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Efficient monitoring of HIV-1 vertically-infected children in Kenya on first-line antiretroviral therapy

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Abbreviations: antiretroviral therapy (ART), antiretroviral drug (ARV), viral load (VL),
reverse-transcriptase (RT), nucleoside reverse-transcriptase inhibitors (NRTI),
non-nucleoside reverse-transcriptase inhibitor (NNRTI).

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ABSTRACT

Background: Worldwide access to antiretroviral therapy (ART) in low- and middle-income countries has significantly increased. Although this presents better treatment options for HIV-infected individuals, the challenge of monitoring ART in these settings still remains.

Objective: To investigate efficient and cost-effective criteria for assessing ART failure among HIV-1-infected children on first-line ART in resource-limited settings.

Study design: Retrospective analysis of 75 HIV-1 vertically-infected Kenyan children with a follow-up period of 24 months after initiating ART. Plasma viral load, peripheral CD4⁺T-cell counts and HIV-1 drug-resistance mutations were monitored biannually.

Results: Plasma viral load (VL) was suppressed to undetectable level or more than 1.5 log₁₀ from baseline levels in 53 (70.7%) children within 24 months. VL in the remaining 22 (29.3%) children was not suppressed significantly. Of the 22 children, 21 were infected with HIV-1 strains that developed drug-resistance mutations; 9 within 12 months and 12 between 12-24 months. Among the 53 who were successfully treated, VL was suppressed in 33 within 12 months and in 20 between 12-24 months. There was no significant difference in VL at baseline and the change of CD4⁺T-cell counts after initiating ART between those treated successfully and the failure groups.

Conclusion: After initiating ART, children may require longer times to achieve complete viral suppression. Plasma viral load testing 24 months after initiating ART could be used to differentiate ART failures among HIV-1 vertically-infected children in resource-limited settings. Additionally, drug resistance testing, if affordable, would be helpful in identifying those failing therapy and in choosing second-line regimens.

Key Words: HIV-1, Children, Antiretroviral therapy, Kenya.

BACKGROUND

Increased availability of antiretroviral therapy (ART) has mitigated HIV-1/AIDS prognoses, especially in resource-poor settings.¹⁻² However, many factors such as bad adherence, treatment interruption, and importantly, the emergence of antiretroviral drug (ARV)-resistance mutations could lead to ART failure.³⁻¹³ According to the world health organization (WHO) treatment guidelines, ART failure is defined as: 1) clinical failure, indicated by new or recurrent WHO stage 3 and 4 conditions after 24 weeks of ART; 2) immunologic failure, indicated by CD4⁺T-cell count of less than 200 cells/mm³ or percentage CD4⁺T-cell count of less than 10 for children between two and five years of age and CD4⁺T-cell count of less than 100 cells/mm³ for children at the age of five years or older, and 3) virologic failure, indicated by a persistent plasma viral load (VL) above 5,000 copies/ml after at least 24 weeks of ART.¹⁴ Once a patient is found to be failing ART, change of regimen should be considered, but choice of second line regimens is limited in resource-poor settings.¹⁵⁻¹⁷ Therefore, monitoring of response to ART is important to determine treatment success. Although plasma VL is the main index for determining treatment failure in developed countries, it is rare in resource-poor settings because of high cost. This leaves the clinical and immunologic markers as the only means of assessing treatment failure.¹⁸⁻²¹

In children, ART effectively reduces morbidity and hospital admissions, and increases long-term survival.²² However, monitoring ART is more challenging than in adults. Children in sub-Saharan Africa seem to be at higher risk for treatment failure, with reported prevalence of 31.6%, 19.7%, 26%, 23-50% in Tanzania, KwaZulu-Natal (South Africa), Uganda and Cote d'Ivoire, respectively.²³⁻²⁷ In addition, clinical and immunologic markers have been reported to be poor predictors of virologic suppression.^{16, 28-30} There is need to investigate efficient and cost-effective criteria for assessing treatment failure among HIV-1-infected children in resource-limited settings.

