Ann Med Res

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Annals of Medical Research

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Evaluation of bone mineral density in tenofovir disoproxil fumarate-treated patients with chronic hepatitis B

Burcu Ozdemir[a,*], Adalet Altunsoy[b], Imran Hasanoglu[c], Esragul Akinci[b], Rahmet Guner[c]

[a] *Ankara City Hospital, Department of Infectious Diseases and Clinical Microbiology, Ankara, Türkiye*
[b] *University of Health Sciences, Ankara City Hospital, Department of Infectious Diseases and Clinical Microbiology, Ankara, Türkiye*
[c] *Ankara Yıldırım Beyazıt University, Ankara City Hospital, Department of Infectious Diseases and Clinical Microbiology, Ankara, Türkiye*

ARTICLE INFO

Keywords:
Bone mineral density
Dual x-ray absorptiometry
Fracture risk assessment tool
Chronic hepatitis B
Tenofovir disoproxil fumarate

Received: May 25, 2023
Accepted: Sep 05, 2023
Available Online: 27.09.2023

DOI:
10.5455/annalsmedres.2023.05.119

Abstract

Aim: Evaluate the impact of Tenofovir Disoproxil Fumarate (TDF) treatment on bone mineral density (BMD) for patients with chronic hepatitis B (CHB) with Dual X-ray absorptiometry (DEXA) and the Fracture Risk Assessment Tool (FRAX) score.

Materials and Methods: A total of 38 CHB patients treated with TDF were included in this retrospective study. To estimate BMD, DEXA, demographic details, and laboratory values were examined in patients. The BMD measurements were compared after dividing the patients into 3 groups as normal BMD, osteopenic, and osteoporotic. FRAX scores (before and after DEXA) were calculated.

Results: Twenty-one of the 38 (55.3%) cases of CHB infection were male, the median age was 51 (min-max: 29-71) years, and seven patients (18.4%) were over 60 years of age. The median TDF duration was median 6 (min-max: 3-13) years. Osteopenia, defined by DEXA results, was found in 17 patients (44.7%) at the lumbar spine site, 11 (28.9%) at the femoral neck, and 10 (26.3%) at the total hip. Osteoporosis was detected in six patients (15.8%) at the lumbar spine site. Pre-DEXA FRAX score and post-DEXA FRAX score were calculated. Only one patient (1/33, 3%) had a score over the intervention threshold, by post-DEXA FRAX score. None of the patients classified as low risk based on the pre-DEXA FRAX score had a post-DEXA FRAX score above the intervention threshold. When the TDF duration, serum phosphorus level, vitamin D levels, and ALP levels were compared, no significant differences were found between the groups.

Conclusion: There are concerns about the negative effects of TDF used in CHB infection on BMD. However, BMD loss may not increase as the duration of TDF use increases. FRAX score is useful in identifying to risk of osteoporotic fracture. FRAX score can also eliminate the need for DEXA in most CHB cases. The FRAX score should be used in clinical practice to determine cases at risk.

Introduction

Chronic hepatitis B (CHB) infection is an important global health threat and a major cause of cirrhosis and hepatocellular carcinoma (HCC) worldwide [1]. Tenofovir disoproxil fumarate (TDF) has a high genetic barrier to resistance, which is suggested as a first-line treatment for chronic hepatitis B (CHB) in international guidelines [2-4]. The treatment is usually life-long due to low rates of Hepatitis B Surface Antigen (HBsAg) loss. Although generally considered safe and well tolerated [5,6], the major concerns of long-term TDF treatment are related to side effects and costs [7].

Clinical trials have found that TDF treatment in HIV-infected patients leads to bone disorders such as osteopenia, osteoporosis, and bone fractures [8-10]. Evaluation of TDF in randomized, controlled clinical trials reported that it decreased BMD in HIV-infected patients [5,11,12].Impaired kidney function and reduced bone mineral density (BMD) were reported in cases firstly with human immunodeficiency virus (HIV) and then in patients with HBV in long-term TDF treatment [7,13,14]. Data are limiting on the effect of TDF therapy on BMD in cohorts with chronic hepatitis B virus (HBV) infection.

The mechanisms of bone toxicity remain unclear; possible mechanisms include Intracellular accumulation of TDF causes proximal tubular dysfunction and Fanconi Syndrome and results in hypophosphatemic osteomalacia [15-17]. This study aimed to evaluate the impacts of TDF treatment on BMD with CHB, using Dual-Energy X-Ray

*Corresponding author:
Email address: burcubagci17@hotmail.com (Burcu Ozdemir)

Absorptiometry (DEXA). Additionally, we evaluated the advantage of the FRAX score as an alternative to DEXA.

