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Original Article

Antiretroviral therapy for HIV-1 infected adolescents in Uganda: Assessing the impact on growth and sexual maturation

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Abstract. There is a paucity of knowledge about perinatally infected human immunodeficiency virus (HIV) positive children surviving into their adolescent years, especially from sub-Saharan Africa. Although studies have described the effects of the disease on the physical and sexual maturation of this population, their response to highly active antiretroviral therapy has not been systematically studied. At the pediatric infectious diseases clinic in Mulago hospital, Kampala, Uganda, we evaluated the effect of antiretroviral therapy (ART) on 118 treatment-naive, perinatally-infected HIV positive adolescents between the ages of 10–19 for 12 months. We monitored physical growth using The Centers for Disease Control and Prevention and recently published World Health Organization (WHO) reference growth standards for height and weight measurements as well as sexual maturation using Tanner staging. Laboratory tests including: complete blood count, absolute CD4 cell count and percentage, and HIV-1 RNA viral load, were performed at baseline and at 3-month intervals. Of 118 children, 64% were female; the median age was 13.6 years old. At baseline, 75% were classified as WHO clinical stages III and IV, with a median CD4 count of 124 cells/ul. Apart from four adolescents, all were on first-line antiretroviral therapy with 2 nucleoside reverse transcriptase inhibitors and 1 non-nucleoside reverse transcriptase inhibitors. After 6 months, the median CD4 count was 304 cells/μL, increasing to 370 cells/μL, by 12 months. Antiretroviral therapy was virologically suppressive (HIV-1 RNA viral load <400 copies/mL) in 79% of the adolescents at 6 months and in 89% at 12 months. Six (5%) patients died during the 12-month study. The median baseline height for age Z score was -2.41 which improved to a median of -1.96 by 12 months ($P < 0.0001$). The median baseline weight for age Z score was -2.61 and improved to -1.26 by 12 months ($P < 0.0001$). The median body mass index Z score increased from -1.39 to -0.47 by 12 months ($P < 0.0001$). At baseline, 63% of the adolescents were noted to have delayed pubertal maturation; this only reduced slightly to 60% after 12 months. Adolescents with predominantly perinatally-acquired HIV infection and significant disease burden showed appropriate virologic and immunological response to ART in addition to having clinically significant improvements in growth and some improvement in sexual maturation.

Keywords: Adolescents, HIV, Africa, antiretroviral therapy

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1. Introduction

The survival of human immunodeficiency virus (HIV)-infected children has been dramatically in-

creased by the access to antiretroviral therapy. Pediatric HIV infection is now considered a preventable and treatable disease in the United States and Europe with the feasible goal of completely eliminating perinatally-acquired HIV. However, this is not the case for the majority of the world, particularly in sub-Saharan Africa with one of the largest burdens of pediatric HIV cases. Historical cohorts before the introduction of highly active antiretroviral therapy showed that almost 50% of children with perinatally-acquired HIV died by age 2 and the majority by 5 years of age without treatment [1–3]. Median survival has been shown to be 32 months from time of diagnosis [1]. The number of HIV infected children in sub-Saharan Africa surviving into adolescence is unknown, although it has been estimated at 10–15% [4]. With the increased access to antiretroviral therapy (ART) we expect that this will be a rapidly growing cohort.

Studies show that the immune dysfunction in HIV-infected children is partially related to the delay in somatic growth and onset of pubertal maturation [5–8]. Lessons from European and US. Pediatric AIDS Clinical Trials Group Cohorts showed that ART not only decreased mortality in comparison to the pre-ART era but also improved physical growth [5–9].

There are increasing numbers of published studies on the effectiveness of pediatric ART programs in resource-limited settings where there are high rates of poverty, malnutrition and tropical diseases [10–13]. The majority of the studies undertaken in African settings have focused on infants and young children or simply grouped all children together. As a result, there are several gaps of knowledge concerning the response to ART and its impact on physical and sexual maturation in adolescents with longstanding HIV disease. In Uganda, no study so far has been done to carefully monitor physical growth and sexual maturation of HIV-infected children surviving into adolescence while on ART.