In Kenya, we have longitudinally followed up HIV-1 vertically-infected children, monitored CD4⁺T-cell counts quarterly and plasma VL biannually since 1999 and 2000, respectively, and reported the emergence of ARV-resistance mutations among the infecting HIV-1 strains together with evolutionary changes in the HIV-1 envelope gene.^{31,32}

OBJECTIVE

To examine the response to ART, the proportion of treatment failure, and the profile of HIV-1 drug resistance-associated mutations among HIV-1 vertically-infected Kenyan children in order to determine appropriate marker(s) and frequency of monitoring children on ART in a resource-limited setting.

STUDY DESIGN

This was a retrospective study involving HIV-1 vertically-infected children from Nyumbani children's home in Nairobi, Kenya. The study subjects have been described previously.³¹ Consent was obtained from the caretaker board of the orphanage, and the study proposal approved by the ethical committees of Kenya Government and Kanazawa University, Japan. Seventy-five children were included in this study. Their baseline characteristics are shown in Table 1. None of them had prior ART exposure. No death occurred during the follow-up.

The ART initiation dates varied from March 1999 to October 2007. First-line ART consisted of two nucleoside reverse-transcriptase inhibitors (NRTI): mostly zidovudine and didanosine or lamivudine, and one non-nucleoside reverse-transcriptase inhibitor (NNRTI): nevirapine or efavirenz, as described in the WHO guidelines.¹⁴ In the current study, "ART success" was defined as plasma VL suppression to undetectable level or 1.5 log₁₀ decrease

from baseline at 24 months after ART initiation, and “ART failure” was defined as two consecutive plasma VL above 5,000 copies/ml by the 24th month.

Peripheral CD4⁺T-cell counts were determined using the FACSCOUNT (Becton-Dickinson, Beiersdorf, Germany) quarterly and plasma HIV-1 RNA quantified biannually using the Amplicor HIV-1 Monitor kit version 1.5 (Roche Diagnostics, Alameda, CA) with detection limit of 400 copies/ml according to the manufacturer’s instructions. Absolute CD4⁺T-cell count was used instead of percentage CD4⁺T-cell count because it was the only available option during study period.

HIV-1 RNA was extracted from plasma using SMITEST EX-R and D (Genome Science Laboratories, Fukushima, Japan) according to the manufacturer’s instructions. The HIV-1 reverse-transcriptase (RT) gene was amplified and population sequencing was done as previously described.³¹⁻³⁴ The HIV-1 RT nucleotide sequences obtained from each child biannually during the first 24 months of ART were analyzed for previously-reported drug-resistance mutations using the Stanford university HIV database (<http://hivdb.stanford.edu/pages/algs/HIVdb.html/>). The REGA HIV-1 subtyping tool (<http://hivdb.stanford.edu/>) was used to determine the HIV-1 subtype. Pairwise comparisons were done in demographic, virologic, and immunologic parameters between treatment success and treatment failure groups using the student’s t-test.

RESULTS

Plasma VL changes before and after ART initiation is shown in Figure 1. Of the 75 children studied, 53 (70.7%) suppressed plasma VL to undetectable level or more than 1.5 log₁₀ from baseline after 24 months of ART [Treatment success group]. Of the 53 treatment success children, 33 suppressed VL to undetectable level within 12 months [rapid responders] and 16 between 12-24 months (nine children within 18 months and seven within 24 months),

and four had detectable VL but suppressed their VL more than 1.5 log₁₀ from baseline at 24 months without any known HIV-1 RTI-resistance mutations [slow responders]. There was no significant difference in plasma VL at baseline between treatment success and failure groups (Table 1).

The remaining 22 children (29.3%) did not suppress their VL more than 1.5 log₁₀ from baseline after 24 months of ART [Treatment failure group], though four of them suppressed plasma VL to undetectable level but their VL rebounded to above 5,000 copies/ml by the end of 24 months. In the 22 children, 21 subsequently developed HIV-1 RTI-resistance mutations; cumulatively, seven children by six months, nine by 12 months, 13 by 18 months, and 21 by 24 months. One female child failed therapy, but no known HIV-1 RTI-resistance mutation was detected in this child after 24 months of ART (Table 2).

Changes of CD4⁺T-cell counts before and after ART initiation are shown in Figure 2. CD4⁺T-cell counts increased in the first 12 months after ART initiation and kept the level thereafter irrespective of children's ART outcome. Although CD4⁺T-cell counts showed higher tendency in slow responder group than in rapid responder group, which was significant at baseline and after 24 months of ART ($p<0.02$ and $p<0.05$, respectively), there was no significant difference in CD4⁺T-cell counts at any time point between the treatment success and failure groups (data not shown).

