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Antenatal Care and Neonatal Outcome

Antiretroviral therapy use during pregnancy and adverse birth outcomes in South African women

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Abstract

Background: Studies of antiretroviral therapy (ART) use during pregnancy in HIV-infected women have suggested that ART exposure may be associated with adverse birth outcomes. However, there are few data from sub-Saharan Africa where HIV is most common, and few studies involving the World Health Organization's (WHO's) recommended first-line regimens.

Methods: We enrolled consecutive HIV-infected pregnant women and a comparator cohort of uninfected women at a primary-level antenatal care facility in Cape Town, South Africa. Gestational assessment combined clinical history, examination and ultrasonography; outcomes included preterm (PTD), low birthweight (LBW) and small for gestational age (SGA) deliveries. In analysis we compared birth outcomes between HIV-infected and -uninfected women, and HIV-infected women who initiated ART before vs during pregnancy.

Results: In 1554 women (mean age 29 years) with live singleton births at time of analysis, 82% were HIV-infected, 92% of whom received a first-line regimen of tenofovir, emtricitabine and efavirenz. Overall, higher levels of PTD [22% vs 13%; odds ratio (OR) 1.94, 95% confidence interval (CI): 1.34, 2.82] and LBW (14% vs 9%; OR 1.62, 95% CI: 1.05, 2.29) were observed in HIV-infected vs uninfected women, although SGA deliveries were similar (9% vs 11%; OR 1.06, 95% CI: 0.71, 1.61). Adjusting for demographic characteristics and HIV disease measures, HIV-infected (vs HIV-uninfected) women had persistently increased odds of PTD [adjusted odds ratio (AOR) 2.03; CI 1.33, 3.10]; associations with

LBW were attenuated (AOR 1.47; CI 0.90, 2.40). Among all HIV-infected women, there appeared to be no association between the timing of ART initiation (before or during pregnancy) and adverse birth outcomes.

Conclusions: These findings suggest that current WHO-recommended ART regimens appear relatively safe in pregnancy, although more data are required to understand the aetiology of preterm delivery in HIV-infected women using ART.

Key words: HIV, antiretroviral therapy, perinatal outcomes, prematurity, low birthweight, small for gestational age

Key Messages

- Several studies have suggested that antiretroviral therapy (ART) use in pregnancy may contribute to adverse birth outcomes, but there are few data from sub-Saharan Africa, where HIV is most prevalent.
- In this cohort of 1554 women enrolled in routine public sector care in Cape Town, HIV-infected women had higher incidence of adverse birth outcomes (preterm and low birthweight delivery) compared with HIV-uninfected women. There appeared to be no associations between the timing of antiretroviral initiation before or during pregnancy and birth outcomes, although some of the comparisons may have been limited by lack of power.
- Whereas these data suggest that first-line ART regimens (TDF + FTC + EFV) appear to be safe during pregnancy, the high incidence of preterm delivery among HIV-infected women on ART remains a significant public health problem.

Background

Use of triple-drug antiretroviral therapy (ART) during pregnancy is the central intervention promoting the health of HIV-infected women and their children. Widespread ART access has significantly reduced the number of new paediatric HIV infections and improved the long-term health of HIV-infected mothers, representing one of the greatest successes of the public health response to the HIV epidemic.[1]

However, there are persistent questions regarding the potential adverse effects of *in utero* ART exposure. Whereas the association between untreated, advanced HIV disease and adverse birth outcomes is well documented,[2,3] a number of studies have suggested increased levels of preterm (PTD),[4–7] low birthweight (LBW)[8–10] and/or small for gestational age (SGA)[4,11] deliveries among women receiving ART. Findings vary by the class of antiretroviral (ARVs) agents used, with protease inhibitors (PIs) more commonly implicated than nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs, respectively).[12–15] However, overall findings for the putative association between antenatal ART use and adverse birth outcomes are highly mixed, with many studies finding no evidence of associations with PTD, LBW and/or SGA.[11,16–21]

