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Research of Effectiveness of Tenofovir used in Treatment of Chronic Hepatitis B Infection on Bone Mineralization Tenofovir using and Bone Mineralization

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Abstract:

Objectives: *Two billion individuals worldwide have exposed to hepatitis B virus (HBV) and Nowadays, nearly three hundred and fifty millions individuals are chronically infected with HBV. Risk of chronicity is about 2-5% in adult age. Tenofovir is an agent that causes inhibition of HBV polymerase activity and early chain termination after introduced into the DNA. In HIV infected patients, a reduction in bone mineral density was determined under the treatment of tenofovir. In this study,we aimed to investigate the effect of tenofoviron bone mineralization in chronic HBV infection patients.*

Material and Methods: *Thirty patients who were admitted to our hospital and who were started tenofovir treatment admitted to this study. Sixty individuals, whose age- and sex-matched with patient group and have no history of systemic disease, werealso determined as control group for bone densitometry measurements. ALT, AST,HBeAg, anti-HBe, HBV DNA levels and bone densitometry measurements were performed for each patient before treatment and first, second and third years. Differences between years were compared statistically.*

Results: *As a result of statistical analysis, we found a correlation between high HBV DNA (>20 copies/ml) and high ALT (>40 IU/ml) levels ($p<0.05$). When pretreatment levels and third year levels were compared, bone mineral density reduction (both for T ($p=0.02$) and Z ($p=0.04$) scores) was observed. Also, as a result of statistical analysis, a significance was determined between decrease in bone mineral density and Vitamin D ($p<0.05$).*

Conclusion: *In conclusion; a reduction in bone mineral density was determined in chronic hepatitis B patients who were treated withtenofovir,Because of these side effects, these patients should be followed periodically and vitamin D supplementation should be done ifrequires.*

Keywords: *Chronic hepatitis B, Tenofovir, Bone mineralization, Vitamin D.*

1. INTRODUCTION

Hepatitis B virus (HBV) infection represents a major public health problem worldwide. The estimated incidence of serious conditions; such as life threatening liver failure and hepatocellular carcinoma in individuals with chronic HBV infection is around 40%. Also, 5- 10% of individuals diagnosed with HCCare scheduled for liver transplantation, with five hundred thousand lives claimed by HCC each year (1).

The current treatment of chronic hepatitis B infection involves Pegylated interferon alpha and nucleos(t)ide analogues (NA) such as; lamivudine, telbivudine, adefovir, entecavir, and tenofovir(1).

Tenofoviris an oral antiviral agent and an acyclic phosphonate nucleotide analogue with a wide spectrum of selective activity on retroviruses and hepadnaviruses(2).It has been used in the treatment of HIV infection since 2001and has been approved by the FDA in 2008 for chronic hepatitis B treatment(3).

Tenofovir disoproxil fumarate is a prodrug, which is converted into tenofovir in the intestines following oral intake. After two steps of phosphorylation reactiontenofovir is converted in to the active metabolitetenofovir diphosphate which is an obligate chain terminator. Tenofovir diphosphate competes with the natural substrate for deoxyadenosine 5-triphosphate to inhibit the activity of HBV

polymerase. After incorporation into DNA it terminates the DNA chain. Currently, 300 mg tenofovir tablets equal to 245 mg of tenofovirdisoproxilfumarate are available. It is administered once daily. Almost 70 to 80% is excreted through the urine therefore dose adjustment is required in individuals with renal failure. Side effects associated with its use include nephrotoxicity, hypophosphatemia, lactic acidosis, nausea, allergic reactions or rashes. Since its first introduction seven years ago, no resistance against tenofovir has been reported in patients with chronic HBV infection treated with tenofovir(2,3).

