

Clinical and immunologic characteristics and therapeutic interventions in children born to human immunodeficiency virus-infected mothers in southern Taiwan

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Since the late 1990s, the epidemic of human immunodeficiency virus (HIV) infection in Taiwan has expanded dramatically. Pediatric HIV infection has also increased at an alarming pace. Nearly 40% of the HIV-infected children (<10 years) contracted infection through mother-to-child transmission (MTCT). The aims of this study were to evaluate the effects of interventions to prevent MTCT of HIV infection, and to describe the clinical and immunologic characteristics of children born to HIV-seropositive mothers in southern Taiwan. From 1995 to 2003, an observational, longitudinal study of 8 children born to HIV-infected mothers was carried out at a tertiary care university hospital. The median age at enrollment was 0.4 years (range, 1 day-7.5 years), and the mean duration of follow-up was 2.7 years. Four mothers were immigrants from southeastern Asia. Due to antenatal diagnosis of maternal HIV infection, 3 children underwent interventions, including cesarean section, prophylactic use of zidovudine, and bottle-feeding in order to prevent vertical transmission. Five children were born without interventions because of delayed diagnosis of maternal HIV infection. During follow-up, 2 children were found to be HIV-infected and 6 were not infected. The rate of MTCT was lower among patients with interventions (0% vs 40%). In HIV-exposed/non-infected children, the clinical and immunologic assessments were normal during follow-up. Both HIV-infected children progressed to the stage of acquired immunodeficiency syndrome. Early identification of HIV-seropositive pregnant women, implementations to reduce vertical transmission, and introduction of antiretroviral therapy permit optimism in the prevention and treatment of pediatric HIV infection.

Key words: Antiretroviral therapy, HIV, treatment outcome, vertical disease transmission

In 2003, an estimated 40 million people were infected with human immunodeficiency virus (HIV) worldwide, and 3 million people died of acquired immunodeficiency syndrome (AIDS)-related disease complications [1]. Increasing numbers of their offspring are also HIV-infected as a consequence of mother-to-child transmission (MTCT). More than 90% of pediatric HIV infection is acquired through MTCT [1]. In Taiwan, nearly 40% of HIV-infected children (<10 years of age) contracted the infection in this manner. The rate of MTCT in HIV-seropositive pregnant women was estimated to be 15-40% [2-4], but decreased to under 2% after the implementation of recommendations for universal prenatal HIV counseling and testing, widespread use of highly active antiretroviral therapy

(HAART), elective cesarean delivery in HIV-infected pregnant women, and avoidance of breast-feeding [5]. HIV infection in children occurs during the development of the immune system, and has more accelerated progression than in adults. Without interventions, about one-fourth of perinatally-acquired HIV-infected children become symptomatic within the first 2 years of life, and the rest do so in the next several years [6,7]. Combination therapy with antiretroviral drugs and prophylaxis against opportunistic infections can substantially slow the disease progression, and improve survival in HIV-infected children.

The objectives of this observational, longitudinal study were to evaluate the effects of interventions for preventing MTCT of HIV infection, to describe the clinical and immunologic characteristics of children born to HIV-seropositive mothers, and to elucidate the role of therapeutic interventions for HIV-infected children.

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Materials and Methods

Patient enrollment and setting

From January 1, 1993 to December 31, 2003, a total of 8 children born to HIV-seropositive mothers were enrolled. These children were followed in the Department of Pediatrics of National Cheng Kung University Hospital, a tertiary care center in southern Taiwan. Patients were excluded if they had history of transfusion of blood-related products or sexual exposure. Gestation, birth weight, mode of delivery, and feeding types were recorded. Maternal medical conditions near delivery and obstetric factors were also recorded, if available. Only 3 children were followed up since birth. The other children did not have follow-up since birth because the maternal HIV infection was detected after delivery.

Children were categorized as intervention group if their mothers had taken more than 2 types of prophylactic interventions, including use of prenatal, perinatal, and postnatal antiretroviral therapy (ART), elective cesarean delivery, and avoidance of breast-feeding. Those who did not receive any prophylactic intervention were categorized as non-intervention group. Clinical and immunologic evaluations of the patients were carried out at regular intervals.

