Factors associated with viral load non-suppression among treatment-experienced pre-teenage children living with HIV in Kenya: a nationwide population-based cohort study, 2015–2021

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Summary

Background Viral load non-suppression (VLNS) in children is a major public health concern because of attendant HIV disease progression and risk of morbidity and mortality. Based on a nationally representative database we present estimates of the prevalence, trends and factors associated with VLNS in Kenyan pre-teenage children between 2015 and 2021.

Methods Kenya National AIDS & STI Control Program's (NASCOP) maintains an early infant diagnosis and viral load (EID/VL) database for all persons living with HIV who are enrolled in the country's primary care clinics for purposes of monitoring progress towards achievement of the 95% viral suppression goals. Participants were eligible if they were children living with HIV (CLHIV), on combination ART (cART) treatment, and ≤12 years old. The modified Mann–Kendall trend test for serially correlated data was used to identify VLNS trends. Generalized estimating equations (GEE) with a logit link was used to assess the effects of covariates on the odds of VLNS (VL ≥1,000 copies/mL) over repeated points in time, allowing for the correlation among the repeated measures.

Findings Between January 2015 and December 2021, 508,743 viral load tests were performed on samples collected from 109,682 pre-teenage children. The prevalence of VLNS decreased from 22.9% (95% CI 22.4–23.3) to 12.5% (95% CI 12.1–12.9), $p < 0.0001$, and mean age increased from 3.1 (4.2) to 8.0 (3.2) years in 2015 and 2021 respectively. A modified Mann–Kendall trend test for serially correlated data denotes a statistically significant decreasing trend ($\tau = -0.300$, $p < 0.0001$) over the study period. In the multivariable GEE analysis adjusted for covariates, the odds of VLNS decreased by 11% per year during the study period, (GEE-aOR 0.89, 95% CI 0.88–0.90; $p < 0.0001$). Factors positively associated with VLNS were EFV/NVP-based first-line cART regimen (GEE-aOR 1.74, 95% CI 1.65–1.84, $p < 0.0001$), PI-based cART regimen (GEE-aOR 1.82, 95% CI 1.72–1.92, $p < 0.0001$), and children aged 1–3 years (toddlers) (GEE-aOR: 1.84, 95% CI 1.79–1.90, $p < 0.0001$). On the contrary, DTG-based cART regimen, were negatively associated with VLNS (GEE-aOR 0.70, 95% CI 0.65–0.75, $p < 0.0001$).

Interpretation There is a strong evidence of decreasing viremia between 2015 and 2021. To sustain the decreasing trend, accelerating the switch from the suboptimal EVP/NVP first-line regimen to optimised DTG regimen is warranted.

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Keywords: Antiretroviral therapy (ART); Generalized estimating equations (GEE); HIV; Kenya; Pre-teenage children; Viral load non-suppression (VLNS)

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Research in context

Evidence before this study

To maximize the benefits of ART for children living with HIV, optimised ART and continuous viral load monitoring are necessary. A search was conducted in PubMed, and google scholar using the following search terms "HIV", "AIDS", "antiretroviral therapy", "viral load suppression", "viral load non suppression", "virological failure", "children" and "Kenya", searching for articles published from January 1st 2010, to December 1st 2022. UNAIDS, and WHO reports were searched for using their websites. We found studies mainly focused on viral suppression in adults restricted to the scope of the supporting partners mainly AMPATH-plus, and Elizabeth Glaser Paediatric AIDS foundation (EGPAF). According to various published articles and reports, there has been an increase in paediatric ART coverage since 2006, though coverage has lagged behind adult ART coverage, especially in resource-limited settings. The published articles had inherent limitations of sample size and there was no nationwide study focusing on pre-teenage children in Kenya.

Added value of this study

To the best of our knowledge, this study is the first comprehensive, nationwide population-based study of VLNS on pre-teenage Kenyan children who most likely acquired HIV through vertical transmission. It utilized 508,743 viral load tests data from 109,682 CLHIV collected from all the 47 counties in Kenya extracted from NASCOP EID/VL database. Findings from this study highlight that, during 2015–2021, there was a decline in VLNS from 22.9% to 12.5%. In the multivariable GEE analysis adjusted for covariates, the odds of VLNS decreased by 11% per year during 2015–2021, which is partly attributed to transition from sub optimal NVP to optimised DTG-based regimens, and the successful implementation of the *Linda mama* program as well as support from 60 non-governmental organizations referred as partners. Among the different age groups, toddlers (1–3 years) had the highest odds of VLNS. Despite the gains, VLNS remained disproportionately high in 7 poor and marginalized counties eight arid and semi-arid (ASA) counties of Baringo, Elgeyo Marakwet, Garissa, Isiolo, Mandera, Samburu, Tana River and Turkana.

Implications of all the available evidence

The major implications of our findings are two-fold. First, policymakers in the Ministry of Health should continue early infant diagnosis, linkage to care, and viral load monitoring, which has resulted in a 50% decrease in VLNS over a seven-year period. To lower the disproportionally high VLNS, the eight ASA counties that are geographical VLNS hotspots require dedicated mitigation interventions. Second, our data provides evidence that ART-related issues were associated with VLNS. The optimization of the ART regimen through continual switch from NVP-based to DTG-based regimen should be intensified in order to achieve VLNS, which is necessary for epidemic control.