Tenofovir is an organic anion that is excreted into the urinary space through an efflux system. Presence of an impairment in this system results in increased intracellular concentrations of tenofovir and cause DNA loss and dysfunction. Subsequently, proximal tubulopathy, which is characterized by acute kidney injury and Fanconi syndrome, develops(4).Fanconi syndrome is characterized by the dysfunction of proximal tubule without primary glomerular involvement. Patients with this syndrome experience varying degrees of phosphate, glucose, amino acid, and bicarbonate loss from the proximal tubules. Additionally; polyuria, renal salt loss, hypokalemia, acidosis, hypercalciuria, and low molecular weight proteinuria may be seen. The major finding in Fanconi syndrome is loss of phosphate. This situation causes systemic reduction in serum phosphate levels along with reduced tubular reabsorption of phosphate. Excessive loss of urinary phosphate along with reduced 1-alpha hydroxylation of 25-OH vitamin D3 in the proximal tubular cells lead to the development of rickets and osteomalacia(5,6). Deficiency of vitamin D and particularly of the active D vitamin calcitriol result in the impairment of intestinal calcium and phosphorus absorption leading to the occurrence of secondary hyperparathyroidism. Subsequently, bone fragility increases and bone density decreases. Therefore, determination of bone densitometry may be important before initiating treatment with tenofovir. Also, dose reduction or discontinuation of the tenofovir treatment may be needed(7).

In the present study, the effect of tenofovir treatment on bone mineralization was examined in patients with chronic hepatitis B infection. For this purpose, pre-treatment bone densitometry results were compared to those determined during treatment with tenofovirin patients with chronic HBV infection.

2. MATERIALS AND METHODS

After obtainment of study protocol approval from the Ethics Committee of our University's Medical Faculty,University, the study procedures were commenced. A total of thirty treatment naive patients with chronic HBV infection,who applicant to the Department of Clinical Microbiology and Infectious Diseases between January 2010 and May 2014 with a HAI ≥ 6 or fibrosis score ≥ 2, and HBV DNA copy of $> 10^7$ copies/ml, were placed on tenofovir treatment. Patients with osteopenia or osteoporosis at the initiation of treatment were advised to have adequate exposure to sunlight on a daily basis. Also,sixtyindividuals whose age- and sex-matched with patient group and have no history of systemic disease served as controls for bone densitometry measurements.

Age, gender, serum ALT, AST, urea, creatinine, calcium, phosphorus (P), parathyroid hormone (PTH), vitamin D, PTT-INR, AFP, CEA, CBC, HBeAg, anti-HBe, HBV DNA level were recorded in each patient. These tests were repeated at first, second and third years after initiation of the treatment. All biochemical, hematological, serologic tests and HBV DNA levels were studied at the Central Laboratory of FU University Hospital. Blood biochemistry tests were performed in an ADVIA 2400 device, while complete blood counts were performed in a Horiba ABX Pentra DX 120 device. PTZ-INR levels were performed with a Siemens BCS XP device, HBeAg and anti-HBe with an Abbotti 1000 SR device using macro-ELISA method, HBV DNA with QIAGEN QIA SYMPHONY device, and vitamin D levels with a SHIMADZU HPC device.

Bone densitometry measurements were performed in the Department of Nuclear Medicine. Weight and height were recorded as well as the age and gender data. Bone densitometry measurements were performed in a Hologic Discovery QDR 4500 W Elite device before treatment and first, second and third years.

Dual energy was applied at 100/140 Kv, 40 mA. The daily calibration of the device was performed using a phantom (lumbar vertebrae, QC Phantom) that contained adequate amount of calcium hydroxyapatite crystals to reflect the bone mineral content of the four lumbar vertebrae. The bone mineral density measurements were performed in the AP position at L1, L2, L3, L4, L1-L4, L2-L4 lumbar vertebrae, femoral neck, greater trochanter, intertrochanteric area, and Ward's triangle after removal of any metallic objects that could interfere with the acquisition of the images. Care was

practiced to maintain the correct patient positioning during measurements.Fifteen minutes were allowed to each patients for measurement. Z score is expression of the standard deviation of the value above or below the age- and sex-matched BMD measurements. Thus, patients and controls were compared with respect to Z score at L1-L4 and L2-L4 before the treatment. In the patient group, T and Z scores at L1-L4 and L2-L4 were compared during the treatment process.

Statistical Analyses

Data were analyzed using SPSS 18.0 software package. Kalmagorov-Smirnov test was used to assess the normality of the data distribution. Data were expressed as mean ± standard deviation. Data without normal distribution and with a wide range of upper and higher limits were expressed as median (upper and lower limits). Between-group comparisons were performed using Kruskal Wallis analysis of variance and pairwise comparisons were performed using Mann-Whitney U test. Spearman's correlation analysis was used to assess the associations between parameters.*p* value less than 0.05 was considered to show statistical significance.