In 2005, the estimated number of adults and children living with HIV in Uganda was 1,000,000 persons; 110,000 being children between the ages of 0 to 14 years old [14]. Furthermore, an estimated one million children were orphans with either one or both parents deceased from acquired immune deficiency syndrome (AIDS) [14].

Since the initiation of ART start-up programs, the Ugandan Ministry of Health reports that 80,000 people are currently being treated for their HIV disease [15]. The Pediatric Infectious Diseases Clinic (PIDC) at Mulago Hospital in Kampala, Uganda cares for an estimated 5000 HIV infected children and adolescents, of which 500 are adolescents between the ages of 10–19 years old. We report on the clinical outcomes after initiating ART in 118 treatment-naive, HIV-infected adolescents. This is a particularly unique cohort as none of them had been exposed to antiretrovirals prior to entering the study.

2. Materials and methods

2.1. Study objective and study population

The objective of this observational study conducted at the Pediatric Infectious Diseases Clinic (PIDC) at Mulago Hospital in Kampala, Uganda was to evaluate the efficacy in treating HIV-1 infected adolescents between ages 10–19 years old and the impact of treatment on growth and sexual maturation. Data was collected at baseline and during each monthly visit. The efficacy of the intervention was assessed by analyzing survival, CD4 cell and HIV-1 RNA responses, changes in height, weight and body mass index (BMI), and sexual development.

During the study period of June 2004 to March 2006, 118 adolescents with HIV infection were enrolled into the observational cohort. The adolescents had been referred by either public or private health-care providers or had been longstanding PIDC patients before ART scale-up was available. All adolescents who were approached to enroll into the study needed a guardian or caretaker to provide consent. HIV status was confirmed after caregiver consent for HIV testing was obtained. In addition, all study participants had to be aware of their own HIV status.

The study population consisted of 118 ART-naive, HIV-infected adolescents (10–19 years). The majority of participants were presumed to have perinatally-acquired infection, given that their mothers with either infected or died from HIV/AIDS. Study participants were also asked about their sexual history at baseline and were excluded if felt to have recent sexually-acquired infection. Adolescents were consecutively enrolled into the study for the initiation of ART during the study period. HIV-infected adolescents not meeting the requirement to initiate ART or those previously started on ART were excluded from this study. Due to termination of funding, the study was stopped earlier than anticipated. Consequently, 33 (28%) adolescents were not followed for the complete 12 months as initially planned.

2.2. HIV therapy and other services

The decision to start participants on ART was based on the World Health Organization (WHO) pediatric ART guidelines. ART was initiated in patients with WHO clinical stage III-IV disease as well as in adolescents with absolute CD4 cell counts $<$200 cells/μL, or CD4% percentage $<$15%. Both absolute and percentage CD4 cell counts were used to assess patients for this study. Patients were also initiated on ART if they were 2 Z-scores below the expected height for age Z-score (HAZ) or weight for age Z-score (WAZ), using the Centers for Disease Control and Prevention (CDC) and WHO growth references [16–18]. Before the initiation of ART, all adolescents were required to attend three adherence-counseling sessions and had to demonstrate family support to emphasize the importance of adherence. Other criteria for inclusion into the study included geographic vicinity within a 50 kilometers radius of Kampala and attendance record of at least 2 times in the previous 6 months. Patients who did not fulfill the study criteria but needed ART received such treatment during non-cohort visits at the PIDC.

Patients were seen by the physician every 2 weeks for the first month and then on a monthly basis for the remainder of the study. Adolescents were also evaluated and treated for concurrent diseases during study visits. As part of continuity of care, the adolescents agreed to come to the clinic for any illness during follow-up. Those who presented with severe malnourishment or an opportunistic infection were assessed in the clinic and admitted to the Mulago Hospital Pediatric Wards if necessary.

Antiretroviral medications were dispensed by the clinic's pharmacist at each monthly clinic visit. Antiretroviral regimens and drug dosing followed WHO guidelines.

The standard first-line treatment included 2 NRTIs [either zidovudine (AZT) or stavudine (D4T), plus lamivudine (3TC)], in combination with 1 NNRTI [either nevirapine (NVP) or efavirenz (EFV)]. Four patients were on a protease-inhibitor based regimen with lopinavir/ritonavir, in combination with AZT or D4T, plus 3TC. The choice of these regimens depended mainly on availability through the funding agency. The combination of ZDV, 3TC, EFV and the protease-inhibitor-based regimens were provided by the President's Emergency Program for AIDS Relief (PEPFAR). The combination of D4T, 3TC, NVP was provided by the Global Fund Program.

