with chronic liver disease (mainly due to alcoholism and HCV infection) have hypovitaminosis D [18]. Recent studies conducted in HCV-infected Caucasians found that low serum levels of 25(OH)D were positively correlated with severe liver fibrosis in patients with HCV genotype 1 [19] and in HIV–HCV-coinfected patients [20]. Little is known about hypovitaminosis D in patients infected with CHB, with or without HIV coinfection. There has been one case report of severe vitamin D deficiency in an HIV–HBV-coinfected patient treated with tenofovir disoproxil fumarate (TDF) [21].

Approximately 22% of HIV-infected patients treated with TDF were reported to have subclinical tubular dysfunction [22]. Furthermore, TDF-related bone loss and/or osteomalacia have been reported [23–27]. Antiretroviral treatment [28,29] and chronic liver disease [30] may contribute to further bone loss. In HIV–HBV coinfection, long-term TDF treatment is required. Interestingly, efavirenz has also been implicated in vitamin D deficiency [31,32].

Unfortunately there are no data on vitamin D status and its role in HIV–HBV coinfection. Our primary objective was to evaluate serum levels of 25(OH)D in a large, longitudinal cohort of HIV–HBV-coinfected patients from Thailand, who have been in follow-up for a median duration of 10 years. Serum 25(OH)D levels were determined prior to and following TDF use. The correlation of serum 25(OH)D levels and significant liver fibrosis/cirrhosis was also assessed.

Methods

Study design and participants

We performed a cross-sectional study among participants enrolled in the HIV Netherlands Australia Thailand Research Collaboration (HIV-NAT), Bangkok, Thailand. They were a part of the HIV-NAT 006 cohort, a prospective longitudinal cohort study initiated in 1996. Bangkok is located at 13°45'N and 100°30'E in the middle of Thailand. The city has a tropical climate, with high temperatures and high humidity levels. Thailand basically has two distinct seasons: the dry season (from November to April) and the rainy season (from May to October). This study took place between July 2011 and 30 September 2012.

All clinical details including the antiretroviral therapy (ART) regimen, $CD4^+$ T-cell counts, HIV RNA viral load, serum creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were extracted from the HIV-NAT 006 database. Other clinical data collected included: use of steroids 6 months before sample collection, smoking, alcohol use, menopausal status, use of vitamin D/calcium supplements and fracture. All patients had stored plasma and/or serum samples at cohort entry and yearly thereafter. Patients had been asked to fast for ≥10 h prior to having their blood drawn. All patients had provided appropriate consent. Additional samples were collected for 25(OH)D testing from patients who visited our clinic during 1 July 2011 to 30 September 2012. The 25(OH)D levels were quantified from the stored samples prior to TDF initiation.

HIV-infected adults (aged ≥18 years) with CHB were selected from the database. CHB was defined as hepatitis B surface antigen-positive on at least two occasions >6 months apart. Patients were excluded if they had chronic diarrhoea, or a history of corticosteroids, anticonvulsants or rifampicin use 6 months prior to the time of blood collection. People who reported injected drug use were also excluded.

This cohort was reviewed and approved by the Institutional Review Boards of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Serum 25(OH)D measurement

Blood was drawn from after ≥10 h of fasting to determine the levels of 25(OH)D. Whole blood samples were centrifuged at 2,500 rpm for 20 min and plasma was stored at -80°C until use. 25(OH)D was tested in serum by the Architect chemiluminescent microparticle immunoassay (CMIA; Abbott, Barcelona, Spain) to quantitate the levels of 25(OH) D according to the manufacturer's instruction. Serum levels of 25(OH)D<30 ng/dl were defined as

hypovitaminosis D. The Architect 25(OH)D assay has an imprecision of <10% within laboratory (total) coefficient variation.

Liver fibrosis and cirrhosis assessment

Liver fibrosis was assessed by transient elastography (TE; FibroScan, Echosens, Paris, France) at the King Chulalongkorn Memorial Hospital. TE was performed by one FibroScan specialist who was unaware of patient participation in this study. Abdominal ultrasound was performed in parallel with TE.

The AST/platelet ratio index (APRI) score was calculated using the formula presented by Wai *et al.* [33] as follows: (AST/upper limit of normal considered as 40 IU/l)/(platelet count $\times 10^9$/l). The Fibrosis 4 score (Fib-4) was calculated using the formula presented by Sterling *et al.* [34] as follows: (age ×AST)/(platelet count $\times[ALT]^{1/2}$). Significant liver fibrosis was defined as fibrosis score >7.1 kPa, APRI>0.5 or Fib-4>1.45.

The diagnosis of cirrhosis was established by the combination of definitive clinical or biochemical evidence (Fib-4>3.25), fibrosis score >14 kPa or by abdominal ultrasound of the liver showing cirrhosis or splenomegaly with portal hypertension.

