Case report

Study on changes in bone metabolism in a cohort of HIV-infected pregnant women and their uninfected children

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Summary

Osteopenia and osteoporosis are frequent in HIV-infected patients, because of disease itself and therapy. This study evaluated the incidence of bone disorders in 18 infected pregnant women treated with antiretroviral drugs and in their uninfected children.
Biochemical markers of bone metabolism were obtained every three months in pregnant women and at 1, 6 and 12 month of age in children. Serum levels of calcium, inorganic phosphate, bone specific alkaline phosphatase were collected; moreover serum levels of osteocalcin and urinary CTX concentrations were evaluated as bone synthesis and bone resorption index respectively. Ultrasonographic bone densitometry was performed in women once in pregnancy and in children at 1, 6, 12 months of age. In order to critically analyze these data, a normal line was generated from data obtained from a cohort of 80 italian children aged 0-12 months: this is the first study ever performed on newborns and infants, in the aim to identify the normal values of reference in this cluster of age.
Lower bone density values, compared with control subjects, were detected in 7/18 women (38.8%): osteopenia was found in 3 women and osteoporosis in 4. Normal serum levels of osteocalcin were detected in osteopenic patients while the serum concentrations were low (< 2.5 ng/ml) all over pregnancy in the four cases of osteoporosis. High CTX urinary concentration was observed in 3 cases of osteoporosis.
In just 2 children (born to women who had started an antiretroviral therapy with PIs before conception and have been treated for more than 30 months) low bone density was diagnosed by ultrasonographic densitometry at the first month, but it was found normal both at 6 and 12 months of age. Only in these two cases high CTX urinary concentration was observed at the first month, and was found normal at the following evaluations.
The pathogenesis of low bone density observed in pregnant women is multifactorial, involving HIV- and HAART- related factors. Bone metabolism resulted normal in most children perhaps because of the lack of HIV infection.

KEY WORDS: HIV, HAART, bone metabolism, bone density, pregnancy, children.

Introduction

In both HIV-infected adults and children skeletal abnormalities, including decreased bone mineral content and bone mineral density are frequently reported (1-4), although the mechanisms of the pathogenesis of these alterations have not completely assessed.
Several studies have concluded that, at least in infected adults, bone disorders are strongly associated with HIV itself that can infect osteoblasts or indirectly alter osteoclast and osteoblast function through T-cell activation and increased production of bone-resorbing cytokines like IL-1, IL-6 and TNF-alfa (5-7).
Besides, the duration of infection, often causing physical inactivity and poor nutrition, can contribute to decrease bone mineral content and increase bone turnover in HIV-infected patients (8-11).
On the other hand, in HAART (Highly Active Antiretroviral Therapy) era many reports on bone disorders in HIV-infected adults and all of those in children suggest that prolonged administration of protease inhibitor (PI)-containing regimens plays a role in the pathogenesis of abnormal bone metabolism, by inducing osteoclast and osteoblast dysfunction.
With regard to HIV-infected HAART-treated pregnant women, the evolution of bone mineralization and metabolism has not thoroughly studied yet.
The lack of data about the bone changes occurring over time in these subjects and about the risk of consequences in their newborns and infants (infected or not) who are exposed "in utero" to the same antiretroviral drugs prompted us to study the bone metabolism in a cohort of HIV-infected pregnant women treated with different therapeutic protocols and in their children, followed from birth to the end of the first year of life with bone mineral measurements and with serial controls of biochemical markers of bone turnover.

Patients and methods

We evaluated the incidence of bone disorders in 18 HIV-infected pregnant women aged 25-39 years (mean 30 years) and in their 18 children (10 males, 8 females) followed from 0 to 12 months of age at the Department of Infectious Diseases in collaboration with the Department of Orthopaedics and Traumatology, "Città di Pavia" Institute, of the University of Pavia.
The control group of pregnant women included 20 white subjects of the same age, healthy and physically active; none of them had an history of chronic illness or was regularly treated with hormone therapy, vitamin supplement or calcium; the con-

