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Evaluation of initial results of naïve HIV-infected patients regarding bone health

HİV ile enfekte naif hastaların ilk değerlendirme bulgularının kemik sağlığı açısından değerlendirilmesi

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Abstract

Aim: HIV-infected patients have increased risk of osteoporosis due to both HIV and the treatment regimens in HIV. Our aim was to reveal the need of screening for bone health in HIV-infected patients, and to reveal the relationship between indirect serum markers of bone-condition, $CD4^+$ T lymphocyte counts and HIV RNA viral loads and DEXA screening results.

Methods: Naïve HIV positive patients over 18 years old who were under follow-up in our hospital between January 2014 and December 2018 were included in this retrospective cohort study. $CD4^+$ T cell counts, HIV RNA viral loads, body mass indexes (BMI), 25 (OH) vitamin D, serum calcium and corrected calcium (cCa) levels and DEXA screening results of these patients were recorded. For statistical analysis and interpretation, e-picos (https://www.e-picos.com) and SPSS (version 20.0; SPSS Inc., Chicago, IL, USA) were used.

Results: A total of 101 naive HIV-infected patients were included in the study. Vitamin D levels were within normal limits in only 9 (10.8%) patients, while 42 (50.6%) patients had insufficiency and 32 (38.5%) patients had deficiency. Serum calcium and cCa values were significantly lower in patients with < 40 years of age (P=0.04). According to the T-score assessment in DEXA screening, 19 patients (47.5%) had osteopenia findings in at least one of three regions (femoral neck, total hip and lumbar spine). A total of three male patients (7.5%) had osteoporosis. In terms of viral load, only BMD and T-score in women with viral load > 100,000 IU/mL were significantly lower in lumbar spine (P=0.01 and P=0.01, respectively). In terms of CD4 counts, only Z-scores in only lumbar spine and femoral neck were statistically lower in women with CD4 counts > 200 cells/µl (P=0.04 and P=0.03, respectively). There were not any significant differences in any other groups and region in terms of viral load and CD4 count. None of the factors including high viral load, low CD4 + count, low 25 (OH) vitamin D level or low cCa levels were directly related to T, Z-score and low BMD.

Conclusion: Osteopenia and osteoporosis are observed more frequently and at younger ages in HIV-infected patients than in the general population. Since we cannot make any prediction on bone health using one of the indirect markers in serum including 25 (OH) vitamin D levels and Ca levels or viral loads and CD4 counts in HIV-infected patients, BMD screening at younger ages may be beneficial.

Keywords: HIV, DEXA, Bone mineral density, Serum calcium, 25 (OH) vitamin D

Öz

Amaç: HIV-enfekte hastalarda hem HIV'in kendi etkisi hem de HIV'deki tedavi rejimlerinden dolayı osteoporoz riski artmıştır. Bu çalışmadaki amacımız, HIV ile enfekte hastalarda kemik sağlığı taramasının gerekliliğini ortaya koymak ve kemik durumuyla ilişkili indirek serum belirteçlerinin, $CD4^+$ T lenfosit sayılarının ve HIV RNA viral yüklerinin DEXA tarama sonuçları ile ilişkisini ortaya koymaktır.

Yöntemler: Bu retrospektif kohort çalışmasına Ocak 2014 ve Aralık 2018 tarihleri arasında hastanemizde takipte olan 18 yaş ve üstü HIV pozitif hastalar dahil edildi. CD4 + T hücre sayısı, HIV RNA viral yükleri, vücut kitle indeksleri (BMI), 25 (OH) D vitamini, serum kalsiyum ve düzeltilmiş kalsiyum (cCa) seviyeleri ve bu hastaların DEXA tarama sonuçları kaydedildi. İstatistiksel analiz ve yorumlama için e-picos (https://www.e-picos.com) and SPSS (version 20; SPSS Inc., Chicago, IL, USA) programları kullanıldı.

