

VIROLOGIC AND IMMUNOLOGIC OUTCOMES IN HIV-INFECTED CAMBODIAN CHILDREN AFTER 18 MONTHS OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART)

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Abstract. This observational cohort study was conducted among HIV-infected, antiretroviral therapy (ART) naive children in Phnom Penh, Cambodia, to evaluate the feasibility and efficacy of highly active antiretroviral therapy (HAART) delivered using a modified directly observed therapy (MDOT) protocol. From August 2004 to March 2006, 26 children were enrolled and started on a first-line HAART regimen, which was continued for 18 months. The study included a directly observed therapy phase (months 1-3) and a medication self-administration phase (months 4-18). CD4 percentage (CD4%) and HIV-1 RNA plasma viral load (PVL) were measured at baseline and at months 6, 12, and 18. At baseline, the median age was 5.5 years (range: 13 months-12 years), the median CD4% was 4, and the median PVL was 7.5×10^5 copies/ml. At 18 months, 23 (88%) children were alive and participating in the study. Of these children, 20 (87%) had a PVL <400 copies/ml and 12 (52%) had PVL <50 copies/ml. The median CD4% increased to 23, while the median change in height-for-weight z-score was 0.64. Genotypic resistance typing in 2 children with PVL >400 copies/ml at 18 months demonstrated mutations associated with resistance to lamivudine (M184V) and non-nucleoside reverse transcriptase inhibitors (Y181C and G190A). The virologic and immunologic outcomes achieved in this study compare favorably with those reported by other pediatric HIV treatment programs worldwide. The study results suggest that MDOT may be effective for HAART administration in limited-resource settings like Cambodia.

Key words: HIV-infected children, ART, HAART, PVL, genotypic resistance

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INTRODUCTION

HIV treatment access in Cambodia has improved significantly in recent years as a result of decreasing drug costs and organized efforts by the Cambodian gov-

ernment and the NGO community. According to the Cambodian National Center for HIV/AIDS, Dermatology and STDs (NCHADS), the number of healthcare facilities in Cambodia providing antiretroviral therapy (ART) to HIV-infected individuals increased from 30 facilities in 2005 to 48 in 2007, with an estimated 25,353 patients receiving ART as of September 2007. Access to treatment for the roughly 4,400 children in Cambodia living with HIV/AIDS also improved over this time period. As of September 2007, 20 facilities in Cambodia were providing pediatric ART and 2,372 HIV-infected children under 15 years of age were receiving ART (UNAIDS, 2008).

Despite these advances, pediatric HIV treatment efforts in Cambodia and in other limited-resource settings continue to lag behind those focused on adults (WHO, 2008). Although the outcomes reported by pediatric HIV treatment programs in limited-resource settings are promising, documentation of outcomes remains limited, as does experience in the best practices for treatment delivery (Fassinou *et al*, 2004; Kline *et al*, 2004; Janssens *et al*, 2007; Myung *et al*, 2007; Song *et al*, 2007).

Highly active antiretroviral therapy (HAART) reduces HIV/AIDS-related morbidity and mortality in children, regardless of disease stage at time of treatment initiation (Mocroft *et al*, 1998,2000; Palella *et al*, 1998; CDC, 2001). HAART also has a beneficial effect on clinical parameters in HIV-infected children, including the incidence of opportunistic infections, weight and height z-scores, and muscle mass (Gortmarker *et al*, 2001; Resino *et al*, 2006). However, achieving and sustaining these positive outcomes is dependent on effective treatment delivery. Even with an appropriate HAART regimen, patients may experience treatment failure. Regular

medical follow-up is needed to monitor response to treatment, medication side effects, and concurrent medical conditions. HIV treatment programs must also identify and address potential barriers to adherence with treatment, including the complexity of the multidrug regimens involved, medication side effects, and the acceptability of treatment for patients and their families (Watson and Farley, 1999; Descamps *et al*, 2000).

