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RISK FACTORS OF VIROLOGIC FAILURE AND SLOW RESPONSE TO ART AMONG HIV-INFECTED CHILDREN AND ADOLESCENTS IN NAIROBI
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ABSTRACT

***Background*: Antiretroviral therapy (ART) in resource-limited settings is effective when backed up with adequate clinical, immunological, and virologic monitoring. Undetected, virologic failure results in increased HIV-1 drug resistance mutations (DRMs), morbidity and mortality, or the need for costly second-line and third-line ART.**

***Objective:* To evaluate the prevalence, patterns, and risk factors of virologic failure and slow response to ART, among children and adolescents in resource-limited settings in Nairobi, Kenya.**

***Design:* A Retrospective study.**

***Setting:* The 8 Lea Toto Programme (LTP) Clinics in Dagoretti, Dandora, Kangemi, Kariobangi, Kawangware, Kibera, Mukuru, and Zimmerman areas of Nairobi. Subjects: One hundred and forty-six HIV-infected children and adolescents aged 1 month to 19 years of the LTP in Nairobi Kenya. Medical and demographic data including, HIV-1 viral loads, information on adherence to ART, HIV-1 DRMs and other key determinants of virologic failure, collected over a period of 2 years, was used for this study.**

Results: **A threshold of 1,000 HIV RNA copies/ml was used to determine treatment outcome. The virologic failure rates in this cohort were 43.8% after 6 months, 32.2% after 12 months, 28.8% after 18 months, and 24.0% after 24 months of first-line ART. Twelve (8.2%) of 146 children showed a slow response to ART: they initially failed ART at 12 months, but had treatment success after 18 to 24 months. The rates of virologic rebound were 4 (2.7%) after 18 months and 3 (2.1%) after 24 months of ART. Multivariate Cox proportional hazards regression revealed that children with suboptimal adherence to ART were 37 times more likely to experience virologic failure (P = 0.000003).**

Conclusions: **This study showed that ART implementation in resource-limited settings is effective when regular virologic monitoring, adherence counselling, and HIV-DR testing are available. Secondly, adherence to ART is a strong predictor of treatment outcome for children and adolescents in resource-limited settings. Therefore, methods of optimizing adherence levels should be explored and implemented.**

INTRODUCTION

Sub-Saharan Africa bears the greatest burden of the Human immunodeficiency virus (HIV) in the world. The region accounts for 71% of all HIV-infected individuals, and 91% of all HIV-infected children below the age of 15 years (1). The prevalence of HIV in 2013 among individuals between 15 and 49 years of age in Kenya was estimated at 6%, with approximately 190,000 HIV-infected children aged between 0 and 14 years (15).

Antiretroviral therapy (ART) has greatly reduced HIV-related morbidity and mortality (2). HIV virologic suppression is usually achieved within the first year of ART initiation in 40% to 81% of HIV-infected children from Sub-Saharan Africa (3-5). The success of ART is reliant on effective clinical, immunological, and especially virologic monitoring (6-8). In many low- and middle-income countries (LMICs), inadequate laboratory infrastructure and costly laboratory reagents, limits viral load and HIV drug resistance mutation monitoring (6). In LMICs, the most widely available options for clinical evaluation of HIV-infected children on ART are, World Health Organization (WHO) clinical staging, CD4+T cell counts and CD4% (9,10).

Lack of HIV virologic monitoring is accompanied by the risk of treatment or virologic failure going undetected, potentiating the emergence of HIV-1 drug resistance (HIV-DR), increasing morbidity and mortality, enhancing the need for costly second-line or third-line ART, as well as the transmission of drug-resistant HIV strains within the population (11-14). Adherence to ART regimens is a keys determinant of treatment success. ART adherence in children and adolescents is influenced by their 'care' environment, the level of vigilance of their primary caregiver, peer influence, ARV pill burden, and ARV formulation: pill or syrup (25-27,31).