Bulgular: Çalışmaya toplam 101 naif HIV ile enfekte hasta dahil edildi. D vitamini düzeyleri sadece 9 (%10,8) hastada normal sınırlardayken, 42 (%50,6) hastada yetersizlik, 32 (%38,5) hastada eksiklik vardı. Serum kalsiyum ve cCa değerleri 40 yaşın altındaki hastalarda anlamlı olarak düşüktü (P=0,04). DEXA taramasındaki T skoru değerlendirmesine göre, 19 hastada (%47,5) üç bölgeden en az birinde (femur boynu, total kalça ve lomber omurga) osteopeni bulguları vardı. Toplam üç erkek hastada (%7,5) osteoporoz vardı. Viral yük açısından, viral yükü > 100.000 IU/mL olan kadınlarda sadece BMD ve T skoru lomber omurgada anlamlı derecede düşüktü (sırasıyla, P=0,01 ve P=0,01). CD4 sayıları açısından, sadece lomber omurga ve femur boynundaki Z skorları, CD4 sayısı> 200 hücre/µl olan kadınlarda istatistiksel olarak daha düşüktü (sırasıyla P=0,03 ve P=0,04). Diğer grup ve bölgelerde viral yük ve CD4 sayısı açısından anlamlı fark yoktu. Yüksek viral yük, düşük $CD4^+$ sayısı, düşük 25 (OH) D vitamini veya düşük cCa seviyeleri gibi faktörlerin hiçbiri doğrudan T, Z-skoru ve düşük BMD ile ilişkili değildi.

Sonuç: Osteopeni ve osteoporoz, HIV enfekte hastalarda genel popülasyona göre daha sık ve daha genç yaşlarda görülebilmektedir. HIV ile enfekte hastalarda 25 (OH) vitamin D düzeyleri ve Ca seviyeleri gibi kemik sağlığı ile ilgili indirek serum belirteçleri veya viral yükler ve CD4 sayılarını kullanarak kemik sağlığı konusunda herhangi bir öngörüde bulunamadığımız için, daha genç yaşlarda BMD taraması yararlı olabilir.

Anahtar kelimeler: HIV, DEXA, Kemik mineral yoğunluğu, Serum kalsiyum, 25 (OH) D vitamini

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Introduction

Osteoporosis, the most common metabolic bone disease, affects almost one-fourth of postmenopausal women. It is characterized by low bone mineral density (BMD), distortion of bone structure and bone tissue, and increased risk of fracture in bones. The life-long risk of bone fracture is 30-40% in women, while 20% in men [1].

In literature, the prevalence of spinal and hip fractures in patients infected with human immunodeficiency virus (HIV) is concluded to be 60% higher than normal population [2,3]. Low vitamin D levels, low body mass index and alcohol-tobacco use are concluded to be risk factors that increase the development of osteoporosis in HIV-infected populations. In addition, various agents in highly active antiretroviral therapy (HAART) regimen may contribute to the reduction of bone mineral density (BMD) in these patients [4,5].

Owing to successfully treatment of the HIV infection after HAART era, management of the comorbidities in boneskeletal, cardiovascular, neurological system, etc. arose as the main challenges in patients infected with HIV. In terms of bone health in HIV-infected patients, awareness about underlying-factors which reduce BMD, like vitamin D and calcium deficiency, has increased.

The aim of this study was to reveal the relationship between $CD4^+$ T cell counts, HIV RNA viral loads, body mass indexes (BMI), 25 (OH) vitamin D and serum calcium levels and bone mineral density (BMD) results in HIV-positive patients.

Materials and methods

A total of 101 naive HIV positive patients over 18 years old who had been follow-up in our hospital between January 2014 and December 2018 were included in the study. The study was approved by the Institutional Ethics Committee of Health Sciences University with approval number 18/72 on November 30th, 2018.

HIV RNA viral load, CD4 + T cell count, serum albumin, calcium, corrected calcium levels, thyroid stimulating hormone (TSH), T4 and alkaline phosphate (ALP) levels in naive HIV positive patients who were not receiving vitamin D or calcium supplement or any drugs that may affect BMD were recorded retrospectively. In addition, BMD results that were screened by dual energy X-ray absorptiometry (DEXA, Dual Energy X-Ray Absorptiometry) and initial BMIs of patients were retrospectively recorded.

Cases were evaluated in two groups; cases younger than 40 years of age were in the first group and older than 40 years of age were in the second group. The results of CD4 + T cell count, HIV RNA viral load, body mass index (BMI), 25 (OH) vitamin D and serum calcium levels and bone mineral density (BMD) were evaluated separately in two age-groups.

Exclusion criteria

HIV positive patients with endocrine, renal, gastrointestinal or hematological diseases like hyperparathyroidism, subclinical hyperthyroidism, Cushing's syndrome, idiopathic hypercalciuria, celiac disease, multiple myeloma, and patients receiving exogenous corticosteroids, vitamin or calcium supplementation were excluded from the study.

Definitions

The assessment of bone mineral density (BMD) test

BMD measurements were performed with DEXA (HorizonTM Wi S/N) and calculations were performed in software version 13602. The assessments of the results were based on World Health Organization (WHO) criteria.