Proximal tubular function assessment

Tubular parameters were assessed in blood and in 24 h urine. Samples were drawn in the morning after a 10 h overnight fast. Venous blood variables included glucose, creatinine, sodium, potassium, chloride, bicarbonate, phosphorus, total calcium, uric acid, albumin and total protein. Urine variables included glucose, creatine, phosphorus, uric acid, albumin, proteins and β2 microglobulin. Urine was also examined for white and red blood cells, and urine pH. All parameters of renal proximal tubular function were performed at the laboratory of the Division of Nephrology, Chulalongkorn University.

The glomerular filtration rate was estimated (eGFR) using the Modification of Diet in Renal Disease equation [35]. Proximal tubular dysfunction (PRTD) was defined on the basis of the presence of ≥2 out of 5 of the following criteria [22]: fractional tubular absorption of phosphorus: (1-[(urine phosphorus × plasma creatinine)/(plasma phosphorus × urine creatinine)])<0.80 or tubular maximum for phosphorus corrected for eGFR plasma phosphorus-([urine phosphorus × plasma creatinine]/urine creatinine)<2.6 mg/dl; total excretion of phosphorus (urine phosphorus × urine volume)>1,200 mg daily; fractional excretion of uric acid: (1-[(urine uric acid × plasma creatinine)/(plasma uric acid × urine creatinine)]×100)>15%; β2 microglobulin >1 mg/day or β2 microglobulin/urinary creatinine >0.3 mg/l; and non-diabetic glucosuria (urine glucose >300 mg/day or positive urine glucose) with normal glycaemia (plasma glucose <100 mg/dl).

Determination of urine β2 microglobulin was performed using immunonephelometry (BN II DADE, Behring, Barcelona, Spain).

Statistical analyses

Data analyses were performed using STATA version 11.2 (Stata Corp., College Station, TX, USA). For descriptive analyses, the frequencies of the categorical variables were calculated, while medians and IQRs were calculated for continuous variables. Each variable was correlated to vitamin D status. The magnitude of the associations was expressed as an OR and 95% CI. Pearson correlation coefficients were used to explore the association between serum vitamin D levels and potential risk factors. For the multivariate analysis, multiple logistic regression analysis was used. Variables with a *P*-value <0.1 in the univariate analysis were tested in the final model.

Results

Study population and vitamin D status

A total of 178 eligible HIV–HBV-coinfected patients were identified. However, only 158 HIV–HBV-coinfected patients had stored serum for 25(OH)D prior to initiating TDF. None of these patients were taking steroid, vitamin D or calcium supplements at the time of study nor had a history of fracture. The clinical details are summarized in Table 1. Overall, the median age was 43 years, the majority (68%) were male, and 62% had their blood drawn during the rainy season (May–October). Most of them had stored samples prior to and following TDF in the same season. Current minimal alcoholic consumption was reported by 43.1%. All patients were receiving combination antiretroviral therapy (cART). The median $CD4^+$ T-cell count was 513 cells/mm^3 and 91.7% had HIV RNA<50 copies/ml. The median eGFR was 96.9 ml/min/1.73 m^2. Median time on cART was 8 years (IQR 6–13) and 131 (82.9%) patients had been on cART for >5 years. The median time on TDF was 5 years (IQR 4–7). The Fib-4 index was consistent with bridging fibrosis/cirrhosis (index >3.25) in 3.25%. Overall, a fibrosis score of >7.1 kPa and >14 kPa by FibroScan were seen in 13.4% and 2.1% of participants, respectively.

Serum 25(OH)D levels

Patients with hypovitaminosis D were more often female and more often on cART for >5 years when compared to those with normal vitamin D levels. The prevalence of hypovitaminosis D (vitamin D insufficiency defined as 25[OH]D 20–30 ng/ml, and

Table 1. Demographic and clinical characteristics of 158 HIV–HBV-coinfected patients

