Edem Binka
Doris Duke Fellow 2011-2012

Vitamin D levels in severely malnourished HIV-infected children initiating HAART (early versus delayed) in Durban, South Africa

1. Study Purpose and Rationale

Studies on the skeletal health of HIV-infected children in resource rich settings have demonstrated lower bone mass densities and an altered bone metabolism rate compared to healthy controls. HIV-infected children have risk factors such as suboptimal dietary intake and lack of physical activity that predisposes them to poor bone health. HIV infection and the toxicities of antiretroviral drugs have also been implicated in adversely affecting bone health. There is a lack of data describing the bone health of HIV-infected children in resource poor settings. As the number of HIV-infected children growing into adulthood increases, a better understanding of the effect of HIV infection and antiretroviral therapy on bone health is imperative in order to deal with aging-related diseases such as osteoporosis and fractures.

Background:

Studies on the skeletal health of HIV-infected children have demonstrated lower bone mass densities and an altered bone metabolism rate compared to healthy controls (2, 3, 5). Risk factors such as lack of physical activity, suboptimal dietary intake of important nutrients, malabsorption, weight loss and delayed puberty may predispose HIV-infected children to suboptimal levels of peak bone mass (4). HIV infection, in itself, has been shown to also have a direct effect on the bone health of HIV-infected children. Serum levels of RANKL, a cytokine which stimulates osteoclast activity, are higher in HIV-infected children as a result of increased release of RANKL by T cells exposed to soluble HIV-1 envelope glycoprotein gp120 (5, 13). This leads to increased bone resorption and consequently reduced bone mass densities in HIV-infected children (11, 12).

Antiretroviral therapy has also been implicated in adversely affecting bone health in HIV-infected children. In general, reports have indicated lower bone mass densities and increased rate of bone turnover were present in HAART-treated children compared to healthy controls and HIV-infected children naïve to antiretroviral therapy (6, 8). Protease inhibitors such as indinavir and ritonavir have been observed in studies to alter bone metabolism by blocking osteoblast differentiation and preventing osteoclast activation respectively (14). Other classes of ARTs have also been implicated in altering bone health in HIV-infected children. The effect of HIV infection on bone health of HIV-infected children is therefore complex and involves the interactions between bone cells, cytokines and antiretroviral therapy (3).

In the United States, studies have shown that serum concentrations of 25 hydroxyvitamin D levels are consistently decreased among HIV-infected children (4, 7, 11). However, the exact mechanism that leads to the reduced values in 25 hydroxyvitamin D levels is unclear. This is further complicated by the possible effects of ARTs on vitamin D metabolism (1, 14). Efavirenz, for example, has been associated with consistently lower serum concentrations of 25 hydroxyvitamin D levels in HIV-infected children (6).

Vitamin D is a potent immuno-modulator as it affects the development and function of immune system cells such as monocytes and macrophages (7, 9). HIV-infected children are susceptible to tuberculosis co-infection due to impaired cellular

immunity, which is further impaired by the loss of monocyte and macrophage innate immunity due to vitamin D deficiency. Improving serum vitamin D levels could potentially boost immune recovery in HIV-infected children on HAART.

It is well known that vitamin D is also required for bone health maintenance and thus low serum concentrations of 25 hydroxyvitamin D in HIV-infected children may portend poorly for bone health. Therefore, correcting the vitamin D deficiency in HIV-infected children could have a great impact on the bone mineral densities of this population. Based on this concept, a study on HIV-infected children and adolescents from 4 pediatric HIV treatment programs in New York showed that bimonthly supplementation with oral cholecalciferol significantly increased serum 25 hydroxyvitamin D levels in this population (10). Recent data from this group showed, however, that supplementation with Vitamin D did not result in significant improvements in bone mass accrual (Apadi et al Paper #707, CROI, 2011). The authors postulated that failure to maintain high levels of serum Vitamin D may account for their findings. Without knowing the vitamin D status of HIV-infected children in resource poor settings, it is unclear whether such interventions will be beneficial in improving the bone health of HIV-infected children in this setting, but it appears that reconstituting and maintaining adequate levels of Vitamin D (>30 ng/dL) is of great importance.