According to WHO, the T-score is recommended for use in postmenopausal women or in men older than 50 years of age while Z-score is recommended for all other populations [1]. The T-score is a comparison of the patient's bone density with healthy, young individuals (ages between 20 and 29) of the same sex. A negative T-score of –2.5 or less at the femoral neck defines osteoporosis, while T score between –2.5 and –1 defines osteopenia and T score between –2.5 and –1 defines osteopenia and T score between –0.9 and 1 is the normal range [6,7]. The Z-score is a comparison with the bone density of people of the same age and sex as the patient. A negative Z-score of –2.0 or less defines osteoporosis.

Body mass index (BMI)

Patients with a body mass index less than 18.5 kg/m2 were considered to be underweight, 18.5-24.9 kg/m2 were normal or healthy, 25-29.9 kg/m2 were overweight, and ≥ 30 kg/m2 were considered obese. In our hospital, height weight measurements are performed before the measurement of BMD. Patients who have the data input in the HorizonTM Wi S/N system prior to BMD measurements were considered for BMI evaluation.

Corrected calcium (cCa) value

Since calcium is strongly bound to albumin in the blood, it should be considered that serum calcium levels can be mis-measured in patients with hypoalbuminemia. In our study, cCa values were calculated by Md+ calculator according to the serum calcium values of all the patients in order to prevent bias. In this calculation, cCa (mg/dL) =serum Ca (mg/dL) + 0.8 [4 - serum albumin (g/dL)] formula was used and the serum calcium reference ranges of the laboratory in our hospital were 8.4-10.5 mg/dL.

25 (OH) D vitamin

Serum 25 (OH) vitamin D levels less than ≤ 20 ng/mL was considered as deficiency, levels between 21 - 29 ng/mL was considered as insufficiency and higher than ≥ 30 ng/mL was considered normal.

Statistical analysis

For statistical analysis and interpretation, e-picos (https://www.e-picos.com) and SPSS (version 20.0; SPSS Inc., Chicago, IL, USA) were used. The values of minimum, maximum, mean, median and standard deviation were calculated as descriptive analyzes. Normality analysis was performed with Kolmogorov Smirnov test. Depending on whether parametric or nonparametric tests are applied; Chi-square / Fisher's exact test was used to analyze the relationship between two categorical variables. The Mann-Whitney U test was used to compare two independent samples. Values including $P<0.05$ in 95% confidence interval was considered to be statistically significant.

Results

A total of 101 naive HIV-infected patients were included in the study. Of the patients, 84 (83.1%) were male and 17 (16.8%) were female. The mean age of patients was 38 years of age (Minimum: 19, maximum: 75) (Table 1). Of the male cases, 56 were younger than 40 years-old and 28 were older than 40 years-old. The mean age among males was 37.4 years of age (Table 1). Among 17 female patients, 11 were younger than 40 years-old and 6 were older than 40 years-old. There was no significant difference between mean ages of the male and female cases (P=0.06).

The mean of $CD4^+$ T cell counts in male and female patients with < 40 years of age were 421 (243) and 282.2 (295) cells/µl, respectively. The mean of $CD4^+$ T cell counts in male and female patients with ≥ 40 years of age were 388.3 (286) and 329.7 (282) cells/µl, respectively. There were no significant differences in $CD4^+$ counts between age groups < 40 and ≥ 40 years of age among male and female cases (P=0.608, P=0.781, respectively).

In all patients, 17 (16.8%) had $CD4^+$ counts lower than 200 cells/µl, and the ratio of female and male patients with $CD4^+$ counts lower than 200 cells/µl were 47% and 13%, respectively (Table 1). The mean viral loads in males with < 40 and ≥ 40 years of age were 518784 (1184879) IU/mL and 1189756 (3744786) IU/mL, respectively. There was no significant difference in mean viral load and also in log2 mean viral load in male patients compared to age groups (P=0.798 and P=0.798, respectively). The mean viral load and mean viral loads in the log2 system were similar in the female cases compared to the age groups (P=0.940 and P=1, respectively) (Tablo 1).

The mean BMI in all cases was 23.7 (3.6) kg/m2. In terms of BMI, 70% of the patients were in normal or low-weight category; only two female patients with < 40 years of age were in the low-weight category. The mean BMI in < 40 and ≥ 40 age-groups was 22.1 (1.66) and 21.8 (1.1), respectively (Table 1). There were four cases in the obese category, and all were male. The number of patients with normal/low-weight and overweight/obese in the < 40 age-group was 22 to three, respectively, while it was six to nine in the ≥ 40 age-group, respectively. The ratio of overweight or obese patients was significantly higher in the ≥ 40 age-group (P=0.03) (Table 1).