This pilot study used a modified directly observed therapy (MDOT) protocol to administer HAART to HIV-infected children followed in an outpatient pediatric HIV clinic. The MDOT model is based on directly observed therapy (DOT) programs, which have been shown to improve patient adherence with HAART and reduce the incidence of drug resistance and treatment failure (Farmer *et al*, 2001; Koenig *et al*, 2004; Macalino *et al*, 2004). Like DOT programs, MDOT programs require direct observation of at least some doses of medication, but allow for adjustment in the intensity of observation and the degree of patient/caregiver autonomy based on demonstrated response to and adherence with treatment (Kagay *et al*, 2004; Mitty *et al*, 2005). The MDOT model is therefore well suited to long-term treatment regimens, such as HAART for pediatric HIV infection, and may be particularly appropriate in limited-resource settings, like Cambodia, where access to medical care remains limited. The study findings thus contribute to Cambodia's documented experience in treating pediatric HIV and may also have relevance for pediatric HIV treatment programs in similar settings worldwide.

MATERIALS AND METHODS

Study site

This study was conducted at the out-

patient Child Health Improvement Clinic (CHIC) at the National Pediatric Hospital (NPH), Phnom Penh, Cambodia.

The NPH is a public pediatric hospital staffed by Cambodian physicians and healthcare workers. The CHIC is the referral site for stable HIV/AIDS patients from the NPH outpatient and inpatient departments, and also provides treatment and care for children referred from other facilities.

The study was approved by the Institutional Review Boards of Miriam Hospital (Providence, Rhode Island, USA) and the Cambodian Ministry of Health, National Center for HIV/AIDS, Dermatology and STDs (NCHADS). Written informed consent was obtained from primary caregivers with assent from children over 7 years of age before enrollment in the study. All study subjects were immunized according to the US National Immunization Schedule. Coded patient samples were sent for analyses at the University of Massachusetts Medical School (Worcester, Massachusetts, USA).

Patients

A cohort of 26 HIV-infected children was enrolled between August 2004 and March 2006 and followed for 18 months. The following inclusion criteria were required for enrollment: age <15 years old and naive to ART; meet the criteria for initiating ART as outlined in the NPH Guidelines for the Use of ART in Pediatric HIV Infection (Working Group on Antiretroviral Therapy and Medical Management of HIV-infected Children, 2009), summarized as follows: 1) positive HIV ELISA test (if <18 months of age at enrollment, HIV status re-confirmed by ELISA testing at 18 months of age); 2) if <18 months of age, CDC category C, irrespective of CD4%, or CDC categories A and B with CD4% <20;

and 3) if ≥ 18 months of age, CDC category C, irrespective of CD4%, or CDC categories A and B with CD4% <15 (CDC, 2008); meet the following social and geographic criteria: 1) child lived with a family; 2) family lived within 20 km of NPH; 3) primary caregiver was capable of understanding the informed consent document; and 4) if the child lived with the biological mother, the mother was receiving medical evaluation for HIV infection prior to the child's enrollment in the study.

Procedures

All children were initially prescribed a first-line HAART regimen of stavudine, lamivudine, and nevirapine (Government Pharmaceutical Organization, Thailand). Efavirenz was later substituted for nevirapine in 5 patients receiving treatment for tuberculosis. Dosing of study medications was based on NPH Guidelines for the Use of ART in Pediatric HIV Infection, consistent with WHO recommendations (DAIDS, 1994; WHO, 2002, 2006). Children ≥ 15 kg were prescribed a pill-based HAART regimen; those <15 kg were prescribed a liquid-based regimen, although stavudine was available only in capsule form. Medication side effects were graded using the US National Institutes of Health Division of AIDS (DAIDS) Toxicity Table for Children (DAIDS, 1994).

The 18-month study was divided into two phases. During the intensive adherence phase (months 1-3), caregiver administration of antiretroviral drugs to children was observed at the clinic. In month 1, patients/caregivers came to the clinic 5 days a week and administration of the morning dose of a twice-daily antiretroviral regimen was observed by the study nurse. Caregivers were then provided with the evening dose to be administered at home unsupervised. Self-reported adherence with the evening

dose, a pill count, and any adverse events were recorded by the study nurse on the following day. At the Friday morning visit, caregivers were provided with sufficient medication to administer all weekend doses at home unsupervised. In month 2, children and caregivers came to the clinic 3 days per week, and in month 3, visits were biweekly.