MATERIALS AND METHODS

Study Setting: This was a nested retrospective cohort study conducted in the Lea Toto Programme (LTP). The LTP is a community outreach Programme, with 8 centres in Nairobi, Kenya providing free medical care to 2,800 HIV-infected children and adolescents. The study included HIV-positive children or adolescents already enrolled in the LTP who were initiated on first-line ART between January 2011 and

December 2011, and who were subsequently followed for 24 or more months while on ART. The study excluded children or adolescents in the Programme not on ART, those who had been on first-line ART for less than 24 months of follow-up and HIV-exposed children below the age of 2 years.

HIV Treatment Guideline: During the period of study, the Kenyan ART guideline for children was as follows: the Nucleoside Reverse Transcriptase Inhibitors (NRTIs) combination chosen was either Zidovudine and Lamivudine (AZT/3TC) or Abacavir and Lamivudine (ABC/3TC). Tenofovir and Lamivudine (TDF/3TC) could also be used, especially for older children or adolescents; the Non-Nucleoside Reverse Transcriptase Inhibitors (NNTRI) selected depended on the child's exposure to Nevirapine (NVP) during the mother's pregnancy; those exposed were to be put on a Protease inhibitor (PI) namely Lopinavir boosted with Ritonavir (LPV/r); for those not NVP-exposed, either NVP or Efavirenz (EFV) was to be used according to the age and/or weight of the child.

Data Collection: The following primary data was collected from each study participant: demographic characteristics including age, height, weight, gender, and caregiver status; medical variables collected included ART regimen the study participant was initiated on, the viral loads over time, ART adherence data, the date of first-line ART initiation, CD4+ T cell counts over time, and information from clinical evaluation for opportunistic infections. The following was determined from the primary data: the WHO clinical stage, weight-for-height Z score and height-for-age Z score at baseline (19). The duration on first-line ART for each child was calculated by subtracting their first-line ART initiation date from 31st December 2013.

Data Analysis: The data analysis was done using SPSS software version 22(IBM Corporation, Armonk, New York). The Pearson $\chi 2$ test was used to analyse categorical variables, and the Mann-Whitney U-test was used to analyse nonparametric variables. Multivariate Cox proportional hazards regression analysis was used to establish the effect of different variables on virologic failure and slow ART response. A threshold of $P < 0.05$ for statistical significance was set. The WHO threshold of 1,000 HIV-1 RNA copies/ ml, was used to determine treatment success or failure after 12 months of ART(33).Optimal adherence to ART was defined as the child or adolescent ingesting 95% - 100% of the pills, as per caregiver report at the LTP Centre. Suboptimal adherence to ART meant that less than 95% of the pills were ingested (23,31).

RESULTS

Descriptive statistics: The LTP had 2,800 HIV-infected children and adolescents at the time of the study. Between January 2011 and December 2013, 301 (10.8%) were initiated on first-line ART; of this group, 12 (0.4%) were lost to follow-up; 57 (2%) had less than 12 months of follow-up; 146 (5.2%) had at least 24 months of follow-up. The baseline characteristics of this cohort of 146 individuals are shown in Table 1

Treatment outcomes: The rate of treatment success increased over time: 56.2% after 6 months of ART, 67.8% after 12 months, and 71.2% after 18 months of ART (Table 2). After 24 months of ART, 111 (76.0%) children had experienced treatment success, while 35 (24.0%) had virologic failure. After 18 months of ART, 4 (2.7%) children had experienced virologic rebound, while 3 (2.1%) had experienced virologic rebound after 24 months of ART (Table 3). Two children (1.4%) had virologic failure after both 18 and 24 months of ART.

Drug regimen efficacy: In this cohort, 2 Nucleoside Reverse Transcriptase Inhibitors (NRTIs) were prescribed to each child.

