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Antiretroviral therapy and bone mineral measurements in HIV-infected youths

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ABSTRACT

Reduced bone mass measurements are often found in HIV-infected youths. Both in vitro and human studies demonstrated a role of antiretroviral treatment in determining bone mass alteration. Nevertheless, the data regarding the responsibility of different antiretroviral drugs on bone health in children and adolescents are highly controversial. The purpose of the current study was to relate antiretroviral treatment to bone mass measurements in a large cohort of HIV-infected children and adolescents. Bone mineral content (BMC) was measured in 86 HIV-infected youths (aged 4.8–22.1 years), and in 194 healthy controls (aged 4.9–21.9 years). Fifteen patients were naive to antiretroviral treatment, 11 were receiving a dual nucleoside reverse transcriptase inhibitor (NRTIs) combination, 32 a protease inhibitor (PI)-based antiretroviral treatment, and 28 a non-nucleoside reverse transcriptase inhibitor (NNRTIs)-based regimen. Comparisons between healthy and HIV-infected children and adolescents have been performed by multiple regression analyses to correct for differences in age, sex, and anthropometric measurements. Patients receiving a PI-based treatment had lumbar spine and whole body BMC values significantly lower than healthy children ($P<0.05$). BMC measurements of patients on other therapeutic regimens or naive to antiretroviral treatment did not differ significantly from those of healthy children. Among patients receiving a PI-based regimen, those receiving full dose Ritonavir had significantly lower lumbar spine BMC values compared to other patients. Lumbar spine and whole body BMC measurements of patients receiving a Stavudine-containing regimen were lower compared to healthy controls, naive patients, and patients on other antiretroviral regimens. Multivariate analyses showed that patients receiving both Stavudine and full dose Ritonavir had significantly lower BMC values both at the lumbar spine ($P=0.0033$), and in the whole skeleton ($P=0.05$). In conclusion, antiretroviral treatment may have a detrimental effect on bone health of HIV-infected youths: the use of Ritonavir full dose alone or in combination with Stavudine is associated to lower bone mass measurements. The use of antiretroviral regimens including these drugs should thus be monitored closely in HIV-infected youths.

Introduction

Human immunodeficiency virus (HIV) infects many cell types in the body, leading to diverse immunologic and metabolic effects. Much attention has been deserved to the complications related to the use of potent antiretroviral treatment in HIV-infected patients: the main focus has been on metabolic disorders associated with cardiovascular disease such as insulin resistance, dyslipidemia, and abnormal fat redistribution [1,2]. However, with the increasing life expectancy related to the new therapies, disease of bone and mineral metabolism is becoming increasingly apparent [3].

The current data on skeletal health of HIV-infected children and adolescents indicate a reduced bone mass and an altered bone metabolism rate [4–11]. The causes of impaired skeletal health are currently largely unclear. A still open issue is the role of antiretroviral treatment in the genesis of poor bone health in HIV-infected youths. The available studies are not aimed to the investigation of a possible relationship between the type of treatment and bone mass measurements. The aim of the present study was therefore to assess the role of different antiretroviral treatments on skeletal health in a cohort of HIV-infected children and adolescents.

Subjects and methods

Subjects

Eligible for this cross-sectional study were HIV-infected youths on long-term highly active antiretroviral treatment and HIV-infected children naive to antiretroviral treatment. We studied 86 HIV-infected patients (47 girls), aged from 4.8 to 22.1 years. Eighty-two patients were

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vertically infected, while 4 were horizontally infected in the first year of life. Recruitment started in December 2002, and the last patient was studied in May 2008. In relation to antiretroviral treatment, 15 patients were naive to antiretroviral treatment, 11 were receiving a dual nucleoside reverse transcriptase inhibitor (NRTI) combination, 32 were receiving a protease inhibitor (PI)-based treatment, and 28 were receiving a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based antiretroviral therapy. The NNRTI was Efavirenz in all cases. The characteristics of the patients are shown in Table 1.

As a control group, we studied 194 healthy controls (90 girls and 104 boys) of comparable age (4.9–21.9 years). None had a history of endocrine, nutritional, growth or renal problems. Their weight ranged from 17.5 to 100 kg, height form 109.4 to 186.0 cm, and BMI from 12.4 to 29.3 kg/m^2.

Informed consent was obtained from each patient's and healthy controls legal guardian and from the patients and healthy controls when appropriate, before enrolment. The study was approved by the ethical committee of the L. Sacco Hospital.

Clinical and anthropometric assessment

All subjects enrolled in this study underwent physical examination to obtain anthropometric measures. Body weight was measured to the nearest 0.1 kg on a balance beam scale (Seca, Hamburg, Germany) and height was measured to the nearest millimeter using a wall-mounted stadiometer (Holtain Ltd., Crosswell, U.K.). Body mass index (BMI) was then calculated as weight on height^2 (kg/m^2). Standard deviation scores of anthropometric measurements were calculated using specific Italian standards [12]. Pubertal stage of HIV-infected patients and healthy controls was defined according to Tanner criteria [13].

Bone mineral measurements

Bone mineral content (BMC) was measured at the L2–L4 vertebrae level and in the whole skeleton. The data were analyzed with proper pediatric software (version 1.5 h). BMC measurements were obtained with a dual-energy X-ray absorptiometer (DPX-L®, GE-Lunar Radiation Corp., Madison, WI). The instrument was calibrated on a daily basis according to the manufacturer's instructions. Reproducibility was calculated as coefficient of variation (CV) by weekly measurements of a standard phantom on the instrument, and by repeated measurements obtained in children of different ages. The CV of our instrument is 0.6% with the standard phantom; in vivo we calculated a CV of 1.4% for the lumbar spine and 1.5% for the whole skeleton.