In the self-administration phase of the study (months 4-18), visits were tapered as deemed appropriate to once weekly, twice monthly, and then once monthly. Where it was perceived necessary, the frequency of visits was increased to ensure adherence with treatment. Throughout the study period, patient complaints and medication side effects were documented and addressed as soon as possible.

Potential barriers to adherence were assessed at the outset using a caregiver questionnaire administered by the study nurse. Children and caregivers were provided with educational materials developed by the National Pediatric and Family HIV Resource Center (Newark, New Jersey, USA) and translated into Khmer. Adherence with treatment was monitored using patient/caregiver self-reports, pill counts, and questionnaires administered by the study nurse. All drugs and tests prescribed as part of the study, as well as treatment for medication side effects, complications, or other medical issues arising during the study, were provided to patients free of charge. Families were also given an icebox for storage of medicines at home and money to cover transportation costs to and from the clinic.

Measurement of virologic, immunologic, and clinical responses

Blood (5 ml) was drawn at baseline for complete blood counts, liver enzyme tests, CD4 percentage (CD4%), and HIV-1 RNA plasma viral load (PVL). CBC and liver en-

zyme tests were evaluated every three months during the study; CD4 counts and PVL were measured at 6, 12, and 18 months. Blood samples were transported promptly to the laboratory, where they were either used for assays (CBC, liver function tests, CD4 counts) or cryopreserved (plasma for PVL) within 6 hours of being drawn. Following centrifugation of the blood, the plasma was removed, placed in vials, and promptly frozen at -70C. Plasma samples were batched, packed in compliance with IATA regulations, and shipped on dry ice by overnight courier to the UMMS Pediatric Immunology Laboratory for plasma HIV-1 RNA PVL quantitation.

Each child also received a monthly physical exam that included measurement of weight and height. Additional testing was performed as needed for complications, medication side effects, or opportunistic infections.

CBC and liver enzyme testing were performed at the NPH clinical laboratory. The CD4% was measured at the Pasteur Institute (Phnom Penh, Cambodia) using monoclonal antibody staining (Becton-Dickenson Multitest) and flow cytometry analysis (Becton-Dickenson FACSCalibur) to determine T cell subset profiles. The CD4% was calculated based on total lymphocyte CD45+. The Pasteur Institute Cambodia Laboratory participates in the United Kingdom National External Quality Assessment Service quality assurance program.

The PVL was determined using RT-PCR (Roche Amplicor HIV-1 V.1.5 assay, Basel, Switzerland) at the UMMS Pediatric Immunology Laboratory (Worcester, Massachusetts, USA). The dynamic range of the assay was 50 to 100,000 RNA copies/ml; samples were diluted and re-run if the initial assay result exceeded the upper

limit of the assay. The UMMS Pediatric Immunology Laboratory participates in the US National Institutes of Health, Department of AIDS Virology Quality Assurance program.

Genotypic resistance typing was performed at the UMMS Pediatric Immunology Laboratory. Mutations were initially evaluated using the Visible Genetics TruGene HIV-1 Genotyping Kit and the OpenGene DNA Sequencing System (Bayer HealthCare, Diagnostics Division, Tarrytown, New York, USA) according to the manufacturer's protocol. HIV-1 RNA was extracted from 140 μ l of patient plasma using the QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, California, USA). Sequencing primers labeled with Cy5.0 or Cy5.5 were used to sequence the HIV-1 reverse transcriptase gene (nt35 to nt244) located within 1.3 kb of the HIV-1 polymerase region. The sequences were obtained, aligned, and analyzed for the presence of drug-resistance mutations using GeneObjects DNA analysis software and the GeneLibrarian reference tool (GenBank accession GU082395-GU082420).

Statistical analysis

This study used an as-treated analysis in which patient data were censored at the time of death. Height and weight z-scores were calculated using EpiNut (Epi Info, CDC, Atlanta, Georgia, USA and WHO, Geneva, Switzerland). Due to the small sample size, data were analyzed prospectively using percentages, frequency tables, and descriptive statistics including odds ratios, χ^2 analysis, and *t*-tests. A *p* value of <0.05 was considered significant.