Abacavir and Lamivudine (ABC/3TC) were prescribed to 50 (34.2%) children, while Zidovudine and Lamivudine (AZT/3TC) were prescribed to 93 (63.7%) of the children (Table 1). Moreover, 1 Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) or 1 Protease inhibitor (PI) was prescribed to each child. These drug permutations resulted in 7 unique ART regimens being prescribed to children in this cohort. These ART regimens did not show a statistically significant difference in treatment outcome (Pearson χ^2 test P = 0.48) (Table 1).Additionally, the difference in treatment outcome amongst the 7 ART regimens was not statistically significant at any time point (Figure 1).

Slow response to treatment: In this study, 12 (8.2%) of the 146 children were slow responders: they had virologic failure after 12 months of ART, but they had treatment success after 18 to 24 months of ART (Table 3). There was a statistically significant difference between the Log10 viral load of the success group, and the Log10 viral loads of the 12 slow responders after 0, 6, and 12 months of ART (Figure 2). Moreover, the failure group – the group of children with viral loads of 1,000 HIV-1 RNA copies/ml or more after 24 months of ART – was compared to the success group. The differences between these two groups were statistically significant after 6, 12, 18, and 24 months of ART (Figure 2).

Table 1
Baseline characteristics of the study patients

Characteristics	Overall cohort, n = 146	Treatment Success α, n= 111	Virologic Failure, n= 35	*P*β
Age at baseline (years)	8.2 (3.7 to 11.5)ψ	7.9 (4.1 to 11.7)	8.5 (2.9 to 10.9)	0.39
Gender				0.007
• Female	70 (47.9%)	60 (54.1%)	10 (28.6%)	
• Male	76 (52.1%)	51 (45.9%)	25 (71.4%)	
Primary caregiver				0.08
• Both parents	75 (51.4%)	63 (56.8%)	12 (34.3%)	
• One parent	51 (34.9%)	33 (29.7%)	18 (51.4%)	
• Guardian	20 (13.7%)	15 (13.5%)	5 (14.3%)	
• HIV-1 RNA, $\log_{10}$ cop-ies/ml	4.82 (4.12 to 5.49) ψ	4.82 (4.16 to 5.43)	4.72 (3.96 to 5.90)	0.38
Adherence to ART Regi-men φ				6.53E-15
• Optimal	93 (63.7%)	90 (81.1%)	3 (8.6%)	
• Suboptimal	53 (36.3%)	21 (18.9%)	32 (91.4%)	
• Duration on ART (months)	30 (26 to 33) ψ	29 (27 to 33)	30 (26 to 34)	0.28
WHO Clinical stage				0.25
• II	70 (47.9%)	51 (45.9%)	19 (54.3%)	
• III and IV	76 (52.1%)	60 (54.1%)	16 (45.7%)	
• Weight-for-height *Z* score	-0.8 (-2.7 to -0.2) ψ	-0.8 (-2.7 to -0.2)	-0.3 (-2.7 to -0.1)	0.24
• Height-for-age *Z* score	-0.8 (-2.3 to -0.3) ψ	-0.8 (-2.3 to -0.3)	-0.9 (-2.2 to 0.4)	0.33
1st Line ART regimen	72 (49.3%)	53 (47.8%)	19 (54.3%)	0.48

• AZT/3TC/NVP						
• AZT/3TC/EFV	20	(13.7%)	17	(15.3%)	3	(8.6%)
• ABC/3TC/NVP	23	(15.7%)	17	(15.3%)	6	(17.1%)
• ABC/3TC/EFV	24	(16.4%)	17	(15.3%)	7	(0.2%)
• TDF/3TC/EFV	3	(2.1%)	3	(2.7%)	0	(0%)
• ABC/3TC/LPV/r	3	(2.1%)	3	(2.7%)	0	(0%)
• AZT/3TC/LPV/r	1	(0.7%)	1	(0.9%)	0	(0%)