Case definitions and therapeutic interventions

HIV type 1 (HIV-1) infection was confirmed in all patients. Children <18 months were considered HIV infected if they had positive results of HIV polymerase chain reaction (PCR) or viral culture on 2 separate determinations of peripheral blood; children ≥18 months were considered to be HIV-infected if anti-HIV antibody (Ab) was detected and confirmed by the Western blot method [8]. The Centers for Disease Control and Prevention (CDC) classification systems of HIV infection were used to define clinical and immunologic conditions of HIV-infected children and their mothers [9,10].

Formula feeding and prophylactic zidovudine (AZT; 2 mg/kg/day for 6 weeks) were given to children born to HIV-infected mothers within 24 hours after birth. The indication for initiation of ART was based on the CDC guidelines for pediatric HIV-infected patients [11]. Combination therapy with a triple-drug regimen is recommended, including 2 nucleoside analogue reverse transcriptase inhibitors (NRTIs) plus 1 protease inhibitor (PI), or 2 NRTIs and 1 non-nucleoside analogue reverse transcriptase inhibitor.

Clinical and laboratory monitoring

Children visited our outpatient department regularly every 1-3 months, and received detailed evaluations, including physical examination, growth, and development. Scheduled laboratory studies included complete blood cells (CBC), lymphocyte subsets, immunoglobulin G (IgG), IgA, IgM, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Lymphocyte subsets of CD4⁺ T cells, and CD8⁺ T cells were enumerated using direct immunofluorescence with fluorescein isothiocyanate. Samples were analyzed by flow cytometry (Beckman-Dickinson Immunocytometry Systems). Anti-HIV Ab was detected by enzyme-linked immunosorbent assay. Plasma HIV-1 RNA quantification was assessed every 3 months in HIV-infected children receiving ART. HIV-PCR was analyzed using primers detecting LTR-*gag*, *pol*, and *env* as previously described [12]. Plasma HIV-1 RNA quantification was performed using quantitative reverse transcriptase-PCR assay (Cobas Amplicor HIV-1 Monitor test, version 1.5, Roche Diagnostic systems, USA). The lower limit of detection was 400 copies/mL.

Results

Clinical features and demographic data

During the 8-year study, 8 children (3 males, 5 females) born to HIV-seropositive mothers were enrolled. The clinical characteristics and demographic data of these children and their mothers are summarized in Table 1 and Table 2. The median age at enrollment was 0.4 years (range, 1 day to 7.5 years). The mean duration of follow-up was 2.7 ± 1.9 years. Four mothers were immigrants from southeastern Asian countries, including 2 from Cambodia, and 2 from Indonesia.

In the intervention group, there were 3 children. They were born with interventions to prevent MTCT due to antenatal diagnosis of HIV-1 infection during pregnancy in their mothers. Maternal CD4⁺ counts before delivery were 82/ μ L, 155/ μ L, and 277/ μ L, respectively. Maternal viral load was only available for case 2 (1869 copies/mL) and case 3 (20,798 copies/mL). Only the mother of case 2 received prenatal ART due to development of the AIDS-defining disease, *Pneumocystis carinii* pneumonia. The other 2 mothers were clinically asymptomatic. All 3 children were born via elective cesarean section, commenced prophylactic AZT, and received formula feeding. At follow-up, they were all negative for HIV infection. The MTCT rate of the intervention group was 0%.

Table 1. Demographic and clinical characteristics of 8 children born to human immunodeficiency virus (HIV)-seropositive mothers

Case	Gender	Age at enrollment	Mode of delivery	GA (weeks)/ BW (kg)	Prophylactic ART ^a	Breast-feeding	HIV infection
Intervention group							
1	Male	1 day	C/S	39/3.1	(+)/AZT	(-)	(-)
2	Female	1 day	C/S	37/2.7	(+)/AZT	(-)	(-)
3	Male	1 day	C/S	39/3.2	(+)/AZT	(-)	(-)
Non-intervention group							
4	Male	6.0 months	NSD	37/3.1	(-)	(+)	(-)
5	Female	3.5 months	NSD	38/2.9	(-)	(+)	(+)
6 ^b	Female	7.5 years	NSD	39/3.0	(-)	(+)	(+)
7 ^b	Female	5.0 years	NSD	38/2.9	(-)	(+)	(-)
8 ^b	Female	4.0 years	NSD	38/3.2	(-)	(+)	(-)

Abbreviations: GA = gestational age; BW = birth weight; ART = antiretroviral therapy; C/S = cesarean section; NSD = normal spontaneous delivery; AZT = zidovudine

^aAZT (2 mg/kg/day) initiated within 48 hours after ART.