Bone mineral measurements are expressed as BMC (in grams), and as areal bone mineral density (aBMD) *z*-scores. *Z*-scores were calculated using the DXA manufacturer's database.

Biochemical analyses

Blood was drawn between 08:00 and 10:00 h after an overnight fast for the determination of serum bone specific alkaline phosphatase (BAP) concentration. Each sample was allowed to clot, and serum was separated by centrifugation, aliquoted and stored at −80 °C until analysis. Urine specimens were collected between 10:00 and 12:00 h as the second voiding of the day, to minimize the effect of circadian rhythm of excretion of collagen degradation products [14]. Samples were aliquoted immediately and stored at −80 °C until analysis.

BAP is a specific marker of bone formation, and its concentration was measured using a commercial immunoassay (Metra™ BAP EIA kit, Quidel Corp., San Diego, CA). Intra-assay reproducibility in our laboratory was less than 4%, and inter-assay variation was less than 7%. Sensitivity of the assay was 0.7 U/L.

Bone resorption rate was assessed by measurements in urine of N-terminal telopeptide of type I collagen (NTx). NTx was measured using an enzyme-immunosorbent assay (Osteomark® NTx Urine, Wampole Laboratories®, Princeton, NJ). Assay values were standardized to an equivalent amount of bone collagen, and were expressed in nanomoles bone collagen equivalents (BCE) per liter (nmol BCE/L). The sample results from a single urine collection were normalized for urine dilution by urine creatinine analysis, and were reported as nmol BCE/mmol creatinine. In our laboratory, the intra-assay variation was less than 10%. The inter-assay precision was less than 9%, and sensitivity was 20 nmol BCE/L.

Table 1
Characteristics of the 86 HIV-infected children and adolescents grouped according to the antiretroviral regimen.[a]

Variable	Antiretroviral therapy naive	Dual NRTI[b]	PI-based treatment[c]	NNRTI-based treatment[d]	Healthy controls
Girls (*n*)	10	7	17	13	90
Age (year)	10.9 (1.6)	10.3 (1.5)	14.3 (0.8)	15.5 (0.8)	13.0 (0.5)
Weight (kg)	35.6 (5.2)	39.3 (7.0)	44.9 (2.9)	49.4 (2.1)	42.8 (1.6)
Height (cm)	136.1 (7.7)	133.6 (8.8)	148.9 (2.9)	157.2 (2.3)	146.0 (1.9)
BMI (kg/m^2)	18.1 (0.9)	20.5 (1.5)	19.8 (0.8)	19.9 (0.6)	19.3 (0.3)
CDC clinical	**A** 3, **B** 2, **C** 0, **N** 5	**A** 2, **B** 2, **C** 1, **N** 2	**A** 4, **B** 10, **C** 3, **N** 0	**A** 7, **B** 3, **C** 3, **N** 0	
CDC immunol	**1** 1, **2** 6, **3** 3	**1** 3, **2** 3, **3** 1	**1** 5, **2** 5, **3** 7	**1** 4, **2** 5, **3** 4	
CD4 (*n*)	267 (94–710)	875 (483–1305)	840 (289–1798)	863 (411–1121)	
CD4 (%)	15.8 (5.9–28.3)	30.2 (18.7–45.0)	34.6 (15.6–49.4)	38.2 (27.3–49.8)	
HIV-RNA	1429 (266–250,155)	49 (49–72,224)	49 (49–1760)	49 (49–173)	
ARV duration (month)		62.6 (9.3)	39.2 (6.0)	43.2 (3.6)	
Boys (*n*)	5	4	15	15	104
Age (year)	13.1 (2.5)	13.5 (3.2)	15.1 (0.8)	15.8 (1.1)	13.0 (0.4)
Weight (kg)	39.6 (8.1)	50.9 (12.7)	54.4 (3.8)	52.5 (4.0)	41.8 (1.9)
Height (cm)	145.8 (12.1)	153.8 (14.8)	160.3 (4.1)	159.9 (3.5)	146.1 (2.2)
BMI (kg/m^2)	17.5 (1.1)	20.1 (2.2)	20.7 (0.7)	20.0 (0.8)	18.4 (0.3)
CDC clinical	**A** 3, **B** 1, **C** 0, **N** 1	**A** 2, **B** 2, **C** 0, **N** 0	**A** 5, **B** 2, **C** 8, **N** 0	**A** 2, **B** 4, **C** 8, **N** 1	
CDC immunol	**1** 2, **2** 1, **3** 2	**1** 1, **2** 1, **3** 2	**1** 1, **2** 7, **3** 7	**1** 4, **2** 3, **3** 8	
CD4 (*n*)	491 (15–902)	417 (359–736)	845 (303–1528)	796 (283–38,647)	
CD4 (%)	12.5 (2.1–35.5)	17.2 (16.9–24.5)	27.5 (15.3–44.3)	32.8 (23.3–46.3)	
HIV-RNA	51424 (4525–500,000)	7424 (1666–95,652)	49 (49–569)	49 (49–37,317)	
ARV duration (month)		43.3 (3.8)	51.2 (7.9)	40.9 (5.1)	

[a] Data are presented as mean (SE), or median (min-max).
[b] NRTI: nucleoside reverse transcriptase inhibitor.
[c] PI: protease inhibitor.
[d] NNRTI: non-nucleoside reverse transcriptase inhibitor; NNRTI-based treatment included Efavirenz.