Materials and Methods

In this retrospective single-center study, thirty-eight CHB cases (aged >18 years) who were treated on TDF (21 males/17 females) in Ankara City Hospital Infectious Diseases and Clinical Microbiology outpatient clinic between 1 March - 31 April 2022 were screened. A special form was created for cases with viral hepatitis B infection who were treated with tenofovir, containing information about patients at admission to collect the study data. The parameters included in this form were age, gender, BMI, smoking, and patients' laboratory results including ALT, AST, ALP, albumin, urea, creatinine, calcium, phosphorus, 25-hydroxy vitamin D, HBsAg, Anti-Hbe, and HBeAg levels were noted. Impaired kidney function was established on an eGFR<60 mL/min/1.73 m^2 [18,19]. All the laboratory records in patient files were completed from the hospital database.

Bone biochemistry was evaluated with laboratory values that could be used to predict BMD changes. Measurements for determined serum calcium level (normal range, 8.7-10.4 mg/dL), serum phosphate level (normal range, 2.4-5.1 mg/dL), and serum ALP level (normal range, 53-141 U/L) were noted. In addition, serum 25-hydroxyvitamin D (vitamin D) level (normal range, 75-150 nmol/L); vitamin D insufficiency is identified as 50-75 nmol/L, and vitamin D deficiency is identified as <50 nmol/L) was noted for its effect on BMD measurements.

Bone mineral density (BMD) measurements of the lumbar spine and hip were determined via dual-energy x-ray absorptiometry (DXA). T values between 1 and -1 were considered to be normal, while T values between -1 and -2.5 were considered to be osteopenic, and values of -2.5 and lower indicated osteoporosis as defined in World Health Organization guidelines for bone health [20].

FRAX score

An estimated 10-year major osteoporotic and hip fracture probability was calculated using an automatic data entry program from the FRAX web calculator [21]. All patients were accepted to have a secondary reason for osteoporosis due to chronic liver disease (i.e., chronic hepatitis B). The clinical FRAX scores were calculated using the Internetbased tool, initially without the DEXA-measured BMD (the pre-DEXA FRAX score). Patients were risk-categorized by the age-adjusted National Osteoporosis Guideline Group (NOGG) recommendations. This allows categorized into low, medium, or high risk for developing a major osteoporotic status or fracture over 10 years. FRAX scores were then recalculated with the BMD T score (the post-DEXA FRAX score). We used the NOGG recommendation with the post-DEXA FRAX score, which stratifies patients as low risk (ie, individuals who are not currently requiring treatment for osteoporosis) or high risk (ie, individuals for whom treatment for osteoporosis is recommended).

Pre-DEXA FRAX score and post-DEXA FRAX score were calculated because we aimed to evaluate the clinical utility of evaluating BMD with the FRAX score without DEXA.

Statistical analysis

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (Version 22, SPSS Inc., Chicago, IL, USA) package program. Descriptive statistics of categorical variables were reported as numbers and percentages (%). Descriptive statistics of continuous variables were reported as mean ± standard deviation or median ± interquartile range (minimum-maximum) according to data normality distribution. Relationships between categorical variables were evaluated using Fisher's exact test. Kruskal-Wallis tests were applied to compare the continuous variables. Two-tailed p-values of <0.05 were defined as statistically significant. The study protocol and procedures for informed consent were approved by the Ankara City Hospital Ethical Committee (approval number: E1-23-3383, date: 26/04/2023).

Results

Thirty-eight patients who were treated with TDF were included in the study, of whom 21 (55.3%) were male. The median age of patients was 51 (min-max: 29-71) years, and 18.4% were over 60 years of age. All patients received TDF treatment for a minimum of 3 years. The median duration of TDF treatment was found to be 6 years (min-max: 3-13 years). Hypocalcaemia was detected in 4.3% (1/23) cases and hypophosphatemia was detected in 4.3% (1/23) cases. Impaired kidney function was not detected. The related characteristics are given in Table 1.