3. RESULTS

A total of thirty patients who received tenofovir treatment in the Department of Clinical Microbiology and Infectious Diseases were included in this study. Also, sixty healthy individuals were included as controls for bone densitometry measurements.

The mean age was 41 ± 12 years with twenty two male and eight female patients. *Table 1*shows the demographic characteristics of the patients. Control group comprised sixty healthy individuals, forty four male and sixteen female, with a mean age of 41 ± 12 years.

Table1. *Demographic Characteristics and Disease Status of the Patients*

Demographiccharacteristics	**Patients**
Age	
Average ±SS (Minimum –Maximum)	41±12 (20-67)
Sex n (%)	
Female	8 (26.6)
Male	22 (73.3)
HBeAg status n (%)	
Positive	14 (46.6%)
Negative	16 (53.3%)

Fourteen patients were positive for HBeAg, while sixteen were anti-HBe positive (Table 1). Pre-treatment measurements were done for ALT, P, PTH and Vitamin D. ALT levels were <40 IU/ml in seven patients, while they were >40 IU/ml in twenty three patients. Four patients have hypophosphatemia (<4.5 mg/dl), twenty six patients have <4.5 mg/dl P levels.PTH levels<65 pg/mL in twenty two patients and >65 pg/mL in eight patients. Vitamin D levels were10-150 microgr/L at six patients and <10 microgr/L at twenty four patients.All other biochemical and hematological parameters including urea, creatinine, calcium, PTT-INR, AFP-CEA, and CBC were normal at pretreatment evaluation.

Bone densitometry measurements at lumbar vertebrae were performed in sixty healthy controls and Z scores were estimated. Forty-four healthy males in the control group have an average Z score of -0.7 ± 0.6, while sixteen healthy female controls had a score of -0.5 ± 0.7. In contrast, the corresponding figures in male and female individuals in the patient group were -1.1 ± 0.8 and -0.2 ± 0.9, respectively (Table 3). When bone densitometry according to Z scores (L1-L4) was considered, twenty seven patients have normal bone mass before treatment, while three patients have low bone mass. Pretreatment average lomber Z score at L1-L4 was -0.8 ± 0.9. Statistical analyses showed no significant differences between pre-treatment lumbar Z scores in the patient group and those of healthy controls ($p > 0.05$). Similarly, no significant gender differences also existed between healthy controls and patients in terms of lumbar Z scores ($p > 0.05$). Pre-treatment lumbar Z scores in the patient group in female and male subjects were -0.2 ± 0.9 and -1.1 ± 0.8, respectively, although the lower Z score in male individuals did not statistical significance ($p=0.05$). T scores (L2-L4) among study participants showed normal bone densitometry in twelve patients, osteopenia in sixteen patients and osteoporosis at two patients. Pre-treatment average T score at L2-L4 was -1.2 ± 0.8.

There were no significant associations between pre-treatment bone densitometry for Z scores (L1-L4) and pre-treatment PTH and vitamin D levels (p=0.9 and 0.6, respectively). In addition, pre-treatment T scores (L2-L4) were not significantly associated with pre-treatment PTH and vitamin D levels (p=0.8 and 0.4, respectively).Datas are shown at table 4.

The rate of ALT normalization among HBeAg positive patients in first year of treatment was 64.2% (9/14), while it was 81.2% (13/16) in HBeAg negative subjects.Among the fourteen HBeAg positive patients, four patients (28.5%) were HBV DNA (-) in first year of treatment. Among the sixteen patients with negative HBeAg, eight were HBV DNA (-) in first year of treatment and six patients have a HBV DNA < 20 copy/ml. HBV DNA was undetectable in fourteen patients overall in first year (93.7%).

In first year, HBV DNA was detected negative in four patients (28.5%) of fourteen HBeAg positive individuals, while it was detected negative in eight of sixteen HBeAg negative individuals. Also, HBV DNA was detected < 20 copy/ml in six of HBeAg negative patients. HBV DNA was undetectable in fourteen patients (93.7%) according to first year's results.

