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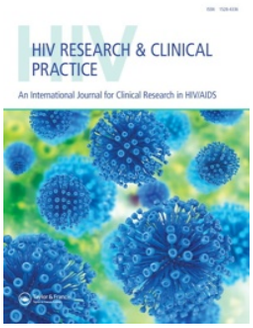

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RESEARCH ARTICLE

Longitudinal analysis of viral suppression before, during, and after pregnancy among women on antiretroviral therapy in Uganda: six-year real-world experience

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ABSTRACT

Introduction: This study evaluated the effectiveness of antiretroviral therapy (ART) and associated factors on viral suppression before, during, and after pregnancy (maternal timeline).

Methods: We conducted a cohort study, retrospectively reviewing records of 1291 pregnant and breastfeeding women on ART. Descriptive statistics summarised the demographics and clinical characteristics. Chi-square, Fisher's exact, and generalised estimating equations were used to assess variations in viral suppression across the maternal timeline.

Results: ART regimens comprised 62.5% dolutegravir (DTG)-, 28.8% efavirenz (EFV)-, 4.5% nevirapine (NVP)-, and 4.2% protease inhibitor (PI)-based therapy. Viral suppression rates before, during, and after pregnancy were DTG- (95.0%, 94.6%, 95.7%), EFV- (94.9%, 94.2%, 93.6%), NVP- (93.1%, 94.7%, 93.5%), and PI-based (79.6%, 88.0%, 85.7%). ART regimens varied in effectiveness, with statistical significance observed before ($p < 0.001$) and after ($p = 0.018$), but not during pregnancy ($p = 0.678$). PI-based regimens showed higher risk of non-suppression in the non-adjusted model (IRR = 3.20, 95% CI: 1.63–6.30, p = 0.001). In the adjusted model, poor adherence (aIRR = 7.80, 95% CI: 2.54–23.90, $p < 0.001$), fair adherence (aIRR = 5.03, 95% CI: 1.11–22.86, $p = 0.036$), second-line ART (aIRR = 3.14, 95% CI: 1.75–5.62, $p < 0.001$), and third-line ART (aIRR = 8.48, 95% CI: 1.82–39.43, $p = 0.006$) remained significant.

Conclusion: ART effectiveness showed variation before and after, but not during pregnancy. EFV- and NVP-based regimens achieved suppression rates comparable to DTG across maternal timelines, with the exception of PI-based regimens. Adherence and ART drugs influence outcomes more than regimen choice alone, with good adherence essential for optimal maternal outcomes.

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Antiretroviral therapy; pregnancy; Uganda; real-world experience; ART effectiveness; viral suppression

Introduction

Viral suppression is a key indicator of antiretroviral therapy (ART) effectiveness and is particularly crucial in the prevention of mother-to-child transmission (PMTCT) of HIV [1–3]. While ART has significantly improved maternal and infant health outcomes, pregnancy-related physiological changes can alter ART pharmacokinetics [4,5], potentially leading to suboptimal viral suppression [6]. This, in turn, increases the risk of virologic failure, which may be influenced by factors such as ART regimen choice, adherence, drug resistance, and the complexities of managing HIV during pregnancy and postpartum [7–9].

Uganda's ART regimens have evolved to improve treatment outcomes. Prior to 2010, the standard first-line regimens included zidovudine (AZT), lamivudine (3TC), and nevirapine (NVP). Owing to concerns

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about toxicity and resistance, it was replaced with tenofovir (TDF), 3TC, and NVP. By 2010, the preferred regimen shifted to TDF/3TC/efavirenz (EFV), and with the introduction of dolutegravir (DTG) in 2013, TDF/3TC/DTG was adopted as the WHO-recommended first-line regimen in 2016. This regimen has shown superior viral suppression and a high genetic barrier to resistance [3,10,11].