Characteristic	Value
Female gender, *n* (%)	51 (32.28)
Menopause, *n* (%)	5 (9.8)
Median current age, years (IQR)	43 (37–47)
Route of HIV transmission	
Heterosexual, *n* (%)	100 (63.3)
MSM, *n* (%)	48 (30.4)
IDUs, *n* (%)	1 (0.6)
MSM and IDU, *n* (%)	2 (1.3)
Others/unknown, *n* (%)	7 (4.4)
Season	
Dry, *n* (%)	60 (37.97)
Rainy, *n* (%)	98 (62.03)
Median CD4$^+$ T-cell count at time of TDF initiation, cells/mm^3 (IQR)	283 (150–430)
Median current CD4$^+$ T-cell count, cells/mm^3 (IQR)	513 (388–667)
Median HIV RNA at time of TDF initiation, $\log_{10}$ copies/ml (IQR)	2.60 (1.7–4.7)
Current HIV RNA<50 copies/ml, *n* (%)	144 (91.72)
d4T exposure, *n* (%)	59 (37.3)
Current cART-based regimen	
Nevirapine	11 (7.0)
Efavirenz	89 (56.3)
PI/boosted PI	51 (32.3)
Median duration of ART, years (IQR)	8 (6-13)
ART>5 years, *n* (%)	131 (82.9)
Diabetes, *n* (%)	16 (10.1)
Hypertension, *n* (%)	70 (44.3)
APRI	
<0.5, *n* (%)	114 (74.03)
0.5–1.5, *n* (%)	37 (24.03)
>1.5, *n* (%)	3 (1.95)
Fib-4	
≤1.45, *n* (%)	121 (78.57)
>1.45–3.25, *n* (%)	28 (18.18)
>3.25, *n* (%)	5 (3.25)
Median FibroScan score, kPa (IQR)	4.9 (4.3–6.1)
FibroScan score	
>7.1–14 kPa, *n* (%)	13 (13.4)
>14 kPa, *n* (%)	2 (2.1)
Median baseline ALT, IU/l (IQR)	43 (28–63)
Median current ALT, IU/l (IQR)	41 (31–51)
Median baseline eGFR, ml/min/1.73 m^2 (IQR)	90.6 (77.5–104.3)
Median current eGFR, ml/min/1.73 m^2 (IQR)	96.9 (82.6–109)
Median vitamin D level, ng/ml (IQR)	22.8 (18–27.7)
Vitamin D status	
>30 ng/ml, *n* (%)	25 (15.82)
20–30 ng/ml, *n* (%)	81 (51.27)
10–20 ng/ml, *n* (%)	51 (32.28)
<10 ng/ml, *n* (%)	1 (0.63)

Total *n*=158. ALT, alanine aminotransferase; APRI, aspartate aminotransferase/platelet ratio index; ART, antiretroviral therapy; cART, combination antiretroviral therapy; d4T, stavudine; eGFR, estimated glomerular filtration rate; Fib-4, Fibrosis 4 score; IDUs, intravenous drug users; MSM, men having sex with men; PI, protease inhibitor; TDF, tenofovir disoproxil fumarate.

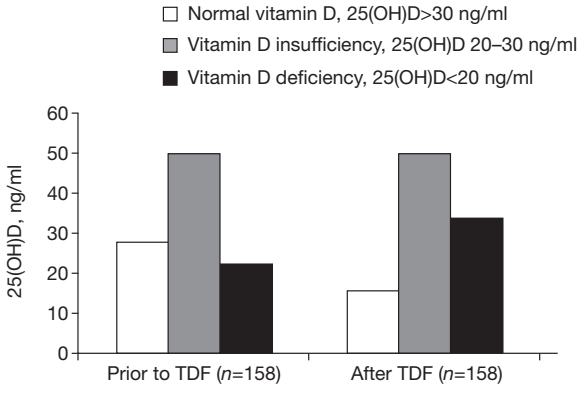
deficiency defined as 25[OH]D<20 ng/ml) prior to and following TDF are presented in Figure 1. The median serum 25(OH)D level prior to taking TDF was 24.8 ng/ml (IQR 21.3–30.6). The median serum 25(OH)D level after taking TDF for a median of 5 years was 22.8 ng/ml (IQR 18.0–27.7). The median change of serum 25(OH)D level was -3.1 ng/ml (IQR -7.2–2.4; *P*<0.001). After initiating TDF, the median (IQR) serum 25(OH)D levels changed each year by 0.04 ng/ml (IQR -0.4–0.5). The proportion of patients with hypovitaminosis D significantly increased from 72.2% (95% CI 64.7, 78.6) prior to taking TDF to 84.2% (95% CI 77.7, 89.0) after taking TDF (*P*=0.01). One-third (33.5%) of patients had vitamin D deficiency after taking TDF. The median serum 25(OH) D levels were 26.4 ng/ml (IQR 21.8–28.2) and 23.1 ng/ml (IQR 18.0–28.6) for cirrhotic and non-cirrhotic patients respectively (*P*=0.235). Only three patients switched their ART regimen after using TDF due to renal toxicity.

Renal function, proximal tubular dysfunction and hypophosphatemia

eGFR at TDF initiation was 90.6 ml/min/1.73 m^2 (IQR 77.5–104.9) and it was 90.4 ml/min/1.73 m^2 (IQR 78.1–101.9) at the last follow-up visit. There was no significant change of eGFR prior to and after long-term TDF therapy (*P*=0.726).

Of 119 (75.3%) HIV–HBV-coinfected patients with available serum phosphate, 8 (6.72%) had hypophosphatemia (PO4<2.5 mg/dl). The median phosphate

Figure 1. Summary of prevalence of vitamin D insufficiency and deficiency prior to and after TDF



TDF, tenofovir disoproxil fumarate; 25(OH)D, 25 hydroxyvitamin D.

was 3.5 mg/dl (IQR 3.1–3.8). Tubular function was assessed in 90 (57%) subjects, among whom 13 (14.4%) had proximal tubular dysfunction.

Identification of risk factors for hypovitaminosis D

Only female gender and duration of cART>5 years were significantly associated with hypovitaminosis D by univariate analysis (Table 2). In the multivariate analysis, female gender (adjusted OR 3.8 [95% CI 1.1, 13.7]; *P*=0.038); and duration of cART>5 years (adjusted OR 3.3 [95% CI 1.2, 8.8]; *P*=0.017) remained statistically significant.

