Predictors of Virologic Failure and Genotypic Resistance Mutation Patterns in Thai Children Receiving Non-Nucleoside Reverse Transcriptase Inhibitor–Based Antiretroviral Therapy

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Background: Nonnucleoside reverse transcription inhibitor (NNRTI)-based antiretroviral therapy (ART) has been widely used as a first-line regimen for the treatment of HIV. This study aimed to determine the rate and predictors of virologic failure and describe patterns of resistance mutation.

Methods: The inclusion criteria were children who were <18 years and receiving NNRTI-based ART. Plasma HIV-1 RNA and CD4 were monitored every 6 months. Virologic failure was defined as plasma HIVRNA >1000 copies/mL.

Results: Forty (20%) of 202 children had virologic failure, of whom 33 (16%) failed in the first year of therapy. By multivariate analysis, the children who received nevirapine were 3.7 times more likely to develop virologic failure than those receiving efavirenz ($P = 0.006$). The prevalence's of patients with ≥1 major mutations conferring drug resistance to nucleoside reverse transcription inhibitors (NRTIs) and NNRTIs were 89% and 97%, respectively. The common NNRTI mutations were Y181C/I (58%) and K103N (34%). The NRTI mutations were M184V/I (84%), K65R (11%), Q151M (5%), and ≥3 TAMs (3%).

Conclusions: The virologic failure rate in children was high and mostly occurred in the first year of treatment. The most common resistance mutations were those conferring resistance to NNRTIs and lamivudine. There were few instances of multiNRTI resistance. Early detection of virologic failure might allow more options for second-line regimens.

Key Words: HIV-infected children, NNRTI-based ART, genotypic resistance mutations, virologic failure

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The widespread use of antiretroviral therapy (ART) for the treatment of HIV-infected patients has improved the course of the HIV disease with successful viral suppression and immune restoration leading to reductions in morbidity and mortality.[1-6] The recommendation for antiretroviral agents in HIV infection is a triple combination including 2 nucleoside reverse transcription inhibitors (NRTIs) and one of the Nonnucleoside reverse transcription inhibitor (NNRTIs) or Protease inhibitors (PIs). An NNRTI-based regimen is preferred for treatment of HIV-infected patients, adults,[7,8] and children[9,10] because it has good efficacy, is well tolerated, has fewer long-term toxicities, and is relatively less expensive.

In Thailand, about 610,000 adults and children were living with HIV/AIDS by the end of 2007.[11] The National access to Antiretroviral Program for people living with HIV/AIDS provides antiretroviral drugs free of charge to all Thai HIV-infected patients since 2002. GPOvir, a fixed-dose combination of antiretroviral drugs composed of stavudine (d4T), lamivudine (3TC), and nevirapine (NVP), which is produced by the Thai Government Pharmaceutical Organization (TGPO), has been used as the major NVP-based treatment regimen. The efavirenz (EFV)-based regimen also has been used in children older than 3 years of age. In pediatric patients, the first-line NNRTI-based ART has shown satisfactory outcomes.[12-15]

Incomplete viral suppression can lead to mutation of the reverse transcription gene of HIV and confers drug resistance to NNRTIs or NRTIs which subsequently causes immunologic and clinical deterioration. Pediatric patients have additional barriers to viral suppression including high viral loads and adherence challenges.

When virologic failure occurs, genotypic resistance testing is recommended if available to choose a second-line regimen.[7,9] There are some reports of genotypic resistance patterns in adults[16-18] but there are very few data regarding genotypic patterns in pediatric patients.[15,19]

The aims of this study were: to determine rate of virologic failure in HIV-infected children who received NNRTI-based regimens, to define predictors of virologic failure, and to characterize patterns of drug resistance mutations in children who had early virologic failure.