Over time, the above-mentioned ART regimens have demonstrated effective viral suppression within PMTCT programs and have significantly reduced the number of infants testing positive at the first PCR test [3,12,13]. However, cases of seroconversion thereafter highlight the need to understand how ART regimens perform across maternal physiological changes before pregnancy, throughout gestation, and after birth. Understanding the association between ART regimens and viral suppression across the maternal timeline is therefore vital for improving maternal and child health outcomes beyond the antepartum stage [10]. Given that ART prescription follows a public health approach, utilising real-world data from HIV program implementation enhances translational research and helps to refine HIV treatment protocols.

This study assessed ART effectiveness, measured by viral load suppression across the maternal timeline, before, during, and after pregnancy, among women receiving different ART regimens at the Mildmay Uganda PMTCT clinic. The findings aim to inform healthcare providers and policymakers in terms of optimising maternal HIV treatment strategies. This research supports efforts to reduce mother-to-child transmission, improve maternal and infant health outcomes, and contribute to global targets for achieving HIV epidemic control by 2030.

Methods

Study design

This cohort study retrospectively reviewed the medical records of pregnant and breastfeeding women living with HIV enrolled in the PMTCT clinic at the Mildmay Uganda Hospital (MUgH) from 2018 to 2023.

Study site and population

This study was conducted at MUgH, a peri-urban facility in Wakiso District with 50-bed capacity, providing integrated TB–HIV care and ART to nearly 15,000 people living with HIV [14]. The study population included pregnant and breastfeeding mothers registered at the PMTCT clinic between 2018 and 2023. We included participants with at least one viral load result during the pre-pregnancy period. Participants who were enrolled in the clinic before 2018 or after 2024 were excluded.

Data collection and management

Data collection and participant recruitment were conducted over a three-month period (April to June 2024). Data on participants' socio-demographic characteristics and medical history were extracted from PMTCT registers, patient cards, and electronic medical records (EMRs). Data entry was performed using Microsoft Excel, with regular accuracy checks against hard copy records to ensure data integrity. A total of 1291 participant records were retrieved, distributed by year as follows: 226 in 2018, 215 in 2019, 165 in 2020, 205 in 2021, 235 in 2022, and 245 in 2023. Viral load results for each time point were considered at any time within the defined period: before pregnancy (preconception), during pregnancy (any trimester), and postpartum. A flowchart illustrates the data collection process (Figure 1).

Statistical analysis

Statistical analyses were performed using Stata 17. Descriptive statistics summarised demographic and clinical characteristics as frequencies and percentages. Categorical variables were compared using the Chi-square or Fisher's exact test as appropriate.

The indicator of ART effectiveness was viral load suppression in copies/mL, which was categorised into three groups: suppressed (≤200), low viremia (201–999), and high viremia (≥1000) [3], and later