Correlation of liver fibrosis, cirrhosis and serum 25(OH)D levels

Compared to those without cirrhosis, patients with cirrhosis were more often male (93% versus 62%), and more often had Fib-4>1.45 (43% versus 16%). Only Fib-4>1.45, APRI>0.5 and male gender were significantly associated with significant liver fibrosis by univariate analysis. However, in the multivariate analysis, only Fib-4>1.45 (OR 3.7 [95% CI 1.05, 13.1]; *P*=0.042) and male gender (OR 8.3 [95% CI 1.01, 67.4]; *P*=0.049), remained statistically significant. Vitamin D levels were not associated with significant liver fibrosis.

Discussion

In this study, we analysed serum 25(OH)D levels in HIV–HBV-coinfected patients prior to and after long-term cART, and evaluated predictors for hypovitaminosis D and the association of 25(OH)D and liver fibrosis/cirrhosis. The most striking findings of our data included: the high prevalence of hypovitaminosis D in HIV–HBV coinfection (84.2%, 95% CI 77.7, 89.0) compared with HIV–HCV coinfection (61.5%, 95% CI 52.3, 69.9) [36], HIV monoinfection (71.7%, 95% CI 64.9, 78.0) [37] and the general population (64.6%, 95% CI 62.8, 66.4) [38] from Bangkok, Thailand; the decline in serum 25(OH)D after a median of 5 years on TDF treatment and 8 years of cART; the lack of association between serum 25(OH)D levels and cirrhosis/liver fibrosis; the lack of association between serum 25(OH)D levels and efavirenz or TDF; and low prevalence of severe liver fibrosis/cirrhosis in our HIV–HBV-coinfected patients (5.9% had fibrosis score >9.5 kPa) compared to our HIV–HCV-coinfected patients (48.8%) [36] using the same FibroScan machine.

Although Thailand is a tropical country where sunshine is abundant all year, we found a high prevalence of hypovitaminosis D in HIV–HBV-coinfected patients. There are limited data regarding vitamin D levels in CHB. A recent study from New York, NY, USA, reported that 73% of HBV-monoinfected patients had hypovitaminosis D [39]. Vitamin D status in HIV–HBV-coinfected patients has not been systematically explored in either high latitude or tropical countries. Only one case of severe vitamin D deficiency in an HIV–HBV-coinfected patient treated with TDF has been reported so far [21].

It has been well established that certain types of seasons are associated with lower vitamin D levels [40]. However in this study, this was not the case. Seasonality was not associated with vitamin D levels. There were no differences in vitamin D levels during the dry (November–April) and the wet/rainy (May–October) seasons. This finding contradicts reports from Western countries where low levels of vitamin D were more commonly found during the winter season [41].

The weather in Thailand and nature of Thai people might in part explain these discordant results. Indeed, Thailand has only two seasons: the dry season (November–April) and wet season (May–October). Although the sun ray is extremely strong in the dry season compared to in the wet season, the majority of Thai people try to avoid sun exposure by using sun protection gears such as sunscreen and umbrellas. Aside from the behavioural changes and increased use of sun protection, increased urbanization and office-based work could contribute to hypovitaminosis D. Polluted air in Bangkok may have blocked the sun rays from reaching the skin resulting in lower production of vitamin D [42,43]. In addition, fortified food with vitamin D and food naturally rich with vitamin D are less affordable [38]. TDF-based cART may be associated with proximal tubular dysfunction, resulting in prolonged phosphate wasting and osteopenia/osteoporosis [44]. In this study, the serum levels of 25(OH)D noticeably declined after a median of 5 years of TDF treatment and 8 years of cART. However, it is possible that age could have contributed to the decreased levels of vitamin D. Other factors such as ART regimen and consumption of vitamin D/calcium were constant throughout follow-up. From the multivariate analysis, only female sex and taking cART for >5 years were independently associated with vitamin D levels. Females were 3.8× more likely than males to develop hypovitaminosis D. In addition, cART use of >5 years increased the risk of developing hypovitaminosis D by 3.3-fold.

When the use of TDF was further investigated, it was discovered that serum phosphate and tubular function were not correlated with vitamin D levels and TDF. Only 6.7% and 14.4% of our HIV–HBV-coinfected subjects had serum phosphate <2.5 mg/dl and PRTD, respectively.