The interest in bone health in HIV-infected children is growing because of increasing evidence for the role of bone accrual in predicting fracture risk in adult life (3). As the number of HIV-infected children growing into adulthood increases, a better understanding of the effect of HIV infection and antiretroviral therapy on bone health is imperative in order to deal with aging-related diseases such as osteoporosis and fractures. HIV-infected children have several risk factors that put them at great risk for poor bone health. HIV-infected children in resource poor settings are often severely malnourished including vitamin D deficiencies. Darker skin pigmentation in HIV-infected children in Southern Africa may also contribute to vitamin D deficiency. Moreover, severely malnourished HIV-infected children are often very inactive due to poor caloric intake and often display delayed puberty which are risk factors for suboptimal levels of bone mass. There is however a paucity of data describing the bone health and serum vitamin D levels of HIV-infected children in resource poor settings. Such information will be useful in addressing fracture risks and possible osteoporosis in HIV-infected children that survive into adulthood.

2. Study Design and Statistical Procedures:

This study is a prospective observational study describing vitamin D levels among malnourished HIV-infected children initiating HAART immediately upon diagnosis versus delayed initiation after nutritional rehabilitation. We hypothesize that at baseline, severely malnourished HIV infected ART naïve children will have low serum vitamin D levels (compared to healthy controls). Re-feeding should lead to an increase in serum vitamin D levels and BMD and an improvement in bone formation markers. Initiation of HAART will blunt the initial improvements in bone formation seen with nutritional regeneration. Therefore, after 6 weeks, the children within the early HAART initiation arm should have reduced levels of serum vitamin D compared to children in the delayed HAART initiation arm.

Descriptive statistics will be calculated for all the variables and all statistical analysis will be conducted at $\alpha = 0.05$. Multivariate analyses will be performed to evaluate the differences between the severely malnourished HIV-infected children with simultaneous re-feeding and HAART initiation versus delayed initiation of HAART after re-feeding. Multivariate analyses will control for confounding variables such as sex, age, tanner stage and anthropometric measurements.

Each study arm will consist of 75 participants making a total of 150 participants. This number came about as a result of the constraints associated with patient recruitment and retention at the King Edwards VIII hospital. The 150 will be stratified according to baseline vitamin D levels and then randomly selected into each arm. Based on this sample size and approximations for 80% power testing at P=0.05, the study should be able to pick up differences between the two groups to within 0.5 standard deviations.

3. Study Procedures:

A member of the investigation team will approach the pediatrics admitting team and inform them of the inclusion and exclusion criteria for the study. Children identified by the pediatrics admitting team who meet the inclusion criteria will be approached by a member of the investigation team. Details of the study will be explained fully to the potential participants and their families after which informed consent will be sought for. Once the family has consented, the patient will be enrolled into the study.

At the time of admission, various anthropometrical assessments such as height, weight and body composition (skin fold thickness at the waist) will be evaluated. These parameters will be re-measured at day 7 of management, at discharge, at day 15, day 30 and day 45. Once acute symptoms resolve, the child will be transferred to a step-down facility for inpatient rehabilitation for up to 30 days. At the step-down facility, the children will be followed up in a ward clinic. Laboratory assays measuring 25 hydroxyvitamin D levels will be done on admission and again at 6 weeks.

Table of Study Evaluations

Evaluation	Day 0	Day 7	At Discharge	Day 15	Day 30	Day 45
Location of evaluation	KEH	KEH	KEH	Clairwood	Clairwood	Clinic
Demographic information (including DOB, date of admission & discharge	X		X			
Weight	X	X	X	X	X	X
Height	X	X	X	X	X	X
Skin fold over waist	X	X	X	X	X	X
Evaluation of diarrhea, edema	X	X	X	X	X	X

KEH = King Edwards VII Hospital
Clairwood Hospital = inpatient rehabilitation step-down facility
Clinic = ward follow-up clinic

Laboratory Assays:
Both HAART Arms

25 Hydroxyvitamin D levels will be measured at baseline and at 6 weeks. The 25 hydroxyvitamin levels will be detected using high performance liquid chromatography (HPLC) with UV detection. While awaiting analysis, the blood samples will be stored at -80°C.