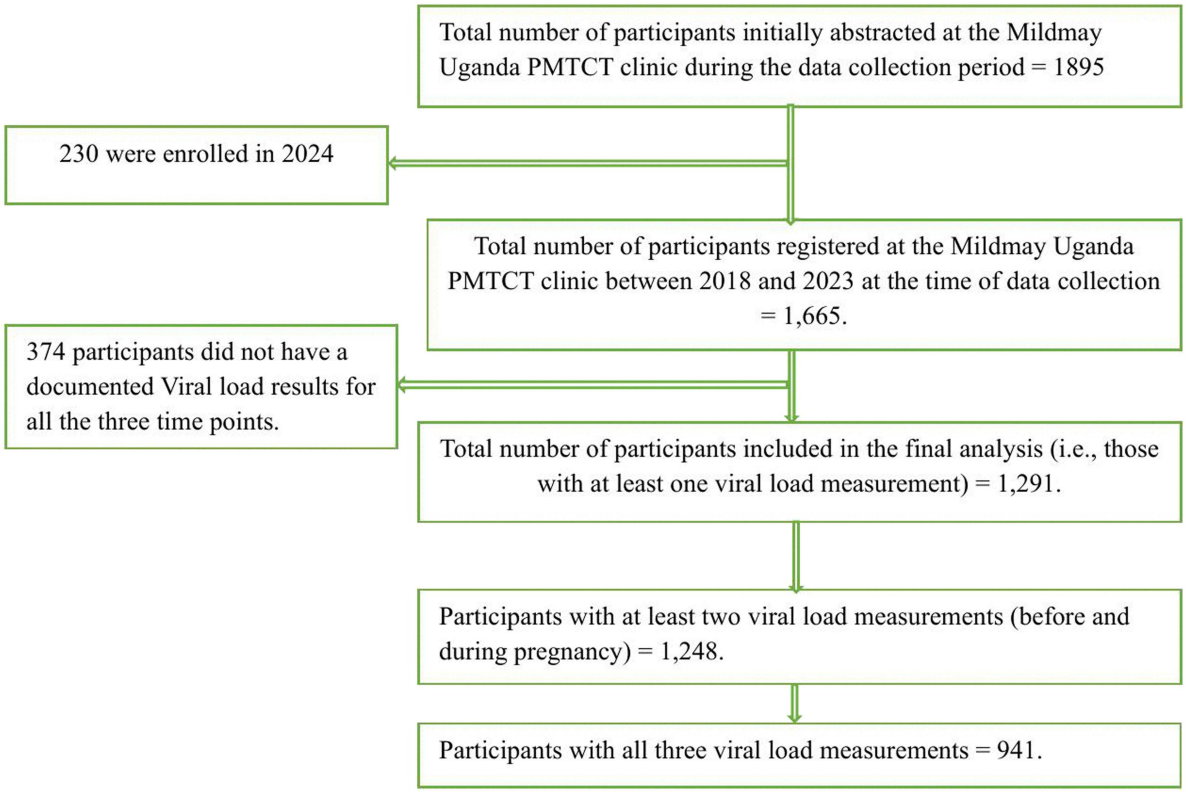

Figure 1. A flowchart illustrating the data collection process.

reclassified as suppressed (≤200) and unsuppressed (≥201) for the assessment of associational factors. Adherence was categorised as good (100%), fair (95%–99%), and poor (<95%), according to the Uganda HIV Care and Treatment Guidelines [3].

In the descriptive analysis, 1291 participants were included. To compare viral suppression before and during pregnancy, 1248 participants were analysed, while 941 participants were considered for longitudinal analysis using generalised estimating equations (GEE).

GEE was used to assess the effects of ART regimens on viral-suppression over time across the maternal timeline. The overall statistical significance was set at $p < 0.05$.

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the Mildmay Uganda Research Ethics Committee (#REC REF 0201-2024) and then the Uganda National Council for Science and Technology (HS3873ES). A waiver of informed consent was granted by the Mildmay Uganda Research Ethics Committee for the use of de-identified retrospective data. Administrative clearance was sought from MUgH administration to access participant data.

Results

The sociodemographic characteristics of the 1291 participants included in the study are summarised in Table 1. The majority were on DTG (807, 62.5%), followed by EFV (372, 28.8%), NVP (58, 4.5%), and PI (54, 4.2%). DTG was predominant at ages 25–34 (466, 63.9%), while EFV was common in >35 years (125, 32.2%). The participants with a normal BMI mostly used DTG (338, 63.3%) and EFV (149, 28.6%) ($p = 0.449$). WHO Stage 1 participants mainly used DTG (761, 62.4%), while stage 3 participants used EFV more (5, 55.6%). First-line ART was most common (1,175, 91.1%), mainly DTG (743, 63.2%). All other characteristics were significant ($p < 0.05$) except age, BMI, and ART experience.

Table 1. Social demographic and clinical characteristics of study participants by ART regimens.