When the mean age at start of ART was compared between the treatment success and failure groups (8.3 years and 7.3 years, respectively), the difference was not statistically significant despite some trend showing younger age as being associated with treatment failure. In addition, mean age at start of ART was higher in rapid responders (9.1 years) than in slow responders (7.1 years), though the difference was not statistically significant (Table 1).

HIV-1 genotypic analysis based on RT sequences showed that all the children were infected with non-B subtype HIV-1. There was no significant difference in the distribution of HIV-1 subtypes between treatment success and failure groups (Table 1). Treatment failure was higher in the nevirapine group (16/52, 30.8%) than in the efavirenz group (4/20, 20.0%), though the difference was not significant.

The profiles of RTI-resistance mutations are summarized in Table 2. Of the 22 children who failed treatment 21 developed mutations. None of the treatment success children had such mutations. Seven children developed drug-resistance mutations within 6 months after initiating ART, two between 6 and 12 months, four between 12 and 18 months, and eight between 18 and 24 months. Of the 21 children, 20 had NRTI-resistance mutations, and 16 had NNRTI-resistance mutations. The common NRTI-resistance mutations were M184V, which appeared in 11 children, and thymidine analogue-associated mutations (TAMs): TAM 1 (M41L, L210W, and T215Y/F) and TAM 2 (D67N, K70R, and K219Q/E), which appeared in 13 and 13 children, respectively. Two of them had both TAM 1 and TAM 2 profiles detected together. M184V appeared as the first primary NRTI-resistance mutation in six of the 20 children, with TAMs in five, TAMs without M184V in eight children, and T69N in one child. The most common NNRTI mutation was K103N, which appeared in 13 of the 21 children.

The sequences obtained in this study were deposited at the Genebank under accession numbers: HQ586062-HQ586272.

DISCUSSION

In the current study, we followed up 75 HIV-1 vertically-infected children who were on first-line ART for at least 24 months. After 24 months of ART, the proportion of treatment success was 70.7% (53 children). During the two-year follow-up, the cumulative treatment

success rate was 29%, 44%, 56% and 71% at 6, 12, 18 and 24 months, respectively. It is of note that 20 “slow responders” were identified among the 53 treatment success children; their VL was suppressed to undetectable level or more than 1.5 log₁₀ decrease from baseline between 12 and 24 months. These results suggest that HIV-1-infected children may need longer periods of ART to control virus replication comparing with adults.

Twenty-two children (29.3%) failed therapy at 24 months after ART initiation. They maintained high VL: less than 1.5 log₁₀ decrease from baseline, or rebounded after an initial good response during follow-up. The treatment failure rate in this study is within the range of previous reports from sub-Saharan Africa: 19.7% - 50%.²³⁻²⁷ Thus, ART failure appears to be more frequent in children than in adults, possibly due to the difference in the prevalence and profile of drug-resistance mutations in children compared with adults.³⁵⁻³⁸ In this study, at the time ART was initiated, ARV dosing was based on body weight and body surface area of children. Though children grow constantly hence it may be advisable to change dosing regularly,^{36,37} this was not possible in our settings. Furthermore, in this setting, all the children were carefully monitored by the staff of the children’s home and were therefore ART compliant. However, by mid-2000, ARVs were acquired through charity and prescription depended on drug availability. This might have affected ART compliance hence the higher failure rate.

Of the 22 children who failed treatment, 21 developed drug-resistance mutations. Cumulatively, they were detected in 7, 9, 13 and 21 children at 6, 12, 18 and 24 months of ART, respectively. In seven children, TAMs emerged as first mutations without M184V, which is known to be associated with the use of lamivudine. Two of them had both TAM 1 and TAM 2 profiles detected together. Despite being on a lamivudine-containing regimen, six children developed NRTI-resistance mutations devoid of M184V (Table 2). These findings may indicate a pathway different from what has been previously suggested.³¹ Nonetheless,