Adherence to ART was assessed on a monthly basis through pill counts, 3-day recall and 30-day visual analogue scale. The visual analogue scale is an instrument that has been validated for assessing adherence in resource-limited settings and consists of a calibrated line measuring 0-100% (19). The patient was asked to point to the nearest estimated percentage correlating with their adherence to therapy in the previous month.

Prophylaxis with co-trimoxazole was given to all the participants. All patients underwent testing for active pulmonary tuberculosis (TB) consisting of a chest x-ray and sputum analysis for acid-fast bacilli. In all cases, both tests did not reveal abnormalities suggestive of TB disease, such as infiltrates, nodules or cavitary lesions.

2.3. HIV diagnosis and clinical measurements

For inclusion into the study, patients needed the diagnosis of HIV-1 infection confirmed by two positive rapid enzyme-linked immunosorbent assay HIV-1 tests.

Baseline laboratory testing included a complete blood count, absolute CD4 cell count and percentage, HIV-1 RNA viral load, renal and liver function tests. The CD4 cell count and percentage were calculated using the FACS Count (Becton Dickinson, San Jose, CA, USA) and HIV-1 RNA viral load was estimated using the Amplicor HIV-1 Monitor polymerase chain reaction test, version 1.5 (Roche Diagnostic, GmbH Molecular Systems, Pleasanton, California, USA). A complete blood count, CD4 cell count and percentage, and HIV-1 RNA viral load were repeated every 3 months. Virologic failure was defined as an HIV-1 RNA level $\geqslant$400 copies/mL. Renal and liver tests were repeated every 6 months. Antiretroviral drug resistance testing was not routinely performed. All laboratory testing was performed at Makerere University Johns Hopkins University Core Laboratory, which follows Good Laboratory Practice guidelines and is certified by the College of American Pathologists.

The sexual maturation rating included breast development in females, genital development in males, and pubic hair development in both females and males. The sexual maturation rating used for this study was the Tanner staging scale [20].

Body weight was measured at each visit. Height and Tanner sexual maturation staging were measured every 3 months. The HAZ and WAZ scores were calculated by subtracting the median weight/height of a reference population of the child's age from the child's height/weight and dividing by the standard deviation of the reference population at that age [16]. Body mass in-

dex Z scores were also calculated in this way. The reference values were based on data from the US National Center for Health Statistics and the recently published WHO child growth standards [18].

2.4. Data collection and statistical analysis

At the initial study visit, demographic data, CDC/WHO disease staging, past medical history and current medications were collected on a standardized form. Heights and weights, current ART regimen, adverse drug events, and adherence were collected on a standardized monthly data collection form. Data was entered into Access (version 2003, Microsoft Corp., Redmond, WA). Quality Assurance of data entry was performed routinely by the co-investigators to minimize data entry discrepancies.

The data was analyzed using SAS (version 9.1, SAS Institute Inc., Cary, NC), and Epi-info (version 6.0; Centers for Disease Control and Prevention, Atlanta, GA) using an 'intention-to-treat-analysis'. A two-sided t-test with P values below 0.05 was considered significant.

The HAZ and WAZ at baseline were compared to the closest 3, 6, 9, and 12-month data points. CD4 cell counts and HIV-1 viral load at baseline were compared to the closest 3, 6, 9, and 12-month data points. For variables with approximately symmetrical distributions (normal), the follow-up means were compared to baseline using a paired t-test, with data reported as means and standard deviations. For variables with non-symmetrical distributions (skewed), paired comparisons were made with the Wilcoxon signed rank test, with data reported as medians and 25th and 75th percentiles (interquartile range). Patients who died or were lost to follow-up were censored at that time. Factors associated with viral suppression were also examined using regression analysis.

Ethical and institutional review boards at Makerere Medical School Research Committee, the Uganda National Council of Science and Technology, and the University of Utah Health Sciences Center approved the study.