Variable	Categories	DTG (*N* = 807, 62.5%)	EFV (*N* = 372, 28.8%)	NVP (*N* = 58, 4.5%)	PI (*N* = 54, 4.2%)	*P*- value
Age in years (1291)	15–24	115(66.1)	42(24.1)	7(4.0)	10(5.8)	0.294
	25–34	466 (63.9)	205 (28.1)	33 (4.5)	25 (3.4)	
	≥35	226 (58.3)	125 (32.2)	18 (4.6)	19 (4.9)	
BMI (1166)	<18.5	39(68.4)	15(21.1)	2(3.5)	4(7.0)	0.449
	18.5–24	338(63.3)	149(28.6)	23(4.4)	19(3.7)	
	25–30	238(65.9)	91(25.2)	19(5.3)	13(3.6)	
	>30	158(69.6)	51(22.5)	6(2.6)	12(5.3)	
WHO stage (1252)	Stage 1	761(62.4)	354(29.0)	56(4.6)	48(3.9)	<0.001
	Stage 2	9(37.5)	11(45.8)	1(4.2)	3(12.5)	
	Stage 3	1(11.1)	5(55.6)	0(0.0)	3(33.3)	
	Stage 4	–	–	–	–	
Adherence (1287)	Good (100%)	802(62.7)	367(28.7)	58(4.5)	52(4.1)	0.020
	Fair (95–99%)	1(50.0)	0(0.0)	0(0.0)	1(50.0)	
	Poor (<95%)	2(33.3)	3(50.0)	0(0.0)	1(16.8)	
Start regimen (1252)	DTG	156(90.8)	15(8.7)	0(0.0)	1(0.6)	<0.001
	EFV	474(57.3)	332(39.81)	4(0.5)	20(2.4)	
	NVP	124(59.6)	10(4.8)	54(26.0)	20(9.6)	
	PI	22(57.9)	3(7.9)	0(0.0)	13(34.2)	
Drug line (1291)	First line	743(63.2)	360(30.6)	56(4.8)	16(1.4)	<0.001
	Second line	63(54.8)	12(10.4)	2(1.7)	38(33.0)	
	Third line	1(100.0)	0(0.0)	0(0.0)	0(0.0)	
Duration on ART in years (1277)	<1	22(59.7)	14(37.8)	1(2.7)	0(0.0)	<0.001
	1–2	77(70.6)	32(29.4)	0(0.0)	0(0.0)	
	>2–5	20.5(62.5)	115(35.1)	3(0.9)	5(1.5)	
	>5	493(61.4)	208(25.9)	53(6.6)	49(6.1)	
History of regimen change during follow up(1291)	Yes	668(59.5)	347(30.9)	55(4.9)	53(4.7)	<0.001
	No	139(82.7)	25(14.9)	3(1.79)	1(0.6)	
ART experience at Conception (1277)	Naive	22(59.5)	14(37.8)	1(2.7)	0(0.0)	0.394
	Experienced	775(62.5)	355(28.6)	56(4.5)	54(4.4)	

Viral load suppression before conception, during pregnancy, and postpartum by ART regimen

The viral load suppression rate was highest with the DTG-based regimen, showing 95.0% before pregnancy, 94.6% during pregnancy, and 95.7% after pregnancy. The EFV-based regimens had suppression rates of 94.9%, 94.24%, and 93.6%, respectively. The NVP-based methods resulted in 93.1%, 94.6%, and 93.5%, while PI-based methods resulted in the lowest suppression, at 79.6%, 88.0%, and 85.7%, respectively. Statistical significance was observed before ($p < 0.001$) and after pregnancy ($p = 0.018$) time points but not during pregnancy ($p = 0.678$) (Figure 2).