In this study, the patients were 146 children and adolescents in the Lea Toto Programme (LTP), who started first-line ART between January 2011 and December 2011, and who were followed-up for24 months while on ART. ψMedians and interquartile ranges (IQRs). αTreatment success was defined as a viral load below 1,000 RNA copies/ml after 24 months of ART. βThe P values shown are a comparison between the children with treatment success and virologic failure. φAdherence to ART Regimen throughout the period of follow-up. Optimal adherence means that 95% - 100% of the pills were ingested. Suboptimal adherence means that less than 95% of the pills were ingested. IQR:- Interquartile range; WHO:- World Health Organization; ART:- Antiretroviral therapy; NRTI:- Nucleoside Reverse Transcriptase Inhibitor; NtRTI:- Nucleotide Analogue Reverse Transcriptase Inhibitor; NNRTI:- Non-Nucleoside Reverse Transcriptase Inhibitor; Pi:- Protease inhibitor; ABC:-Abacavir; 3TC:- Lamivudine; AZT:- Zidovudine; TDF:- Tenofovir; NVP:- Nevirapine; EFV: Efavirenz; LPV/r:- Lopinavir/ritonavir.

Figure 1

Treatment success rates over 24 months for the antiretroviral therapy (ART) regimens prescribed within the cohort. ABC:- Abacavir; 3TC:- Lamivudine; AZT:- Zidovudine; TDF:- Tenofovir; NVP:- Nevirapine; EFV: Efavirenz; LPV/r:- Lopinavir/ritonavir. The P values were derived when the ART regimen outcomes were compared, and were calculated using the Pearson $\chi 2$ test.

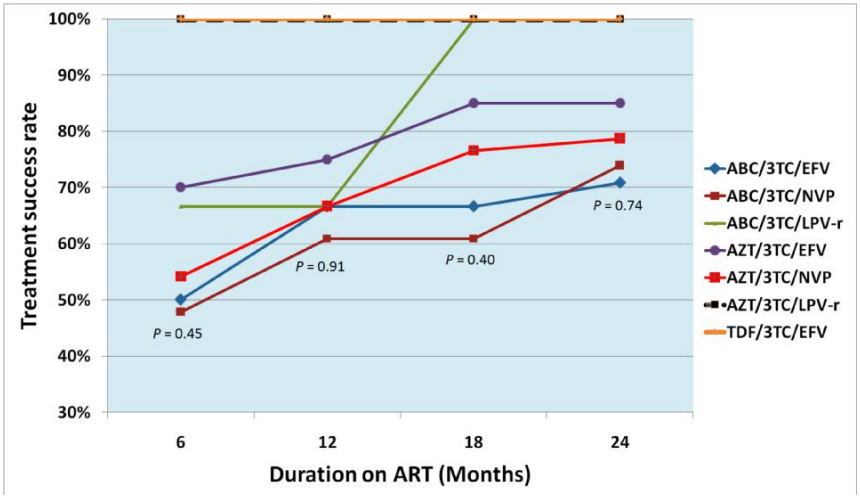

Figure 2
Comparison of HIV-1 viral load over 24 months of first-line ART for the treatment success group, virologic failure group and the slow responders group. The P-values with an asterisk are comparisons between the treatment success and slow responder groups