Introduction

Paediatric HIV is a global public health concern since children living with HIV (CLHIV) have a higher risk of mortality and morbidity compared to adults living with HIV.[1] As at 2021, 2.73 million [2.06–3.47] children aged 0–15 years were HIV-positive. As of 2021, Kenya had 100,000 CLHIV, or about 10% of the population, living with HIV.[2] The Dar es Salaam Declaration, signed in February 2023, is a commitment by 12 high-burden African countries to end AIDS in children by 2030, because all paediatric HIV infections are preventable and no child should contract AIDS.[3]

The majority of paediatric infections are transmitted from mother to child due to high viral loads caused by new maternal infection and poor adherence to cART or lack of it during pregnancy and breastfeeding in chronically infected women.[4,5] To win the war on VLNS in children, HIV clinicians must provide optimised treatment while providers ensure children remain in care and adhere to treatment. This is because paediatric HIV poses unique challenges because HIV RNA levels remain high throughout infancy due to immature immune systems, which impair viral control and the rapid progression to death.[6] Once on cART, medication adherence issues, cART pharmacokinetic differences, and unsuitable formulations particularly for younger children in resource-limited settings can limit viral suppression. This is critical because ART initiated within the first few hours to days of life reduces the time to viremia suppression and the reservoir size.[7] Paediatric HIV is also adversely affected by lower access to cART, which is estimated to be 52% in 16 Sub-Saharan African countries, with approximately 668,500 CLHIV lacking access to cART.[8]

Kenya has adopted the HIV test- and treat policy and is committed to achieving elimination of mother to child transmission (MTCT) of HIV. Infants born to mothers living with HIV should start infant prophylactic ART within 72 h of delivery and be tested for HIV using PCR no later than two weeks. Those who have a positive PCR result should continue to receive ART, while those who have a negative initial result should continue to receive infant ARV prophylaxis and be monitored as HIV-exposed uninfected infants. A confirmatory PCR should be performed at the point of initiating ART. The preferred first-line ART for infants, and children prior to

2017 was AZT + 3TC + NVP and ABC+3TC + EFV which has been gradually transitioned to DTG-based cART. DTG-based cART has been prescribed in Kenya to CLHIV since 2018, with dolutegravir (DTG) 10 mg scored, dispersible tablets, also known as paediatric DTG, being prescribed since 2021.[9,10] Children who fail to achieve viral suppression while on first-line treatment are switched to second-line ART after a period of enhanced adherence support.[11] The paediatric testing and linkage to care is supported by *Linda Mama* program, a publicly funded healthcare scheme launched in 2013 whose aim is to "achieve universal cases to maternal and child health services and contribute to the country's progress towards universal health care".[12] Along with *Linda Mama*, 60 PEPFAR- and CHAI-funded partners supported CLHIV across the country gain access to care, maintain continuity of care, and attend clinic appointments in accordance with national ART guidelines.

Despite the significant progress in the prevention of MTCT in recent years, new paediatric infections continue to occur in Kenya. In 2022, it was estimated that 31% of CLHIV had not accessed testing, 41% were not on treatment, and not all children on ART were virally suppressed.[13] Understanding the factors associated with VLNS in treatment-experienced children is critical to accelerating progress toward ending AIDS by 2030. Furthermore, monitoring national viral load trends is critical in determining whether the VLNS is increasing, decreasing, or remaining constant in order to assess the efficacy of cART regimens in controlling the paediatric HIV pandemic. As a result, the goal of this nationwide study was to estimate the marginal longitudinal predictive relationship between use of cART and other covariates among pre-teenage children living with HIV who are not suppressing viremia over a 7-year period (2015–2021). We used the GEE model to model the estimates at the population level in order to account for repeated viral load tests.

Methods

Study design and data sources

This population-based cohort study included viral load data collected from pre-teenage CLHIV and on cART in Kenya between 2015 and 2021. The viral load data was extracted from the NASCOP viral load/early infant diagnosis (EID/VL) monitoring system. NASCOP is a national organization under the Kenyan Ministry of Health whose mandate is to collect, pool, and analyse clinical and epidemiological data gathered by Kenyan health facilities implementing HIV programmes and using Electronic Medical Records (EMRs). The NASCOP's EID/VL database contains data on HIV VL tests performed in the country's testing labs linked to patient's clinical data and socio-demographic characteristics. The database has deduplication algorithm which is defined at both the health facility and NASCOP levels to improve data quality by identifying duplicates for unique patient categorization.

Data collection

This study's dataset included 508,743 viral load tests collected from 109,682 CLHIV patients identified by unique patient IDs. The children were aged 0–12 years old, on cART, and enrolled in any of Kenya's 3,735 primary care clinics between 2015 and 2021. The year 2015 was chosen as the baseline because it was the calendar year in which the NASCOP dashboard, which was created to hold national electronic medical records for HIV testing, care, and treatment in Kenya, became fully operational after its initial launch in 2014. Viral load tests were conducted after 6 months of initiating ART and subsequently after 1 year except for breastfeeding infants where the guidelines recommend the VL test at three month intervals. Viral load tests/year include all tests performed on children aged 0–12 during that calendar year. For example, a child aged 12 in 2015 contributed only one sample to the dataset, whereas a child born in January 2015 and breastfed for 12 months contributed an average of 10 results to the dataset. The choice of 0–12 years, which covers preteenage, was motivated by the need to model only the perinatal infections.