Longitudinal analysis of viral suppression across maternal timelines

In the unadjusted model, PI-based regimens were significantly associated with higher risk of viral non-suppression (IRR = 3.20, 95% CI: 1.63–6.30, $p = 0.001$), but this difference lost significance in the adjusted model. In the adjusted models, poor adherence (aIRR = 7.80, 95% CI: 2.54–23.90, $p < 0.001$), fair adherence (aIRR = 5.03, 95% CI: 1.11–22.86, $p = 0.036$), second-line ART (aIRR = 3.14, 95% CI: 1.75–5.62, $p < 0.001$), and third-line ART (aIRR = 8.48, 95% CI: 1.82–39.43, $p = 0.006$) were significant predictors of non-suppression. The other variables were not statistically significant (Table 2).

Discussion

Viral suppression is a key indicator of ART effectiveness, particularly in the context of PMTCT and other adverse pregnancy outcomes. Our study provides real-world evidence on the longitudinal patterns of viral suppression among 1291 pregnant women living with HIV in Uganda and the impact of different ART regimens. The findings highlight key determinants of viral suppression across maternal timelines, emphasising the role of ART regimen choice, adherence, and patient characteristics in influencing treatment success.

Across the maternal timeline, DTG-based regimens demonstrated the highest viral-suppression rates, followed by EFV-based, NVP-based, and PI-based regimens. This aligns with WHO recommendations that prioritise DTG-based regimens for their strong performance in achieving suppression [3,15–21]. This

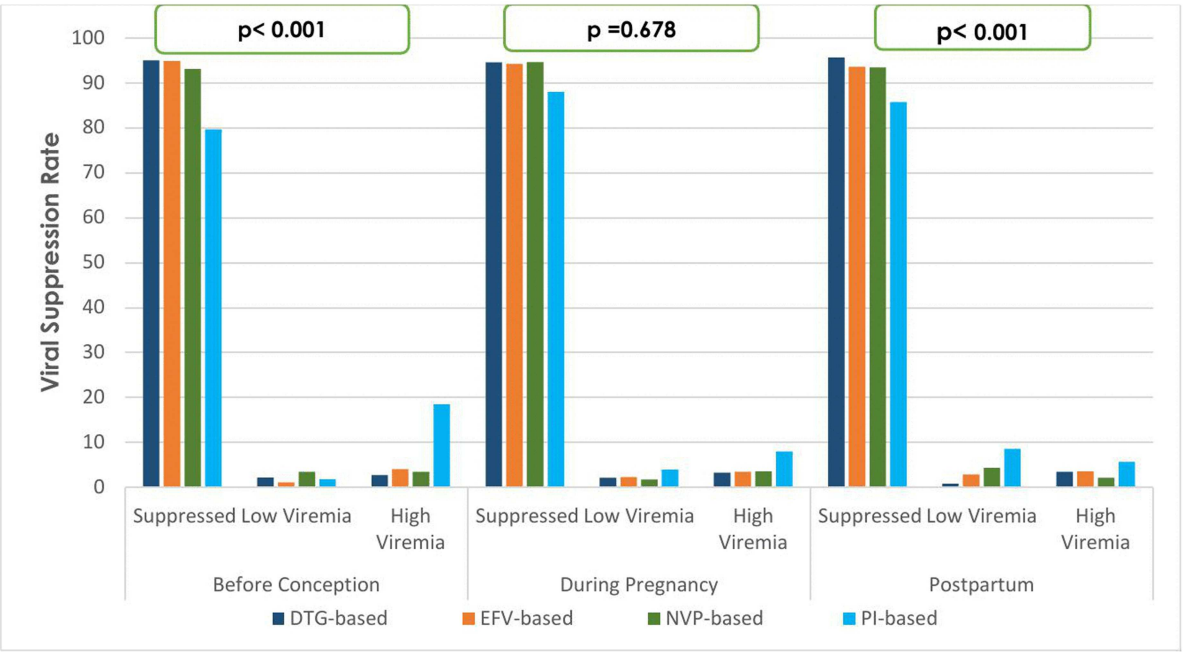

Figure 2. Viral load suppression before conception, during pregnancy, and postpartum by ART regimen.

Table 2. Bivariable and multivariable analysis of factors associated with viral load supression across all time points.