trol group of children included 80 Italian healthy males and females aged from 0 to 12 months.
The characteristics of the pregnant women are shown in Table I.
In 14 cases the pregnancy lasted 38 weeks and an elective caesarean section was performed; 4 women delivered prematurely (after 32-36 weeks of pregnancy) by emergency caesarean section.
Biochemical markers of bone metabolism were obtained every three months in pregnant women and at 1, 6 and 12 months of age in children.
The following metabolic parameters were collected: serum levels of calcium, inorganic phosphate, bone specific alkaline phosphatase, serum levels of osteocalcin (evaluated as bone synthesis index), urinary CTX (C-terminal telopeptide of type 1-collagen) concentration (evaluated as bone resorption index).
Blood was collected by venipuncture and serum samples, separated by centrifugation, were evaluated at once. Urine samples were collected from the second voiding of the day, with the aim of standardizing the results by reducing the effect of circadian rhythm of CTX urinary elimination; the samples were stored at -30°C until analysis effected by an enzyme-immunosorbent assay.
CTX is an eight aminoacid sequence found on the C-terminal end of type 1-collagen. When the collagen molecules are broken down, the presence of this sequence specifically indicates bone breakdown. The rate of bone resorption is proportional to the urinary level of CTX. This parameter is a reliable index of degradation and not of biosynthesis of molecules deriving from collagen.
In this study, urinary excretion of these breakdown products of type 1-collagen was determined by the Osteosal (Provalis Ltd, Deeside, UK) quantitative immunochromatographic assay, which employes high affinity monoclonal antibodies specific for CTX. All determinations were corrected for creatinine.
Osteosal results are expressed as a T-score, similarly to the bone density measurements. A population of 200 pre-menopausal women were tested with Osteosal to assess their CTX levels; a mean CTX value was calculated for this population and was indicated as T-score of 0. The standard deviation was also calculated and this value was indicated as T-score of 1. Osteosal T-scores are defined as the number of standard deviations from the mean of a normal pre-menopausal population (12).
A positive or negative T-score is equivalent to a higher or lower rate of bone turnover. It is to be emphasized that the bone density T-score increases as bone density increases, while the Osteosal T-score decreases as the rate of bone turnover decreases.
Bone mineral density (BMD) was measured by ultrasonographic densitometry with an Omnisense device (Sunlight Technologies, Rehovot, Israel), performed once in pregnancy in women and at 1-6-12 months of age in children. This device measures non-invasively the velocity of ultrasound waves (speed of sound, SOS, in m/sec) propagating along the bone.
The ultrasonographic densitometry has been chosen as diagnostic method because of its high sensitivity and its safety, allowing to use it even more than once in pregnant women, in newborns and infants. Moreover, Omnisense device allows to perform a "multisite" bone density measurement that result in a more accurate diagnosis. In addition, Omnisense is a small, lightweight and easy to handle device, and their results are immediately available.
In women measurements were performed at proximal phalanx of the medium finger, at tibia and at distal radius, while in newborns and infants tibia site only was considered, due to the bone size. According to the criteria established by the WHO for the diagnosis of osteoporosis, in this study patients with a T-score between +1 and –1 SD from the mean of healthy young adults were considered as normal, patients with a T-score between –1 and –2,5 SD were consideres as osteopenic, patients with a T-score under –2,5 SD were considered as osteoporotic.
In order to critically interpret the sonographic results of the children included in our study, we have for the first time drawn a diagram of reference with the data obtained by the same device in the control group.
Speed of sound values, indicatory of bone density, obtained in children born to HIV-infected women, were compared with those obtained by means of the same device in a control group of 80 italian healthy newborns and infants aged 0-12 months.
A detailed reference curve for this age interval is not provided by the Omnisense device: only a "general" pediatric reference curve from 0 to 21 years is provided, without making any distinction in months inside the first year of age. Therefore, an Italian diagram of reference for this age, based on the values obtained in the control group, was drawn for the first time.
The availability of this diagram (taking into account the eating habits of Italian newborns and infants) allowed us to make an accurate diagnosis of bone density alterations in children included in this cluster of age. We could use the diagram of reference also for black children, because their mothers had lived in our country for years and were keeping the alimentary habits of italian people. Two diagrams (for males and females) were obtained. In each diagram 3 lines are shown: the central line indicates the means of the measured SOS values in the control group, while the dotted lines show the standard deviations (+1 and –1 SD respectively).
The female diagram shows a tendency to increase until the 3rd month, followed by a decrease with a minimum peak at the 4th month, a definite increase of the values until the 6th month and a further increase from the 6th month on. The male diagram is quite different, with a decrease of the values from the birth to the 4th month and a progressive increase in the following period.
Values obtained from children born to HIV-infected women,

Table I - Characteristics of 18 HIV-infected pregnant women.

