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Bone and kidney toxicity induced by nucleotide analogues in patients affected by HBV-related chronic hepatitis: a longitudinal study

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Objectives: Nucleotide analogues may promote renal and bone toxicity. The aim of the present study was to evaluate markers of osteorenal toxicity in patients affected by hepatitis B virus-related chronic hepatitis treated with lamivudine plus adefovir who were switched to tenofovir.

Patients and methods: We evaluated 60 consecutive patients at the time of the switch of treatment and after 1, 3, 6, 9 and 12 months. The mean baseline estimated glomerular filtration rate (eGFR) was 89.3 ± 19.0 mL/min/1.73 m^2.

Results: During the study period we observed a reduction in mean eGFR up to 6 months after switching to tenofovir, and this remained stable for the last two timepoints. At the end of study, the mean eGFR was 82.6 ± 21.5 mL/min/1.73 m^2, a reduction of 7.5%. The mean baseline proteinuria was 202.6 ± 237.6 mg/24 h. Microhaematuria was observed in 22.6% of patients and hypophosphataemia in 18.6%. After 1 month of tenofovir, we observed a worsening of serum phosphate and parathyroid hormone levels, haemoglobinuria and 24 h proteinuria. After 3 and 12 months of tenofovir, these data tended to recover to baseline levels. A total of 92.6% of patients at baseline had hypovitaminosis D. After supplementation with cholecalciferol, this percentage decreased significantly. We observed a reduced bone mineral density (BMD) in 52.7% of patients at baseline; this increased to 77.8% after 6 months of tenofovir, but at the last timepoint the percentage of patients with a reduced BMD had fallen to a level above the baseline.

Conclusions: In conclusion, patients exposed to lamivudine plus adefovir showed relevant osteorenal damage. The switch to tenofovir provoked a slight reduction in eGFR that stabilized after 6 months. The reduced BMD at baseline did not worsen under tenofovir treatment.

Keywords: hepatitis B, tenofovir, adefovir, bone toxicity

Introduction

Purine nucleotide analogues are reverse transcriptase inhibitors used in the treatment of HIV and hepatitis B virus (HBV) infections. Adefovir is phosphorylated in the host cells and converted to its active intracellular diphosphate metabolite, which inhibits HBV viral polymerases, acting as a chain terminator of viral DNA synthesis. Adefovir is primarily eliminated by the kidney via both glomerular filtration and tubular secretion. Nephrotoxicity is a major concern and is dose related: an impairment in parameters of renal function is observed at higher doses and occurs after 6–8 months of treatment.[1]

Tenofovir disoproxil fumarate, a pro-drug, is hydrolysed to tenofovir and subsequently phosphorylated to tenofovir diphosphate (the active metabolite) by cellular kinases. Tenofovir is excreted by the kidney via a combination of glomerular filtration and active tubular secretion. The major mechanism of renal excretion involves the uptake of tenofovir on the basolateral side of the proximal tubular cell via human organic anion transporter-1; other protein carriers, namely multidrug resistance protein-2 and multidrug resistance protein-4, are responsible for the cellular extrusion of tenofovir into the urinary space at the apical level.[2,3] In the selected populations enrolled in clinical trials with rigorous monitoring of renal function, tenofovir appears to be associated with a low risk of nephrotoxicity,

and this adverse event occurs mostly in patients with predisposing kidney disease or comorbidities.[4–7] However, several case reports and various observational studies have found evidence of potential renal toxicity induced by tenofovir treatment, mostly producing proximal tubulopathy with Fanconi's syndrome and nephrogenic diabetes insipidus. Tenofovir is the antiretroviral agent most commonly associated with Fanconi's syndrome, which can potentially lead to calcium and phosphorus dysregulation, acute renal failure, osteomalacia and fractures. Aside from this overt modality of renal toxicity, analyses of large datasets from clinical trials of tenofovir have shown that a mild decrease in estimated glomerular filtration rate (eGFR) may sporadically occur.[2,8–10] Although it is well known that the use of nucleotide analogues in HIV-positive patients may promote renal tubular toxicity and bone damage, few data are available on kidney and bone toxicity among HBV-infected patients. In two trials, tenofovir remained safe and effective over a 7 year treatment period, with a low rate of renal events.[11] In addition, in a 4 year prospective study in adefovir-experienced patients with chronic hepatitis B, high rates of tenofovir dose adjustment were required.[12]