With approximately 1.4 million HIV-infected women becoming pregnant annually,[22] the possibility of an increased risk of adverse birth outcomes has generated considerable concern. The current evidence base is subject to several notable limitations. Few studies have focused on African populations where most pregnant women using ART live, and where rates of PTD are often high.[23,24] In addition, most studies investigate ARVs not widely used in low- and middle-income countries (LMIC), and there are few data examining the World Health Organization (WHO) recommended regimen of two NRTIs [tenofovir (TDF) and emtricitabine (FTC)], with the NNRTI efavirenz (EFV)]. Accurate pregnancy dating is critical for defining adverse outcomes in perinatal epidemiology, but the quality of gestational dating in the existing literature is variable, and subsequent potential for bias poorly understood. Finally, the choice of comparison groups varies between studies, and in studies without HIV-negative or HIV-infected, ART-unexposed comparator groups, it can be difficult to attribute adverse effects to ART exposure rather than HIV disease.[8]

Given the large numbers of ART-exposed pregnancies around the world and the conflicting evidence to date, better understandings of the potential associations between commonly used ART regimens and adverse birth outcomes are critical.[25] In particular, with national treatment programmes in high-burden countries implementing a first-line regimen of TDF + FTC + EFV for all HIV-infected women regardless of disease status or CD4 cell count, data on how this regimen may affect major birth outcomes are

urgently required. Therefore, we examined the associations between ART use and birth outcomes in a well-characterized cohort of women seeking routine public sector antenatal care in Cape Town, South Africa.

Methods

Study setting

This prospective cohort study was conducted among consecutive HIV-infected and HIV-uninfected women seeking antenatal care (ANC) at a large, community-based public sector primary care facility in Cape Town, South Africa, enrolled between April 2013 and August 2015. The facility serves a catchment population of approximately 350 000 where ANC uptake is high (95%); in 2014, the antenatal HIV seroprevalence was estimated at 30%.[26]

All women in this setting have gestational age estimated based on last menstrual period (LMP) and symphysis-fundal height (SFH) at the first ANC visit, as part of routine clinical care at their first ANC visit.

All women without a previous HIV diagnosis underwent HIV testing, with ART eligibility based on CD4 cell count <350 cells/μl or WHO stage III/IV disease (from April to June 2013) or universal ART eligibility, regardless of CD4 cell count or disease stage (July 2013 onwards). HIV-infected women conceiving while on ART continued their current regimen throughout pregnancy; regimens included PIs (used in this setting predominantly after failure of first-line therapy) or NNRTIs such as EFV or nevirapine (NVP, used in previous first-line regimens). For women initiating ART in pregnancy, a fixed-dose combination of TDF + FTC + EFV was used throughout. Following ART initiation, clinical follow-up was through an integrated primary care service providing antenatal and HIV care.

Study procedures

This analysis draws on data from a larger multicomponent study of antiretroviral services for HIV-infected women during pregnancy and postpartum [https://clinicaltrials.gov/ct2/show/NCT01933477].[27] HIV-uninfected women were enrolled consecutively into a separate comparator cohort with identical study procedures. The parent study was reviewed and approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee and Columbia University Medical Center Institutional Review Board. Written informed consent was obtained from all participants at their first ANC visit, and this consent included access to their clinical records for this birth outcomes analysis.

Inclusion and exclusion criteria

Consecutive women (aged ≥ 18 years) attending their first antenatal care visit, who were identified as HIV-infected through routine rapid antibody tests, were eligible for enrolment into the HIV-infected cohort. Women not eligible for ART at their first ANC visit (receiving zidovudine prophylaxis) were excluded from this analysis. For the comparator HIV-uninfected cohort, women were eligible for enrolment based on the same criteria and a negative test on the same routine rapid antibody test.