In the third year, twenty four patients have<40 IU/ml ALT and six patients with >40 IU/ml ALT levels. The rate of ALT normalization among HBeAg positive patients was 64.2% (9/14) and 93.7% (15/16), respectively (Table 2). HBV DNA was undetectable in sixteen patients (100%). HBV DNA (-) in thirteen patients and < 20 copy/ml in three patients. All of these parameters are presented at *table 2*. Statistical analyses showed a positive correlation between elevated HBV DNA and elevated ALT ($p < 0.05$).No patients have HBeAgsero conversion after three years. Also, there was no loss of HBsAg.

Three years after treatment starting P, PTH and Vitamin D levels were measured again. Five patients have hypophosphatemia(P<4.5 mg/dl). PTH levels were detected <65 pg/mL levels in twenty two patients, and >65 pg/mL levels in eight patients. Vitamin D levels were 10-150 microgr/L in eight patients and <10 microgr/L in twenty two patients inthird year of treatment (Table 2). No significant associations between P, PTH, and vitamin D levels which were measured before treatment and three yearsafter starting tenofovir($p > 0.05$).

Table2. *The Measured Levels of AST, ALT, HBV DNA,P, PTH,Vitd and ALT Normalization Rates and HBV DNA Negativity in Pretreatment, First and Thirdyears*

Years	***Pretreatment***	***1.year***	***3.year***
AST (IU/ml)			
Normal <40IU/ml n (%)	*10 (33.3)*	*No determined*	*28 (93.3)*
High >40 IU/ml n (%)	*20 (66.7)*		*2 (6.7)*
Average ±SS	*94 ±18*		*26± 9*
ALT (IU/ml)		*No determined*	
Normal <40IU/ml n (%)	*7 (23.3)*		*24 (80)*
High>40 IU/ml n (%)	*23 (76.6)*		*6 (20)*
Average ±SS	*162 ± 39*		*31± 16*
HBeAg*n (%)*	*14 (46.6)*		
ALT< 40IU/ml		*64.2%*	*64.2%*
HBV DNA (-) and< 20copy/ml		*28.5%*	*71.4%*
HBeAg negative *n (%)*	*16 (53.3)*		
ALT< 40IU/ml		*81.2%*	*93.7%*
HBV DNA (-) and<20copy/ml		*93.7%*	*100%*
HBVDNA (*copies/ml)*	$3.8x10^8 \pm 8.5x10^7$	*ND*	*52±20*
P *(mg/dL)*	*2.6-4.5*	*ND*	
Normal *n (%)*	*26 (86.6)*		*25 (83.3)*
Low *n (%)*	*4 (13.3)*		*5 (16.6)*
Mean±SS	*3,2 ±0,6*		*3,1 ± 0,6*
PTH *(12-65pg/mL) n (%)*		*ND*	
Normal	*22 (73.3)*		*22 (73.3)*
High	*8 (26.6)*		*8 (26.6)*
Average ±SS	*64,1 ±37*		*64,1 ±37*
VİT D *(10-150 microgr/ L)*	*6 (20)*	*ND*	*8 (26.6)*
Normal	*24 (80)*		*22 (73.3)*
High	*15,8±9,2*		*16,6 ± 9,4*
Average ±SS			

ND: No determined

As a result of performed bone densitomery T scores (L2-L4) in third year, thirteen patients have normal bone densitometry fourteen have osteopenia and three have osteoporosis. All three patients with osteoporosis were male. According to Z scores (L1-L4), twenty five patients have normal bone mass and five have low bone mass (Table 3). All five patients with low bone mass were male.

Table3. *Pre-Treatment Z Scores in Patient Group and Controls and T-Score Results in Bone Densitometry and Bone Mass Assessment (L2-L4)*

	Pretreatment		***3.Year results***
	Control Group	***Patient Group***	***Patient Group***
Z Score			
Female	-0.5 ± 0.7	-0.2 ± 0.9	ND
Male	-0.7 ± 0.6	-1.1 ± 0.8	ND
T Score n (%)			
Normal	12 (40)		13 (43.3)
Osteopenia	16 (53.3)		14 (46.6)
Osteoporosis	2 (6.6)		3 (10)
Normal bone	27 (90)		25 (83.3)
MassLow Bone Mass	3 (10)		5 (16.6)

The average T and Z scores according to treatment-years in the patient group is shown in Table 4. Three years after starting tenofovir, average T and Z scores weredetected -1.2 ± 0.9 and0.1 ± 1.0, respectively. A statistically significant was observed between the first and third year's values (p=0.02, p=0.04, respectively). Both for T and Z scores, there were no significant associations between pre-treatment values and values at first and second years of treatment (p > 0.05). Statistical *p* values were shown in Table 5.