Osteopenia was recorded in 17 patients (44.7%) at the lumbar spine site, 11 (28.9%) at the femoral neck, and 10 (26.3%) at the total hip. Osteoporosis was found in 6 patients (15.8%) at the lumbar spine site. Osteoporosis was not found at the femoral neck and the total hip site (Table 2). A total of 14 patients had osteopenia or osteoporosis at any site and 9 had osteopenia or osteoporosis at all 3 anatomical sites.

Complete FRAX scores were calculated for 33 patients. Pre-DEXA FRAX scores ranged between 2.8% and 17.0% (median 4.8%), and post-DEXA FRAX scores ranged between 2.4% and 11.0% (median 4.1%). Using the pre-DEXA FRAX score, cases may be categorized to be at low, intermediate, or high risk for major osteoporotic fracture, with age-dependent NOGG cutoffs. In this study 16 of 33 patients (48.5%) were classified as medium risk, and 51.5% were classified as low-risk. It was determined that these low-risk patients (51.5%) could be evaluated with the FRAX score without DEXA. There were no high-risk patients (Table 3). In this study, 1 of 33 patients (3%) had a score over the intervention threshold, by post-DEXA FRAX score. No patients who had low risk based on pre-DEXA FRAX scores had post-DEXA FRAX scores over the intervention threshold.

The BMD measurements were compared after dividing the patients into 3 groups as follows: normal BMD, osteopenic, and osteoporotic (Table 4). No significant difference was detected when the groups were compared in terms of age, BMI, gender, and TDF treatment duration. No significant

Table 1. Characteristics features of the 38 patients enrolled.

Characteristics	
Age (median,min-max)	51 (29-71)
Age, years, (n, %)	
18-30	2 (5.3)
31-40	5 (13.2)
41-50	12 (31.6)
50-60	12 (31.6)
>60	7 (18.4)
Sex, (n,%)	
Female	17 (44.7)
Male	21 (55.3)
TDF duration, years (median,min-max)	6 (3-13)
TDF duration, years (n,%)	
0-3 years	4 (13.8)
4-6 years	13(44.8)
>6 years	12(41.4)
BMI (median,min-max)	27(18.6-36.7)
BMI >25 kg/m2 (n,%)	26 (86.6)
Smoking (n,%)	9 (23.7)
Serum ALT level, U/L(median,min-max)	24 (10-125)
Serum AST level, U/L (median,min-max)	23.5 (15-102)
Serum ALP level, U/L (median,min-max)	103 (64-141)
Vitamin D level, nmol/L (mean,SD)	32.45±11.3
Vitamin D deficiency (<50 nmol/L) (n,%)	20 (86.95)
Vitamin D insufficiency (50-75 nmol/L) (n,%)	3 (13.05)
Serum albumin level, mg/dL (mean, SD)	45.05±4
Serum phosphorus level, mg/dL (mean, SD)	3.29±0.62
Serum calcium level, mg/dL (mean, SD)	9.5±0.5
Serum urea level, mg/dL (mean, SD)	32.9±8.3
Serum creatinine level, mg/dL (mean, SD)	0.86±0.16
eGFR level, mL/min (mean, SD)	90±17.2
Anti-Hbe, n (%)	
Positive	29 (76.3)
Negative	5 (13.2)

BMI: Body Mass Index; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; ALP: Alkaline Phosphatase. Body mass index (BMI) is calculated as the weight in kilograms divided by the height in square meters.

differences were found between the biochemical variables in serum phosphorus, serum ALP, serum calcium levels, and vitamin D levels (Table 4).

Discussion

Most patients require life-long treatment because of the risk of recurrence after the discontinuation of the antiviral treatments in CHB. For this reason, it is important to follow up on the side effects of long-term use of drugs [22,23]. TDF is a nucleotide analog reverse transcriptase inhibitor and is a part of HIV therapy. Clinical studies that evaluated the impact of TDF on BMD in cases with CHB are limited. Pathological bone fractures and a decrease in BMD (osteopenia, osteoporosis) were reported in previous studies that evaluated the impact of TDF on bone metabolism in HIV-infected cases [8,9,24-27].

The use of TDF therapy with CHB cases may be correlated with BMD changes, in previous studies on BMD loss in HIV-infected cases. In the study by Gill et al., cases with CHB infection TDF therapy were compared with healthy controls, and only a decrease in hip BMD score and an increase in fracture risk score were reported [28]. In a cohort report by Wong et al., the bone and renal side effects of nucleo(t)ide analogs were examined. It was reported in a 3-year follow-up that included 53.500 patients (46.454 non-treated and 7.046 treated) with a total of 53.500 CHB in-

Table 2. Comparison of the Normal, Osteopenia, and Osteoporosis Classification According to Bone Mineral Density Scores by Dual X-ray Absorptiometry (DEXA).