MATERIALS AND METHODS

Study Design

This was a substudy of a prospective longitudinal study assessing clinical, immunologic, and virologic outcomes of ART in HIV-infected children[12,13] in the National access to Antiretroviral Program for people living with HIV/AIDS program at Chiang Mai University hospital, Chiang Mai provincial hospital, Lamphun provincial hospital, and Sanpatong district hospital. The patients were enrolled to receive ART from August 2002 to October 2006. Patients were included in this analysis if they: (1) were HIV-infected at less than 18 years of age; (2) were antiretroviral drug-naive before initiation of ART except for exposure to antiretroviral prophylaxis for mother-to-child transmission; and (3) had been receiving NNRTI-based ART.

Patients received either NVP- or EFV-based regimens at the discretion of the attending physicians. An adult fixed-dose combination tablet GPOvir (30 mg of d4T, 150 mg of 3TC, and 200 mg of NVP; TGPO) was used for the NVP-based regimen. The dosage was calculated to deliver a NVP dose of 150 to 200 mg/m^2 q12

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hours. During the first 14 days, the NVP was given once daily (lead-in period). This was done by using GPOvir in the morning dose and separate pills or syrup of d4T and 3TC in the evening dose. After the first 14 days, GPOvir was given as a quarter, one-third, one-half, three-fourths of a tablet, and an entire tablet q12 hours for children with body weights of 6 to 9 kg, >9 to 12 kg, >12 to 18 kg, >18 to 25 kg, and >25 kg, respectively. The formulations used for the EFV-based regimen were 3TC (150-mg tablets or syrup; TGPO) plus d4T (15- and 30-mg capsules or syrup; TGPO), or zidovudine (100-mg capsules or syrup; TGPO) as the NRTI backbone of the regimen. The dosage of d4T, 3TC, and zidovudine ranged from 0.9 to 1.3 mg/kg, 4.0 to 6.3 mg/kg, and 180 to 240 mg/m^2 q12 hours, respectively. The dose of EFV (50-mg and 200-mg capsules; Bristol-Meyers Squibb) was 200, 250, 300, 350, 400, and 600 mg q24 hours in the evening for children with body weights of 10 to <15 kg, 15 to <20 kg, 20 to <25 kg, 25 to<32.5 kg, 32.5 to <40 kg, or ≥40 kg, respectively.

The study was approved by the research ethics committee, Faculty of Medicine, Chiang Mai University.

Assessment and Definition

Children were evaluated every 3 months for clinical assessments using the clinical CDC category system.[20] CD4 cell counts and plasma HIV RNA values were done every 6 months. The adherence was captured by pill count method in the first 48 weeks of ART.

Virologic failure was defined as plasma HIV-RNA >1000 copies/mL after at least 24 weeks of treatment. Virologic failure was classified as incomplete viral suppression or viral rebound. Incomplete viral suppression was defined as having never achieved HIV plasma viral load <50 copies/mL after the initiation of ART. Viral rebound was defined as increased plasma HIV RNA to >1000 copies/mL after having been previously undetectable below 50 copies/mL. The HIV genotypic resistance testing was performed on the first stored blood specimen of each patient at or nearest to the time of virologic failure. The immunologic failure at week 48 was defined as CD4 increase less than 5 percentage points above baseline or less than 50 cells/mm^3 above baseline for children more than 5 years of age.[9] Clinical failure was defined as disease progression from one clinical category to a more severe one, or if a new AIDS-defining disease was diagnosed.[9]

CD4 Cell Count

CD4 cell counts were assessed with a FACS Count apparatus (Becton-Dickinson, Mountain View, CA).

HIV-1 RNA Quantifications

Plasma HIV RNA was measured using the Roche Ultrasensitive Amplicor assay, version 1.5 (Cobas Amplicor HIV-1 Monitor, version 1.5; Roche Diagnostics GmbH).The lower limit of detection for the assay was 50 copies/mL.