The aim of the present longitudinal study was to evaluate markers of kidney and bone toxicity in a group of patients affected by HBV-related chronic hepatitis who had been treated with adefovir-based therapy and were subsequently switched to tenofovir therapy.

Patients and methods

From November 2010 to April 2011, we evaluated 60 consecutive patients in our outpatient facility who were undergoing antiviral treatment for hepatitis B with two nucleotide analogues (adefovir and tenofovir) in two sequential phases. More specifically, the patients had been initially treated with lamivudine monotherapy, and adefovir was now being given with the lamivudine. To reduce the potential occurrence of stable bone or renal adverse effects, all the patients undergoing treatment with lamivudine plus adefovir were switched to a tenofovir-only regimen. The patients were evaluated at the time of switching from lamivudine plus adefovir to tenofovir (T0) and subsequently after 1 month (T1), 3 months (T2), 6 months (T3), 9 months (T4) and 12 months (T5). Dual-energy X-ray absorptiometry (DEXA) of the spine and both femurs was performed at T0, T3 and T5.

The objective of the study was to evaluate the impact on the kidney and bones of the two sequential treatments with nucleotide analogues. Standard doses of lamivudine (100 mg/day), adefovir (10 mg/day) and tenofovir (245 mg/day) were administered. Renal function was assessed by calculating the eGFR using the chronic kidney disease epidemiology collaboration (CKD-EPI) formula[13] at the time of the switch from lamivudine plus adefovir to tenofovir. Patients were stratified by eGRF into categories of eGFR >90 mL/min/1.73 m^2, 60–89 mL/min/1.73 m^2 and <60 mL/min/1.73 m^2, representing a normal GFR, CKD stage II and CKD stage III, respectively (National Kidney Foundation Kidney Disease Outcomes Quality Initiative). Serum phosphate, quantitative proteinuria on a 24 h collected urine sample, haematuria at urinalysis, serum 25(OH)-vitamin D levels and serum parathyroid hormone levels were determined in the hospital's laboratory. Normal values of phosphataemia were considered to be >2.5 mg/dL, normal values for proteinuria did not exceed 200 mg/24 h, while serum parathyroid hormone levels were considered to be within the normal range if they did not exceed 65 pg/mL. Normal (sufficient) vitamin D levels were taken to be >30 ng/mL, while insufficiency was defined as serum 25(OH)-vitamin D levels >10 to <30 ng/mL. Levels <10 ng/mL defined vitamin D deficiency.

Bone mineral density (BMD) was assessed by DEXA scanning of the spine and both femurs using a Hologic system. *T*-scores below −1.0 were considered to be indicators of reduced BMD (osteopenia or osteoporosis).

Table 1. Summary of patients' general and clinical characteristics

Total, *n* (%)	60 (100)
Male, *n* (%)	48 (80)
Female, *n* (%)	12 (20)
Age (years), mean ± SD	57.5 ± 11.4
BMI (kg/m^2), mean ± SD	25.9 ± 3.6
Overweight patients (BMI >25 kg/m^2 to <30 kg/m^2)	30 (50)
Obese patients (BMI >30 kg/m^2)	5 (8.3)
Hepatitis C virus co-infection, *n* (%)	1 (1.6)
HDV co-infection, *n* (%)	1 (1.6)
HBeAg positive, *n* (%)	0 (0)
HBV DNA (>2000 copies/mL), *n* (%)	0 (0)
Liver cirrhosis, *n* (%)	19 (31.7)
Oesophageal varices, *n* (%)	9 (15)
Hepatocellular carcinoma, *n* (%)	7 (11.7)
HTN, *n* (%)	7 (11.7)
DM, *n* (%)	2 (3.2)
Hypothyroidism, *n* (%)	1 (1.6)
Glomerulonephritis, *n* (%)	1 (1.6)

DM, diabetes mellitus; HBeAg, hepatitis B envelope antigen; HDV, hepatitis D virus; HTN, hypertension.