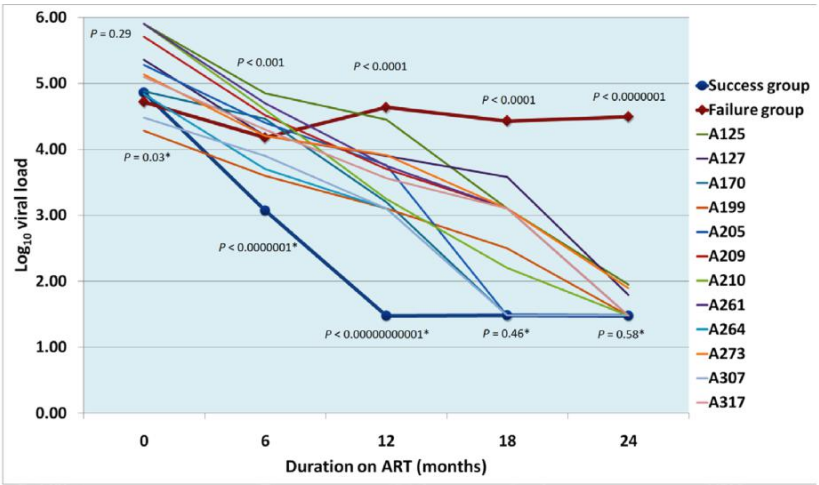

Table 2
Treatment outcome during 24 months of first-line ART

Duration on ART	Total δ, N(%)	Treatment SuccessΨ, N (%)	Virologic Failure, N (%)
6 months	146 (100%)	82 (56.2%)	64 (43.8%)
12 months	146 (100%)	99 (67.8%)	47 (32.2%)
18 months	146 (100%)	104 (71.2%)	42 (28.8%)
24 months	146 (100%)	111 (76.0%)	35 (24.0%)

Table 3
Slow responders to ART and Virologic rebounders during 24 months of first-line ART

ART Treatment Outcome	Totalα, N (%)	Proportion with ART Treatment Outcome, N (%)
Slow responders$^{\beta}$	146 (100%)	12 (8.2%)
Virologic rebound after 18 months	146 (100%)	4 (2.7%)
Virologic rebound after 24 months	146 (100%)	3 (2.1%)
Virologic rebound after 18 and 24 months	146 (100%)	2 (1.4%)

Number of children in the cohort. $^{\beta}$ This group had virologic failure (a viral load of 1,000 HIV-1 RNA copies/ml or higher) after 12 months, then treatment success after 18 to 24 months of first-line ART.

A multivariate Cox proportional hazards regression analysis was conducted to determine the risk factors associated with treatment failure. For this analysis, various variables were simultaneously compared between the treatment success group (N=111) and virologic failure group (N = 35), after 24 months of ART (Table 4). The risk factors associated with treatment failure were considered to be predictive and statistically significant when 3 conditions were met: the Cox proportional hazard ratio (HR) had to be greater than 1.96, both the lower and upper limits of the 95% confidence interval (CI) had to be greater than 1, and the P value had to be less than

0.05(16). The analysis revealed that children and adolescents with suboptimal adherence to ART, were 37 times more likely to develop treatment failure when compared to individuals with optimal adherence (HR= 36.99, CI 8.21 to 166.66, P value of 0.000003) (Table 4). Secondly, the NRTI combination of ABC/3TC was 3.5 times more likely to lead to treatment failure than AZT/3TC (HR= 3.45, CI 1.39 to 8.51, P value of 0.007) (Table 4).

Table 4

Risk factors of virologic failure$^{\alpha}$ among 146 children and adolescents after 24 months of ART, as determined by Multivariate Cox proportional hazards regression

Variable	Cox Proportional Hazard Ratio (HR)	95% Confidence Interval(CI)	*P*
Adherence to ART: Optimalφ	1.0		
Suboptimal	36.99	8.21 to 166.66	0.000003 β
NRTI drug combination: AZT/3TC φ	1.0		
ABC/3TC	3.45	1.39 to 8.51	0.007
CD4 T cell count at baseline: ≥ 200 cells/μlφ	1.0		
≤ 199 cells/μl	3.38	0.77 to 14.89	0.11
CD4% at baseline: ≤ 14%φ	1.0		
≥ 15%	2.24	0.28 to 17.86	0.046
Primary Caregiver: Two parents φ	1.0		
One parent	2.08	0.44 to 9.90	0.36
Primary Caregiver: Two parents φ	1.0		
Guardian	1.27	0.34 to 4.72	0.72
WHO stage at baseline: I or II φ	1.0		
III or IV	1.17	0.48 to 2.85	0.73
Weight-for-height *Z* score at baseline: ≥ -2φ	1.0		
<-2	1.10	0.33 to 3.73	0.88
Height-for-age *Z* score at baseline: ≥ -2 φ	1.0		
<-2	1.04	0.33 to 3.28	0.95
Age at baseline: 36 months or more φ	1.0		
35 months or less	0.85	0.17 to 4.31	0.85
Gender: Male φ	1.0		
Female	0.73	0.26 to 2.07	0.56
HIV RNA at baseline:Log_{10} copies/ml <5.0 φ	1.0		
Log_{10} copies/ml ≥5.0	0.46	0.17 to 1.21	0.12