	Median (min/max)	Normal BMD n (%)	Osteopenia n (%)	Osteoporosis n (%)
L1 spine T score	0.785 (1.3/-2.5)	22 (57.9)	15 (39.5)	1 (2.6)
L2 spine T score	0.65 (2.4/-2.9)	20 (52.6)	13 (34.2)	5 (13.2)
L3 spine T score	0.6 (2.4/-2.5)	23 (60.5)	14 (36.8)	1 (2.6)
L4 spine T score	-1 (1.8/-2.9)	18 (47.4)	17 (44.7)	3 (7.9)
Total hip T score	-0.3 (1.6/-1.9)	28 (73.7)	10 (26.3)	0
Femoral neck T score	-0.7 (1.2/-2)	27 (71.1)	11 (28.9)	0

Table 3. The 10-year probability of fracture due to pre-DEXA FRAX and post-DEXA FRAX scores.

FRAX score	
Pre-DEXA FRAX score	
Major osteoporotic fracture (median,min-max)	4.8 (2.8-17)
Hip fracture (median,min-max)	0.6 (0.1-6.5)
Post-DEXA FRAX score	
Major osteoporotic fracture (median,min-max)	4.1 (2.4-11)
Hip fracture (median,min-max)	0.4 (0-1.4)
Pre-DEXA FRAX (n,%)	
Low risk	17 (51.5)
Medium risk	16 (48.5)
High risk	0
Post-DEXA FRAX (n,%)	
Low risk	32 (97)
High risk	1 (3)

FRAX: Fracture Risk Assessment Tool Score.

Table 4. Comparison of demographic, and laboratory results according to their BMD measurements as normal BMD, osteopenic and osteoporotic.

	Normal	Osteopenia	Osteoporosis	p values
	(n:15)	(n:17)	(n:6)	
Age	51.87±11.24	49.65±11.03	50.17±12.09	0.902
Age, years, (n, %)				
18-30	1 (6.7)	1 (5.9)	0 (0)	0.861
31-40	1 (6.7)	3 (17.6)	1(16.7)	
41-50	6 (40)	4 (23.5)	2 (33.3)	
51-60	3 (20)	7 (41.2)	2 (33.3)	
>60	4 (26.7)	2 (11.8)	1 (16.7)	
Sex, (n,%)				
Female	5 (33.3)	8 (47.1)	4 (66.7)	0.420
Male	10 (66.7)	9 (52.9)	2 (33.3)	
TDF duration, years	6.73±3.69	6.69±2.69	5.83±1.83	0.872
BMI	27.29±3.64 1	26.45±4.36	27.75±5.98	0.621
BMI > 25 kg/m^2 (n,%)	2 (80)	10 (58.8)	4 (66.7)	0.404
Vitamin D deficiency (<50 nmol/L) (n,%)	5 (83.3)	10 (83.3)	5 (100)	1.000
Vitamin D insufficiency (50-75 nmol/L) (n,%)	1 (16.7)	2 (16.7)	0 (0)	
Serum ALT level, U/L	31.13±24.73	30.71±27.11	31±20.69	0.732
Serum AST level, U/L	26.13±12.89	26±9.44	35.33±32.8	0.985
Serum ALP level, U/L	105.29±19.39	98.67±25.12	118±19.18	0.347
25 OH vitamin D level, nmol/L	30.5±12.58	34.08±12.4	33.8±9.47	0.722
Vitamin D deficiency (<50 nmol/L) (n,%)	5 (83.3)	10 (83.3)	5 (100)	1.000
Vitamin D insufficiency (50-75 nmol/L) (n,%)	1 (16.7)	2 (16.7)	0 (0)	
Serum albumin level, mg/dL	46.22±2.28	45.75±3	41±7.81	0.454
Serum phosphorus level, mg/dL	2.97±0.52	3.35±0.67	3.42±0.48	0.422
Serum calcium level, mg/dL	9.38±0.53	9.68±0.44	9.33±0.56	0.440
Serum urea level, mg/dL	35.31±8.08	32.27±8.53	28.6±7.5	0.269
Serum creatinine level, mg/dL	0.91±0.2	0.83±0.14	0.75±0.08	0.145
eGFR level, mL/min	85.14±15.92	92.65±17.15	96.5±14.15	0.262
Anti-Hbe, n (%)				
Positive	10 (76.9)	14 (87.5)	5 (100)	0.545
Negative	3 (23.1)	2 (12.5)	0 (0)	