Data collection

All women (HIV-infected and HIV-uninfected) completed questionnaires including demographics and obstetric and medical history. HIV-infected women provided 5 ml of blood for viral load (VL) testing using Abbot Realtime HIV-1 assay (Abbot Laboratories, Waltham, MA). At their first visit, an obstetric ultrasound (US) was performed on all women by an experienced research sonographer using a standardized assessment protocol and blinded to other clinical details. Follow-up study interviews, separate from routine clinical care, were scheduled around the second ANC visit, late third trimester and within 7 days postpartum. Obstetric outcomes, including date and mode of delivery and birthweight, were abstracted from obstetric records at delivery facilities.

Variables and outcomes

In analysis, gestation was based on completed weeks using the best available measure (US or combination of LMP/SFH at later gestations). HIV/ART status (the exposure of interest) was categorized as: (i) HIV-uninfected; (ii) ART initiated before pregnancy; and (iii) ART initiated during pregnancy in the (a) first trimester (<14 weeks), (b) first half of the second trimester (14–20 weeks), (c) second half of the second trimester (21–27 weeks) or (d) third trimester (≥28 weeks). Regimens were categorized as either PI or NNRTI; NNRTI regimens were either EFV-based [TDF + 3TC (lamivudine) + EFV], NVP-based (TDF + 3TC + NVP) or involving other NNRTIs.

All deliveries before September 2015 were included in analysis. PTD was defined as delivery at <37 weeks' gestation, categorized as late preterm (34–37 weeks), moderately preterm (32–34 weeks) or very preterm (<32weeks). LBW was defined as birthweight < 2500 g and very low birthweight (VLBW) as <1500 g. Using the INTER GROWTH-21st Project Standards, infants with birthweights <10th percentile for gestational age were classified SGA; those between 10th and 90th percentiles were

classified appropriate for gestational age (AGA); and those >90th percentile were classified large for gestational age (LGA).[28,29] Composite pregnancy loss was defined as any loss before delivery, and included: ectopic pregnancies as determined by the research sonographer; miscarriages defined as pregnancy loss <28 weeks;[30] and stillbirths defined as fetal death occurring before/during labour and delivery (based on a 1-min APGAR score of 0).

Data analysis

Statistical analyses (STATA 14.0, Stata Corporation, College Station, TX, USA) focused on three exposure comparisons: HIV-infected vs HIV-uninfected women (Comparison A); among HIV-infected women, those initiating ART before pregnancy vs those initiating during pregnancy (Comparison B); and among women initiating ART during pregnancy, comparisons across gestational ages at ART initiation (Comparison C). Pregnancy outcome analyses were restricted to live singleton births. In bivariable analyses, proportions were compared using chi-square and rank sum tests. Birth outcomes (PTD, LBW and SGA) were compared using unadjusted and adjusted logistic regression; results are presented as odds ratios (OR) with 95% confidence intervals (CI). Confounders identified a priori included age, maternal height, parity and previous PTD; and among HIV-infected women, pre-ART CD4 count and pre-ART viral load (VL). Subgroup analyses involved restrictions by EFV or PI use, and by gestation at first ANC visit. Model fit was assessed using likelihood ratio tests and Akaike's Information Criterion; throughout, statistical tests were two-sided (alpha = 0.05).

Results

A total of 1793 women who had delivered at the time of analysis were included: 1494 (83%) HIV-infected and 299 (17%) HIV-uninfected. Among HIV-infected women, 572 (38%) initiated ART before the current pregnancy and 922 (62%) initiated during pregnancy: 186 during the first trimester, 289 during the first half and 256 during the second half of the second trimester and 191 during the third trimester (Figure 1). TDF + FTC + EFV was the most commonly used regimen and 6% reported PI use.