Statistical analysis

This was a longitudinal study in which the same dependent variable (viral load) was measured on the same children over time (between 2015 and 2021). The data on viral load (VL) is dichotomized into two categories: VLNS (≥1,000 copies/mL) or viral load suppression (VLS) (<1,000 copies/mL). For descriptive analyses, categorical and continuous variables were reported as proportions and medians with interquartile range (IQR), respectively. One-way repeated measures ANOVA, was used to analyse changes in VLNS proportions over different years. It was utilized because same individuals are measured on the same outcome variable in different years. Prior to performing pairwise comparisons, the data was checked for outliers, normality, and sphericity functions, inbuilt in the "rstatix" R package. Prevalence of VLNS was calculated by calendar year (point prevalence) and further by county using the first VL result per year to avoid bias from multiple results within the same year. The proportion of VLNS was calculated as follows:

% VLNS = (Viral load tests with ≥1,000 copies/mL in a given year)/(All viral load tests in a given year)?

The VLNS trends were calculated using the Modified Mann–Kendall test for serially correlated data using the Yue and Wang variance correction approach using the "modifiedmk" R package.[14] The prevalence of VLNS stratified per year and associated 95% confidence intervals (CI) were estimated using "*epiR*" R package. To

account for multiple viral load tests per child at different years over the study period, we used generalized estimating equations (GEE) with logit link using a first-order autoregressive (ar1) correlation matrix (assuming equal correlations between observations within person) and robust standard errors (reduces risk of error due to mis-specified matrix) to examine changes in the dependent variable using "geepack" R package. The ar1 correlation matrix was used because we expect correlation between time points to degrade with time, so viral load tests conducted earlier in the study are more correlated than those conducted later in the study due to the influence of cART regimen and other support programmes. Equations were adjusted for socio-demographic factors (sex, age category, and county HIV prevalence status), and clinical factors (cART regimen., which is further categorized as DTG-based regimen, NVP/EFV based regimen, PI based regimen and other regimen (any cART that does not include either DTG, EFV, NVP or PI drug), sample type, and treatment duration. Three multivariable models were created: Model 1 includes clinical variables and treatment duration; Model 2 adds sex, age category, and county HIV prevalence variables while omitting treatment duration from Model 1; and Model 3 adds an interaction term for sample type and county prevalence to Model 2 to see if VLNS results differed significantly by sample type and county HIV prevalence. Covariates were included based on the most parsimonious model accounting for existing knowledge on possible risk factors *a priori*. The most parsimonious model was then found by comparing the GEE fit criteria's Quasi-likelihood under the Independence model Criterion scores, with lower scores indicating greater parsimony. Missing values were excluded from statistical analyses. All statistical analyses were performed using the R statistical package (version 4.2.3).

Ethical statement

The protocol for viral load assays was approved by the Kenya Medical Research Institute's Scientific and Ethics Review Unit (KEMRI/SERU/CIPDCR/031/3707). Waiver of informed consent was granted by Kenyatta National Hospital-University of Nairobi Ethics and Research Committee (KNH-UON-ERC: P87/02/2023), for secondary data analysis because the data was collected during the course of routine viral load monitoring by Kenya's Ministry of health via NASCOP. The data obtained complied with relevant data protection and privacy regulations and individual identifiers were removed. The study was conducted in accordance with the 1964 Helsinki declaration.

Role of the funding source

The study funders (CHAI and PEPFAR) had an indirect role in viral load assays (funding viral load assays). The funders had no role in the design of the study, data analysis, data interpretation, or report writing. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit the manuscript for publication.

Results

Sociodemographic and clinical characteristics of the participants are shown (Table 1). Overall, 508,743 viral load tests were conducted on 109,682 CLHIV enrolled in 3,735 clinics in all 47 counties between January 2015 and December 2021. The early infant diagnosis and viral load monitoring program under NASCOP was supported by 60 partners spread across the country (Supplementary Figure S1). The notable missing's were sex 19,619 (3.8%), and ART regimen 20,893 (4.1%). The median age of the children was 7.0 (IQR 3.0–10.0) years, with a majority aged between 7 and 12 years (middle childhood) (61.7%). Overall, 55% of the VL tests were conducted on girls, with 61,542/267,463 (23% having VLNS) compared to 58,786/221,661 (27%) in boys. TDF + 3TC + EFV was the most prescribed ART in 2015 at 29% (8,183/28,254) which declined to 1.7% (532/31,264) in 2021. At the same time, there was a gradual increase of DTG-based regimens, TDF + 3TC + DTG from 0% in 2015 to 21% (6,695/31,264) in 2021. Notably, the use of NVP-containing regimens such as ABC + 3TC + NVP decreased from 11% (3,055/28,254) in 2015 to 0.5% (169/31,264) in 2021. A similar decline in prescription was noted for AZT + 3TC + NVP which decreased from 24% (6,647/28,254) in 2015 to 2% (51/31,264) in 2021 as shown in Fig. 1.

The prevalence of VLNS decreased from 22.9% (95% CI 22.4–23.3) to 12.5% (95% CI 12.1–12.9), $p < 0.0001$ in 2015 and 2021 respectively. Despite a downward trend, there was a notable spike in the proportion of VLNS between 2016 and 2017. After accounting for repeated viral load tests conducted within and between years, the proportion of VLNS showed statistically significant difference over the years, ANOVA F (1.95, 70.2) = 74.08, $p < 0.0001$, generalized eta squared = 0.82. A post-hoc analyses with a Bonferroni adjustment revealed that all the 21 pairwise differences, between the years, were statistically significantly different at $p < 0.002$ (Fig. 2). A modified Mann–Kendall trend test for serially correlated data using the Yue and Wang variance correction approach for all 47 counties from 2015 to 2021 showed a statistically significant decreasing trend, ($\tau = -0.300$, $p < 0.0001$) over the seven-year period with the greatest effect observed after 2017. However, the scenario differs significantly for seven poor and marginalised counties in Kenya's arid and semi-arid (ASA) regions, which account for 6.5% of HIV viral load tests and where VLNS remains disproportionately high (Fig. 3).