Efavirenz-based cART has also been associated with low serum 25(OH)D levels [32,45,46]. It is thought that efavirenz induces the 24 hydroxylase enzyme,

Table 2. Logistic regression analysis of vitamin D insufficiency/deficiency and associated factors

Characteristic	Vitamin D group: Normal (25[OH]D >30 ng/ml; *n*=25)	Vitamin D group: Insufficient/deficient (25[OH]D≤30 ng/ml; *n*=133)	Unadjusted OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
Median present age, years (IQR)	38 (33–45)	43 (38–48)	–	–		
Age<50 years	22 (16.92)	108 (83.08)	Ref	0.419		
Age≥50 years	3 (10.71)	25 (89.29)	1.7 (0.5, 6.1)	–		
Median current age, years (IQR)	32 (28–37)	33 (29–39)	–	–		
Current age<33 years, *n* (%)	13 (16.67)	65 (83.33)	Ref	0.774		
Current age≥33 years, *n* (%)	12 (15.00)	68 (85.00)	1.1 (0.5, 2.7)	–		
Gender						
Male, *n* (%)	22 (20.56)	85 (79.44)	Ref	0.027	Ref	0.038
Female, *n* (%)	3 (5.88)	48 (94.12)	4.1 (1.2, 14.6)	–	3.8 (1.1, 13.7)	–
Body mass index						
<25 kg/m^2, *n* (%)	18 (14.52)	106 (85.48)	1.5 (0.5, 4.3)	0.413		
≥25 kg/m^2, *n* (%)	6 (20.69)	23 (79.31)	Ref	–		
EFV-containing regimen						
No, *n* (%)	11 (15.94)	58 (84.06)	Ref	0.971		
Yes, *n* (%)	14 (15.73)	75 (84.27)	1.0 (0.4, 2.4)	–		
Ritonavir-containing regimen						
No, *n* (%)	21 (16.03)	110 (83.97)	Ref	0.875		
Yes, *n* (%)	4 (14.81)	23 (85.19)	1.1 (0.3, 3.5)	–		
TDF-based ART						
No, *n* (%)	4 (21.05)	15 (78.95)	Ref	0.508		
Yes, *n* (%)	21 (15.11)	118 (84.89)	1.5 (0.5, 5.0)	–		
Duration of TDF						
≥5 years, *n* (%)	14 (15.56)	76 (84.44)	1.1 (0.4, 2.7)	0.905		
<5 years, *n* (%)	8 (14.81)	46 (85.19)	Ref	–		
Duration of ART						
<5 years, *n* (%)	9 (33.33)	18 (66.67)	Ref	0.009	Ref	0.017
≥5 years, *n* (%)	16 (12.21)	115 (87.79)	3.6 (1.4, 9.3)	–	3.3 (1.2, 8.8)	–
Advanced fibrosis[a]						
No, *n* (%)	13 (14.29)	78 (85.71)	Ref	0.231		
Yes, *n* (%)	2 (33.33)	4 (66.67)	0.3 (0.1, 2.0)	–		
eGFR						
<60 ml/min/1.73 m^2, *n* (%)	1 (12.50)	7 (87.50)	1.3 (0.2, 10.9)	0.823		
≥60 ml/min/1.73 m^2, *n* (%)	23 (15.44)	126 (84.56)	Ref	–		
HBV genotype						
Genotype C, *n* (%)	13 (15.48)	71 (84.52)	2.3 (0.7, 7.5)	0.179		
Non-genotype C, *n* (%)	5 (29.41)	12 (70.59)	Ref	–		
d4T exposure, *n* (%)	7 (11.86)	52 (88.14)	1.7 (0.6, 4.2)	0.296		
Season						
Dry, *n* (%)	13 (21.67)	47 (78.33)	Ref	0.120		
Rainy, *n* (%)	12 (12.24)	86 (87.76)	2.0 (0.8, 4.7)	–		
Proximal tubular dysfunction						
No, *n* (%)	11 (14.67)	64 (85.33)	1.1 (0.2, 5.4)	0.946		
Yes, *n* (%)	2 (15.38)	11 (84.62)	Ref	–		

[a]FibroScan>9.5 kPa. ART, antiretroviral therapy; d4T, stavudine; EFV, efavirenz; eGFR, estimated glomerular filtration rate; Ref, reference; TDF, tenofovir disoproxil fumarate.

which hydrolyzes 25(OH)D and 1,25(OH)2D to the inactive form, resulting in low serum 25(OH)D levels. However, only one-half of our patients were on efavirenz at the time of study and no correlation was detected between efavirenz and 25(OH)D levels. The lack of association between serum 25(OH)D levels and efavirenz or TDF was also unexpected, but may be due in part to the small sample size.

Chronic liver disease has been implicated in vitamin D deficiency. The liver plays an important role in vitamin D metabolism by converting vitamin D from the skin and diet into 25(OH)D [10]. There is evidence that the prevalence of vitamin D deficiency is high among patients with advanced liver disease [18,20]. However the findings in this study did not support this. We found that the prevalence of advanced fibrosis in our HIV–HBV-coinfected patients was low. The lower liver fibrosis is partly explained by early HBV-active cART treatment at an early phase of chronic hepatitis B disease. Thus, low serum 25(OH)D in our HIV–HBV-coinfected patients could not be explained by advanced liver disease.