BMI: Body Mass Index; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; ALP: Alkaline Phosphatase. Body mass index (BMI) is calculated as the weight in kilograms divided by the height in square meters.

fections that exposure to nucleotide analogs increased the risk of hip fractures when compared to nucleoside analogs [29]. It was reported in another study that there was an increase in hip osteopenia and major FRAX Score and a significant decrease in L3 lumbar spine BMD in the TDF group when the subjects who received entecavir and TDF were compared. No significant changes were detected in BMD and major FRAX scores in the entecavir group [30]. Seto et al. showed that the marker of bone resorption was significantly higher in TDF patients than in the tenofovir alafenamide group in 96 weeks of follow-up [31]. Osteopenia was most common in the lumbar spine region in the DEXA results of our study (17/38, 44.7%), followed by the hip (11/38, 28.9%). Osteoporosis was detected only in the lumbar spine area (6/38, 15.8%) and no osteoporosis was detected in the hip site and femoral neck. Unfortunately, the BMD changes could not be reported because of a lack of baseline BMD.

The data on when changes in BMD begin are limited because most studies did not report serial BMD measurements. A 96-week prospective study that evaluated the changes in BMD in patients with CHB infection by using serial DEXA scans showed a plateau for hip BMD loss at week 72 [32]. Cassetti et al. reported that osteopenic changes were shown in the hip and lumbar spine during the first 48 weeks, but did not progress in the 288-week follow-up [33]. In another study, annual BMD evaluations of CHB cases treated with TDF were investigated

between the fourth and seventh years of the treatment. In this study, no statistically significant osteopenia and osteoporosis were detected [34]. In our study, when the cases with and without BMD loss (osteopenia, osteoporosis) were compared in terms of the duration of TDF use, no significant differences were detected. It was concluded that the BMD loss may not increase as the duration of TDF use increased. When evaluated together with previous studies, it supports that the treatment-related decrease in BMD occurs right after the start of the treatment, but does not continue in the future.

Our study was compared after dividing the patients into 3 groups as follows: normal BMD, osteopenic and osteoporotic. No significant differences were found between the biochemical variables in serum phosphorus, serum ALP, serum calcium levels, vitamin D levels. These results did not support the use of serum phosphorus, serum ALP, and serum calcium levels as an indicator for BMD loss. Vitamin D deficiency is another significant factor in the loss of BMD [35]. However, this study detected that vitamin D deficiency was not significantly correlated with decreased BMD.

The risk of osteoporotic fracture is also associated with many other factors, such as age, sex, weight, height, alcohol use, and smoking. The 10-year fracture risk can be evaluated with the FRAX score based on basic clinical factors with the FRAX score and can be a guide in terms of treatment management in clinical practice [21]. Previous studies reporting that the FRAX score was beneficial in the evaluation of fracture risk in HIV and CHB-infected patients receiving TDF treatment were reported [28,36]. Classical risk factors are important for the development of osteoporotic fractures, and cases with CHB who receive TDF or those who are scheduled to initiate TDF must be evaluated in this regard. In the present study, the FRAX score was compared before and after DEXA. When the FRAX score before and after DEXA was calculated, we defined that none of the cases that had a low-risk pre-DEXA FRAX score fell under the high-risk classification which osteoporosis therapy would be needed. Only one case that had intermediate risk according to the FRAX score before DEXA was identified as a high-risk patient according to the FRAX score after DEXA. These findings underscore the clinical utility of evaluating BMD with the FRAX score without DEXA. Also, according to the pre-DEXA FRAX score, 51.5% of the patients in this study were found in the low-risk group in terms of major osteoporotic and hip fractures. This ultimately eliminated the need for DEXA and the cost associated with DEXA in low-risk patients.

Conclusion

In the present study, similar to the previous studies, TDF-treated patients with CHB increased concerns about the negative effects of BMD. The present study that the FRAX score is an inexpensive and effective tool in determining the risk of osteoporotic fractures in TDF-treated patients with CHB, and might eliminate the need for DEXA in most cases. The FRAX score should be detected in clinical practice to identify cases that have risks. This study's limitations were the small number of patients and their unknown baseline DEXA results. Another limitation is that there was no comparison with the control group which does not use TDF. Further studies with large multi-centers are needed for TDF to have a direct significant effect on BMD loss.