HIV RNA Separation and Reverse Transcription Gene Amplification

HIV RNA was extracted from EDTA-treated plasma by the guanidinium isothiocyanate and isopropanol precipitation technique. HIV reverse transcription RNA was reverse transcribed with B887–3 primer (59-ATAGGCTGGACTGTCCATCTGT CAGG-39). The first round polymerase chain reaction (PCR) was carried out with primers A-35 (59 GGTTGTACTTTAAATTTC CCAATTAGTCC-39) and B887–3 and then nested with primers B887–2 (59-CTGTACCAGTAACATTAAAGCCAGG-39) and B887–3. The 711 base pair PCR products were purified by Qiagen and then the sequencing reaction was commenced. The sensitivity of the assay was >95% in plasma samples with HIV-1 RNA >1000 copies/mL.[21]

Reverse Transcription Gene Sequencing

The purified PCR products were sequenced by the ABI PRISM dideoxy Dye Terminator Cycle Sequencing Kit (BigDye, Applied Biosystems, Foster City, CA), and were analyzed on an ABI.

PRISM 310 automatic sequencing system. Sequence Navigator Software (Applied Biosystems) was used for analysis.[21] The determination of NRTI and NNRTI mutations was based on the guidelines published by the International AIDS Society–United States (IAS-USA) Drug Resistance Group 2007.[22]

Statistics

Baseline characteristics such as age, gender, HIV clinical staging, CD4 cell count and percentage, plasma HIV viral load, and antiretroviral drug regimen were reported with mean (standard deviation) or proportion as appropriate. The Kaplan–Meier estimate was used to describe the cumulative probability of virologic failure over time. To identify predictors of virologic failure, univariate and multivariate analyses were performed using Cox regression analysis. To detect the virologic failure, the sensitivity, specificity, and predictive values of the immunologic failure at week 48 was calculated. The proportion of each major drug resistance mutation was presented. The SPSS software, version 12.0 (SPSS), was used, and $P \leq 0.05$ was considered to be statistically significant.

RESULTS

Baseline Characteristics and Rate of Virologic Failure in Children

From August 2002 through October 2006, 202 HIV-infected children were initiated on NNRTI-based ART at Chiang Mai University hospital (137), Lamphun provincial hospital (35), Chiang Mai provincial hospital (10), and Sanpatong district hospital (20). The data was censored as of October 31, 2007. The median follow-up time was 200 weeks (interquartile range: 167–241 weeks). Four children died (herpes encephalitis 1, malabsorption 1, acute renal failure 1, and autoimmune hemolytic anemia 1). All of them had virologic suppression. Seven cases were transferred to other hospitals. The data was censored at the time of death or transfer. The baseline characteristics of the patients are shown in Table 1.

Forty children (20%) met the criteria of virologic failure, 27 children (22%) at Chiang Mai University hospital, 5 (11%) at Lamphun Provincial hospital, 3 (30%) at Nakornping provincial hospital, and 5 (20%) at Sunpatong district hospital ($P = 0.65$). Among 20 children who initiate ART before 2 years of age, 1 of 4 (25%) children who had history of exposure to NVP had virologic failure compared with 4 of 16 (25%) children who did not have history of exposure to NVP ($P = 1.0$).

Of these, 32 had received NVP-based regimens and 8 had received EFV-based regimens. At the time of virologic failure, the median age was 7.6 years (range: 1.1–18.9 years), with a median CD4 of 280 (range: 74–2126) cells/mm^3 or 10% (range: 2–29) and a median HIV-RNA of 4.2 (range: 3.03–5.1) $\log_{10}$ copies/mL.