Statistical analysis

Variations in continuous variables between the different timepoints were analysed by paired *t*-test for each timepoint versus T0. Categorical variables were compared using the χ^2 test. The evolution of CKD from stage I to stage II or from stage II to stage III at timepoints T1 and T5 versus baseline was analysed by the Bowker test.

Results

A total of 60 patients receiving treatment with lamivudine plus adefovir and then switched to tenofovir were enrolled. All of them had initially been treated with lamivudine monotherapy for a mean time of 43 ± 26.8 months; they subsequently underwent add-on treatment with adefovir, and the combination of lamivudine plus adefovir was continued for a mean of 114 ± 27.1 months.

The general and clinical characteristics of the cohort studied are shown in Table 1.

The mean baseline eGFR was 89.3 ± 19.0 mL/min/1.73 m^2. The prevalence of patients with an eGFR <90 mL/min/1.73 m^2 was 40%, and only 6.7% had an eGFR <60 mL/min/1.73 m^2 (Table 2). The mean eGFR tended to decline during the study period, with a more marked reduction after 6 months on tenofovir (T3 or T4 versus T0, $P=0.003$). However, after this timepoint, eGFR values stabilized and did not decline further at 9 and 12 months (T5 versus T0, $P=0.001$) (Figure 1). At the end of study, the mean eGFR was 82.6 ± 21.5 mL/min/1.73 m^2, which represented on average a 7.5% reduction with respect to baseline values. At this timepoint, the percentage of patients with a reduced eGFR (<90 mL/min/1.73 m^2) had increased to 63%, and 13% belonged to CKD stage III. At T1, 14.7% of patients with a baseline eGFR >90 mL/min/1.73 m^2 had evolved to CKD stage II, while 5.0% of those who had a reduced eGFR at baseline and were CKD stage II evolved to stage III. Moreover, at T5, 37.1% of patients who had an eGFR >90 mL/min/1.73 m^2 showed a

reduction in eGFR typical of CKD stage II. Finally, 15.0% of patients with CKD stage II at baseline evolved to CKD stage III at the final timepoint (Table 3) ($P<0.05$ using the Bowker test).

One patient categorized as CKD stage II was taking adefovir, and subsequently tenofovir, on alternate days. Another patient who had received 6 months of tenofovir at full dose was then treated on alternate days because of a decrease in eGFR to <50 mL/min.

No premature discontinuations were registered.

The mean value for proteinuria at baseline was 202.6 ± 237.6 mg/24 h, slightly above the cut-off value for normal proteinuria (200 mg/24 h). A total of 26.9% of patients had a urinary excretion of protein above the normal range; in the majority of these patients, the proteinuria was mild (<1000 mg/24 h), and only one patient had moderate proteinuria (>1000 to <3000 mg/24 h) at baseline. During the study period, the mean value for proteinuria did not vary significantly, with the average being close to the cut-off for normal proteinuria. At T5, the mean proteinuria was 194.1 ± 168.4 mg/24 h and 32% of patients showed values higher than the normal cut-off (Table 4).

Microhaematuria at baseline was observed in 22.6% of patients. During the study period, the prevalence of proteinuria was 23.6%, 11.9%, 13.0%, 17.5% and 19.0% at T1, T2, T3, T4 and T5, respectively ($P=0.432$) (Table 4).