25 (OH) vitamin D levels were screened in 83 patients. Vitamin D levels were within normal limits in only 9 (10.8%) patients, while 42 (50.6%) patients had insufficiency and 32 (38.5%) patients had deficiency. There was no significant difference in vitamin D levels between the age groups (P=0.796). In addition, there was no significant difference in serum albumin, TSH, T4 and ALP levels between the age groups (P=0.951, P=0.682, P=0.522, P=0.868, respectively) (Table 2).

However, mean serum calcium levels in < 40 and ≥ 40 age-groups was 8.9 (0.6) and 9.3 (0.7) mg/dL, respectively, and patients in < 40 years-old group had significantly lower levels of serum calcium (P=0.04). Accordingly, cCa values were significantly lower in patients in <40 years-old (Table 2). Only one patient had a slightly higher calcium level.

According to the T-score assessment in DEXA screening, 19 patients (47.5%) had osteopenia findings in at least one of three regions (femoral neck, total hip and lumbar spine). Of the patient, 15 (37.5%), nine (22.5%) and 17 (42.5%) had osteopenia findings in femoral neck, total hip and lumbar spine, respectively. A total of three male patients (7.5%), all of whom were younger than 50 years-old, had osteoporosis in the lumbar spine region.

We evaluated the differences in DEXA results according to HIV RNA and CD4+ counts in female and male patients. In terms of viral load, only BMD and T-score were significantly lower in lumbar spine in females with viral load > 100,000 IU/mL (P=0.01 and P=0.01, respectively). In terms of CD4 counts, only Z-scores in lumbar spine and femoral neck were significantly lower in women with CD4 counts > 200 cells/µl (P=0.03 and P=0.04, respectively). There were not any significant differences in any other groups and region in terms of viral load and CD4 count (Table 3).

In our study, we also investigated the effect of high viral loads (> 100000 IU/mL), low $CD4^+$ counts (< 200 cells), 25 (OH) vitamin D and cCa levels on DEXA parameters in patients with < 40 and ≥ 40 years of age. None of the factors including high viral loads, low $CD4^+$ counts, low 25 (OH) vitamin D levels and low cCa levels were directly related to T, Z-score and low BMD (Table 4).

Table 1: Baseline characteristics of the patients in the study regarding age-groups

Total n: 101	Age <40	Age ≥40	*P*-value
Patients (n)	67	34	N/A
Age (Median)	31	51	N/A
Male (n: 84; 83.1%)	56 (67.8%)	28 (%32.1)	N/A
Mean (SD)	29.1 (5.7)	52.1 (10.6)	N/A
Median	30	47	N/A
Female (n: 17; 16.8%)	11 (64.7%)	6 (35.2%)	N/A
Mean (SD)	31.4 (5.1)	56 (10.3)	N/A
Median	31	56	N/A
Body Mass Index			
Underweight or Normal (n: 28)	n:22 (88%)	n:6 (40%)	0.03
Overweight or obese (n: 12)	n:3 (12%)	n:9 (60%)	
$CD4^+$ T-cell count (cell/µl)			
Male (n: 73) Mean (SD)	421 (243)	388.3 (286)	0.608
Female (n: 17) Mean (SD)	282.2 (295)	329.7 (282)	0.781
HIV RNA (IU/mL)			
Male (n: 73) Mean (SD)	518784 (1184879)	1189756 (3744786)	0.798
Female (n: 17) Mean (SD)	151812 (234721)	62884 (50611)	0.940
Log_2 HIV RNA			
Male (n: 73) Mean (SD)	0.72 (0.03)	0.07 (0.02)	0.798
Female (n: 17) Mean (SD)	0.08 (0.03)	0.06 (0.01)	1

SD: Standard deviation, N/A: Non applicable

Table 2: Baseline laboratory results of the patients in the study

	Age <40 Mean (SD)	Age ≥40 Mean (SD)	*P*-value
25 (OH) vitamin D (ng/mL)	21.9 (11.7) (n:57)	22.9 (15.7) (n:26)	0.796
Serum Ca (mg/dL)	8.9 (0.6) (n:56)	9.3 (0.7) (n:25)	0.04
cCa (mg/dL)	8.7 (0.4) (n:56)	9.2 (0.7) (n:23)	0.006
ALP (U/L)	78.9 (46) (n: 61)	80.5 (30) (n:28)	0.868
Albumin (g/dL)	4.19 (0.6) (n:62)	4.16 (0.5) (n:25)	0.951
TSH (microIU/mL)	1.48 (0.9) (n:50)	1.35 (0.6) (n:25)	0.682
T4 (ng/dL)	0.99 (0.2) (n:38)	1.04 (0.1) (n:18)	0.522