174 this is consistent with previous observations on the response to ART in children comparing
175 with adults.^{38,39} Besides, one child failed therapy, but did not harbor HIV-1 strains with
176 known RTI-resistance mutations. This may be due to unknown factors, such as low
177 compliance, or other unknown drug-resistance mutations located outside of the RT region
178 that was examined. The possible cause of treatment failure in this child is under investigation.
179 The most observed NNRTI-resistance mutation was K103N, which is easily transmitted and
180 persistent, and has become the most common mutation in patients failing first-line
181 regimens.⁴⁰

182 The WHO recommends early initiation of ART for all HIV-1 vertically-infected
183 children.⁴¹ To get a better clinical outcome, plasma VL and drug-resistance testing should be
184 done before starting ART and as many times as possible during therapy to allow timely and
185 optimized therapeutic change. However, most infected children reside in resource-limited
186 countries where such monitoring is nonexistent.⁴¹ So far, clinical and immunological markers
187 have been used for this purpose.⁴² However, there was no significant difference in the change
188 of CD4⁺T-cell counts before and after ART initiation between the treatment success and
189 failure groups. In addition, there was no difference in the plasma VL at baseline between the
190 two groups. From these findings, it would be advisable that plasma viral load testing at 24
191 months of ART be done to differentiate failing ART regimens. Additionally, drug-resistance
192 testing, if affordable, at 24 months after initiating ART would also be helpful in choosing
193 second-line regimens.

195 **ACKNOWLEDGEMENTS AND CONFLICT OF INTEREST**

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FIGURE LEGENDS

Figure 1: Changes in plasma viral load after initiating antiretroviral therapy among HIV-1–infected children.

Treatment success (TS12,): undetectable viral load within 12 months after initiating antiretroviral therapy; Treatment success (TS24,): viral load suppressed to less than 400 copies/ml or more than 1.5 log from baseline between 12–24 months; Treatment failure (TF,): failed therapy within 24 months. 0 = baseline.

Figure 2: Changes in peripheral CD4⁺ T-cell counts after initiating antiretroviral therapy among HIV-1–infected children.

Treatment success (TS12,): undetectable viral load within 12 months after initiating antiretroviral therapy; Treatment success (TS24,): viral load suppressed to less than 400 copies/ml or more than 1.5 log decline from baseline between 12–24 months; Treatment failure (TF,): failed therapy within 24 months. 0 = baseline.

TABLE1: The characteristics of vertically-infected Kenyan children on ART

Variable	All (n=75)	Treatment Success (TS)			Treatment failure (n=22)
		All TS (n= 53)	TS12 [†] (n= 33)	TS24 [‡] (n= 20)	
Gender					
Male, n (%)	41 (54.7)	28 (52.8)	19 (57.6)	9 (45.0)	13 (59.1)
Female, n (%)	34 (45.3)	25 (47.2)	14 (42.4)	11 (55.0)	9 (40.9)
Age at ART start (years)					
Mean (SD)	7.8 (3.9)	8.3 (4.1)	9.1 (4.3)	7.1 (3.3)	7.3 (3.1)
Baseline viral load (log₁₀copies/ml)					
Mean (SD)	5.1 (0.7) ^a	5.0 (0.8)	5.0 (0.8) ^c	4.9 (0.7) ^d	5.2 (0.5) ^d
Baseline CD4⁺T-cell counts (/mm³)					
Mean (SD)	431 (310) ^b	445 (270)	376 (224) ^{e*}	584 (309) ^{f*}	469 (376) ^g
HIV-1 subtype, n (%)					
A1	59 (78.7)	44 (83.0)	27 (81.8)	17 (85.0)	15 (68.2)
C	3 (4.0)	2 (3.8)	2 (6.1)	0 (0)	1 (4.55)
D	12 (16.0)	7 (13.2)	4 (12.1)	3 (15.0)	5 (22.7)
CRF02_AG	1 (1.3)	0 (0)	0 (0)	0 (0)	1 (4.55)
Drug Combinations, n (%)					
AZT/3TC/NVP	50 (66.7)	36 (67.9)	22 (66.7)	14 (70.0)	14 (63.6)
AZT/ 3TC/ EFV	16 (21.3)	14 (26.4)	9 (27.3)	5 (25.0)	2 (9.1)
AZT/3TC/ABC	3 (4.0)	1 (1.9)	0 (0)	1 (5.0)	2 (9.1)
AZT/ddI/EFV	3 (4.0)	1 (1.9)	1 (3.0)	0 (0)	2 (9.1)
AZT/ddI/NVP	2 (2.7)	0 (0)	0 (0)	0 (0)	2 (9.1)
d4T/3TC/EFV	1 (1.3)	1 (1.9)	1 (3.0)	0 (0)	0 (0)