In terms of phosphate homeostasis, the patients enrolled in the study showed mean serum phosphate levels in the normal range at baseline (2.9 ± 0.5 mg/dL), with 18.6% of patients presenting phosphate levels that were below the normal cut-off (<2.5 mg/dL). The mean serum phosphate values did not vary during the study period, and even at the end of the study we recorded an average serum phosphate concentration of 3.0 ± 0.5 mg/dL. At T5, the percentage of hypophosphataemic patients declined to 14.8%.

In relation to 25(OH)-vitamin D plasma levels, only 7.4% (5) of patients at T0 showed sufficient 25(OH)-vitamin D plasma levels, while 72.2% (43) and 20.4% (12) showed insufficient (<30 ng/mL) and deficient (<10 ng/mL) levels, respectively. Patients with hypovitaminosis D received oral cholecalciferol supplementation. Of the remainder, one developed vitamin insufficiency at T1 and one at T2, and were consequently supplemented. The mean values of plasma 25(OH)-vitamin D increased significantly during the study period ($\chi^2=28.063$, $P=0.002$), even though they did not on average reach the cut-off for normal values of 30 ng/mL (Figure 2). At T5, the percentage of patients with normal plasma vitamin D levels had increased to 30.4%, while 65.9% and 8.6%, showed insufficiency and deficiency, respectively. The percentage of patients with sufficient or deficient vitamin D levels decreased significantly, while the number of patients with normal levels increased significantly, at T3 and T5 compared with T0 ($P<0.004$ for both) (Figure 2).

The mean plasma concentration of parathyroid hormone fluctuated during the study period. Increased parathyroid hormone levels (>65 ng/mL) were present in 29.6%, 50.0%, 50.0%,

Table 2. Percentage of CKD stage II and III in patients treated with tenofovir at different timepoints

	T0	T1	T2	T3	T4	T5
Normal eGFR (%)	60.0	56.2	50.0	38.7	51.6	37.0
CKD II (%)	33.3	38.6	41.3	48.4	45.2	50.0
CKD III (%)	6.7	5.2	8.7	12.9	3.2	13.0
P versus T0		0.799	0.798	0.089	0.531	0.027

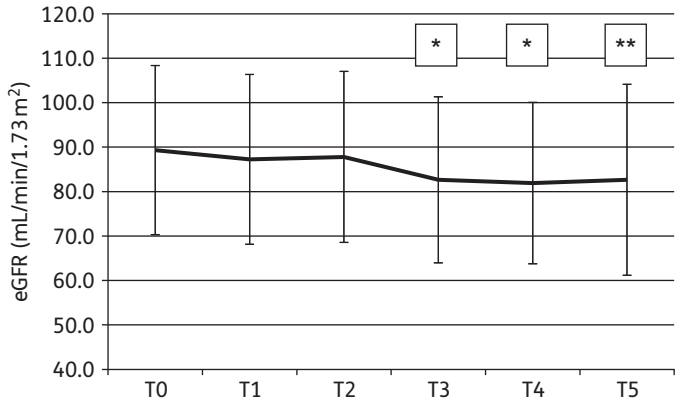

Figure 1. Profile of eGFR variations during the study period. Data represent mean ± SD. A comparison of the mean values at each timepoint versus T0, by paired *t*-test, yielded statistical significance at T3 and T4 (*$P=0.003$) and at T5 (**$P=0.001$).

Table 3. Percentage variation of CKD stage at different timepoints

	T0	T1	T5
Stage I (%)	60	43.8	37
Stage I to CKD II (%)		14.7	37.1
CKD II to CKD III (%)		5.0	15.0

Table 4. Percentage of patients showing renal and bone pathophysiological findings at different timepoints

	T0	T1	T2	T3	T4	T5	χ^2/P
Proteinuria (%)	26.9	34.7	28.6	25.0	23.1	32.0	2.078/0.838
Microhaematuria (%)	22.6	23.6	11.9	13.0	17.5	19.0	5.435/0.432
Hypophosphataemia (%)	18.6	26.8	19.1	20.0	6.2	14.8	4.449/0.487
Increased parathyroid hormone (%)	29.6	50.0	50.0	30.0	40.0	33.3	6.431/0.267

Cut-off values are as follows: proteinuria, >200 mg/24 h; microhaematuria, any positive value; hypophosphataemia (phosphate <2.5 mg/dL); and increased parathyroid hormone, >65 pg/mL.