We fitted GEE models with a first-order autoregressive (ar1) correlation matrix to identify variables

Characteristic	N = 508,743 n (%)
VLNS	123,825 (24%)
Year of VL test	
2015	31,405 (6.2%)
2016	132,960 (26%)
2017	75,654 (15%)
2018	77,689 (15%)
2019	95,758 (19%)
2020	63,920 (13%)
2021	31,357 (6.2%)
Sex	
Female	267,463 (55%)
Male	221,661 (45%)
Missing data	19,619
Age	7.0 (3.0–10.0)
Age category (years)	
Infants (<1)	97,414 (19%)
Toddlers (1–3)	46,070 (9.1%)
Early childhood (4–6)	51,451 (10%)
Middle childhood (7–12)	313,808 (62%)
Justification for the VL test	
Baseline	6,823 (1.4%)
Breastfeeding	773 (0.2%)
Clinical failure	857 (0.2%)
Confirmation of PLLV	396 (<0.1%)
Other reason	2,502 (0.5%)
Pregnant mother	332 (<0.1%)
Repeat VL	33,849 (7.0%)
Routine VL	432,132 (90%)
Single drug substitution	4,226 (0.9%)
Missing data	26,853
Sample type	
Dried blood spot	136,756 (27%)
Plasma	296,876 (58%)
Whole blood	75,111 (15%)
cART Regimen	
ABC + 3TC + ATVr	3,242 (0.7%)
ABC + 3TC + DRVr + RAL	104 (<0.1%)
ABC + 3TC + DTG	12,929 (2.7%)
ABC + 3TC + EFV	94,073 (19%)
ABC + 3TC + LPVr	72,553 (15%)
ABC + 3TC + NVP	47,170 (9.7%)
ABC + 3TC + RAL	449 (<0.1%)
AZT + 3TC + ABC	253 (<0.1%)
AZT + 3TC + ATVr	17,105 (3.5%)
AZT + 3TC + DRVr + RAL	68 (<0.1%)
AZT + 3TC + DTG	872 (0.2%)
AZT + 3TC + EFV	10,153 (2.1%)
AZT + 3TC + LPVr	20,762 (4.3%)
AZT + 3TC + NVP	66,345 (14%)
TDF + 3TC + ATVr	5,628 (1.2%)
TDF + 3TC + DTG	17,066 (3.5%)
TDF + 3TC + DTG + ATVr	23 (<0.1%)
TDF + 3TC + DTG + DRVr	9 (<0.1%)
TDF + 3TC + DTG + ETV + DRVr	5 (<0.1%)
TDF + 3TC + EFV	74,908 (15%)
TDF + 3TC + LPVr	3,579 (0.7%)
TDF + 3TC + NVP	17,237 (3.5%)
TDF + 3TC + RAL + DRVr	1 (<0.1%)
Other regimen	23,316 (4.8%)
Missing data	20,893
cART regimen class	
PI-based regimen	123,042 (25%)
DTG-based regimen	30,904 (6.3%)
EFV/NVP-based regimen	309,886 (64%)
Other regimen	24,018 (4.9%)
Missing data	20,893
Treatment duration (Months)	49 (24–79)
VL tests in a given year	
>1	368,912 (73%)
2	88,648 (17%)
≥3	51,183 (10%)

n (%); Median (IQR). cART, combination antiretroviral therapy. ABC, abacavir. 3TC, lamivudine. EFV, efavirenz. ATVr, ritonavir boosted atazanavir. DRVr, ritonavir boosted darunavir. RAL, raltegravir. DTG, dolutegravir. LPVr, ritonavir boosted lopinavir. NVP, nevirapine. AZT, Zidovudine. TDF, tenofovir disoproxil fumarate. ETV, Etravirine. Other, ART not based on 3TC backbone or different combination from what is provided by Ministry of Health. PLLV, persistent low level viremia. VL, viral load. VLNS, viral load non-suppression (HIV viral load ≥1,000 copies per mL).

Table 1: **Patient sociodemographic and clinical characteristics.**

associated with VLNS. The outputs of the three GEE models that include the QICu statistic are presented in Table 2. Model III had the best fit and the lowest QICu. Using this model, the odds of VLNS decreased by 11% per year during the study period, (GEE-aOR 0.89, 95% CI 0.88–0.90; p < 0.0001) after adjusting for covariates included in the model. The independent risk factors positively associated with VLNS included: EFV/NVP-first line ART regimens, PI-based regimens; male sex; and use of dried blood spot (DBS) samples. After adjusting for covariates, the odds of VLNS in children on EFV/NVP-based first-line cART regimens were 74% higher than those on other regimens (reference) (GEE-aOR 1.74, 95% CI 1.65–1.84, p < 0.0001). Similarly, the odds of VLNS on children on double boosted PI-based cART regimens were 82% higher than the reference group (GEE-aOR 1.82, 95% CI 1.72–1.92, p < 0.0001). Boys had 19% higher odds of being VLNS compared to girls (GEE-aOR 1.19, 95% CI 1.16–1.21, p < 0.0001). Utilization of DBS was associated with 72% higher odds of VLNS compared to the use of plasma. There were no significant associations found between whole blood and VLNS. In addition, when an interaction effect between sample type and county HIV prevalence status, use of DBS in high prevalence counties was associated with 38% higher odds of VLNS (GEE-aOR 1.38, 95% CI 1.27–1.50, p < 0.0001); and 11% lower