Predictor variable	Category	Incidence rate ratio (IRR)	95% confidence interval	*p*-value	Adjusted incidence rate ratio (aIRR)	95% confidence interval	*p*-value
PMTCT ART regimen	DTG	Ref			Ref		
	EFV	1.38	0.89–2.13	0.147	1.18	0.70–1.98	0.533
	PI	3.20	1.63–6.30	**0.001**	1.31	0.56–3.05	0.533
	NVP	1.58	0.70–3.53	0.269	1.46	0.53–3.98	0.460
Age category in years	15–24	1.48	0.87–2.52	0.151	0.70	0.16–3.08	0.638
	25–34	0.70	0.45–1.09	0.117	0.53	0.15–1.91	0.330
	>35	Ref			Ref		
WHO stage	Stage 1	Ref			Ref		
	Stage 2	0.37	0.03–4.18	0.424	0.58	0.06–6.02	0.651
	Stage 3	2.99	0.88–10.12	0.079	1.70	0.36–8.16	0.506
BMI category	<18.5	1.88	0.95–3.73	0.072	1.38	0.56–3.40	0.481
	18.5–24	Ref			Ref		
	25–30	0.62	0.36–1.08	0.093	0.64	0.33–1.23	0.184
	>30	0.82	0.04–0.08	0.511	0.61	0.15–2.51	0.489
Adherence	Good (100%)	Ref			Ref		
	Fair (95%–99%)	13.06	3.97–43.01	**<0.001**	5.03	1.11–22.86	**0.036**
	Poor (<95%)	14.70	6.55–32.98	**<0.001**	7.80	2.54–23.90	**<0.001**
ART start regimen	DTG	Ref					
	EFV	0.86	0.47–1.56	0.620	–	–	–
	PI	1.85	0.64–5.35	0.256	–	–	–
	NVP	1.12	0.56–2.25	0.755	–	–	–
History of regimen change during follow up	No	Ref					
	Yes	0.78	0.44–1.37	0.382	–	–	–
ART drug line	First line	Ref			Ref		
	Second line	4.07	2.68–6.20	**<0.001**	3.14	1.75–5.62	**<0.001**
	Third line	11.49	2.97–44.51	**<0.001**	8.48	1.82–39.43	**0.006**
ART duration in years	<1	2.35	0.79–7.01	0.126	1.11	0.20–6.09	0.906
	1–2	2.07	0.98–4.38	0.058	1.41	0.62–3.23	0.413
	>2–5	Ref			Ref		
	>5	1.62	0.96–2.73	0.073	1.00	0.58–1.74	0.995
ART experience at conception	Experienced	Ref			–	–	–
	Naive	1.58	0.58–4.33	0.373	–	–	–

observation could be attributed to the fact that DTG-based regimens have been introduced more recently than NNRTI- and PI-based regimens have been in use for more than two decades [22,23]. Moreover, in Uganda, pretreatment resistance to NNRTI regimens exceeds the ten percent threshold noted in the WHO guidance, which may also help explain the observed patterns in suppression [22].

Analysis of the effectiveness of ART regimens showed a variation across maternal timelines. Variation in the effectiveness of ART regimens was observed before and after but not during pregnancy. Similar viral-suppression effects were observed for the DTG-based, NVP-based, EFV-based and PI-based methods during pregnancy. This could be attributed to the maternal perceived risk of non-suppression to the unborn child, which potentially increases adherence irrespective of regimen type [24]. This highlights the need for regimen consistency during pregnancy.

PI-based regimens were associated with a higher incident risk of non-suppression in the unadjusted models, although this association was not sustained in the multivariable analysis. This may reflect confounding factors related to adherence challenges driven by pill burden. Their twice-daily dosing further increases the likelihood of missed doses, adding to the already substantial pill burden for individuals on these regimens [11,25]. Although PIs are known to have a high genetic barrier to resistance [26], the combined influence of pill burden and dosing frequency can negatively affect adherence, which in turn may compromise overall treatment effectiveness.