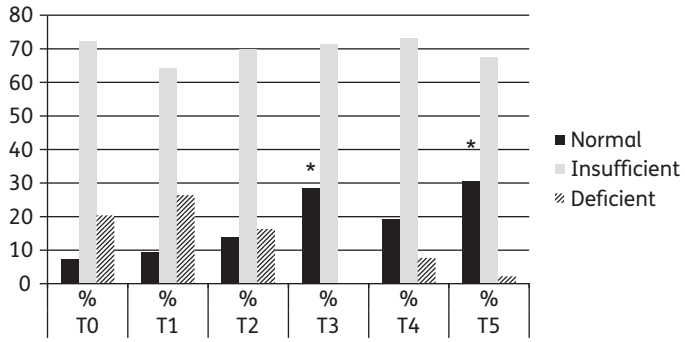

Figure 2. The percentage of patients with normal, insufficient or deficient plasma levels of vitamin D at different timepoints. A statistical comparison between different timepoints of the prevalence of sufficient, insufficient or deficient levels of vitamin D generated a significant difference ($\chi^2 = 28.063$, $P = 0.002$). *$P < 0.004$ versus T0 (after implementation at baseline).

Table 5. Summary of DEXA evaluation

	T0	T3	T5	χ^2/P
Femoral BMD	52.7	77.8	60.6	4.186/0.123
Spinal BMD	28.6	26.3	37.5	2.646/0.221

Data represent the percentage of patients with reduced BMD of either the femur or spine at different timepoints

30.0%, 40.0% and 33.3% of patients T0, T1, T2, T3, T4 and T5, respectively (Table 4) ($P = 0.267$).

Bone density measurements using DEXA scanning revealed that, at baseline, 52.7% of patients had femoral osteopenia or osteoporosis; while the prevalence of a reduced BMD of the spine was 28.6%, all of these patients also had a reduced femoral BMD. However, only 10.5% of patients at baseline manifested an altered BMD of the spine with a normal density of the femur. At T3, the prevalence of reduced BMD of the femur increased to 77.8%, while the reduced spinal BMD measurements stayed stable at 26.3%. Finally, falls in BMD to below the cut-off values at T5 were present in 60.6% and 37.5% of patients on femoral and spinal DEXA, respectively (Table 5) ($P = 0.123$ and $P = 0.221$ for the femur and spine, respectively).

Discussion

In the present study, data collected at the time of the switch of treatment from lamivudine plus adefovir to tenofovir (T0) showed a high prevalence of kidney and bone impairment among our patients. Although a control group was not available and the exact role of hepatitis B infection in renal alterations could not be analysed in the present study, a comparison of our data with those obtained from huge cohort studies in the general population showed significant differences. Indeed, 33.3% of our patients had an eGFR between 60 and 89 mL/min/1.73 m^2 and 6.7% an eGFR <60 mL/min/1.73 m^2, versus 3% and 4.6% in the general population, respectively.[13] A total of 18.6% of patients had hypophosphataemia, while Brown and Greenwood[14] observed a prevalence of hypophosphataemia among the general population that ranged from 2.5% to 3.1%. Urinary abnormalities of proteinuria and microscopic haematuria were present in 26.9% and 22.6% of patients, respectively. Data from the US population show a prevalence of proteinuria of 11% and microscopic haematuria of 4.2%.[15,16] Suboptimal plasma levels of vitamin D were present in 92.6% of our patients, while Bettica *et al.*[17] reported a value of 38.5% in a selected population of postmenopausal women evaluated from December to May, a period of the year associated with a lower production of vitamin D. In addition, DEXA scans of the femur showed a disproportionate rate of osteopenia (52.7%) and osteoporosis (10.5%) in comparison to the normal values for patients of the same mean age.