Statistical analyses showed a negative correlation between bone densitometry T score (L2-L4) and vitamin D level inthird year of treatment (p < 0.05). In other words, while T scores were reduced, there was a slight increase in vitamin D levels. Similarly, a negative correlation between the Z scores (L1-L4) and vitamin D levels was observed.

All other biochemical and hematological parameters including urea, creatinine, calcium, PTT-INR, AFP-CEA, and CBC were normal in third year.

Table4. *The Average T and Z Scores according to Treatment-Years in the Patient Group and Hhe Association of T and Z Scores with Vitamin D and PTH before Treatment and in Third Year*

Treatmentyear	T-score (L2-L4) *	Z –score (L1-L4)*	VitD* (microg/L)	PTH (pg/ml)*
Year 0	-1,2 ±0,8	-0,8 ±0,9	15.8± 9.2	64,1± 37.0
Year 1.	-1,2 ±0,8	-0,9 ±0,9	ND	ND
Year 2.	-1,2 ±0,9	-0,9 ±0,9	ND	ND
Year 3.	-1,2 ±0,9	-0,9 ±1,0	16.6± 9,4	64,9± 37,4

** Mean ± standard Deviation*

Statistical comparisons of all parameters and p values were presented at table 5.

Table5. *Statistical Comparisons of all Parameters and P Values*

STATISTICAL COMPARISON OF RELATIONSHIP	***p* VALUE**
Corelation between HBV DNA>20 copies/ml and ALT >40	**p < 0.05***
Gender and lumbar Z scores in healthy controls and patients groups	P > 0.05
Pre-treatment lumbar Z scores in the patient group and healthy controls	p > 0.05
Z scores in pre-treatment and first year of treatment groups	p > 0.05
Z scores in pre-treatment and second year of treatment groups	p > 0.05
Average Z score in pre-treatment and third year of treatment	**p = 0.04***
Pre-treatment Z scores (L2-L4) and PTH elevation	P = 0.9
Pre-treatment Z scores (L2-L4) and vitamin D decreasing	P = 0.6
T scores in pre-treatment and first year of treatment groups.	p > 0.05
T scores in pre-treatment and second year of treatment	p > 0.05
Pre-treatment and third year's average T score	**p = 0.02***
T score (L2-L4) increasing and vitamin D level decreasing in third year of treatment	**p < 0.05***
Pre-treatment T scores (L2-L4) and PTH elevation	p = 0.8
Pre-treatment T scores (L2-L4) and vitamin D decreasing	P = 0.4

** Statistically significance*

4. DISCUSSION

The number of individuals with chronic hepatitis B infection worldwide is nearly three hundred and fifty million and approximately 10% individual die due to HBV-associated chronic disease. The aims of the treatment are significantly reducing in HBV replication and prevent the development of cirrhosis, liver failure, and HCC. Although the primary target of the virological response is to achieve anti-HBs seroconversion, it is very difficult. So, the other targets were determined for assessment of treatment response such as,HBeAgseroconversion to anti HBe and even suppression of HBV DNA. Tenofovir is a nucleotide analogue and currently used for the treatment of chronic HBV infection owing to low genotypic resistance rates and high anti-viral efficacy(3).

Previous studies assessed the HBV DNA levels in patients who are treated with 245 mg/day of TDF and found significant reductions in HBV DNA in both HBeAg positive and negative patients within weeks. These studies reported an ALT normalization rate around 75 to 80%. Theseroconversion rates inHBeAg positive patients correlate with the duration of treatment and may reach from 20% to nearly 50%. The reported rate of HBsAg loss is around 3.2-8% in these studies(8-10).