Consistently, second- and third-line ART regimens are associated with a greater risk of viral non-suppression, even after adjusting for potential confounders. Individuals on third- and second-line ART regimens are likely to have a history of virologic failure or drug resistance, poor drug adherence track records, and a lack of social support networks, necessitating closer clinical monitoring and strengthened adherence support [27–29]. In addition, second- and third-line therapies typically involve a higher pill burden because they rely on individualised drug selection guided by resistance profiles rather than fixed-dose combinations commonly used in first-line ART [30]. This increased pill burden can further compromise adherence, ultimately contributing to suboptimal viral suppression.

Adherence emerged as a strong predictor of suppression, with both fair and poor adherence significantly associated with an increased risk of viral non-suppression even after adjustment for confounding factors. Although adherence is well established as a key determinant of ART effectiveness and poor adherence is associated with poor outcomes [29], fair adherence in this aspect as well showed a similar phenomenon. This could be due to physiological changes during pregnancy, including increased body fluid volume and altered pharmacokinetics, which potentially reduce drug concentrations [4,5]. Under these conditions, the impact of fair adherence may approximate that of poor adherence, resulting in a higher likelihood of viral non-suppression. Additionally, pregnancy-related challenges, such as nausea, stigma, and increased healthcare demands, may hinder adherence [9,31], emphasising the need for targeted counselling and support programs tailored to pregnant women.

Notably, longitudinal analysis did not reveal significant differences in suppression between DTG-based and other regimens except for PI-based regimens across the maternal timeline. After adjustment for cofounders, there was no significant influence of the ART regimen on viral suppression, which suggests that treatment success depends on more than regimen choice. Factors such as treatment fatigue, long-term toxicity from older regimens [22,23], and emerging resistance may reduce efficacy over time, highlighting the need for enhanced adherence support, resistance monitoring, and strategies to address ART-related toxicities.

Strengths and limitations

This study benefits from a large sample size, real-world clinical data, and robust methods, including GEE for repeated measures. However, the retrospective design introduces inherent limitations, including missing data due to documentation gaps, which may have impacted the identification of key predictors. Additionally, the reliance on self-reported adherence poses risks of recall, yet it is a critical factor in viral load suppression and social desirability bias, while residual confounding cannot be ruled out. Moreover, the exclusion of certain patient groups may limit generalisability, making our findings most applicable to settings with similar ART programs and patient populations.

Conclusion

Our study highlights that ART effectiveness varied before and after pregnancy, but no notable differences were observed during pregnancy. PI-based regimens were associated with non-suppression while EFV-based, NVP-based, and DTG-based regimens exhibited comparable viral-suppression rates across all time points. Adherence and ART drug line of therapy may play a more significant role in long-term outcomes than regimen choice alone. A good adherence to ART is warranted across the maternal timeline for better outcomes.

These findings emphasise the need for timeline-specific interventions, optimising ART and good adherence. Future research should investigate pharmacokinetics, pharmacogenetics, adherence strategies, and socio-behavioural factors to enhance maternal and neonatal outcomes.

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CA, JE, SN, BK, and JN conceived and designed the study. CA acquired funding. CA, JE, SN, BK, CS, IM, and JN collected and interpreted the data. CA and JE participated in the analysis. All authors participated in the interpretation of the results and writing of the final manuscript. All authors read and approved the final version of the manuscript.

Author contributions

CRediT: **Collins Ankunda:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing; **Jude Emunyu:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing; **Sharon Namasambi:** Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing; **Brendah Kyomuhangi:** Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing; **Conrad Sserunjogi:** Conceptualization, Data curation, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing; **Iving Mumbere:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing; **Jane Nakawesi:** Conceptualization, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

Disclosure statement

All authors report no conflicts of interest in this work.

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Data availability statement

The data sets for this study are available upon reasonable request from the corresponding author.

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