RESULTS

Characteristics of study population

Twenty-six HIV-infected, ART-naïve

children were enrolled between August 2004 and March 2006. Characteristics of the children before initiating HAART are presented in Table 1. The median age at baseline was 5.5 years (range: 13 months-12 years); only one child was <18 months old. All 26 children had a baseline CD4% <20 and a PVL >400 copies/ml. At 18 months, 23 children (88%) were alive and participating in the study. The 3 children who died during the study were not included in this analysis.

Virologic outcomes

Examination of patient viral sequences in *gag* and/or *env* genes indicated the children were infected with non-Clade B (CRF01_AE) viruses, while one child was infected with a Clade B virus (data not shown). Fig 1 shows the log decrease in PVL in 23 children over 18 months of HAART. At baseline, the median PVL was 7.5×10^5 copies/ml. At month 6, the PVL was <400 copies/ml in 20 (87%) children. In 3 children, the PVL was >400 copies/ml at 6 months, although the log decrease from baseline in those subjects ranged from 1.2 to 2.7 log. At month 12, 19 (83%) children had a PVL <400 copies/ml and 8 (35%) children had a PVL <50 copies/ml. At month 18, 20 (87%) children had a PVL <400 copies, and 12 (52%) children had a PVL <50 copies/ml.

Immunologic outcomes

At baseline, all 26 children enrolled in the study had a CD4% <20. Twenty-two children (85%) had CDC Stage 3 disease (AIDS) with a CD4% <14, and 4 patients (15%) had Stage 2 disease with a CD4% of 14-28 (http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5710a1.htm?s_cid=rr5710a1_e). Increased CD4 counts were observed in the 23 alive children participating in the study at 18 months. The median change in CD4% from baseline to 18 months was 14.4 (Fig 2a and 2b).

Table 1
 Characteristics of HIV-infected Cambodian children before initiating HAART.

Characteristic	Value
Demographic	
All patients	26
Female, <i>n</i> (%)	13 (50)
Male, <i>n</i> (%)	13 (50)
Age in years, median (range)	5.5 (1.1-12)
Family status	
Both parents living, <i>n</i> (%)	13 (50)
Lost one parent, <i>n</i> (%)	9 (34.6)
Lost both parents, <i>n</i> (%)	4 (15.4)
Primary caregiver	
^a Mother, <i>n</i> (%)	14 (53.8)
Father, <i>n</i> (%)	3 (11.5)
Grandparent, <i>n</i> (%)	7 (26.9)
Aunt, <i>n</i> (%)	2 (7.7)
Clinical	
Weight-for-age z score, mean	-2.97
Height-for-age z score, mean	-3.32
Weight-for-height z score, mean	-1.57
Immunologic	
CD4 cell percentage, median (IQR)	4 (2-12)
^b CDC category	
Stage 1, <i>n</i> (%)	0 (0)
Stage 2, <i>n</i> (%)	4 (15)
Stage 3, <i>n</i> (%)	22 (85)
Virologic	
HIV-1 RNA plasma viral load (copies/ml), median (IQR)	7.5×10^5 (2.2×10^5 - 2.4×10^6)

CDC, Centers for Disease Control and Prevention

IQR, inner quartile range

^aIncludes one case in which both the mother and a maternal aunt were listed as the primary caregiver.

^bBased on the CDC 2008 revised classification system for HIV/AIDS, in which: Stage 1 – absence of AIDS-defining condition and CD4+ T-lymphocyte count ≥ 500 cells/ μ l or CD4% ≥ 29 ; Stage 2 – absence of AIDS-defining condition and CD4+ T-lymphocyte count of 200-499 cells/ μ l or CD4% of 14-28; and Stage 3 (AIDS) – CD4+ T-lymphocyte count < 200 cells/ μ l, CD4% < 14 , or presence of an AIDS-defining condition (Kagay *et al*, 2004).