Ca: Calcium, cCa: Corrected calcium, ALP: Alkaline phosphatase, TSH: thyroid stimulating hormone, T4: Thyroxine, SD: Standard deviation

Table 3: DEXA screening results of the patients in the study regarding sex, viral loads and CD4 counts

Region	Sex	Scores	HIV RNA (IU/mL) (n) Mean (SD) < 100,000	> 100,000	*P*-value	$CD4^+$ T (cell/µl) (n) Mean (SD) <200	≥ 200	*P*-value
Femoral neck	Female	T score	(4) 0.17 (1.2)	(4) -1.17 (0.9)	0.11	(2) 0.2 (2.4)	(6) -0.7 (0.7)	0.68
		Z score	(4) 0.35 (1.2)	(4) -0.57 (1.0)	0.28	(2) 1.20 (1.3)	(6) -0.55 (0.7)	0.04
		BMD	(4) 0.86 (0.1)	(4) 0.71 (0.1)	0.10	(2) 0.86 (0.3)	(6) 0.76 (0.1)	0.39
	Male	T score	(14) -0.49 (1.2)	(13) -0.02 (0.9)	0.28	(9) -0.9 (0.9)	(23) -0.1 (1.0)	0.05
		Z score	(14) -0.25 (1.2)	(13) 0.36 (0.9)	0.15	(9) -0.43 (1.1)	(23) 0.20 (1.0)	0.12
		BMD	(14) 0.85 (0.2)	(14) 0.85 (0.3)	0.95	(9) 0.79 (0.1)	(24) 0.87 (0.2)	0.35
Total Hip	Female	T score	(4) 0.50 (1.1)	(4) -0.92 (0.7)	0.06	(2) 0.6 (1.9)	(6) -0.4 (0.8)	0.27
		Z score	(4) 0.67 (1.0)	(4) -0.47 (1.1)	0.17	(2) 1.40 (0.9)	(6) -0.33 (0.9)	0.05
		BMD	(4) 1.00 (0.1)	(4) 0.82 (0.1)	0.06	(2) 1.01 (0.2)	(6) 0.88 (0.1)	0.26
	Male	T score	(15) -0.42 (0.8)	(14) 0.01 (0.7)	0.13	(9) -0.5 (0.1)	(25) -0.17 (0.8)	0.25
		Z score	(14) -0.35 (0.8)	(13) 0.29 (0.8)	0.05	(9) -0.20 (0.9)	(23) -0.06 (0.8)	0.71
		BMD	(14) 0.95 (0.1)	(13) 1.01 (0.1)	0.26	(9) 0.91 (0.1)	(23) 1.00 (0.1)	0.06
Lumbar spine	Female	T score	(4) 0.15 (0.9)	(4) -1.5 (0.1)	0.01	(2) 0.05 (0.2)	(6) -0.9 (0.7)	0.62
		Z score	(4) 0.45 (0.7)	(4) -0.92 (1.1)	0.08	(2) 1.15 (0.6)	(6) -0.70 (0.8)	0.03
		BMD	(4) 1.07 (0.1)	(4) 0.088 (0.0)	0.01	(2) 1.05 (0.2)	(6) 0.95 (0.1)	0.65
	Male	T score	(14) -0.70 (1.1)	(13) -0.41 (1.2)	0.52	(9) -0.4 (1.1)	(23) -0.8 (1.3)	0.38
		Z score	(14) -0.59 (1.1)	(13) -0.13 (1.3)	0.35	(9) -0.10 (1.1)	(23)-0.73 (1.4)	0.23
		BMD	(14) 0.99 (0.1)	(13) 1.04 (0.1)	0.34	(9) 1.01 (0.1)	(23) 0.98 (0.1)	0.61

SD: Standard deviation, BMD: Bone mineral density

Table 4: DEXA screening results of the patients in the study regarding age-group, viral loads and CD4 counts