^bCase 6, 7, 8 were siblings.

In the non-intervention group, there were 5 children without interventions to prevent MTCT. Among them, cases 6, 7, and 8 were siblings. The median age at enrollment for these children was 4.0 years. Only the mother of case 5 had HIV-related symptoms (oral candidiasis) during the third trimester of pregnancy. The other mothers were clinically asymptomatic. Because of undetermined maternal HIV infection before delivery, these children were born via vaginal delivery, did not commence prophylactic use of AZT, and all had breast-feeding. Two of these children (cases 5 and 6) were confirmed to be HIV-1-infected. The MTCT rate of the non-intervention group was 40%.

In this study, all children were born at term and their birth body weights were appropriate for gestational

age, and did not have physical findings associated with HIV infection during the newborn period. During follow-up, HIV-uninfected children had normal growth and development. Serial laboratory evaluations, including CBC, CD4⁺ count, CD4⁺:CD8⁺ ratio, blood biochemistry, and immunoglobulins, were all normal. The median weaning time of maternal anti-HIV Ab was 18 months (range, 15 to 18 months). Two of the children of the non-intervention group were HIV-1-infected, and both of them were female. During serial investigations, clinical symptoms related to HIV-1 infection and progressive immunologic dysfunction developed in the 2 HIV-infected children. Their clinical and immunologic features are outlined in the following descriptions.

Table 2. Clinical and laboratory characteristics of human immunodeficiency virus (HIV)-seropositive mothers

Case	Maternal condition near delivery					Nationality	Paternal status of HIV infection
	Viral load (copies/mL)	CD4 ⁺ cell count (/ μ L)	CD4 ⁺ :CD8 ⁺ ratio	Clinical category ^a / condition	Prenatal ART		
Intervention group							
1	NA	155	0.23	A/asymptomatic	(-)	Indonesia	(+)
2	1869	82	0.25	C/PCP	(+)	Taiwan	(+)
3	20,798	277	0.20	A/asymptomatic	(-)	Taiwan	(-)
Non-intervention group							
4	NA	15	0.50	NA	(-)	Cambodia	(-)
5	NA	163	0.16	C/oral candidiasis	(-)	Cambodia	(+)
6 ^b	NA	NA	NA	A/asymptomatic	(-)	Indonesia	(-)
7 ^b	NA	NA	NA	A/asymptomatic	(-)	Indonesia	(-)
8 ^b	NA	NA	NA	A/asymptomatic	(-)	Indonesia	(-)

Abbreviations: ART = antiretroviral therapy; NA = not available; PCP = *Pneumocystis carinii* pneumonia

^aAccording to the CDC 1993 revised classification system for HIV infection and expanded surveillance case definition for acquired immunodeficiency syndrome among adolescents and adults.

^bCases 6, 7, 8 were siblings.

Case 5

This 6.5-year-old girl was referred to our service at the age of 3.5 months because maternal HIV infection was detected at that time. Initially, physical findings were normal. Laboratory investigations showed white blood cell (WBC) count 11,800/ μ L, IgG 934 mg/dL, IgA 104 mg/dL, IgM 158 mg/dL, AST 437 U/L, and ALT 405 U/L. HIV-1 infection in the child was established by positive results of HIV-1 PCR on 2 separate blood samples. The baseline level of HIV-1 RNA was $>10^6$ copies/mL. The percentage and counts of CD4⁺ cells were 20% and 1664/ μ L, respectively, before HAART was initiated.

She had poor drug compliance during ART due to failure in cooperation of the parents. Hepatosplenomegaly, lymphadenopathy, parotitis, and growth failure developed within the first 2 years of life. Progressive decrease of CD4⁺ cell counts and fluctuation of plasma HIV-1 viral load (range, 200,000 to 1,428,000 copies/mL) were observed during the initial 200 weeks of ART (Fig. 1). Due to close contact with her tuberculosis-infected grandmother, she developed disseminated *Mycobacterium tuberculosis* infection at the age of 4.5 years. At that time, her CD4⁺ count was 180/ μ L, and HIV-1 viral load was 870,000 copies/mL.