Clinical outcomes

Twenty-three children remained on HAART for at least 18 months, and showed improved growth over this time period (Fig 3). Five children developed

Immune Reconstitution Syndrome (IRS) after a mean of 45 days of HAART. These cases included 1 case of pleurisy tuberculosis, 1 case of pulmonary tuberculosis, 1 case of diffuse lymphadenopathy, and 2

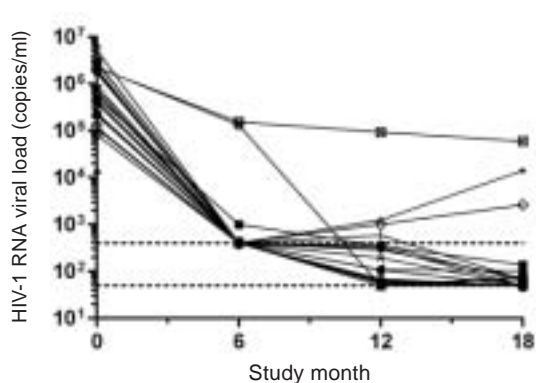


Fig 1—Log change in plasma HIV-1 RNA levels in 23 HIV-infected Cambodian children receiving HAART for 18 months.

cases of TB/MAC. Three children died within the first 5 months of the study. The CD4% on enrollment in the 3 children who died were extremely low (1-2%). The causes of death included intestinal tuberculosis or TB/MAC, cachexia and severe dehydration due to diarrhea and pulmonary tuberculosis. The CD4% and PVL in the children at the time of death are not available.

Genotypic resistance mutation patterns

All children were ART-naive prior to enrollment. At 18 months, 3 children had a PVL >400 copies/ml. Plasma samples collected at baseline and 18 months were available in 2 of these children and used for resistance genotyping (the plasma sample from the third child was insufficient for testing). Mutations associated with resistance to reverse transcriptase inhibitors were not detected in the baseline samples. However, mutations associated with resistance to lamivudine (M184V) and to non-nucleoside reverse transcriptase inhibitors (Y181C and G190A) were detected in samples collected from both children at 18 months. Despite the PVL rebound (in Patient 16) and incom-

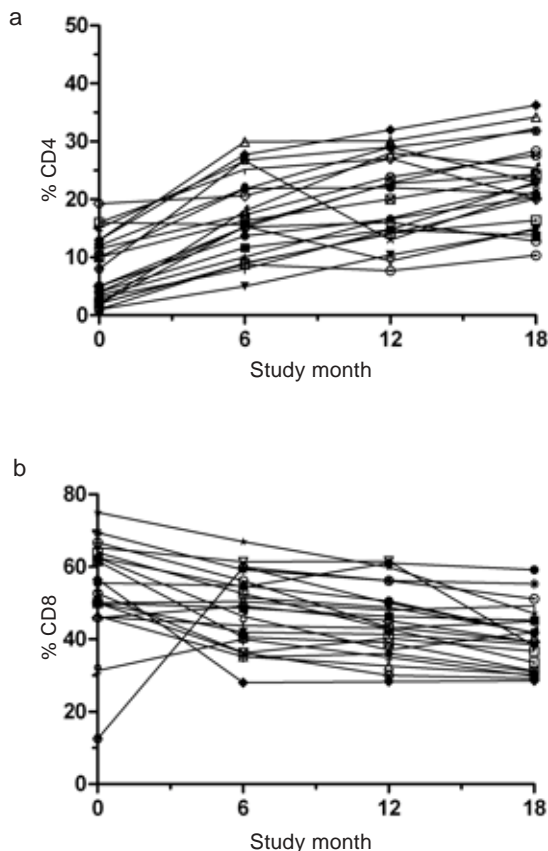


Fig 2a and 2b—Changes in CD4 and CD8 cell percentages in 23 HIV-infected Cambodian children receiving HAART for 18 months.

plete control of viral replication (in Patient 26), sustained increases in CD4% were observed in both children (Fig 4a and 4b).

Adherence assessment

According to patient/caregiver self-reports, 5 children missed at least one dose of medication during the study period. Pill counts performed for children receiving pill-based HAART indicate that 6 children missed at least one dose of medication during the study period. There was a discrepancy between self-reported adherence and pill counts, since only 3 children who were non-adherent according to pill counts also self-reported non-adherence.