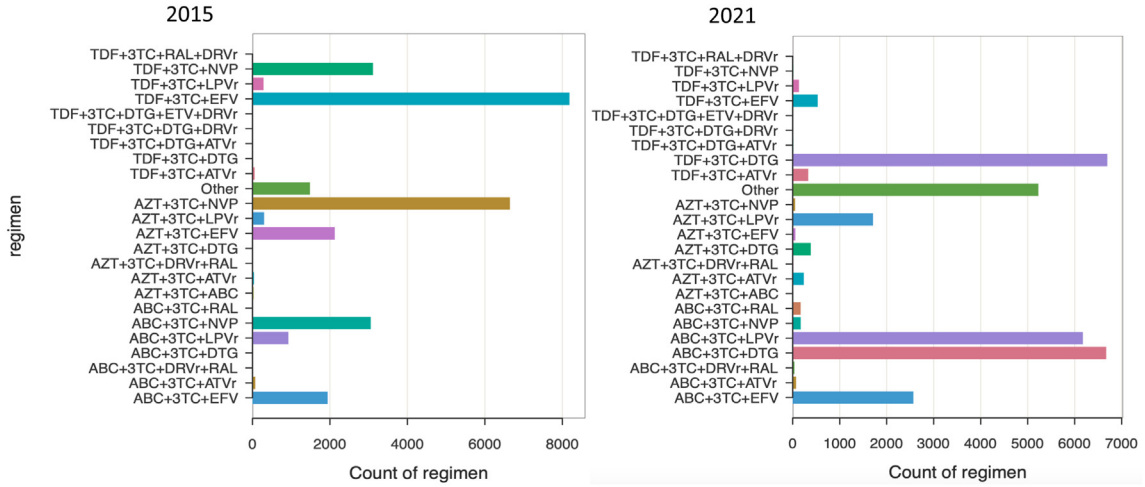

Fig. 1: Bar graph of differences in the absolute counts of ART regimen prescribed to pre-teenage children in 2015 (left) and 2021 (right).

odds of VLNS when DBS was used in low prevalence HIV counties (GEE-aOR 0.89, 95% CI 0.85–0.93, p < 0.0001). The interaction effects did not extend to the use of whole blood samples. Low prevalence counties had a 13% higher odds of VLNS (GEE-aOR 1.13, 95% CI 1.09–1.17, p < 0.0001), compared to medium prevalence counties (reference). We noted substantial variation of VLNS in different age categories. Children aged 1–3 years (toddlers) had 84% higher odds of VLNS (GEE-aOR: 1.84, 95% CI 1.79–1.90, p < 0.0001); while children who were <1 year old (infants) had 51% lower odds of VLNS (GEE-aOR: 0.49, 95% CI 0.47–0.51, p < 0.0001) compared to children aged between 7 and 12 years (middle childhood) (reference). The main determinant for lower VLNS during the study period was DTG containing cART regimens which were associated with a 30% lower odds of VLNS (GEE-aOR 0.70, 95% CI 0.65–0.75, p < 0.0001) compared to other regimens (reference).

Discussion

Our results suggest three key findings. First, the prevalence of VLNS among pre-teenage children has decreased significantly over the seven-year study period, and this trend is expected to continue. This finding is due to

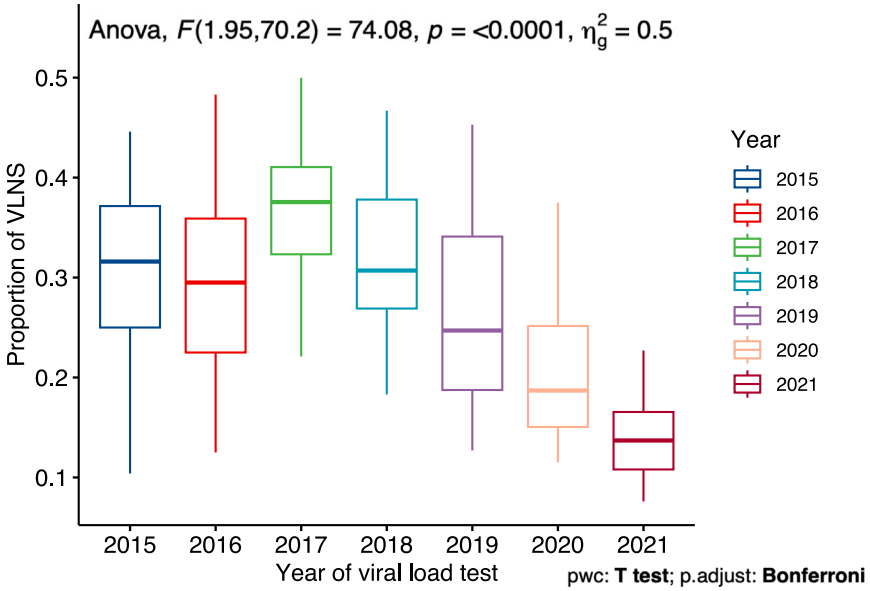

Fig. 2: **A pairwise comparison of viral load non-suppression (VLNS) differences between years using** one-way repeated measures ANOVA. Significant p-values adjusted for multiple comparisons using Bonferroni test and were considered statistically significant at p < 0.002.