Region	Age	Scores	HIV RNA (IU/mL) (n) Mean (SD)			$CD4^+$ T (cell/µl) (n) Mean (SD)			25 (OH) vitamin D (ng/mL) (n) Mean (SD)			cCa (mg/dL) (n) Mean (SD)		
			< 100,000	> 100,000	*P*-value	<200	≥ 200	*P*-value	< 21	≥21	*P*-value	Low	Normal	*P*-value
Femoral neck	<40 years	T score	(9) -0.65 (1.49	(10) -0.56 (1.1)	0.87	(8) -0.98 (0.9)	(13) -0.46 (1.3)	0.35	(10) -0.91 (1.4)	(11) -0.49 (1.0)	0.44	(3) -0.56 (0.9)	(15) -0.61 (1.3)	0.95
		Z score	(9) -0.41 (1.4)	(10) -050 (0.9)	0.52	(8) -0.37 (0.9)	(13) -0.20 (1.3)	0.75	(10) -0.63 (1.3)	(11) -0.45 (0.9)	0.27	(3) 0.06 (0.5)	(15) -0.26 (1.3)	0.68
		BMD	(9) 0.82 (0.2)	(11) 0.76 (0.3)	0.61	(8) 0.77 (0.1)	(14) 0.79 (0.3)	0.90	(10) 0.79 (0.2)	(11) 0.85 (0.1)	0.40	(3) 0.83 (0.2)	(16) 0.77 (0.3)	0.73
	≥40 years	T score	(9) -0.03 (0.9)	(7) 0.08 (0.8)	0.80	(3) 0.06 (1.7)	(16) -0.03 (0.65)	0.85	(10) -0.07 (0.8)	(8) 0.01 (0.9)	0.84	(2) 0.35 (0.5)	(10) 0.02 (0.9)	0.66
		Z score	(9) 0.17 (0.9)	(7) 0.41 (0.9)	0.62	(3) 0.50 (1.9)	(16) 0.25 (0.6)	0.84	(10) 0.21 (0.9)	(8) 0.40 (0.9)	0.66	(2) 0.70 (0.7)	(10) 0.42 (1.0)	0.72
		BMD	(9) 0.89 (0.1)	(7) 0.93 (0.1)	0.52	(3) 0.89 (0.2)	(16) 0.91 (0.1)	0.86	(10) 0.91 (0.1)	(8) 0.89 (0.1)	0.77	(2) 0.93 (0.1)	(10) 0.91 (0.1)	0.85
Total Hip	<40 years	T score	(10) -0.37 (0.9)	(11) -0.30 (0.9)	0.86	(8) -0.56 (0.7)	(15) -0.32 (1.0)	0.85	(10) -0.71 (1.0)	(12) -0.17 (0.8)	0.18	(3) -0.36 (0.5)	(17) -0.33 (0.9)	0.95
		Z score	(9) -0.32 (1.0)	(10) -0.02 (1.0)	0.53	(8) -0.22 (0.8)	(13) -0.25 (1.1)	0.95	(10) -0.60 (1.0)	(11) 0.07 (0.9)	0.13	(3) 0.13 (0.7)	(15) -0.22 (1.1)	0.60
		BMD	(9) 0.94 (0.2)	(10) 0.96 (0.2)	0.83	(8) 0.92 (0.1)	(13) 0.95 (0.2)	0.59	(10) 0.90 (0.2)	(11) 0.99 (0.1)	0.20	(3) 0.95 (0.1)	(15) 0.95 (0.2)	0.99
	≥40 years	T score	(9) -0.07 (0.9)	(7) -0.02 (0.6)	0.90	(3) 0.30 (1.4)	(16) -0.15 (0.6)	0.56	(10) -0.11 (0.6)	(8) -0.10 (0.9)	0.97	(2) -0.20 (0.7)	(10) 0.02 (0.9)	0.75
		Z score	(9) 0.06 (0.9)	(7) 0.30 (0.7)	0.60	(3) 0.93 (1.5)	(16) -0.01 (0.6)	0.39	(10) 0.00 (0.6)	(8) 0.27 (1.0)	0.51	(2) 0.00 (0.7)	(10) 0.28 (1.0)	0.72
		BMD	(9) 0.99 (0.1)	(7) 0.98 (0.1)	0.97	(3) 0.94 (0.2)	(16) 0.99 (0.1)	0.74	(10) 1.00 (0.1)	(8) 0.95 (0.1)	0.32	(2) 0.96 (0.2)	(10) 0.99 (0.1)	0.84
Lumbar spine	<40 years	T score	(9) -0.36 (1.0)	(10) -0.95 (1.2)	0.29	(8) -0.55 (1.2)	(13) -1.01 (1.3)	0.42	(10) -1.20 (0.9)	(11) -0.77 (1.4)	0.43	(3) -1.46 (0.1)	(15) -0.76 (1.3)	0.06
		Z score	(9) -0.18 (1.0)	(10) -0.53 (1.5)	0.57	(8) -0.50 (1.1)	(13) -0.87 (1.4)	0.18	(10) -1.01 (0.9)	(11) -0.40 (1.5)	0.31	(3) -0.76 (1.2)	(15) -0.53 (1.4)	0.80
		BMD	(9) 1.01 (0.13)	(10) 0.97 (0.14)	0.49	(8) 0.99 (0.1)	(13) 0.95 (0.2)	0.59	(10) 0.91 (0.1)	(11) 0.99 (0.2)	0.22	(3) 0.91 (0.0)	(15) 0.97 (0.2)	0.58
	≥40 years	T score	(9) -0.66 (1.2)	(7) -0.27 (0.8)	0.48	(3) 0.23 (1.20)	(16) -0.75 (1.12)	0.18	(10) -0.54 (0.9)	(8) -0.8 (1.4)	0.65	(2) -0.40 (1.7)	(10) -0.57 (1.2)	0.87
		Z score	(9) -0.53 (1.2)	(7) -0.02 (0.9)	0.40	(3) 0.60 (1.2)	(16) -0.60 (1.2)	0.12	(10) -0.42 (1.0)	(8) -0.51 (1.5)	0.88	(2) -0.25 (1.8)	(10) -0.35 (1.3)	0.93
		BMD	(9) 1.01 (0.1)	(7) 1.06 (0.1)	0.40	(3) 1.10 (0.1)	(16) 1.0 (0.1)	0.22	(10) 1.02 (0.1)	(8) 0.99 (0.2)	0.51	(2) 1.03 (0.2)	(10) 1.01 (0.1)	0.89