4. Study Drugs or Devices: HAART medication given will be based on guidelines from the ministry of health in South Africa. No experimental drugs will be used otherwise.

5. Study Questionnaires:
Not applicable.

6. Study Subjects:

Inclusion Criteria:

1. 4 months -12 years
2. HIV infection as defined as a positive ELISA in children over 18 months or a positive DNA PCR in children under 18 months.
3. ART-naïve except for antiretroviral prophylaxis given in the early postnatal period for PMTCT Severe malnutrition as defined by WHO criteria: Presence of severe wasting (<70% Weight for height or <-3 SD) with or without edema, signs of severe visible wasting.
4. Eligible for initiation of HAART by the SA national treatment guidelines/
5. Not on any additional vitamin D and calcium supplementation.

Exclusion Criteria:

1. Enrollment in other interventional studies.
2. Availability of parent/guardian willing and able to adhere to study protocol.

7. Recruitment of Subjects:
The pediatric admitting team at the King Edwards VII hospital will evaluate a child who is admitted to the hospital for severe malnutrition. Once the child is transferred to the floor, the pediatric team will approach the caregivers of the child to gauge their interest participating in the study and willingness to speak to the investigator. Once the caregiver indicates interest in the study, the investigator will then approach the family and explain the study in more detail and seek consent for participation. There will be no written advertisement or recruitment media used in this study.

8. Confidentiality of Study Data:
Personal information such as names and address will be collected from each child mainly for the purpose of follow up. The children in this study will be separated into either the early versus delayed HAART initiation groups and will be given code numbers that will be linked to their personal information. Only the members of the investigation team (Edem Binka, Dr. Moherndran Archary and Dr. Raziya Bobat) will have the ability to link the code numbers to the data collected for each child. Upon completion of the study, all personal identifiable information will be destroyed.

9. Potential Conflict of Interest:

None of the investigators have a proprietary interest in a drug, device or procedure under investigation, or might stand to benefit financially in any other way from the results of the investigation.

10. Location of the Study:
The participants of this study will be recruited from the King Edwards VIII hospital in Durban, South Africa. Participants in the delayed HAART initiation arm will continue their nutritional rehabilitation at the Clairwood hospital in Durban, South Africa. IRB consent will be obtained from these respective institutions.

11. Potential Risks:
Risks include increased morbidity or mortality in the delayed vs. immediate onset of HAART arm. There is also the risk of loss of confidentiality of HIV status, however this is very unlikely as King Edward Hospital has many years of experience in protecting the confidentiality of these patients. Other risks include complications related to the blood draw, including blood loss, infection or discomfort at the venipuncture site. However, the blood draws will be performed by trained nursing and physician staff thus the risk of these complications is very low.

12. Potential Benefits:
Study participants will receive HAART for the length of the study as well as regular medical attention and follow-up. With the information collected in this study, it will be easier to tailor the management guidelines to the subjects' needs and growth progress and handle any complications that may arise in the in or outpatient setting. A more expansive understanding of the bone status of severely malnourished HIV-infected children and whether interventions such as calcium and vitamin D supplementation should be pursued to reduce future risks of osteoporosis and fractures in adulthood.

13. Alternative Therapies:
The participants in this study will only be provided the standard of care; no alternative therapies will be applicable to this study.

14. Compensation to Subjects:
No direct compensation will be provided to the subjects.

15. Costs to the Subjects:
Information for the study will be collected as part of the routine visits for these patients. Therefore there should not be any additional transportation or co-payment costs for the participants.

16. Minors:
Approval from the Department of Pediatrics Committee on Human Investigation has been obtained and will be provided to the IRB review board.

17. Radiation or Radioactive Substances:
Not applicable.

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