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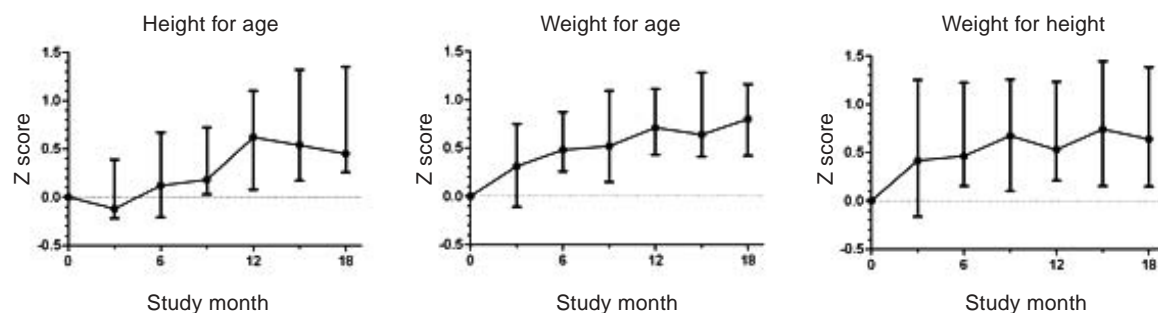


Fig 3—Median change in z-score from baseline in 23 HIV-infected Cambodian children during 18 months of HAART.

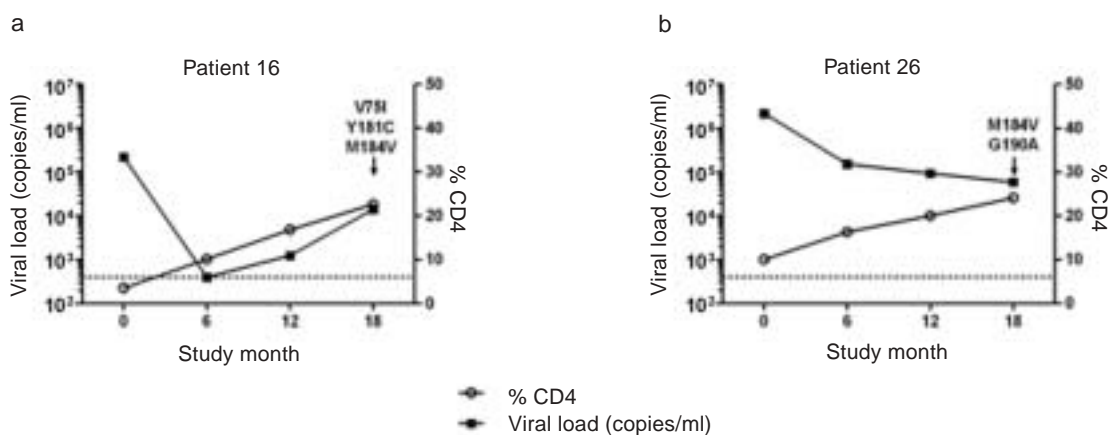


Fig 4a and 4b—Changes in plasma viral load (PVL) and CD4% over 18 months of HAART in 2 children with documented development of ART resistance.

Adverse drug reactions

Medication toxicity and tolerance were monitored during the study and any adverse drug reactions were graded using the DAIDS Toxicity Table for Children (WHO, 2002). Most medication side effects were mild, the most common being nausea and abdominal pain. Rash was observed in one case during the first 2 weeks of nevirapine treatment; the child was continued on nevirapine at the lead-in dose for an additional week, then the dose was increased when the rash subsided. No severe adverse reactions occurred in any patients during the 18 months of the study.

DISCUSSION

The results of this pilot study suggest that HAART is feasible and effective in HIV-infected Cambodian children, with clear virologic, immunologic, and clinical benefits over 18 months of treatment. Although these findings were obtained in a small cohort, the data indicate high rates of virologic suppression with HAART. After 18 months of HAART, the PVL was <50 copies/ml in 12 of the 23 children (52%) enrolled in the study, and <400 copies/ml in 20 (87%) of the children. In addition, the CD4% increased in all the children, with a

median CD4% increase from 4 at baseline to 23 at 18 months. Improved clinical outcomes, including increased weight and height z-scores, were also observed.