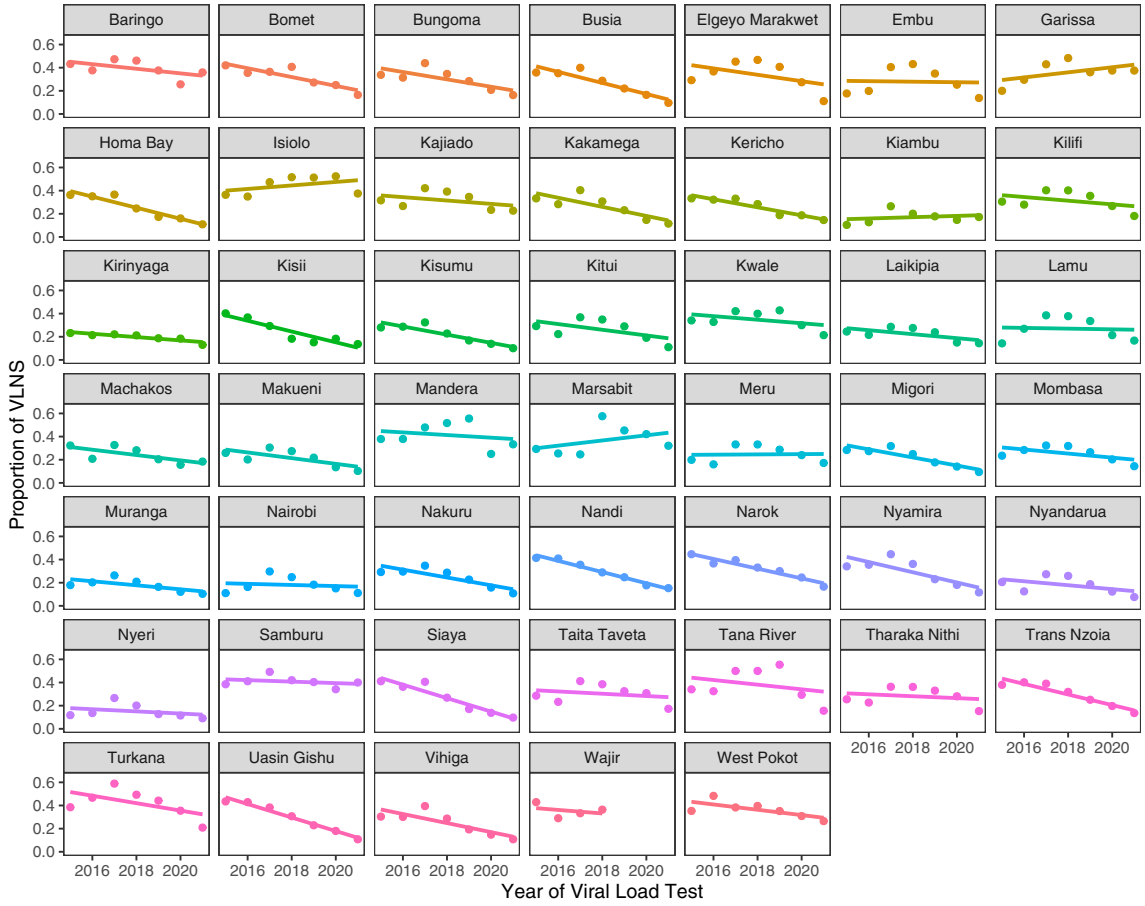

***Fig. 3*: Calendar year trends in Viral load non-suppression (VLNS) for children on cART in all the 47 counties in Kenya.** The dots represent the point estimate of VLNS for that year. Over the study period, there is a discernible decline in the majority of counties. The line-colour schemes generated by R-software have no special meaning.

paediatric-friendly and optimised cART, which are effective in achieving and maintaining viral suppression. This is significant because viral suppression triggers faster immune reconstitution, less illness, and death.[15] The significant increase in age of the children is further validation of these findings. Second, the increased risk of VLNS in paediatric population is due to the convergence of three factors: EFV/NVP-based first-line therapies, PI-based therapies, with the biggest VLNS observed in 1–3 year olds (toddlers). Third, increased scaling-up of VL monitoring and optimised cART during the study period was very effective in lowering VLNS levels across the country, as it brought on board more CLHIV who would be put on optimised cART if they experienced treatment failure. These findings should be interpreted with knowledge that VLNS was based on ≥1,000 copies/mL, the highest cut-off according to WHO guidelines.[16] Our study's strengths include a large and longitudinal dataset collected in validated laboratories in accordance with standard patient care.

In many resource-constrained settings where population-based interventions are the norm, assessing the impact of various HIV treatment interventions and associated sociodemographic characteristics on reducing population level viremia are critical. For viral load datasets that are repeated on an annual basis, there are two approaches that are traditional and widely used in practice: generalised linear mixed-effects (GLME) and GEE.[17] In this study we adopted the GEE, a population-level approach based on a quasi-likelihood function and provides the population-averaged estimates of the parameters.[18] Specifically, since we are interested in whether the available strategies mainly the prescribed cART is effective in reducing viremia across the country, GEE is preferred as it relaxes the distribution assumption and only requires the correct specification of marginal mean and variance, as well as the link function which connects the covariates of interest and marginal means.[19] Moreover, for population-level studies with large datasets, such as ours, authors face the