After using various approaches to management, strict compliance was achieved, and the viral load

was reduced to <400 copies/mL after 12 weeks of HAART (AZT, lamivudine, and efavirens). After 1 year of adherence to therapy, the CD4⁺ count had recovered to 604/ μ L, viral load was persistently suppressed (<400 copies/mL), and clinical condition had greatly improved.

Case 6

This 7.5-year-old girl was admitted to our hospital due to prolonged fever for 2 weeks. She also developed intractable dermatitis, chronic otitis media, and wasting syndrome 6 months prior to admission. Physical findings included growth failure, generalized maculopapular rash, and yellowish discharge from the left ear canal. Laboratory findings showed WBC count 1100/ μ L, hemoglobin 7.6 mg/dL, IgM 158 mg/dL, IgG 1280 mg/dL, IgA 626 mg/dL, IgE 56,200 IU/mL, AST 51 U/L, and ALT 22 U/L. The baseline CD4⁺ cell count was 6/ μ L and HIV-1 viral load was 56,800 copies/mL before initiation of HAART. Familial survey revealed that only her mother was HIV-seropositive. Her mother was asymptomatic at that time with a CD4⁺ count of 85/ μ L and HIV-1 viral load of 78,300 copies/mL. Due to the clinical stage of AIDS, and the presence of severe immunologic suppression in this patient, HAART was initiated with AZT, lamivudine, and zidovudine. After 6 weeks of HAART, the plasma HIV-1 RNA level was reduced to

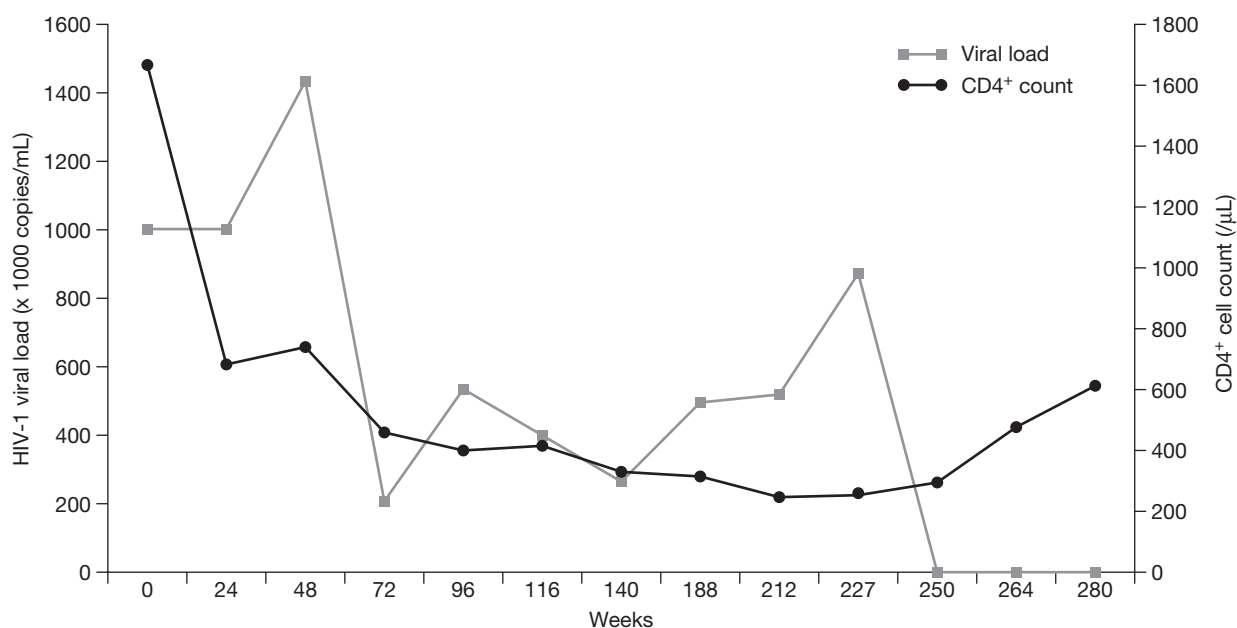


Fig. 1. Dynamic change of CD4⁺ cell counts and plasma human immunodeficiency virus type 1 (HIV-1) viral load of case 5 after initiation of highly active antiretroviral therapy.