$^{\alpha}$ Virologic failure was a viral load of 1,000 HIV RNA copies/ml or more, after 24 months of ART.

$^{\beta}$Suboptimal adherence to ART is the only variable with a predictive Cox proportional hazard ratio (>1.96), with a 95% CI whose lower limit is > 1.96, and that is also statistically significant (P < 0.05).

$^{\varphi}$Reference / baseline variable in Cox Hazards Regression Analysis

ART:- Antiretroviral therapy; NRTI:- Nucleoside Reverse Transcriptase Inhibitor; NRTI:- Nucleotide Reverse Transcriptase Inhibitor; ABC:- Abacavir; 3TC:- Lamivudine.

Multivariate Cox proportional hazards regression analysis was also performed to determine the risk factors that lead to a slow response to ART. For this analysis, various variables were simultaneously compared between the group of 99 children who had treatment success after 12 months of ART initiation, and the group of 12 who attained treatment success only after 18 to 24 months of ART. None of the variables tested met the 3 conditions to be a risk factor of slow response to ART (Table 5).

Table 5

Risk factors of slow response to ART$^{\alpha}$ among 146 children and adolescents after 24 months of ART, as determined by Multivariate Cox proportional hazards regression

Variable	Cox Proportional Hazard Ratio (HR)	95% Confidence Interval(CI)	*P*
Adherence to ART: Optimal φ	1.0		
Suboptimal	3.20	0.55 to 18.72	0.20
NRTI drug combination: AZT/3TCφ	1.0		
ABC/3TC	1.53	0.30 to 7.80	0.61
WHO stage at baseline: I or II φ	1.0		
III or IV	1.51	0.31 to 7.50	0.61
Gender: Male φ	1.0		
Female	1.13	0.19 to 6.62	0.89
Weight-for-height *Z* score at baseline: ≥ -2φ	1.0		
< -2	1.03	0.09 to 11.15	0.98
HIV RNA at baseline: Log_{10} copies/ml <5.0	1.0		
Φ	1.11	0.22 to 5.64	0.90
Log_{10} copies/ml ≥ 5.0			
Age at baseline: 36 months or moreφ	1.0		
35 months or less	1.02	0.07 to 14.47	0.20
CD4% at baseline: ≤ 14%φ	1.0		
≥ 15%	0.20	0.01 to 3.59	0.27
Height-for-age *Z* score at baseline: ≥ -2 φ	1.0		
< -2	0.65	0.07 to 6.53	0.71
Primary Caregiver: Two parents φ	1.0		
Guardian	0.13	0.01 to 2.80	0.19

$^{\alpha}$A slow response to ART is defined as having a viral load of 1,000 HIV RNA copies/ml or more after 12 months of ART, and treatment success after 18 to 24 months of ART.

$^{\varphi}$Reference / baseline variable in Cox Hazards Regression Analysis ART:- Antiretroviral therapy; NRTI:- Nucleoside Reverse Transcriptase Inhibitor; NNRTI:- Non-Nucleoside Reverse Transcriptase Inhibitor; ABC:- Abacavir; 3TC:- Lamivudine.

DISCUSSION

This study evaluated the prevalence and risk factors associated with virologic failure and slow response to ART among children and adolescents in the LTP cohort of Nairobi, Kenya. The findings of this study revealed virologic failure rates found in other paediatric studies done in Sub-Saharan Africa (5,9,17-21,32).