Recently, it has been reported that patients with chronic HCV genotype 1 had low serum levels of 25(OH)D as well as severe liver fibrosis [19,20]. In addition, low vitamin D levels tended to be more common in patients with advanced stage fibrosis and cirrhosis [18,39]. However, in this study, only 2% of the HIV–HBV-coinfected patients had cirrhosis. It is possible that not all patients with cirrhosis in the study were detected because cirrhosis was defined as TE>14 kPa, Fib-4 index >3.25 and imaging of liver due to the lack of availability of liver biopsy. However, fibrosis score and Fib-4 score have been well validated and can differentiate between mild/moderate fibrosis and bridging fibrosis/cirrhosis in HIV–HCV-coinfected populations [34].

For this study, we also identified that low levels of vitamin D could exist in patients who have CHB, but have not yet developed serious liver damage. Thus, vitamin D should always be measured in HIV–HBV-coinfected patients. The high prevalence of hypovitaminosis D in our HIV–HBV-coinfected patients is of concern for both skeletal- and extra-skeletal-related vitamin D deficient conditions. This deficiency in the HIV–HBV-coinfected population can be serious, considering that many patients are treated with long-term TDF-containing ART that can also contribute to future osteopenia/osteoporosis. In addition, as vitamin D is a potent regulator of proliferation, differentiation and migration of fibroblasts and vascular smooth muscle cells, low vitamin D could be a signal for fibrinogenesis, resulting in more liver fibrosis and cirrhosis.

Intact parathyroid hormone levels (iPTH) stimulate the 1-α-hydroxylase enzyme which converts 25(OH)D to 1,25-dihydroxyvitamin D. Elevated iPTH has been found in obese patients which could contribute to low levels of 25(OH)D [10]. Vimaleswaran *et al.* [47] performed bi-directional Mendelian randomization analysis on 42,024 participants from 21 studies; they reported that each 10% increase in body mass index (BMI) will lead 25(OH)D concentrations to decrease by 4.2%. In our study, 26 (16.2%) patients and 4 (2.6%) patients had BMIs of 25–29.9 kg/m^2 and ≥30 kg/m^2, respectively. A total of 22 patients from the former group, and all 4 from the latter group had hypovitaminosis D. However, we could not find a significant association between BMI and hypovitaminosis D, which may havebeen due to the small sample size.

Our study is limited by its cross-sectional design. Factors such as dietary intake of vitamin D and individual exposure to sunlight could not be analysed. In addition, vitamin D levels in HBV- and HIV-monoinfected patients with and without TDF therapy were not available. Hence we cannot conclude whether the high prevalence of hypovitaminosis in the HIV–HBV-coinfected patients was a result of HBV, HIV or cART. In addition, parathyroid hormone, bone markers, bone mass density and liver histology were not available so we could not ascertain the long-term effect of hypovitaminosis D as well as the clinical significance of this finding. Since serum phosphate and tubular function assessment was available in only a subset of the study cohort, we could not confirm the link between PRTD, hypophosphatemia and vitamin D deficiency. Finally, the findings from this study may not be representative of Thai individuals from rural areas outside Bangkok.

In conclusion, although this cohort of HIV–HBV-coinfected patients lived in the tropics, there was a high prevalence of hypovitaminosis D, especially in female patients and with prolonged ART. Given patients with HIV–HBV-coinfection require long-term HBV-active ART including TDF, which can also contribute to bone loss, routine vitamin D assessment and supplementation as necessary should be considered. Furthermore, bone mass density of this population should be further investigated.

Acknowledgements

We would like to thank our patients, HIV-NAT staff members and Piraporn June Ohata.
This study (clinical trial number NCT01138241) was funded by the National Research Council of Thailand (NRCT) under grant number Por Kor/2554-136 (5 September 2011), the Thailand Research Fund (TRF) under grant number RSA5380002, BRG5580005, the Ratchadapisek Sompotch Endowment Fund (Faculty of Medicine, Chulalongkorn University) under grant number RA33/53 (25 June 2010), Research Unit of Hepatitis and Liver Cancer, Chulalongkorn University, and the Aligning Care and Prevention of HIV/AIDS with Government Decentralization to Achieve Coverage and Impact (ACHIEVED Project; The Global fund Project, Thailand).

SRL is a National Health and Medical Research Council (NHMRC) of Australia Practitioner Fellow.

Disclosure statement

RAR is supported by the ART AIDS Foundation. SRL is a recipient of an investigator initiated research grant funded by Gilead Sciences. She is also Chair of Australian Judging Panel for the Gilead Research Fellowships. All other authors declare no competing interests.