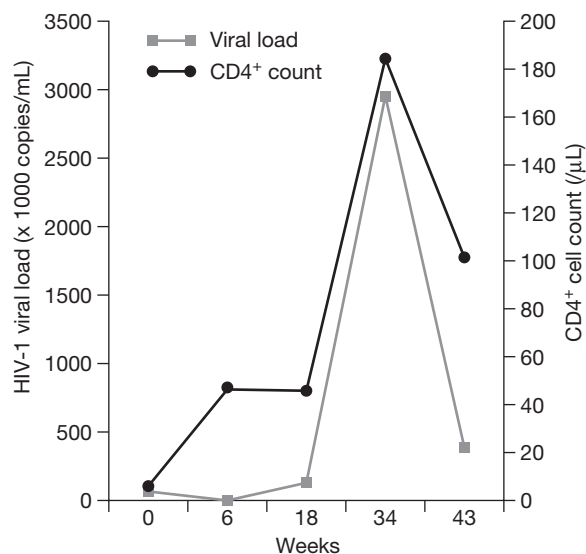


Fig. 2. Dynamic change of CD4⁺ cell counts and plasma viral load of human immunodeficiency virus type 1 (HIV-1) in case 6 after initiation of highly active antiretroviral therapy.

<400 copies/mL and CD4⁺ count had recovered to 46/μL (Fig. 2). However, poor compliance with HAART led to a plasma HIV-1 viral load of 2,940,000 copies/mL, and 375,000 copies/mL at 34 and 43 weeks of therapy.

Discussion

MTCT of HIV infection is preventable by interventions during pregnancy which depend on the availability of routine antenatal screening for HIV. Because MTCT of HIV infection occurs mostly during the perinatal period, avoidance of delivery via the birth canal with planned cesarean section has been demonstrated to reduce the risk of transmission for women at all levels of viremia [13]. The use of AZT in pregnant women with HIV-1 infection and their infants could reduce perinatal HIV-1 transmission by nearly two-thirds (from 25% to 8%) [14]. More recently, with the implementation of HAART for pregnant women and elective cesarean delivery, rates of MTCT have decreased to <2%, and it is estimated that <400 infected infants are currently born each year in the United States, and Europe [15,16]. In this study, the rate of MTCT among infants born without interventions was 40%. This is higher than in reports from the United States [2] and from Europe [3]. In contrast, the MTCT rate was 0% among infants who received interventions. Despite the limited patient number in this study, infants who received the combined

application of elective cesarean section, prophylactic AZT in newborns, and avoidance of breast-feeding had a lower rate of MTCT.

In this study, there was no significant difference in the birth weight, gestation, and physical findings between HIV-infected and uninfected patients during the neonatal period. HIV-uninfected children had normal growth and development and did not develop HIV-1 related symptoms during follow-up. However, hepatosplenomegaly, parotitis, and lymphadenopathy were observed in 1 infected patient (case 5) during the first year of life. Growth failure occurred in early childhood of 2 HIV-infected children. Infants with vertically-acquired HIV infection were usually asymptomatic during the perinatal period. In the European Collaborative Study, the early manifestations of HIV-infection in infancy were non-specific, including lymphadenopathy, growth delay, and hepatosplenomegaly [6]. Without appropriate therapy, most infected infants in the European Collaborative Study progressed to AIDS stage within a few years as a consequence of severe depression of immunologic function [7]. At the severe immunocompromised stage (CD4⁺ count <200/μL), 2 infants in this study developed AIDS-defining illnesses, at the age of 4.5 years (case 5) and 7.5 years (case 6), respectively.

In this study, uninfected children had serial measurements of levels of immunoglobulins, and counts and percentages of lymphocyte and CD4⁺ cells which were appropriate for their age. By contrast, hypergammaglobulinemia was the first feature of the disease at the age of 3.5 months in 1 infected infant (case 5), followed by decreased percentages and counts of CD4⁺ cells during the first year of life before the onset of HIV-related clinical signs. HIV infection in children occurs during the development of the immune system. The immunologic markers differ from those in adults. As a consequence of polyclonal stimulation of B lymphocytes by infectious agents, hypergammaglobulinemia was identified in 77% of HIV-1 infected children at the age of 6 months with 97% specificity in the European Collaborative Study [6]. Analysis of lymphocyte subpopulations in HIV-infected versus non-infected children younger than 2 years revealed no difference in absolute CD8⁺ count but clearly decreased levels of CD4⁺ T cells [17].