Previous studies have shown that the level of adherence to ART in children and adolescents is influenced by a number of factors which include the quality and quantity of nutrition (24), pill burden and the availability of syrup regimens (25), as well as adverse drug side effects (26,27). Other factors that can influence the adherence level are treatment fatigue (28), fear of stigmatization by peers (29), as well as vigilance and support of the primary caregivers (22,23,30,31). Our results confirm that suboptimal adherence to ART is the greatest risk factor for virologic failure, as evidenced by the finding that children with suboptimal adherence in this study were 37 times more likely to experience virologic failure than those with optimal adherence. The importance of adherence in ART outcome is further evidenced by the fact that this result was highly statistically significant (P = 0.000003). Suboptimal adherence to ART carries a 3.2-fold greater risk of slow response to ART than optimal adherence. However, this latter result was invalid because the lower limit of the hazard ratio was less than 1 and the P value was greater than 0.05. Perhaps a larger sample size would clearly reveal if adherence plays a role in slow response to ART.

From our results, we postulate that in the initial 12 months of first-line ART, children and their caregivers were likely to adhere strictly to ART because they were eager to attain an undetectable viral load and freedom from opportunistic infections. Once they achieved these goals, treatment that the prevalence of virologic failure was 43.8% after 6 months of ART, 32.2% after 12 months, 28.8% after 18 months, and 24.0% after 24 months of first-line ART. The virologic failure rate of 24% after 24 months of ART found in this study is within the 20% to 34% range of fatigue set in: they became complacent, even careless in their adherence to ART. In other words, their motivation to maintain an undetectable viral load and good health was not as strong as their initial motivation to attain the same. This is supported by three findings from the current study: firstly, the presence of children with virologic rebound after initial treatment success: 2.7% and 2.1% of the cohort after 18 and 24 months of ART, respectively. Secondly, the difference in adherence levels between the treatment success and virologic failure groups was large, as reflected in the level of statistical significance: P = 6.53E-15. Thirdly, children with suboptimal adherence to ART were 37 times more likely to experience virologic failure after 24 months, than those with optimal adherence to ART. The limitations of this study are: firstly, it was a retrospective study, and for some variables, data was available for only 51 children, instead of the total cohort number of 146. This limited the power for data analysis. Secondly, although data on point mutations in this cohort was available, the complete DNA sequences were not. Therefore, a comprehensive analysis of HIV-DR in this cohort could not be conducted.

The strengths of this study are: firstly, the multivariate Cox hazards regression performed in this study made possible the identification of suboptimal adherence to ART as the most important risk factor for virologic failure. Cox hazards analysis of multiple risk factors interacting simultaneously and over time accurately models the challenges of implementing ART in the real world. Secondly, this cohort comprised children and adolescents living

with their caregivers in 8 low-income areas of Nairobi, thereby allowing us to study the outcome of ART implementation in resource-limited settings. Thirdly, the study had 24 months of follow-up, which facilitated the identification of risk factors for virologic failure during the first 2 years of ART. Fourthly, this study did not exclude those HIV-infected children with chronic illnesses or those with interruptions in ART. This allowed us to factor in opportunistic infections and treatment disruptions in the measurement ART outcome.

This study showed that ART implementation in resource-limited settings is effective when regular virologic monitoring, adherence counselling, and HIV-DR testing are available. Secondly, adherence to ART is a strong predictor of treatment outcome for children and adolescents in resource-limited settings. Therefore, methods of increasing adherence to optimal levels should be explored and implemented.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICAL APPROVAL

The study proposal was approved by the Kenya Medical Research Institute, Scientific and Ethics Review Unit (KEMRI-SERU), SSC 2500. Institutional approval for data collection and access to medical records was granted by the Nyumbani Medical Board (NMB), which oversees the Lea Toto Programme (LTP).

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