Age	26-39 years	
Race	White	12
	African	5
	South-American	1
Clinical stage	Asymptomatic	17
	AIDS	1*
CD4+ cells/mm^3 (medium level)	> 500	3
	> 200 < 500	14
	< 200	1
Antiretroviral therapy during pregnancy**	ZDV or ZDV/3TC	5
	NRTIs (2) + PI (1 or 2)	7
	NRTIs (2) + NVP	6

* AIDS was diagnosed three years before the pregnancy, because of Pneumocystis carinii pneumonia and CMV disseminated infection.
** 7/18 women had started the antiretroviral therapy before the conception and kept on taking the same drugs during pregnancy; the others started the therapy from the second trimester of pregnancy.

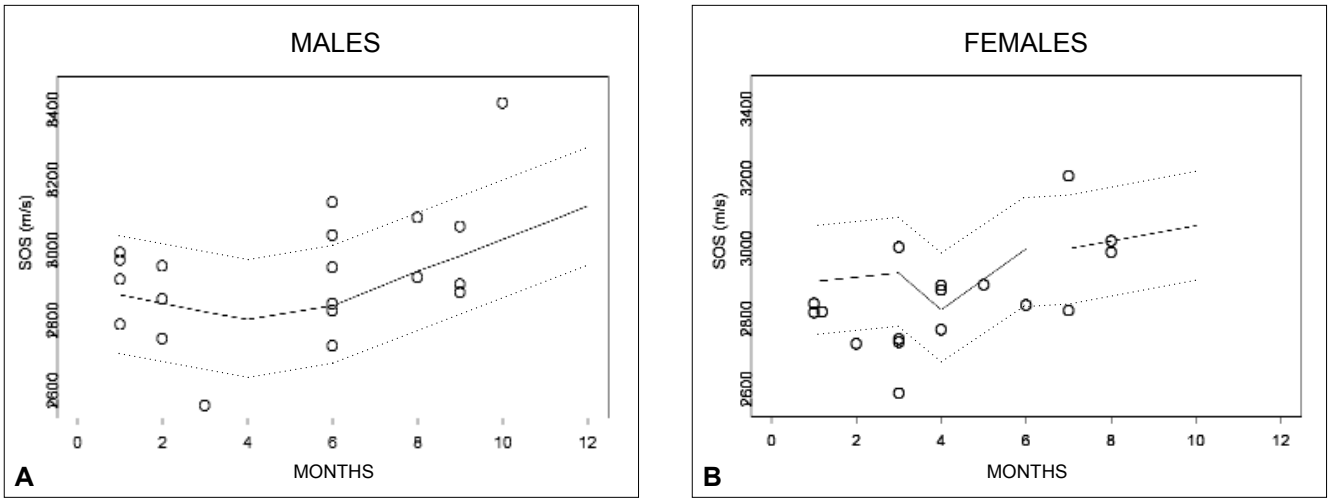

Figure 1 A, B - Male and female diagrams showing the means of the measured SOS values in the control group (central continuous line) with +1 and –1 SD (dotted lines), and values obtained from children born to HIV-infected women (circles).

placed above the lower dotted line were considered as normal; values placed below this line were considered as index of osteopenia (Fig. 1 A,B).
With regard to HIV infection follow-up in pregnant women, CD4+ lymphocytes and HIV viral load (HIV-RNA) were monthly evaluated.
Infants were said to be HIV uninfected either if two negative test results (by PCR or bDNA test) were available with the latest being at 2 months of age or later or if one or more negative test results were available at 3 months of age or later.