The median duration of treatment until virologic failure was 26 (range: 24–168) weeks. The cumulative probability of virologic failure defined as plasma HIV-RNA >1000 copies/mL after at least 24 weeks of ART is shown in Figure, Supplemental Digital Content 1, http://links.lww.com/INF/A154. The cumulative probability of virologic failure at week 48 and 192 were 0.18 and 0.23, respectively. Of 40 children who had virologic failure, 33 children

TABLE 1. Characteristics of 202 HIV-Infected Children Stratified by Virologic Failure

Variable	Total (N = 202)	Children With Virologic Failure (N = 40)	Children Without Virologic Failure (N = 162)	*P*
Age, yr	7.1 (3.5)	7.1 (4.1)	7.1 (3.3)	0.96
<5 yr	49 (24.3%)	13 (32.5%)	36 (22.2%)	0.24
6–10 yr	109 (53.9%)	17 (42.5%)	92 (56.8%)	
>11 yr	44 (21.8%)	10 (25.0%)	34 (21.0%)	
Body weight, kg	17.4 (7.4)	18.1 (9.3)	17.3 (6.9)	0.72
6–9 kg	23 (11.4%)	7 (17.5%)	16 (9.9%)	0.26
>9–12 kg	22 (10.9%)	4 (10.0%)	18 (11.1%)	
>12–18 kg	83 (41.1%)	11 (27.5%)	72 (44.5%)	
>18–25 kg	49 (24.2%)	13 (32.5%)	36 (22.2%)	
>25 kg	25 (12.4%)	5 (12.5%)	20 (12.3%)	
Male gender	97 (48%)	25 (62.5%)	72 (44.4%)	0.04
CDC clinical category				0.87
N	28 (13.9%)	4 (10.0%)	24 (14.8%)	
A	33 (16.3%)	7 (17.5%)	26 (16.1%)	
B	47 (23.3%)	9 (22.5%)	38 (23.5%)	
C	94 (46.5%)	20 (50%)	74 (45.7%)	
$CD4^+$ cell count, cell/mm^3	226 (359)	276 (498)	213 (315)	0.99
$CD4^+$ cell percentage, %	6.7 (7.0)	6.5 (6.7)	6.8 (7.0)	0.86
CD4 cell percentage ≥5	111 (55.8%)	25 (62.5%)	86 (54.1%)	0.34
CD4 cell percentage >5	88 (44.3%)	15 (37.5%)	73 (45.9%)	
HIV RNA level $\log_{10}$ copies/mL	5.4 (0.4)	5.5 (0.4)	5.4 (0.5)	0.09
HIV RNA >5 $\log_{10}$	32 (16.9%)	5 (13.5%)	27 (17.8%)	0.54
HIV RNA ≥5 $\log_{10}$	157 (83.1%)	32 (86.5%)	125 (82.2%)	
ARV regimen,				0.002
EFV-based regimen	79 (39.1%)	7 (17.5%)	72 (44.4%)	
NVP-based regimen	123 (60.9%)	33 (82.5%)	90 (55.6%)	
Adherence >95%	199 (98.5%)	38 (95.0%)	161 (99.4%)	0.10
Adherence ≥95%	3 (1.5%)	2 (5.0%)	1 (1.6%)	

Data presented as number (%) or mean (SD).
CDC indicates Centers for Disease Control and Prevention.

TABLE 2. Predictors of Virologic Failure for 202 HIV-Infected Children Receiving NNRTI-Based First-Line Therapy

Variable	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Male gender	1.90 (1.00–3.60)	0.05	1.60 (0.79–3.25)	0.19
Age (vs. 0–5 yr)				
6–10 yr	0.53 (0.26–1.10)	0.09	0.47 (0.20–1.30)	0.09
>11 yr	0.80 (0.35–1.82)	0.59	0.74 (0.26–2.17)	0.59
CDC clinical category (vs. category N)				
A	1.64 (0.48–5.61)	0.43	1.17 (0.31–4.40)	0.82
B	1.48 (0.46–4.82)	0.51	1.69 (0.49–5.80)	0.41
C	1.67 (0.57–4.89)	0.35	1.38 (0.42–4.50)	0.60
CD4 cell percentage ≤5%	1.42 (0.70–2.89)	0.35	1.71 (0.75–3.91)	0.20
Plasma HIV RNA >5 $\log_{10}$ copies/mL	1.35 (0.53–3.50)	0.53	1.32 (0.45–3.87)	0.61
Adherence <95%	5.13 (1.23–21.41)	0.03	3.19 (0.66–15.42)	0.15
NVP-based ARV regimen vs. EFV-based regimen	3.34 (1.48–7.57)	0.004	3.72 (1.47–9.40)	0.006

CDC indicates Centers for Disease Control and Prevention.