SD: Standard deviation, BMD: Bone mineral density

Discussion

HIV is a multisystemic disease with various complications depending on the affected system. Reduction in BMD is one of the most common complications and emerges as a result of both HIV infection and antiretroviral treatment [1].

Weight loss and low BMI are common signs in HIV-infected patients. However, none of the patients except for two women had low BMI in our study, and mean BMI was 23.7 (3.6) kg/m2 which was within the normal range. According to the study results of Gumuser et al. [8] including 72 naive HIV-infected patients, four patients had underweight, four were obese, and 64 patients had normal or overweight. Aydin et al. [9] revealed a BMI of 24.9 (3.7) kg/m2 in 126 HIV cases in Turkey. Vlot et al. [10] showed that the mean BMI of the patients in their study was 23.4. Cotter et al. [4] revealed that BMI in 210 naive HIV-infected patients was 26 kg/m2. Majority of the patients with HIV infection were normal or over-weighted in studies. Excluding the late presenters, we can conclude that patients with HIV infection do not have any difference from normal population in terms of body weight.

Vitamin D3 is required for the continuity of bone tissue, and its effects on bone structure are carried out through calcitriol (1,25 dihydroxyvitamin D) [11]. The rates for vitamin D deficiency in HIV positive patients vary between 60-90% in literature [12, 13]. In our study, the ratio of patients with vitamin D deficiency and insufficiency in all cases was 50.6% and 38.5%, respectively; total ratio was 89.1%. According to the results of Gumuser et al. [8] from Turkey, the ratio of vitamin D deficiency was 74.6% and the ratio for insufficiency was 14.5%, while the ratio of vitamin D deficiency and insufficiency was 14.6% and 68.8% in the study of Aydin et al. [9].

Dao et al. [13] from the United States revealed a vitamin D deficiency or insufficiency ratio of 70.3% in their observational cohort study including 673 HIV-infected patients, and the ratio of deficiency in the study of Gedela et al. [14] from England was 58.5%. However, the ratio of vitamin D deficiency in the study of Canuto et al. [15] from Brazil was remarkably the lowest in the literature with the rate of 1.6%; the low rate was attributed to the fact that the patients were exposed to sunlight for a long time because of long summer season in Brazil and very low rate of use of sunscreen. The high rate of osteopenia and osteoporosis in our study, despite the fact that Turkey has a long sunny summer season, may be related to the fact that patients were not staying in the sun enough and not wearing clothing that leaves their skin exposed.

Calcium is stored in the body at a rate of 99% combined with phosphate in the bone and is bound to plasma proteins at a rate of 40% [16]. Eight patients in our study had hypocalcemia, and one patient had mild hypercalcemia. Considering the low levels plasma proteins, especially low levels of albumin, in HIV-AIDS cases, cCa values should be calculated. In our study, all of the patients including eight patients with hypocalcemia had cCa levels within the normal range. It should be kept in mind that serum calcium levels may mislead clinicians for patients with low muscle mass, with metabolic disease, with impaired absorption or with AIDS; and cCa levels should be taken into consideration in such kind of patients.