3. Results

3.1. Study population

One hundred and eighteen children were enrolled in the study (Table 1); 64% were female with a median age of 13.6 years old (range 10–19 years). The majority of patients had either one or both parents deceased (84%). The majority of the study participants were presumed to have acquired their infection perinatally as they were orphans of HIV-infected parents, and there was no clear history of sexual activity or abuse prior to testing.

Table 1
Baseline characteristics of adolescents receiving antiretroviral therapy in Uganda

Variable	Baseline value ($n = 118$) n (%)
Age, mean (range)	13.6 (10–19)
Female sex	76 (64)
Status of parents	
Both dead	44 (37)
One dead	55 (47)
Both alive	19 (16)
Hx of active tuberculosis	12 (10)
Initial antiretroviral therapy regimen	
AZT, 3TC, EFV	74 (63)
D4T, 3TC, NVP	27 (23)
AZT, 3TC, NVP	8 (7)
D4T, 3TC, EFV	5 (4)
AZT, 3TC, LPV/RTV	3 (3)
D4T, 3TC, LPV/RTV	1 (1)
Centers for Disease Control and Prevention classification	
A	24 (20)
B	63 (53)
C	28 (24)
Unknown	3 (3)
World Health Organization classification	
I	3 (3)
II	23 (19)
III	72 (61)
IV	17 (14)
Unknown	3 (3)
Delayed sexual maturity*	74 (63)

AZT = Zidovudine; 3TC = Lamivudine; EFV = Efavirenz; D4T = Stavudine; NVP = Nevirapine; LPV/RTV = Lopinavir/ritonavir.
*Sexual maturity based on Tanner staging.

At baseline, 12 % of the adolescents had received treatment for active TB within the previous one year.

One hundred and twelve (95%) adolescents completed 6 months of treatment, 85 (72%) completed 12 months before the study was ended. Six patients (5%) died during the 12 months. The causes of death included renal failure among two patients and pneumonia of unknown etiology among the remaining four patients. Autopsies were not performed.

3.2. Skeletal growth

There were improvements in skeletal growth demonstrated by significant increases in Z scores for height at both 6 months and 12 months, improving from a baseline of -2.41 to -2.17 and -1.96 by 6 and 12 months

respectively (Table 2). Z scores for weight and BMI also improved significantly by 6 and 12 months. Significant improvements were seen using both the CDC and WHO growth standards.

3.3. Pubertal development

The percentage with delayed sexual maturation did not improve statistically by 12 months (Table 2). Sixty-three percent had delayed sexual maturity at baseline. At month 6 and 12, 60% had delayed ratings. This improvement was only significant at 6 months.

3.4. Immunological and virological status

There were significant improvements seen in CD4 cell counts from baseline to 6 and 12 months (Table 2). CD4 cell counts improved from a median of 124 cells/μL to 304 cells/μL and 370 cells/μL, by 6 and 12 months respectively. Hemoglobins also improved from a median baseline of 11.7 g/dL to 13.2 by 12 months

Approximately 79% of the adolescents had undetectable viral loads ($<$400 copies/mL) at 6 months, and 89% had undetectable viral loads at 12 months (Table 2).

Regression analysis was performed to identify factors associated with viral suppression at 6 and 12 months. None of the factors analyzed, which included age, sex, pre-existing TB, or baseline CDC or WHO staging, were statistically associated with viral suppression.

When the immune response was stratified by baseline CD4 count (Fig. 1), there were similar robust improvements seen across the different groups, including the most immunocompromised.

3.5. Adherence

The average level of adherence using the three methods of assessment was between 95–97% ($n = 118$) during the first 3 months; however, this dropped to 90% ($n = 85$) by 12 months. Factors associated with poor adherence included forgetfulness, high pill burden, and lack of interest.