(82.5%) had virologic failure within 48 weeks of treatment; of which 32 of these instances were classified as incomplete viral suppression and one as viral rebound. Seven children (17.5%) had virologic failure after week 48, of whom one was classified as incomplete viral suppression and 6 as viral rebound. During the study period, 26 (65%) children have switched to second-line regimen at the median time of 87 weeks (range: 35–231) after detection of virologic failure.

At week 48 of treatment, 5 of 33 children (15%) who had virologic failure within the 48 weeks of treatment met the criteria of immunologic failure, compared with 32 of 169 children (19%) who did not have virologic failure ($P = 0.61$). To determine virologic failure, the sensitivity, specificity, positive predictive value, and negative predictive value of immunologic failure at week 48 were 15%, 81%,16%, and 83%, respectively. No child met the criteria of clinical failure before or at the time of virologic failure.

Predictors of Virologic Failure

Predictors for virologic failure are shown in Table 2. In the univariate analysis, males had higher risk of virologic failure ($P = 0.05$). Children with poor adherence in the first 48 weeks of therapy were prone to develop virologic failure ($P = 0.03$). Children who received NVP-based regimens were 3.3 times more likely to develop virologic failure than those receiving EFV-based regimens ($P = 0.004$). In the multivariate

analysis, only the use of a NVP-based regimen was a predictor for virologic failure ($P = 0.006$).

Drug Resistance Data

Of the 40 children with virologic failure, 39 specimens were sent for genotypic resistance testing. Twenty-six (67%) specimens were collected at the time of virologic failure, whereas 8 (20%) and 5 (13%) were at 6 months and 12 months after the time of virologic failure. Among the 39 specimens that underwent the genotypic resistance test, 38 specimens had at least one point of mutation.

NRTI Mutations

The frequency of patients with any major mutations conferring drug resistance to NRTIs was 34 (89%). M184V/I, the most common NRTI mutation, was found in 32 (84%) children. Thymidine analogue mutations (TAMs) were found in 7 (18%) children. The distribution for each thymidine analogue mutation was D67N 2 (5%), L210W 1 (3%), T215Y/F 2 (5%), and K219Q/E 3 (8%). There was no M41L or K70R detected. One patient (3%) had ≥3 thymidine analogue mutations. K65R and Q151M were detected in 4 (11%) children and 2 (5%) children, respectively. There was no T69 insertion detected (Fig., Supplemental Digital Content 2, http://links.lww.com/INF/A155).

NNRTI Mutations

There were 37 children (97%) who had any mutations conferring drug resistance to NNRTIs. The NNRTI resistant mutations were L100I 2 (5%), K103N 13 (34%), V108I 5 (13%), Y181C/I 22 (58%), G190S/A 7 (18%), and P225H 2 (5%). There was no V106A/M or Y188C/L/H (Fig., Supplemental Digital Content 2, http://links.lww.com/INF/A155) Y181C/I was found among children on NVP-based regimens.

DISCUSSION

This study showed virologic failure in 20% of children who initiated NNRTI-based ART regimens, usually during the first year of therapy. The strongest predictor of virologic failure was using a NVP-based regimen compared with an EFV-based regimen. Almost all children had NNRTI and 3TC resistance, but few had multiNRTI resistance such as ≥3 TAMs, Q151 complex, or 69 insertion.