QIC_u statistic	Model I			Model II			Model III		
	265,714			262,400			261,035		
Variable	OR	(95% CI)	p-value	OR	95% CI	p-value	OR	95% CI	p-value
Year	0.96	(0.95–0.97)	<0.0001	0.89	(0.88–0.90)	<0.0001	0.89	(0.88–0.90)	<0.0001
cART regimen class									
Other regimen	Ref			Ref			Ref		
PI-based regimen	2.01	(1.90–2.13)	<0.0001	1.82	(1.72–1.92)	<0.0001	1.82	(1.72–1.92)	<0.0001
DTG-based regimen	0.65	(0.61–0.70)	<0.0001	0.70	(0.65–0.74)	<0.0001	0.70	(0.65–0.75)	<0.0001
EFV/NVP-based regimen	1.68	(1.59–1.78)	<0.0001	1.73	(1.64–1.83)	<0.0001	1.74	(1.65–1.84)	<0.0001
Sample type									
Plasma	Ref			Ref			Ref		
Whole blood	1.11	(1.10–1.14)	<0.0001	1.00	(0.97–1.04)	0.78	1.08	(0.99–1.18)	0.050
Dried blood spots	1.88	(1.83–1.92)	<0.0001	1.69	(1.65–1.74)	<0.0001	1.72	(1.66–1.78)	<0.0001
Treatment duration	1.00	(1.00–1.00)	<0.0001						
Sex									
Female				Ref			Ref		
Male				1.18	(1.16–1.20)	<0.0001	1.19	(1.16–1.21)	<0.0001
Age category									
Infant (<1)				0.49	(0.47–0.51)	<0.0001	0.49	(0.47–0.51)	<0.0001
Toddler (1–3)				1.87	(1.81–1.93)	<0.0001	1.84	(1.79–1.90)	<0.0001
Early childhood (4–6)				1.20	(1.16–1.24)	<0.0001	1.20	(1.16–1.24)	<0.0001
Middle childhood (7–12)				Ref			Ref		
County HIV prevalence									
Medium prevalence (5–14.9%)				Ref			Ref		
Low prevalence (<5%)				1.06	(1.04–1.09)	<0.0001	1.13	(1.09–1.17)	<0.0001
High prevalence (≥15%)				1.03	(0.99–1.06)	0.12	0.99	(0.95–1.03)	0.57
Sample type*County HIV prevalence									
Whole blood*low prevalence							1.06	(0.87–1.28)	0.55
DBS*low prevalence							0.89	(0.85–0.93)	<0.0001
Whole blood*high prevalence							0.96	(0.87–1.06)	0.44
DBS*high prevalence							1.38	(1.27–1.50)	<0.0001

For all categorical variables, each category is compared to the base category (Ref). The ORs of <1 indicate a decreased odds of VLNS, whereas ORs of >1 indicate an increased odds of VLNS. cART, combined antiretroviral treatment. Other, ART not containing DTG, EFV, NVP or PI-cART. DBS, dried blood spots. PI, protease inhibitor. OR, Generalized estimating equations adjusted Odds Ratio. CI, Confidence Interval.

Table 2: **Generalized estimating equations model outputs with calculated QICu (with binomial logistic link) fitted with respect to predictors of viral load non-suppression in pre-teenage children.**

conundrum of determining the combinations of determinants with the highest predictive value of VLNS while balancing statistical significance and clinical relevance. In our case, our parsimonious model included year of the test, cART regimen, sex, and sample type–county interaction. The inclusion of counties (47 units) in the final multivariable model resulted in a very complex model with no significant improvement over the one without it, so we used counties classified according to their HIV prevalence's.[20]

Across the country, we noted a steady decline of VLNS among all age groups, with a remarkable decline after 2017. Intriguingly, VLNS increased between 2016 and 2017, coinciding with strategic programme shifts such as the introduction of DTG, structured adherence support for low level viremia, and plasma VL network expansion to routinely monitor patients.[21,22] The decreasing trend of VLNS in pre-teenage children was likely caused by a number of factors. Notably, a substantial decrease in VLNS among the infants (<1 year old children) is attributed to the impact of the *Linda Mama* program a public funded initiative and support from partners including AMPATH-plus, EGPAF, and KCCB-KARP, which offer mother-infant dyads focussed services.[12,23] During the program's successful implementation, we have observed a substantial decrease in VLNS specifically among the infants, as well as fewer vertical transmissions in the later years of the study period. Several enabling factors that have made *Linda Mama* program successful include linkage and retention into care for the mother and infant. This has played a key role in preventing MTCT, and reduction of viremia in infants who access ART very early in life which reduces the reservoir size and thus hastens the time to achieve viremia suppression.[7] The decreasing population level viremia is consistent with data from other

studies conducted in similar settings.[24] Despite the progress made over the study period, VLNS prevalence remained disproportionately high in seven poor and marginalised counties, which had the lowest declines of all the counties analysed over the study period. These counties also have low HIV prevalence based on adult statistics, as well as difficult geographical conditions that may necessitate the use of DBS over plasma. Due to their low HIV prevalence, these counties receive less attention than high prevalence counties, which could explain the observed high VLNS in lower HIV prevalence settings.