Ethical approval

The study protocol and procedures for informed consent were approved by the Ankara City Hospital Ethical Committee (approval number: E1-23-3383, date: 26/04/2023).

References

1. World Health Organization. Guidelines on hepatitis B and C testing, 2017. https://www.who.int/publications/i/item/9789241549981. Accessed August 28, 2023.
2. European Association for the Study of the Liver Electronic address: easloffice @easloffice.eu, European association for the study of the liver. EASL 2017 clinical practice guidelines on the management of hepatitis B virus infection. J Hepatol 2017;67(2):370–98. doi: 10.1016/j.jhep.2017.03.021.
3. Sarin SK, Kumar M, Lau GK, et al. Asian-pacific clinical practice guidelines on the management of hepatitis B: A 2015 update, 10. India: Springer; 2016. doi: 10.1007/s12072- 015- 9675- 4.
4. Terrault NA, Lok ASF, Mcmahon BJ, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance american association for the study of liver diseases. Hepatology 2018;67(4):1560–99. doi: 10.1002/hep.29800.
5. Gallant JE, Staszewski S, Pozniak AL, et al. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naïve patients: a 3-year randomized trial. JAMA 2004; 292:191–201.
6. Gallant JE, DeJesus E, Arribas JR, et al. Tenofovir DF, emtricitabine, and efavirenz vs. zidovudine, lamivudine, and efavirenz for HIV. N Engl J Med 2006; 354:251–60.
7. Hamzah L, Samarawickrama A, Campbell L, et al. Effects of renal tubular dysfunction on bone in tenofovir-exposed HIV-positive patients. AIDS. 2015;29(14):1785-1792.
8. Gafni RI, Hazra R, Reynolds JC, et al. Tenofovir disoproxil fumarate and an optimized background regimen of antiretroviral agents as salvage therapy: impact on bone mineral density in HIV-infected children. Pediatrics. 2006;118(3):e711-e718.
9. Purdy JB, Gafni RI, Reynolds JC, Zeichner S, Hazra R. Decreased bone mineral density with off-label use of tenofovir in children and adolescents infected with human immunodeficiency virus. J Pediatr. 2008;152(4):582-584.
10. Mirani G, Williams PL, Chernoff M, et al. Changing trends in complications and mortality rates among US youth and young adults with HIV infection in the era of combination antiretroviral therapy. Clin Infect Dis. 2015;61(12):1850-1861.
11. Martin A, Bloch M, Amin J, et al. Simplification of antiretroviral therapy with tenofovir-emtricitabine or abacavir-Lamivudine: a randomized, 96-week trial. Clin Infect Dis 2009; 49:1591–601.
12. Stellbrink HJ, Orkin C, Arribas JR, et al. Comparison of changes in bone density and turnover with abacavir-lamivudine versus the tenofovir emtricitabine in HIV-infected adults: 48 week results from the ASSERT study. Clin Infect Dis 2010; 51:963–72.
13. Buti M, Riveiro-Barciela M, Esteban R. Long-term safety and efficacy of nucleo(t)side ana-logue therapy in hepatitis B. Liver Int 2018;38:84–9. doi: 10.1111/liv.13641.
14. Cooper RD, Wiebe N, Smith N, et al. Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV infected patients. Clin Infect Dis 2010.doi:10.1086/655681.
15. Rodríguez-Nóvoa S, Labarga P, Soriano V, et al. Predictors of kidney tubular dysfunction in HIV-infected patients treated with tenofovir:a pharmacogenetic study. Clin Infect Dis. 2009;48(11):e108-e116.
16. Lucey JM, Hsu P, Ziegler JB. Tenofovir-related Fanconi's syndrome and osteomalacia in a teenager with HIV. BMJ Case Rep. 2013;2013.
17. Hamzah L, Samarawickrama A, Campbell L, et al. Effects of renal tubular dysfunction on bone in tenofovir-exposed HIV-positive patients. AIDS. 2015;29(14):1785-1792.