The virologic failure rate in this study was similar to reports from other resource-limited settings, for example 16% in Cambodia[15] and 26% in Uganda.[23] These rates were higher than those reported from studies in adults[23] and may reflect the difficulty of drug administration in young children and the high incidence of noncompliance in adolescents. The median time to develop virologic failure in our study was similar to that in another Thai pediatric study in which the median duration of ART before failure was 6.3 months.[19]

Our study did not show that children exposed to the single dose of NVP for prevention of mother-to-child transmission had a higher rate of virologic failure compared with those who were not. This is contrast to other report[24] and may be because of the small sample size of children with documented history of NVP exposure in our study.

At the time that our study was conducted, the Thai national guideline recommended that patients be changed to second-line ART at the time of immunologic or clinical failure. This could result in accumulation of drug-resistant mutations at the time of switching. In our study, the majority of virologic failure occurred within the first year of treatment in the absence of immunologic or clinical failure. This underlined the importance of access to affordable plasma HIV RNA measurement and ART switching at the time of virologic failure even in resource-limitted setting.

Our study and the one from Uganda[23] showed that children receiving NVP-based regimens were more likely to have virologic failure compared with those taking EFV-based regimens. However, both studies were not randomized. There is a concern that using split adult fixed-dose combination tablets might lead to more virologic failure in the NVP-based regimens due to under dosed NVP in young children. However in our study there was no difference in rate of virologic failure among children who received NVP-based regimen in different weight-band dosing ($P = 0.23$, data not shown). On the other hand, there is a study which provided evidence that the administration of GPOvir fixed-dose combination tablets in fractions to children (body weight: >9 kg) resulted in appropriate NVP exposure.[25] In our study, there was a trend toward higher risk of virologic failure in children with poor adherence, supporting the importance of good adherence in preventing virologic failure.

The present study shows a similar pattern of the emergence of genotypic resistance strains as studies done in adults[17,18] and children[15] with NNRTI-based regimens. The prevalence of patients with ≥1 major mutation conferring drug resistance to NRTIs and NNRTIs ranged from 84% to 95% and 89% to 97%, respectively. The most common NNRTI mutation in our study was Y181C/I, the mutation induced by NVP.[26] This was expected because the majority of children in this study were on a NVP-based regimen.

The majority of our children had M184V/I mutations conferring resistance to 3TC confirming that M184V mutation was always the first mutation to emerge in the regimen containing 3TC.[27] The incidence of multiresistant NRTI (K65R, Q151M, or >3 TAMs) was not high in our study population. This could be explained by the fact that early virologic failure had been detected by regular viral load monitoring in our study and that the presence of M184V delayed the emergence of TAMs.[28] Thus, our patients who have failed first-line ART can be expected to respond well to the current WHO-recommended second-line ART options that include didanosine and sabacavir in combination with boosted protease inhibitor.[10]

The strength of this study is the observational information obtained from a large cohort of children who were monitored for CD4 cells and plasma HIV viral load every 6 months. Therefore we could identify the timing of virologic failure and describe the resistance pattern of early virologic failure in children. Furthermore, because the patients received standardized WHO-recommended first-line regimens, the drug resistance data are useful for public health policy makers in choosing second-line regimens. One of the limitation was 33% of specimens were genotyped 6 to 12 months after detection of virologic failure, therefore some of the mutations may have developed while continuing the failing regimen for the added 6 to 12 months.

In conclusion, 20% of children on NNRTI-based ART developed virologic failure, of which the majority developed within the first year of ART initiation. The immunologic response at week 48 is not sensitive enough to detect virologic failure. Children who received a NVP-based regimen had more chance to develop virologic failure than those on EFV-based regimen. Almost all children had NNRTI and 3TC resistance, but few had multiNRTI resistance. In the resource-limited settings where second-line regimen option is limited, plasma HIV-RNA testing at 1 year after initiation of antiretroviral treatment should be considered for early detection of children with treatment failure.

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