4. Discussion

There is a paucity of knowledge about perinatally-infected HIV positive children in Africa who survive into their adolescent years, in particular with regard to their growth and development and their response to ART [21]. The cohort investigated in this study is particularly interesting, as none of these perinatally-infected adolescents had been exposed to antiretrovirals prior to entering the study, and thus represent a relatively rare group of slow-progressors

Between the period of June 2004 to March 2006, 118 adolescents were initiated on ART and followed prospectively to evaluate their clinical response while on ART. The immune response to ART was remarkable and more similar to what has been described in the younger pediatric population [9–13]. Our cohort compares well with a recently-described Zambian cohort of children ($<$16 years), where the majority were also felt to be perinatally-infected with a low baseline CD4 count [22]. In the Zambian study, children older than 60 months had a mean increase of 351 cells/μL by 12 months. In our cohort, the 12-month mean increase was slightly lower at 258 cells/μL; however, this difference may be the result of our cohort starting with a lower baseline mean CD4 count than the Zambian group.

Although the growth measures did not return to normal by 12 months, there were significant improvements seen in the height, weight and BMI Z-scores. This compares well to a Romanian cohort of adolescents on ART [9] as well as the Zambian cohort of older children [22]. In a large pediatric study in Spain, ART was associated with significant increases in Z-scores of height and weight but not BMI [23]. Long-term investigations need to be conducted to assess the long-term effect of ART on growth and development and investigate whether weight and height can ever recover to the level of uninfected children. Equally important will be to ensure that these children develop mentally, neurologically and psychologically, appropriately reaching physical, emotional and sexual maturation milestones. The goal is to develop treatments that permit HIV-infected children to return to lives that are as normal as possible.

Our study has several limitations. Although most of the adolescents were felt to have acquired HIV perinatally, we cannot definitely rule out that no one acquired their infection sexually. Ninety-nine adolescents (84%) had at least one deceased parent most often due to HIV. However, it is difficult to determine the etiology of HIV

Table 2
Changes in clinical outcomes at 6 and 12 months follow-up after initiation of antiretroviral therapy among adolescents in Uganda

Variable	Baseline		Month 6		P value	Month 12		Pvalue
HIV-1 RNA (copies/mL), median (IQR)	271,840	(96,451–616,848)	<400	(<400–570)	<0.0001	<400	(<400 to <400)	<0.0001
CD4 cell (count/uL), median (IQR)	124	(12–249)	304	(200–488)	<0.0001	370	(21–528)	<0.0001
Z score for weight, median (IQR)*	−2.61	(−3.93 to −1.67)	−1.58	(−2.95 to −0.68)	<0.0001	−1.26	(−2.5 to −0.4)	<0.0001
Z score for height, median (IQR)*	−2.41	(−3.27 to −1.67)	−2.17	(−2.97 to −1.48)	<0.0001	−1.96	(−2.7 to −1.2)	<0.0001
Z score for height, median (IQR)**	−2.69	(−3.57 to −1.78)	−2.59	(−3.45 to −1.72)	0.0006	−2.58	(−3.3 to −1.6)	0.0016
Z score for BMI, median (IQR)*	−1.39	(−2.38 to −0.61)	−0.43	(−1.32 to 0.22)	<0.0001	−0.47	(−1.4 to 0.3)	<0.0001
Z score for BMI, median (IQR)**	−1.61	(−2.49 to −0.81)	−0.76	(−1.58 to −0.10)	<0.0001	−0.68	(−1.3 to 0.1)	<0.0001
Hemoglobin (g/dL), median (IQR)	11.7	(10.6–12.7)	12.4	(11.5–13.5)	<0.0001	13.2	(12.7–14.1)	<0.0001
Delayed sexual maturity***, no. (%)	74	(63%)	65	(60%)	<0.0001	36	(60%)	0.1419

HIV = Human immunodeficiency virus; IQR = Interquartile range; BMI = Body mass index.
*Using Centers for Disease Control and Prevention growth references.
**Using World Health Organization growth references.
***Sexual maturity based on Tanner staging.