References

1. Gust ID. Epidemiology of hepatitis B infection in the Western Pacific and South East Asia. *Gut* 1996; **38 Suppl 2:**S18–S23.
2. Gilson RJ, Hawkins AE, Beecham MR, *et al*. Interactions between HIV and hepatitis B virus in homosexual men: effects on the natural history of infection. *AIDS* 1997; **11:**597–606.
3. Soto B, Sanchez-Quijano A, Rodrigo L, *et al*. Human immunodeficiency virus infection modifies the natural history of chronic parenterally-acquired hepatitis C with an unusually rapid progression to cirrhosis. *J Hepatol* 1997; **26:**1–5.
4. Konopnicki D, Mocroft A, de Wit S, *et al*. Hepatitis B and HIV: prevalence, AIDS progression, response to highly active antiretroviral therapy and increased mortality in the EuroSIDA cohort. *AIDS* 2005; **19:**593–601.
5. Chun HM, Fieberg AM, Hullsiek KH, *et al*. Epidemiology of hepatitis B virus infection in a US cohort of HIV-infected individuals during the past 20 years. *Clin Infect Dis* 2010; **50:**426–436.
6. Law WP, Duncombe CJ, Mahanontharit A, *et al*. Impact of viral hepatitis co-infection on response to antiretroviral therapy and HIV disease progression in the HIV-NAT cohort. *AIDS* 2004; **18:**1169–1177.
7. Sungkanuparph S, Vibhagool A, Manosuthi W, *et al*. Prevalence of hepatitis B virus and hepatitis C virus co-infection with human immunodeficiency virus in Thai patients: a tertiary-care-based study. *J Med Assoc Thai* 2004; **87:**1349–1354.
8. Sungkanuparph S, Wongprasit P, Manosuthi W, Atamasirikul K. Compliance with hepatitis B and hepatitis C virus infection screening among HIV-1 infected patients in a resource-limited setting. *Southeast Asian J Trop Med Public Health* 2008; **39:**863–866.
9. Kiertiburanakul S, Chotiprasitsakul D, Atamasirikul K, Sungkanuparph S. Late and low compliance with hepatitis B serology screening among HIV-infected patients in a resource-limited setting: an issue to improve HIV care. *Curr HIV Res* 2011; **9:**54–60.
10. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007; **357:**266–281.
11. Heaney RP. The Vitamin D requirement in health and disease. *J Steroid Biochem Mol Biol* 2005; **97:**13–19.
12. Mueller NJ, Fux CA, Ledergerber B, *et al*. High prevalence of severe vitamin D deficiency in combined antiretroviral therapy-naive and successfully treated Swiss HIV patients. *AIDS* 2010; **24:**1127–1134.
13. Van Den Bout-Van Den Beukel CJ, Fievez L, Michels M, *et al*. Vitamin D deficiency among HIV type 1-infected individuals in the Netherlands: effects of antiretroviral therapy. *AIDS Res Hum Retroviruses* 2008; **24:**1375–1382.
14. Wasserman P, Rubin DS. Highly prevalent vitamin D deficiency and insufficiency in an urban cohort of HIV-infected men under care. *AIDS Patient Care STDS* 2010; **24:**223–227.
15. Allavena C, Delpierre C, Cuzin L, *et al*. High frequency of vitamin D deficiency in HIV-infected patients: effects of HIV-related factors and antiretroviral drugs. *J Antimicrob Chemother* 2012; **67:**2222–2230.
16. Conrado T, Miranda-Filho Dde B, Ximenes RA, *et al*. Vitamin D deficiency in HIV-Infected women on antiretroviral therapy living in the tropics. *J Int Assoc Physicians AIDS Care (Chic)* 2011; **10:**239–245.
17. Avihingsanon ARR, Praditpornsilpa K, Wongsabut J, *et al*. Prevalence of and risk factors for hypovitaminosis D among HIV-infected adults living in the tropics. *19th Conference on Retroviruses and Opportunistic Infections*. 5–8 March 2012, Seattle, WA, USA. Abstract 882.
18. Arteh J, Narra S, Nair S. Prevalence of vitamin D deficiency in chronic liver disease. *Dig Dis Sci* 2010; **55:**2624–2628.
19. Petta S, Camma C, Scazzone C, *et al*. Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C. *Hepatology* 2010; **51:**1158–1167.
20. Terrier B, Carrat F, Geri G, *et al*. Low 25-OH vitamin D serum levels correlate with severe fibrosis in HIV–HCV co-infected patients with chronic hepatitis. *J Hepatol* 2011; **55:**756–761.
21. Kumar N, Bower M, Nelson M. Severe vitamin D deficiency in a patient treated for hepatitis B with tenofovir. *Int J STD AIDS* 2012; **23:**59–60.
22. Labarga P, Barreiro P, Martin-Carbonero L, *et al*. Kidney tubular abnormalities in the absence of impaired glomerular function in HIV patients treated with tenofovir. *AIDS* 2009; **23:**689–696.
23. Perrot S, Aslangul E, Szwebel T, Caillat-Vigneron N, Le Jeunne C. Bone pain due to fractures revealing osteomalacia related to tenofovir-induced proximal renal tubular dysfunction in a human immunodeficiency virus-infected patient. *J Clin Rheumatol* 2009; **15:**72–74.
24. Garlassi E, Zona S, Vescini F, *et al*. Tenofovir accelerates bone mass loss of the lumbar spine in the first years of menopause in HIV-infected women. *J Int AIDS Soc* 2012; **15 Suppl 4:**18317.
25. Haverkort ME, van der Spek BW, Lips P, *et al*. Tenofovir-induced Fanconi syndrome and osteomalacia in two HIV-infected patients: role of intracellular tenofovir diphosphate levels and review of the literature. *Scand J Infect Dis* 2011; **43:**821–826.
26. Woodward CL, Hall AM, Williams IG, *et al*. Tenofovir-associated renal and bone toxicity. *HIV Med* 2009; **10:**482–487.
27. Brim NM, Cu-Uvin S, Hu SL, O'Bell JW. Bone disease and pathologic fractures in a patient with tenofovir-induced Fanconi syndrome. *AIDS Read* 2007; **17:**322–328.
28. Piso RJ, Rothen M, Rothen JP, Stahl M. Markers of bone turnover are elevated in patients with antiretroviral treatment independent of the substance used. *J Acquir Immune Defic Syndr* 2011; **56:**320–324.
29. Bonjoch A, Figueras M, Estany C, *et al*. High prevalence of and progression to low bone mineral density in HIV-infected patients: a longitudinal cohort study. *AIDS* 2010; **24:**2827–2833.
30. George J, Ganesh HK, Acharya S, *et al*. Bone mineral density and disorders of mineral metabolism in chronic liver disease. *World J Gastroenterol* 2009; **15:**3516–3522.
31. Pasquet A, Viget N, Ajana F, *et al*. Vitamin D deficiency in HIV-infected patients: associated with non-nucleoside reverse transcriptase inhibitor or efavirenz use? *AIDS* 2011; **25:**873–874.
32. Welz T, Childs K, Ibrahim F, *et al*. Efavirenz is associated with severe vitamin D deficiency and increased alkaline phosphatase. *AIDS* 2010; **24:**1923–1928.
33. Wai CT, Greenson JK, Fontana RJ, *et al*. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38:**518–526.