These findings are similar to those reported by pediatric HIV treatment programs in other resource-limited settings. Studies in Romania and Cote d'Ivoire reported a PVL <400 copies/ml and <50 copies/ml, respectively, in 50% of cases after a mean of 70 weeks of treatment (Fassinou *et al*, 2004; Myung *et al*, 2007). A cohort study in Thailand reported a PVL <50 copies/ml in 83% of children after 72 weeks of HAART (Puthanakit *et al*, 2005). Our findings are also consistent with the outcomes reported by other studies in HIV-infected Cambodian children (Kline *et al*, 2004; Janssens *et al*, 2007). A 12-month pediatric HAART program conducted at 2 different sites in Cambodia reported a PVL <400 copies/ml in 81% of children and an increase in the median CD4% to 25 (Janssens *et al*, 2007).

All children enrolled in this study were treated initially with a first-line regimen of stavudine, lamivudine, and nevirapine, with substitution of efavirenz for nevirapine in 5 patients receiving tuberculosis treatment. In general, both regimens were well tolerated, with only mild medication side effects observed. Five children, all of whom had a CD4% ≤ 5 at baseline, developed mycobacteria-associated Immune Reconstitution Syndrome (IRS) within the first 2 months of HAART initiation. The data suggest that mycobacterial co-infections are common in this setting; since mycobacterial infection may be associated with IRS following treatment initiation, efforts should be made to identify and treat co-infected children prior to initiating HAART.

At study month 18, 3 children had a PVL >400 copies/ml. Resistance genotyping

performed in 2 of these patients demonstrated mutations associated with resistance to lamivudine (M184V) and to non-nucleoside reverse transcriptase inhibitors (Y181C and G190A). As drug resistance mutations were not detected in baseline viral samples, these mutations developed while on HAART. However, even in the 3 children with incomplete suppression of viral replication, there was a sustained rise in the CD4% throughout the study period and at 18 months; the CD4% was ≥ 20 in all 3 children. Other studies have also reported dissociation between the CD4% and viral suppression following HAART and suggest the CD4% may be a better predictor of outcomes than PVL in HIV-infected children receiving HAART (Ghaffari *et al*, 2004; Song *et al*, 2007).

Although the study attempted to monitor adherence with treatment, the data collected were insufficient to delineate the impact of adherence or non-adherence on outcomes. The available data suggest high rates of adherence, with some discrepancy between patient/caregiver self-reports and pill counts. Significantly, the 2 patients in whom drug resistance mutations were detected (Patients 16 and 23) missed at least 2 HAART doses during the first 4 months of the study, as assessed by a patient/caregiver self-report and/or pill counts. This is an area that merits further investigation, particularly with regard to the impact of non-adherence on observed treatment failure and the emergence of drug resistance.

Emergence of viral resistance to first-line HAART regimens is a particular concern in limited-resource settings given the additional costs associated with second- or third-line drug regimens. Although the availability of cheaper antiretroviral drugs has been a positive development in improving HIV treatment access, increased drug

affordability has also created new challenges in the form of private, unsupervised antiretroviral use. In this context, there is a need for further evaluation of MDOT programs as a strategy to enhance enrollment of HIV-infected patients in supervised treatment programs that will monitor response to and adherence with treatment. Comparison of the rates of treatment failure with MDOT versus other HIV treatment models is also needed, as is a formal cost-benefit analysis of MDOT programs for pediatric HAART administration.

This pilot study was among the first to examine the outcomes of HAART treatment in HIV-infected Cambodian children. As far as we are aware, it was also one of the first studies to use a modified directly observed therapy (MDOT) protocol for HAART in HIV-infected children. Given the pressing need to develop best practices for HIV treatment delivery, this study provides proof of concept that children in Cambodia and other limited-resource settings can be treated effectively with HAART and will benefit from this treatment. Scale up of pediatric treatment efforts is warranted, along with continued efforts to document outcomes.

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