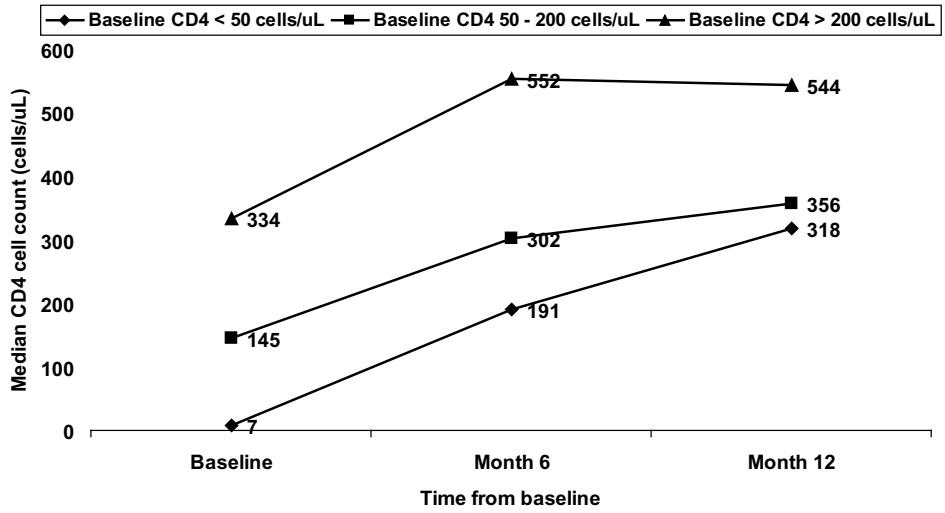

Fig. 1. Response to antiretroviral therapy by baseline CD4 count.

infection for those 16% of adolescents with uninfected parents. Questions regarding sexual activity were part of the baseline assessment but it is possible that the adolescents' response were not always completely reliable.

Another limitation is that we did not have a HIV negative control group of Ugandan adolescents. This would have been extremely helpful in ruling out other causes for growth delay, such as diet, genetics, or other chronic diseases, such as malaria, which are endemic to the region. The Ugandan demographic health survey of 2006 showed that up to 42% of all children less than 12 years (HIV serostatus unknown) are stunted [15].

In our study, we used both the CDC and the recently released WHO growth curves, which are both based on datasets from Western countries. In 2007, the WHO released curves for both height and BMI up to 19 years old. Weight for age was not felt adequate for monitoring growth beyond childhood due to its inability to distinguish between relative height and body mass [17]. Interestingly, the Z scores appeared to be worse using the WHO data sets.

Likewise, sexual maturation reference information is only currently available from Western countries. Therefore, the maturation rates for this cohort may be unfairly measured. The percentage of adolescents with delayed sexual maturation in our study did not improve statistically by 12 months. Sixty-three percent had delayed sexual maturity at baseline. At month 6 and 12, 60% had delayed ratings. This improvement was only significant at 6 months. In the US, immunosuppression has been associated with delayed pubertal onset in perinatally HIV-infected children [24]. In a longitudinal study done on HIV infected children in Italy, however, the age at onset of puberty was not related to the clinical and immunological status of the patients although the study did show that perinatally-acquired HIV does interfere with sexual maturation [8].

The study was also limited in that funding was terminated early before the 12 months of follow-up were completed in all of the patients. This is particularly regrettable as anthropometric changes may take a considerable amount of time to fully manifest after the initiation of ART.

We used three methods to assess the level of adherence to ART, including a three-day pill recall, a visual analogue scale, and returned pill counts. In this study adherence, rates were not quantified by pharmacokinetic studies, and the pill counts were not done on all patients in a consistent manner. Adherence levels were initially good but dropped with longer follow-up. Good adherence has been observed using a more rigorous assessment in another study in the same clinic among children aged 2–18 years [25]. Nevertheless, adolescents should always be considered as a population that requires extra attention and support in order to maintain optimal adherence levels. Adherence rates among adolescents with sexually acquired infection have been very low. A recent study in the US showed that only 24% of the adolescents initiating therapy achieved and maintained undetectable viral loads over 3 years [26]; poor adherence and higher baseline viral loads were obvious predictors of virological failure. The fact that all the adolescents in our study were aware of their HIV status and referred to a counselor and peer support group probably helped improve adherence rates.

In conclusion, in this cohort of predominantly perinatally infected adolescents in sub-Saharan Africa, ART proved to be very effective both immunologically and virologically. Although height, weight and BMI Z scores remained low, these measures improved significantly by one year. Sexual maturation rates only improved slightly, although 12 months may not have been long enough to witness any significant change. A longer-term study needs to be conducted to further assess the impact of ART on growth and development in adolescents with long-standing HIV infection.

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