In our study, ALT normalization rates in HBeAg (+) and HBeAg (-) patients were 64.2% and 81.2%in first year and 64.2% and 93.7%in third year respectively. HBeAg positive five patients (three patients have hepatosteatosis and two patients have HBV DNA levels around 100 copies/ml) could not achieve ALT normalization in third year.HBeAg negative one patient could not achieve ALT normalization in third year. ALT elevation in this single patient could not be explained. This was thought to relate to nutritional habits of the patients, and nutritional optimization along with treatment compliance may be expected to result in better ALT normalization rates.

In a multi-center cohort study by Lampertico*et al*. (11) the virological and biochemical response rates have been found 84.3% - 95% in first year and 77.3% - 83% in third year. In our study,virological response rates in HBeAg positive and negative patients were found 66.3% and 86% in first year and 74% and 98% in third year. After three years follow-up,HBeAgseroconversion rate was 36% and rate of HBsAg loss was 13%.

In our study, there was a significant reduction in HBV DNA levels within weeks of treatment ($p<0.05$) both HBeAg positive and negative patients. Achieved undetectable HBV DNA ratesinHBeAg positive patients were 28.5% in first year and 71.4%third year, respectively. These rates were 93.7% and 100% inHBeAg negative patients. Consistent with previous reports, proportion of patients with undetectable HBV DNA was higher inHBeAg negative patients.

Demir*et al*. (12) has been investigated the statistically significant reduction in HBV DNA with a daily dose of 245 mg forty eight week TDF treatment. Proportions of patients with HBV DNA below 400 copies/ml in HBeAg positive and negative patients were 64.7-81.3% and 85.7-89% at the end of treatment, respectively. ALT normalization with 245 mg/day of TDF occurred in 83-88.2% and 79-100% of HBeAg positive and negative patients, respectively. Only one patient in eighty eight HBeAg positive subjects could achieve anti-HBe, while HBsAg loss did not occur in any patients.

In another study from Turkey, virological HBV DNA response rates in forty nine patients undergoing tenofovir treatment were 29.7%-100% inthree months and four years respectively. ALT normalization rates were found in 74.4% in first year and 100% four years after treatment. HBeAgseroconversion has been observed 8.3% of patients infirst year and this figure has been continued in the following years(13).HBeAg negative patients have higher rates for ALT normalization and undetectable HBV DNA levels. Thus, we believe that a larger sample size and a longer follow up could yield HBsAg loss and HBeAg seroconversion in our sample as well.

Tenofovir is an organic anion and is taken into the cell by the organic anion transporter (OAT-1) at the basolateral membrane of the proximal tubule. Tenofovir is transported by transporter proteins within the cell. Then, it is excreted into the urinary space through multi-drug resistance protein-2 (MRP-2) and MRP-4 via the efflux system. An impairment in this system (increased OAT-1 activity or decreased MRP efflux transport activity) may cause elevated levels of intracellular tenofovir and lead to tubular damage. Early signs of proximal tubular dysfunction include microalbuminuria, glucosuria without diabetes, phosphaturia, and fractional phosphate excretion. The late signs on the other hand are reduced GFR, proteinuria, hypophosphatemia, bone disease, and osteomalacia. Presence of at least two early signs is indicative of clinically significant tubular damage(14).

Although tenofovir was first introduced for use in chronic HBV infection approximately seven years ago, its use in patients infected with HIV dates back even earlier. Considering the seven year history of tenofovir use in chronic HBV infections, the side effect profile of tenofovir in this indication appears to be favorable. Approximately, 4% of HIV positive patients undergoing treatment with tenofovir experience tenofovir associated nephrotoxicity and Fanconi syndrome(14). The primary aim of this study was to examine the effect of tenofovir on bone mineralization and its nephrotoxicity in patients with chronic hepatitis B.

In a study by Marcellin *et al*. (10) whichfour hundred and twenty six patients (using 245 mg/day of TDF) have been evaluated, no renal toxicity has been observed withinforty eight weeks of treatment. In this study, three hundred and eighty nine patients have been followed up for six years, five patients (1.3%) have 0.5 mg/dl increase in serum creatinine compared to baseline and five patients (1.3%) have a phosphorus level below 2 mg/dl, with three additional patients (0.8%) having a creatinine clearance below 50 ml/min. Liaw*et al*. (15) hasinvestigatedforty five patients who received 245 mg/day TDF for forty eight weeks. In four patients (8.9%), there was a 0.5 mg/dl increase in serum creatinine compared to baseline, and one patient (2.2%) had serum phosphorus below 2 mg/dl.