Many factors may cause low BMD in patients with HIV infection. In vitro studies have shown that gp120 in the HIV virus structure increases the activity of osteoclasts, suppresses the function of osteoblasts, and also decreases ALP activity and calcium deposition by increasing primary apoptosis [11,17]. It is recommended to take calcium supplement and vitamin D to improve bone mass, especially in elderly individuals [16]. However, in our study, serum Ca and cCa levels were slightly lower in patients with younger-ages than the older, but the difference was not statistically significant. The results could be different if the age limit considered as 60 years of age instead of 40, but we did not have enough cases to design such a study (>60

age, n: 7). A more comprehensive study is needed to provide clearer conclusions.

ALP levels in our study, one of the bone turnover markers (BTMs), were also analyzed retrospectively. High levels of ALP were detected in only eight cases (7.9%) and there was no significant difference in ALP levels between both age groups. According to the study of Cotter et al. [4] including 210 cases, median ALP was 78 IU/L. And median ALP level was 66 U/L in the study of Vlot et al. [10]. These results were similar to our results.

Since BTMs reflect dynamic and short-term changes, DEXA is considered to be the gold standard for determining osteoporosis by providing to predict changes in BMD over the years [1]. The American National Osteoporosis Foundation recommends DEXA screening for all women over 65 years-old and for men over ≥ 70 years-old. The screening for individuals with HIV infection is recommended for postmenopausal women and for males over 50 years-old, however it is stated that the cost-effectiveness of this approach is not yet determined [18]. There is a contradiction standing out between these screening suggestions and our practices. According to the DEXA results in our study, the rate of osteopenia and osteoporosis were 47.5% and 7.5%, respectively. And the osteopenia was frequently seen in the lumber spine region while the minimum ratio of osteopenia or osteoporosis was in the hip. Only two of the patients with osteopenia had the findings in the regions other than lumbar spine. Hence, we may conclude that bone loss occurs first and highest in the lumbar spine region in both male and female cases. Studies in literature including HIV-infected male patients revealed that the ratio of osteopenia varied between 40% and 60% and the ratio of osteoporosis varied between 12% and 23% [19-22]. Similar to our study results, the ratio of osteopenia was 44.4%, while the ratio of osteoporosis was 11% in the study of Vlot et al. [10] including naive patients with a mean age of 39. The ratio of osteopenia in our study was similar to the results in literature, while the ratio for osteoporosis was lower than the results in literature. A meta-analysis including 10 studies with naïve patients and with a mean age of 31-44 years revealed 12 to 62.5% reduction in BMD [23]. When compared to non-HIV group, the ratio of osteopenia and osteoporosis were 6.4 and 3.6 fold higher in HIV-infected group according to the meta-analysis, respectively. So, even in a young-aged male predominant study group, osteopenia was common and there was a non-negligible risk of osteoporosis in the horizon in this group. Hence, these results may influence on the decision of antiretroviral treatment (ART) and such patients may require a prophylactic approach in terms of bone health.

One of the primary aims of this study was to search for the effect of viral loads, $CD4^+$ count, 25 (OH) vitamin D and cCa levels on the development of osteopenia and osteoporosis in HIV-infected cases. We revealed that only one of the factors including high viral load, low $CD4^+$ count, low 25 (OH) vitamin D level or low cCa levels were not directly related to T, Z-score and low BMD. Santi et al. [24] revealed in 1204 male patients infected with HIV that osteopenia ratio was 63.2%, osteoporosis ratio was 15.1% and 25 (OH) vitamin D deficiency ratio was 60.1%; these results were parallel with but slightly higher than our results. And similarly with our results, they also revealed that the relationship between viral load, $CD4^+$ count, 25 (OH) vitamin D and Ca levels and BMD were weak.

Relatively small size of the numbers of the patients in the study groups may have affected the statistical significance negatively; hence this may be considered as the limitation of the study.

Conclusion

As a result; osteopenia and osteoporosis are observed more frequently and at younger ages in HIV-infected patients than in the general population. Since we cannot make any prediction on bone health using one of the indirect markers in serum including viral load, CD4 count, 25 (OH) vitamin D levels or Ca levels in HIV-infected patients, BMD screening at younger ages may be beneficial. Thus, initial ART regimen can be determined considering the risk factors in terms of bone health. And also we believe that bone health can be better protected in HIV positive patients by proper recommendations like life style change or by providing vitamin D and calcium supplements in the early period if necessary.

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