Table 1 compares demographic and clinical characteristics of women at their first ANC visit. Compared with HIV-uninfected women, women who were HIV-infected were older, less educated, more likely to be unemployed

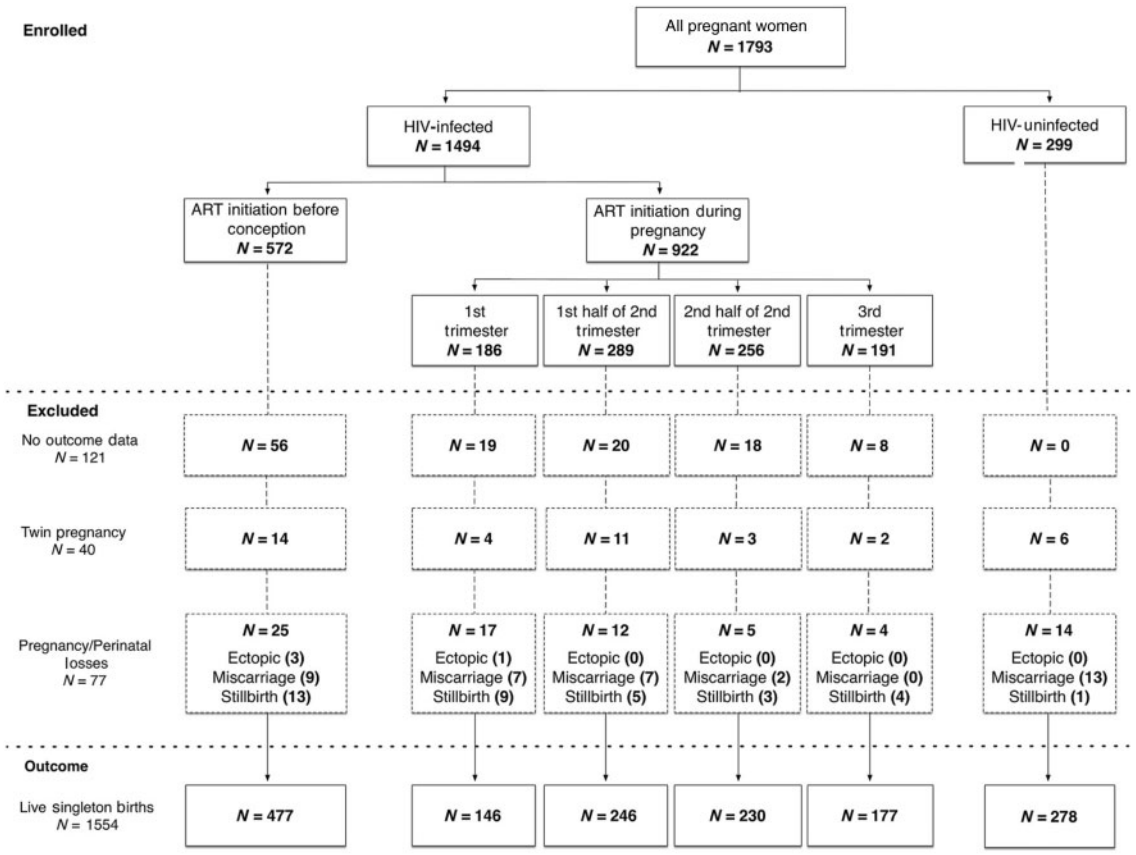

Figure 1. Birth outcomes by HIV/ART exposure status among women in the cohort.

Table 1. Characteristics of pregnant women at first antenatal visit stratified by HIV/ART status