Lampeertico*et al*. (11) has examined three hundred and two patients who received TDF 245 mg/day duringthree years.The mean serum creatinine level before treatment was 0.9 mg/dl, while it was 0.92 mg/dl after three years. Approximately, 2% of the patients have 0.5 mg/dl increase in serum creatinine compared to baseline in third year. The average phosphorus concentration before and three years after treatment were 3.3 mg/dl and 3.1 mg/dl, respectively.

In a study from Turkey, no creatinine elevation or hypophosphatemia has been observed during four years tenofovir treatment. In that study, the baseline creatinine level was 0.87 ± 0.18 mg/dl, and no patients experienced a 0.5 mg/dl increase in serum creatinine levels compared to baseline during the study period (13).

In a studyserum creatinine, P, calcium, and ALP levels have been followed during the forty eight weeks of treatment with 245 mg of TDF dailyand no significant changes have been observed in these parameters (12).

Similarly, in our study calcium, phosphorus, PTH, vitamin D, urea and creatinine levels before and first, second and third years of treatment were assessed and followed the treatment side effects. Although there were some differences between pre-treatment and third year results, the differences were not statistically significant. Urea, creatinine, and calcium were within the normal range both before treatment and third year of treatment. Hypophosphatemia was found in four patientsbefore treatment and in five patients three years after starting tenofovir. There were no significant differences.

Marcellin *et al*. (10) assessed the bone mineral density in chronic HBV patients four and six years after treatment with tenofovir. They found no significant changes in hip and vertebral BMD assessmentsby DEXA T scores (vertebral) from fourth year to year sixth.Nevertheless, these authors still recommended periodic evaluation of chronic HBV patients receiving tenofovir treatment.

Osteoporosis and osteopenia are well known complications of chronic liver disease, with twice higher risk of osteoporosis and higher risk of fractures in those subjects with chronic liver disease(16,17).In a study by Ridruejo*et al*. (18), it has been exhibited that bone mineral loss is more likely due to vitamin D deficiency rather than tenofovir treatment in HBV patients. Galego *et al*. (19) havefound an osteoporosis incidence of approximately 50% in patients with liver cirrhosis due to hepatitis B or C.

In the current study, there were no significant BMD differences between patients and healthy controls before the initiation of treatment. The average pre-treatment Z score in the patient group was lower in males than in females, probably due to the fact that female individuals were in the pre-menopausal period with increased osteoblastic activity of estrogen.

In this study, we examined the effect of tenofovir on bone mineralization in patients with chronic HBV infection and a significant difference was found in T and Z scores between baseline and third year of treatment ($p < 0.05$). Absence of a difference in pre-treatment BMD between patients and healthy controls and a statistically significant decline of BMD inthird year of treatment suggests that tenofovir might have some unfavorable effects on bone mineralization. Normalization of bone

densitometry is an expected outcome of elevated vitamin D levels, and conversely, a lowered bone density may be observed with low vitamin D levels. However, in our study although there was a reduction in bone densitometry results, a slight increase in vitamin D levels was observed. This finding is an odds with previous results and may be due to the once yearly measurement of both BMD and vitamin D which may have occurred during the sunny season and due to the recommendation to have increased exposure to sunlight to those individuals with osteopenia.

Majority of our patients have vitamin D deficiency prior to the initiation of treatment and when one considers the positive effects of vitamin D replacement on bone metabolism, it is plausible to recommend routine replacement of vitamin D to patients with deficient vitamin D. Bone density measurements in larger patient populations with more frequent intervals could yield more elucidating results in this respect.

In conclusion, tenofovir is an effective therapeutic option for the treatment of chronic HBV infection. Tenofovir related nephrotoxicity and decreased bone mineral density observed in patients with HIV may also occur in chronic HBV patients receiving tenofovir. Therefore, patients with chronic hepatitis B who receive tenofovir treatment should be routinely monitored for signs of nephrotoxicity and effects on bone mineralization. If required, vitamin D replacement should be performed. Until now, such effects reported in patients with chronic HBV infection are generally milder as compared to those in patients with HIV infection and do not require discontinuation of treatment. However, further studies with larger sample size are warranted to better define such effects.

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