Results

Both in pregnant women and in children the serum levels of calcium, inorganic phosphate and bone specific alkaline phosphatase were always normal.
By ultrasonographic densitometry lower bone density values, compared with control subjects, were detected in 7/18 women (38.8%).
Osteopenia was found in 3 women and osteoporosis in 4: normal serum levels of osteocalcin were detected in osteopenic patients while the serum concentrations were low (< 2.5 ng/ml) all over pregnancy in the four cases of osteoporosis.
High CTX urinary concentration (more than +2 SD higher T-score) was observed in 3 cases of osteoporosis.
With regard to antiretroviral therapy, 6/7 women with low BMD have been treated for at least two years before the conception with different HAART protocols including the following PIs: Nelfinavir (NFV) (3 cases), Indinavir (IDV) (1 case), Lopinavir/Ritonavir (LPV/RTV) (2 cases).
No relation was observed between BMD alterations and degree of immunodepletion.
In 11/18 pregnant women BMD resulted constantly normal, with normal serum levels of osteocalcin and CTX urinary concentration in 8 of them.
These patients had started antiretroviral therapy during pregnancy: 4 were treated with PIs while the others were just receiving two NRTIs either combined or not with NVP.
In all children the results of biochemical measurements performed on blood and urine samples during the first year of life to evaluate the bone turnover were similar to those of healthy subjects.
In the 11 children born to mothers with normal bone mass during pregnancy, the ultrasonographic data, evaluated in comparison with the diagram of reference, resulted normal all over the first year of life.
In just 2 of the 7 remaining children (born to women who had started an antiretroviral therapy with PIs before conception and have been treated for more than 30 months) osteopenia and, respectively, osteoporosis were diagnosed by ultrasonographic densitometry at the first month of life, but their bone density was found normal both at 6 and 12 months of age.
Only in these two cases high CTX urinary concentration (more than +1,5 and +2 SD higher T-score respectively) was observed at the first month of life; this parameter was found normal at the following evaluations.
No cases of vertically transmitted HIV infection were detected.

Discussion

Although there is evidence for bone metabolism alterations in adult and young patients treated with HAART, data are still lacking with regard to HIV-infected pregnant women, who more and more frequently receive antiretroviral drugs not only to reduce the HIV vertical transmission rate but also to improve the virological and immunological parameters of infection (9, 13, 14).
The incidence of osteopenia and osteoporosis in healthy pregnant women is unclear, because many other elements (such as increase in weight, smoke, alcohol, physical activity), in addition to pregnancy, can influence the bone mass in this period. Longitudinal studies demonstrated that, during pregnancy and breast feeding, a loss of bone mass > 5% can occur, but this loss is reversible (15, 16).
In our cohort of pregnant women the ultrasonographic densitometry proved to be the most effective test for an early diagnosis of bone turnover alterations: by means of this technique we were able to demonstrate osteopenia and osteoporosis in a high number of cases in spite of normal serum indexes of bone formation and resorption like calcium, inorganic phosphate and bone specific alkaline phosphatase. Besides, our data show that just in a few cases serum level of osteocalcin and CTX urinary concentration can be considered like sensitive markers of increased bone synthesis or bone resorption.

The high incidence of osteopenia and osteoporosis found in the women of our cohort seems to be associated with the role of PIs (particularly of NFV, IDV and RTV) in the pathogenesis of bone loss frequently described in HIV-infected adults (1, 17).
We can moreover speculate that the duration of PIs administration can also have effect on the bone metabolism not only in women but also in newborns exposed "in utero" to the same drugs, because in our cases we observed that a higher rate of low bone mass in pregnancy corresponded to a longer duration of PIs administration (at least 24 months) and that the longest duration of maternal treatment (> 30 months) was related to a higher risk of osteopenia and osteoporosis in newborns.
We could rightly interpret the results of ultrasonographic densitometry in the followed newborns and infants because for the first time we were able to use the diagram of reference drawn by us on the basis of the results obtained among the control group of paediatric patients aged from 0 to 12 months.
By this way osteopenia and osteoporosis respectively were diagnosed at birth in two infants; nevertheless bone mass resulted normal in both of them at 6 and 12 months of age, most likely because the lack of HIV-infection strongly protected them against prolonged side effects of maternal antiretroviral therapies.
To confirm this hypothesis, prospective studies about higher number of children both HIV-infected and uninfected born to women treated with highly active combination of antiretroviral drugs during pregnancy are needed to definitely establish the effects of vertically acquired infection and of perinatal exposure to antiretroviral drugs on bone metabolic rate.
As more and more cases of osteonecrosis and fractures among patients receiving antiretroviral drugs are being reported, it is important to assess therapeutic protocols and management programs to minimize from childhood and adolescence the risks of skeletal deformities and pathological fractures and to protect also HIV-infected pregnant women against this metabolic complication of both infection and therapy (1, 18-21).

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