34. Sterling RK, Lissen E, Clumeck N, *et al*. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; **43:**1317–1325.

35. Levey AS, Bosch JP, Lewis JB, *et al*. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Ann Intern Med* 1999; **130:**461–470.

36. Ramautarsing RA, Tangkijvanich P, Jirajariyavej S, *et al*. Predictors for the presence of liver fibrosis among HCV and HIV/HCV infected patients in Thailand. *20th Conference on Retroviruses and Opportunistic Infection*s. 3–6 March 2013, Atlanta, GA, USA. Abstract P-121.

37. Wiboonchutikul S, Sungkanuparph S, Kiertiburanakul S, *et al*. Vitamin D insufficiency and deficiency among HIV-1-infected patients in a tropical setting. *J Int Assoc Physicians AIDS Care (Chic)*; **11:**305–310.

38. Chailurkit LO, Aekplakorn W, Ongphiphadhanakul B. Regional variation and determinants of vitamin D status in sunshine-abundant Thailand. *BMC Public Health* 2011; **11:**853.

39. Tang SJ AR, Schulman A, Jacobson I. Prevalence of vitamin D deficiency in patients with chronic viral hepatitis. *Digestive Disease Week*. 7–11 May 2011, Chicago, IL, USA. Abstract P-121.

40. van der Mei IA, Ponsonby AL, Engelsen O, *et al*. The high prevalence of vitamin D insufficiency across Australian populations is only partly explained by season and latitude. *Environ Health Perspect* 2007; **115:**1132–1139.

41. Milazzo L, Mazzali C, Bestetti G, *et al*. Liver-related factors associated with low vitamin D levels in HIV and HIV/HCV coinfected patients and comparison to general population. *Curr HIV Res* 2011; **9:**186–193.

42. Hosseinpanah F, Pour SH, Heibatollahi M, *et al*. The effects of air pollution on vitamin D status in healthy women: a cross sectional study. *BMC Public Health* 2010; **10:**519.

43. Manicourt DH, Devogelaer JP. Urban tropospheric ozone increases the prevalence of vitamin D deficiency among Belgian postmenopausal women with outdoor activities during summer. *J Clin Endocrinol Metab* 2008; **93:**3893–3899.

44. Gara N, Zhao X, Collins MT, *et al*. Renal tubular dysfunction during long-term adefovir or tenofovir therapy in chronic hepatitis B. *Aliment Pharmacol Ther* 2012; **35:**1317–1325.

45. Welz T, Childs K, Post FA. Do nevirapine and efavirenz affect vitamin D homeostasis similarly? *AIDS* 2011; **25:**875–876.

46. Dao CN, Patel P, Overton ET, *et al*. Low vitamin D among HIV-infected adults: prevalence of and risk factors for low vitamin D Levels in a cohort of HIV-infected adults and comparison to prevalence among adults in the US general population. *Clin Infect Dis* 2011; **52:**396–405.

47. Vimaleswaran KS, Berry DJ, Lu C, *et al*. Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. *PLoS Med* 2013; **10:**e1001383.

Accepted 28 June 2013; published online 23 August 2013