The multivariable GEE analyses showed a strong association between NVP-based cART regimens and higher odds of VLNS, which is in line with previous studies in sub Saharan Africa.[25,26] This observation could be explained by the fact that NNRTIs such as NVP and EFV feature low genetic barriers of resistance, due to the K103N, G190A, and Y181C mutations.[27,28] Pre-treatment drug resistance (PDR) can obscure a regimen effect when the viral load results of PDR and non-PDR subjects are compared in the absence of a genotypic test. Thus, in a country like Kenya, where there is a test and treat policy, the problem of PDR is a challenge, and it was exacerbated in paediatrics by previous practices attributed to single dose nevirapine (sdNVP) for prevention of MTCT of HIV.[22] At the advent of cART therapy in sub-Sahara Africa, the demonstration that sdNVP, that is one tablet to the mother at the onset of labour and less than a teaspoonful to the baby had a 50% efficacy in reducing MTCT of HIV, paved the way for the development of the PMCT services and which have evolved to become robust comprehensive HIV services for pregnant and breastfeeding women.[29] However, it later emerged that previous exposure to NVP even as a single dose for PMTCT was associated resistant strains,[30] which prompted WHO to recommend switching from NNRTI regimens where communal resistance exceeded 10%.[31] The government of Kenya has also adopted a public health approach and progressively transitioned individuals on cART to LPV/r or DTG-based regimen in a process called treatment optimization and which has resulted in an impressive 50% reduction in VLNS from 2015 to 2021. Superior viremia suppression has been reported in Kenyans over the age of 15 living with HIV who transitioned to DTG-based regimen between 2015 and 2021.[21] However, careful monitoring of children on lifelong DTG-based regimen is warranted. This is because data indicates that DTG-regimens in patients of African descent are associated with obesity and metabolic disorders, which increases their lifetime risk of cardiovascular disease.[32]

Children aged 1–3 years (toddlers) had nearly two-fold higher odds of having VLNS compared to infants (<1 year old) and the other age categories in concordance with studies conducted in Malawi, Uganda and Zimbabwe.[24] The increased prevalence of VLNS could be non-adherence due to lack of appropriate drug formulations in the younger age groups.[33,34] This scenario is different in childhood whereby the children might not have any challenges swallowing tablets. These factors constitute risk factors for VLNS irrespective of ART-regimen and therefore confound the association between ART-regimen and VLNS. Therefore there is need for development of child-friendly ART formulation which then enable better adherence to treatment. Paradoxically, infants had lower odds of VLNS among all categories which could be attributed to *Linda Mama* and other programs that ensures follow-up of the mother-infant dyad during immunization programs and keen follow-up during breastfeeding to ensure the mother is fully suppressed, in compliance with Undetectable = Untransmissible.[35] Boys had a higher risk of VLNS than girls in this study, which was consistent with previous research, and this finding can be attributed to sex-specific differences in treatment efficacy, toxicity profiles, and drug pharmacokinetics.[36] For instance, rash with NVP is more common in women than in men, most likely due to sex-based pharmacokinetic differences, which may necessitate frequent changes from sub-optimal NVP regimens and thus better viremia control.[37] The extent to which same is applicable in children is not well established.

The GEE model, which employs a population-level approach based on a quasi-likelihood function to provide population-averaged estimates of the parameters, was the best tool for answering the research question for our nationwide viral load dataset. Despite this, there are some limitations; the first and second are model-specific, while the rest are unique to our dataset. First, as an observational study analysing secondary data, it encountered data quality issues, particularly missing data. Analysis models using GEEs require that the data are missing completely at random (MCAR) which may at times be an unreasonable assumption. However, despite MCAR violations, GEEs have been shown to have acceptable performance even when data are missing at random (MAR) and may depend as well on the amount of missing data, choice of working correlation structure, and level of model misspecification.[38] Second, GEEs do not provide an estimate of the mechanism that generates the data. As a result, we cannot simulate new data using a fitted GEE. Third, since we included data collected from publicly funded clinics, there is a likelihood we excluded an important group of children who access care from non-public funded institutions who might not sharing this data with NASCOP. Fourth, the lower number of viral load assays in 2020 and 2021 are the result of COVID-19 lockdowns, which restricted people's movement and constrained the supply of essential HIV prevention and treatment commodities, with the greatest impact on vulnerable groups such as children.[39] This missingness though well addressed by the GEE model has an effect on one-way

repeated measures ANOVA that require balanced data. Despite the limitations, to the best of our knowledge, this study included the largest sample size in the country, allowing a population-level assessment of the effect of enhance scaling up of optimised cART on reduction of HIV viremia in the study population.

In conclusion, cART scale-up with optimised DTG-based regimen and a scale-up of VL monitoring, facilitated in part by the Linda Mama programme and other partners, resulted in a 50% decrease in VLNS in preteenage children. Our findings highlight geographical variation with regions of relatively low HIV prevalence having high VLNS despite the nationwide decreasing trend. Policymakers should take note of the findings in order to sustain the transition from suboptimal EFV/NVP-based regimens to optimised DTG-based regimens, focus attention on regions identified as geographical hotspots, and thus help mitigate the paediatric pandemic in seven counties that are lagging behind.

Contributors

MMM, and MM conceived and designed the study. MMM, NKK, HK and EOO developed the statistical analysis plan. NKK, JW, PB, MO, and KW collected viral load data. KW uploaded viral load data into NASCOP database. MMM and EOO performed the statistical analyses, interpreted data and created the figures. MM provided managerial and logistical support, and helped with result interpretation. MMM wrote the initial draft. DCW and RWN provided expert input to inform background, context, and paediatric epidemiology. MMM, RWN, and MM reviewed and edited the final manuscript. MMM, NKK, HK, and MM accessed and verified the analysed data and outputs. All authors contributed to and approved the final manuscript. MMM, and MM had final responsibility for the decision to submit the manuscript for publication.

Data sharing statement

De-identified participant data underlying the findings described in this manuscript will be made available upon request to the corresponding author in consultation with the Ministry of Health based on the scientific veracity of the proposal. Upon approval of a proposal, data can be shared through a secure online platform after signing a data access agreement.

Declaration of interests

All the authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2024.102454.

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