	HIV-uninfected $N=299$	HIV-infected $N=1494$	HIV-infected					*P*-value*
			Initiation before pregnancy $N=572$	Initiation during pregnancy $N=922$				
				First trimester $N=186$	First half of second trimester $N=289$	Second half of second trimester $N=256$	Third trimester $N=191$	
Maternal characteristics								
Age, years								<0.001
≤24	103 (34)	285 (19)	55 (10)	51 (27)	69 (24)	62 (24)	48 (25)	
25–29	84 (28)	472 (32)	152 (27)	72 (39)	107 (37)	72 (28)	69 (36)	
≥30	110 (37)	720 (48)	358 (63)	63 (34)	110 (38)	116 (45)	73 (38)	
Median (IQR)	27 (23–32)	29 (26–34)	31 (28–35)	27 (24–31)	28 (25–32)	29 (25–32)	29 (25–33)	
Education (finished high school)	119 (40)	402 (27)	127 (22)	60 (32)	90 (31)	78 (30)	47 (25)	<0.001
Employment status								0.002
Employed	139 (46)	557 (37)	214 (37)	88 (47)	118 (41)	89 (35)	48 (25)	
SES								0.66
Lowest	91 (30)	451 (30)	180 (31)	50 (27)	73 (25)	80 (31)	68 (36)	
Medium	94 (31)	539 (36)	216 (38)	65 (35)	99 (34)	89 (35)	70 (37)	
Highest	95 (32)	504 (34)	176 (31)	71 (38)	117 (40)	87 (34)	53 (28)	
Obstetric characteristics, gestation, weeks								
Median (IQR)	21 (16 – 27)	21 (15–27)	21 (15–28)	10 (8–12)	18 (16–19)	24 (22–25)	32 (26–35)	–
Height, cm								0.9
≤155	85 (28)	444 (30)	163 (29)	60 (32)	92 (32)	79 (31)	50 (26)	
156–161	90 (30)	464 (31)	170 (30)	72 (39)	89 (31)	69 (27)	64 (34)	
≥162	73 (24)	353 (24)	142 (25)	33 (18)	68 (24)	59 (23)	51 (27)	
Mean (SD)	158 (8)	158 (7)	158 (7)	158 (7)	157 (7)	158 (7)	158 (6)	
Gravidity								0.005
1	72 (24)	244 (16)	63 (11)	38 (20)	64 (22)	48 (19)	31 (16)	
2	101 (34)	544 (36)	196 (34)	81 (44)	109 (38)	90 (35)	68 (36)	
≥3	14 (47)	706 (47)	313 (55)	67 (36)	116 (40)	118 (46)	92 (48)	
Median (IQR)	2 (2–3)	2 (2–3)	3 (2–3)	2 (2–3)	2 (2–3)	2 (2–3)	2 (2–3)	
Parity								0.006
0	75 (25)	259 (17)	68 (12)	43 (23)	65 (22)	50 (20)	33 (17)	
1	100 (33)	558 (37)	202 (35)	79 (42)	117 (40)	94 (37)	66 (35)	
≥2	122 (41)	677 (45)	302 (53)	64 (34)	107 (37)	112(44)	92 (48)	
Median (IQR)	1 (0–2)	1 (1–2)	2 (1–2)	1 (1–2)	1 (1–2)	1 (1–2)	1 (1–2)	
Previous miscarriage[a]	8 (3)	204 (14)	104 (18)	36 (19)	34 (12)	20 (8)	10 (5)	<0.001
Previous preterm[a]	6 (2)	107 (7)	47 (7)	11 (6)	21 (7)	8 (3)	20 (10)	0.001
HIV								
Current ART regimen, self-report								<0.001
TDF + 3TC + EFV		1116 (87)	197 (34)	186 (100)	288 (99)	256 (100)	189 (99)	
TDF + 3TC + NVP		57 (4)	56 (10)	0	0	0	1 (0.5)	
Other NNRTI-based regimen		72 (6)	71 (12)	0	1 (0.4)	0	0	
PI-based regimen		33 (3)	32 (6)	0	0	0	1 (0.5)	
CD4 cell count, (cells/μl)								0.38
≤200		213 (14)	65 (12)	29 (16)	44 (16)	47 (19)	28 (15)	
201–350		426 (29)	167 (30)	52 (29)	90 (32)	70 (28)	47 (26)	
351–500		384 (26)	154 (28)	42 (23)	75 (27)	65 (26)	48 (26)	
>500		423 (28)	167 (30)	58 (32)	72 (25)	67 (26)	59 (31)	
Median (IQR)			396 (271–524)	379 (256–552)	361 (246–504)	357 (235–506)	397 (258–576)	

(continued)