18. Stanaway JD, Flaxman AD, Naghavi M, et al. The global burden of viral hepatitis from 1990 to 2013: findings from the Global Burden of Disease Study 2013. Lancet. 2016 Sep 10;388(10049):1081-1088. doi: 10.1016/S0140-6736(16)30579-7.
19. Levey AS, Eckardt KU, Tsukamoto Y, et al. Definition and classification of chronic kidney disease: a position statement from kidney disease: improving global outcomes (KDIGO)z. Kidney Int 2005;67(6):2089–100. doi: 10.1111/j.1523-1755.20 05.0 0365.x.
20. Bonjour JP, Ammann P, Rizzoli R. Importance of preclinical studies in the development of drugs for treatment of osteoporosis: a review related to the 1998 WHO guidelines. Osteoporos Int. 1999;9(5):379-393.
21. Kanis JA, Johnell O, Oden A, et al. FRAX and the assessment of fracture probability in men and women from the UK. Osteoporos Int. 2008;19(4):385-397.
22. Reijnders JG, Perquim ML, Zhang N, et al. Nucleos(t)ide analogues only induce temporary hepatitis B e-antigen seroconversion in most patients with chronic hepatitis B. Gastroenterology 2010; 139:491–8.
23. Zoutendijk R, Hansen BE, van Vuuren AJ, et al. Serum HBsAg decline during long-term potent nucleos(t)ide analogue therapy for chronic hepatitis B and prediction of HBsAg loss. J Infect Dis 2011; 204:415–8.
24. 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(6):322-8,C3.
25. Stellbrink HJ, Orkin C, Arribas JR, et al. Comparison of changes in bone density and turnover with abacavir-lamivudine versus tenofovir-emtricitabine in HIV-infected adults: 48-week results from the ASSERT study. Clin Infect Dis. 2010;51(8):963-972.
26. Bernardino JI, Mocroft A, Mallon PW, et al. Bone mineral density and inflammatory and bone biomarkers after Darunavir-ritonavir combined with either raltegravir or tenofovir-emtricitabine in antiretroviral-naive adults with HIV-1: a substudy of the NEAT001/ ANRS143 randomised trial. Lancet HIV. 2015;2(11):e464-e473.
27. Mirani G, Williams PL, Chernoff M, et al. Changing trends in complications and mortality rates among US youth and young adults with HIV infection in the era of combination antiretroviral therapy. Clin Infect Dis. 2015;61(12):1850-1861.
28. Gill US, Zissimopoulos A, Al-Shamma S, et al. Assessment of bone mineral density in tenofovir-treated patients with chronic hepatitis B: can the fracture risk assessment tool identify those at greatest risk? J Infect Dis. 2015 ;211(3):374-382.
29. Wong GL, Tse YK, Wong VW, et al. Long-term safety of oral nucleos(t)ide analogs for patients with chronic hepatitis B: a cohort study of 53,500 subjects. Hepatology. 2015;62(3):684-693.
30. Kahraman R, Şahin A, Öztürk O, et al. Effects of Long-Term Tenofovir and Entecavir Treatment on Bone Mineral Density in Patients with Chronic Hepatitis B. Turk J Gastroenterol. 2022 Jan;33(1):35-43. doi: 10.5152/tjg.2020.18024. PMID: 35040786; PMCID: PMC9128468.
31. Seto WK, Asahina Y, Brown TT, et al. Improved bone safety of tenofovir alafenamide compared to tenofovir disoproxil fumarate over 2 years in patients with chronic HBV infection. Clin Gastroenterol Hepatol. 2018. [Online ahead of print].
32. Fung S, Kwan P, Fabri M, et al. Randomized comparison of Tenofovir Disoproxil Fumarate vs. Emtricitabine and Tenofovir Disoproxil Fumarate in patients with Lamivudine-resistance Chronic Hepatitis B. Gastroenterology 2014; 146:980–8.
33. Cassetti I, Madruga JV, Suleiman JM, et al. The safety and efficacy of tenofovir DF in combination with lamivudine and efavirenz through 6 years in antiretroviral-naive HIV-1-infected patients. HIV Clin Trials. 2007;8(3):164-172.
34. Buti M, Tsai N, Petersen J, et al. Seven-year efficacy and safety of treatment with tenofovir disoproxil fumarate for chronic hepatitis B virus infection. Dig Dis Sci. 2015;60(5):1457-1464.
35. Christodoulou S, Goula T, Ververidis A, Drosos G. Vitamin D and bone disease. Biomed Res Int 2013; 2013:396541.
36. Calmy A, Fux CA, Norris R, et al. Low bone mineral density, renal dysfunction, and fracture risk in HIV infection: a cross-sectional study. J Infect Dis 2009; 200:1746–54.