The profound differences in osteorenal status between our patients treated with adefovir and members of the general population is further magnified by the fact that the majority of the data extracted from the cited cohort studies comes from US subjects, who are more prone to kidney disease than European populations, or, as with the data for hypovitaminosis D, from a population with special predisposing conditions (postmenopausal women).

The variation in eGFR during the study period was characterized by a slight to moderate reduction in 5h3 mean eGFR up to 6 months after switching to a tenofovir-based regimen. However, this moderate reduction remained stable for the last two timepoints and did not progress. After 1 year of exposure to tenofovir, patients experienced a mean reduction in eGFR of 7.5% compared with baseline, and about half the patients had progressed from stage I to stage II CKD or from stage II to stage III CKD. Several studies conducted in cohorts of HIV patients treated with tenofovir have shown a reduction in eGFR of a similar magnitude that remained stable for a longer follow-up period.[18] The effect of tenofovir on HBV-infected patients has been reported not to significantly affect eGFR in different settings.[19] In our specific cohort of patients sequentially exposed to two potentially nephrotoxic drugs without any washout period, we cannot rule out a potential cumulative effect of the two nucleotide analogues in determining the renal dysfunction we observed.

Evaluating the change in serum phosphate and serum parathyroid hormone levels, haemoglobinuria and proteinuria for 24 h urine samples after 1 month of tenofovir treatment, we observed a worsening of all these parameters versus baseline. After 3 and 12 months of tenofovir treatment, these data tend to recover to baseline levels. Although exposure to tenofovir has been associated with phosphate wasting and hypophosphataemia,[20] we did not observe a worsening of the prevalence and magnitude of hypophosphataemia in the present study. Levels of vitamin D were reduced in the majority of patients at baseline. However, the overall percentage of patients with a normal vitamin D status increased significantly at the later timepoints after supplementation with the vitamin. In HIV-infected patients, tenofovir promotes an elevation of parathyroid hormone levels.[21] However, this effect was not evident in our cohort. The cholecalciferol supplementation probably played a role in the maintenance of serum phosphate and parathyroid hormone levels in the study.

The differences observed between the DEXA data for the femur and spine could be due to other factors affecting spinal measurement in patients over 65 years of age, such as arthrosis, calcifications and lumbar fractures (25% of our patients were more than 65 years old). For this reason, DEXA of the femur is considered to be more accurate after this age.

Data from the present cohort revealed a reduced BMD in more than half of the patients at baseline; this prevalence increased to 77.8% after 6 months of exposure to tenofovir, but at the last time-point the percentage of patients with a reduced BMD fell to a level slightly above the baseline value. Therefore, the effect of tenofovir in this cohort seemed transient and perhaps in line with the relatively preserved phosphate and vitamin D homeostasis.

In conclusion, this study, conducted with a cohort of patients with HBV who had already been exposed to a regimen of lamivudine plus adefovir and were switched to tenofovir, shows that the exposure to tenofovir provoked a slight reduction in eGFR. However, this seemed to stabilize after 6 months and did not progress. In this cohort, a significant alteration in phosphate or parathyroid hormone homeostasis was not evident, while plasma levels of vitamin D increased significantly during the study period after supplementation with the vitamin; in turn, the reduced BMD at baseline before starting therapy with tenofovir did not worsen under tenofovir treatment.

Because this is a retrospective study, and because of the quality of the indicators of renal dysfunction and potential confounding factors, caution must be used in interpreting the data given here. Other prospective studies are warranted to confirm our results. However, in the light of our findings, we suggest that markers of kidney and bone toxicity should be monitored in patients affected by HBV-related chronic hepatitis who are treated with tenofovir, especially if they have been switched from an adefovir-based therapy, and particular attention should be given to their baseline values.

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Transparency declarations

None to declare.

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