Aggressive ART with at least 3 drugs is recommended worldwide for initial treatment of HIV-infected children because it provides better efficacy than monotherapy or dual therapy to suppress viral

replication, preserve immune function, and delay disease progression [11,18]. In this study, ART with a triple-drug combination was the only regimen prescribed in the 2 children with MTCT of HIV infection. Before treatment, the baseline HIV-1 viral load was $>10^6$ copies/mL in case 5, and 56,800 copies/mL in case 6. Recent data indicate that high baseline HIV-1 RNA levels (i.e., levels of $>100,000$ copies/mL) were correlated with rapid disease progression and mortality, particularly if the percentage of CD4⁺ cells was $<15\%$ [19]. Two-year disease progression was documented in 38% of children aged <30 months with baseline RNA in the high quartile (500,000-1,700,000 copies/mL) [20]. In this study, 1 patient (case 5) had persistently high plasma viremia ($>100,000$ copies/mL) in parallel with decreased CD4⁺ counts during the first 4 years of life due to poor compliance with ART. The reason for the rapid onset of AIDS stage in this patient may have been due to both high baseline plasma HIV-1 RNA level and poor drug compliance.

We observed a rebound in viral load in both patients with MTCT of HIV infection, shortly after lack of compliance with HAART. Treatment failure of HAART has been reported worldwide, and most has been contributed to poor compliance with treatment and resistance to antiviral drugs. The success of ART has shown to be mostly dependent on strict adherence that has also prevented development of drug resistance [21, 22]. After multifaceted approaches and coordination, 1 of the HIV-infected children (case 5) had strict adherence to HAART, and complete suppression of plasma HIV-1 viremia was achieved with 12 weeks of therapy. We also observed clinical improvement, sustained virologic suppression, and a recovery of CD4⁺ cell counts after 1 year of remaining on HAART. Although resistance to antiretroviral drugs was not evaluated in this study, it seemed that compliance with HAART is essential to the success of treatment.

Our data showed that 67% of the HIV-seropositive mothers were immigrants from southeastern Asia, an HIV/AIDS epidemic area since the late 1980s. These individuals did not know their status of HIV infection before immigration. Four husbands of these 6 HIV-infected women did not disclose their HIV-seropositive status until the onset of AIDS symptoms in their spouse. Screening of immigrants from epidemic areas and evaluation of HIV infection before or during pregnancy are essential to prevent MTCT. Prenatal HIV counseling and testing with consent should be considered as the standard care for all pregnant women in Taiwan. The

availability and acceptance of voluntary and confidential HIV counseling and testing services are essential to enabling pregnant women to determine their HIV infection status and access preventive interventions. Additional resources will be required in many settings in order to introduce such programs.

There were several limitations in this study. Because of the limited numbers of patients, statistical analysis of the efficacy of interventions to reduce MTCT in HIV infection could not be performed. Previous studies showed that the risk of MTCT was also correlated with maternal medical conditions during pregnancy, including plasma HIV-1 viral load, CD4⁺ counts, and clinical status [23]. In this study, HIV-1 infection was not detected during pregnancy in 3 mothers, and maternal medical information was not collected completely. The mothers were at the advanced stage of HIV-1 infection according to the maternal CD4⁺ counts near delivery. This led to the assumption that most of the mothers had moderate to severe immunosuppressive status. The impact of maternal medical factors on MTCT could not be fully interpreted in this study.

In conclusion, early identification of maternal HIV infection is important to maximize options and allow optimal timing of therapeutic strategies to prevent MTCT of HIV infection. The threat of MTCT in immigrants with indeterminate status of HIV infection from southeastern Asia seems to be underestimated in Taiwan. In this study, both cases of MTCT of HIV infection were diagnosed after the newborn period. Improvement of antenatal diagnosis of HIV infection in pregnant women and better awareness of the epidemic of HIV infection in Taiwan are needed. Combination therapy with antiretroviral drugs is a potent method to suppress viral replication and disease progression in HIV-infected children. However, the success of ART is dependent on adherence to the regimen.

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