ART: antiretroviral therapy; AZT: zidovudine; 3TC: lamivudine; ddI: didanosine; ABC: abacavir; NVP: nevirapine; EFV: efavirenz; SD: standard deviation. [†] undetectable viral load within 12 months;; [‡] viral load to <400 copies/ml or >1.5log decline from baseline between 12-24 months; ^a n=57; ^b n=59; ^c n=27; ^d n=12; ^e n=26; ^f n=13; ^g n=20; * p value = 0.02

TABLE 2: Characteristics of children who failed therapy and time to emergence of first major RTI drug resistance-associated mutations

ID	GENDER	AGE AT ART START	REGIMEN	6 MONTHS	12 MONTHS	18 MONTHS	24 MONTHS
38	M	8.6	AZT/3TC/NVP	D67N	D67N/K70R/L210W/K219E	D67N/K70R/L210W/K219E	D67N/K70R/L210W/K219E
62	M	5.4	AZT/ddI/NVP	D67N/K70R/G190A	D67N/K70R/G190A / T215F/K219E		D67N/K70R/G190A /T215F/ K219E /Y181C
85	F	4.7	AZT/3TC/NVP	D67N/M184V/K103N	D67N/M184V/K103N		
103	M	3.2	AZT/3TC/NVP	M184V/K103N	M184V /L74V/K103N	L74V/M184V/K103N	L74V/M184V/K103N
98	F	5.3	AZT/3TC/NVP	M184V/K103N	M184V/K103N	M184V/K103N	M184V/K103N
89	F	1.4	AZT/3TC/NVP	M184V/L210W/T215F/ K101E/G190A	M184V/L210W/T215F/K101E/ G190A/D67N/K70R	M184V/L210W/T215F/K101E/ G190A/D67N/K70R	M184V/L210W/T215F/K101E/ G190A/D67N/K70R
36	M	7.4	AZT/3TC/ABC	M184V/T215F/K103N	M184V/T215F/K103N /G190A	M184V/T215F/K103N /G190A	M184V/T215F/K103N /G190A
48	M	11.4	AZT/3TC/NVP	NA	M184V/K103N	M184V/K103N	M184V/K103N
74	F	6.8	AZT/3TC/NVP	NONE	NONE	NONE	T69N /K103N
73	F	6.7	AZT/3TC/NVP	NONE	NONE		K103N
87	M	3.4	AZT/3TC/NVP	NONE	NONE		M41L
80	M	5.5	AZT/3TC/NVP	NONE	NONE		M41L//A62V/T69P/K103N
41	M	10.9	AZT/3TC/NVP	NONE		M184V/K103N	NA
10	M	12.8	AZT/ddI/NVP	NONE		M41L	E44D/V118I
122	M	11	AZT/3TC/NVP		M184V/K103N	M184V/K103N	M184V/K103N
91	F	3.5	AZT/3TC/NVP			M184V/G190A	M184V/G190A
19	M	10	AZT/ddI/EFV			M184V/T215F/K103N/G190A	M184V/T215F/K103N/G190A / M41L/D67N
42	F	6.9	AZT/ddI/EFV				D67N/K70R
33	F	7.6	AZT/3TC/EFV				K219Q/K101Q
11	M	9.9	AZT/3TC/EFV				M41L//T215Y/K103N
65	M	7.6	AZT/3TC/NVP				M41L/K103N/M184V
7	F	11	AZT/3TC/ABC				NONE

ART: antiretroviral therapy; RTI: reverse transcriptase inhibitor; AZT: zidovudine; 3TC: lamivudine; ddI: didanosine; ABC: abacavir; NVP: nevirapine; EFV: efavirenz. F: Female; M: Male; NA: Not amplified; Blank: No sample available for testing; NONE: No major mutation detected.

Figure 1.

Figure 2