Table 1. Continued

	HIV-uninfected $N=299$	HIV-infected $N=1494$	HIV-infected					*P*-value*
			Initiation before pregnancy $N=572$	Initiation during pregnancy $N=922$				
				First trimester $N=186$	First half of second trimester $N=289$	Second half of second trimester $N=256$	Third trimester $N=191$	
Median HIV RNA viral load (log_{10} copies/ml)		3.35	1.59	3.97	4.12	3.99	3.79	<0.001
		(1.59–4.25)	(1.59–1.6)	(3.41–4.49)	(3.44–4.12)	(3.41–4.64)	(3.18–4.42)	

All variables, with the exception of height and ART regimen, had <3% missing data. For height, 16% (n = 284) of data was missing with similar proportions of missing data across all comparison groups. For ART regimen, 14% (n = 216) of data was missing and this was among the women who initiated ART before pregnancy

IQR, interquartile range; SES, socioeconomic status; SD, standard deviation.

[a]Among women with a previous pregnancy.

**P*-values refer to the comparisons across exposure categories: HIV-uninfected, initiation before pregnancy, initiation during pregnancy (all four time periods).

and more likely to have previous adverse birth outcomes, but gestation at first ANC visit did not vary systematically between these groups. Among HIV-infected women, those initiating ART before pregnancy were older and less educated than women initiating during pregnancy. Neither pre-ART CD4 cell count nor pre-ART HIV VL appeared associated with timing of ART initiation in pregnancy among women newly initiating ART.

Figure 1 shows the cohort disposition through delivery. Overall 121 pregnancies (7%) were missing outcome data, principally among those receiving ART before pregnancy. Following exclusion of 40 twin deliveries and 77 pregnancy losses (4%), 1554 live singleton births were available for analysis. No difference was observed in the composite pregnancy loss outcome by HIV status or timing of ART initiation. HIV-uninfected women experienced a higher proportion of miscarriages ($n=13$; 4%) compared with their HIV-infected counterparts ($n=25$; 2%); the opposite was observed with stillbirths, with HIV-infected women experiencing a higher proportion ($n=34$; 2%) compared with HIV-uninfected women ($n=1$; 0.3%) (Figure 1).

Birth outcomes by HIV/ART status

Comparing outcomes overall between HIV-infected ($n=1276$) and uninfected ($n=278$) women (Comparison A), a higher incidence of any PTD (OR 1.94, 95% CI: 1.34, 2.82; 22% vs 13%) and any LBW (OR 1.62, 95% CI: 1.05, 2.29; 14% vs 9%) was observed among the HIV-infected women. SGA deliveries were similar (OR 1.06, 95% CI: 0.71, 1.61; 9% vs 11%) (Figure 2). In both groups, most preterm deliveries were either late (59% and 58%) or moderately preterm (32% and 36%); similarly, most newborns were LBW (87% and 88%) rather than VLBW (Table 2). Following adjustment for age, parity, height and previous PTD, HIV infection was associated with an increased odds of PTD [adjusted odds ratio (AOR) 2.03, 95% CI: 1.33, 3.10] but not LBW (AOR 1.47, 95% CI: 0.90, 2.40) (Table 3).

Among HIV-infected women (comparisons B and C), there was a similar distribution of gestational age and birthweight subgroups, with most being late or moderately preterm and/or LBW (Table 2). Birth outcomes did not vary appreciably in comparison B (initiating ART before pregnancy, $n=477$ vs initiating in pregnancy, $n=799$): PTD (AOR 0.70, 95% CI: 0.45, 1.07); LBW (AOR 0.72, 95% CI: 0.43, 1.21); SGA (AOR 1.05, 95% CI: 0.58, 1.91) (Figure 2, Table 3). Results were similar for comparison C (Figure 2, Table 3). In addition, the findings did not change appreciably when those comparisons were restricted to women who initiated ART after the July 2013 ART eligibility guideline changes.

Among term infants, similar proportions were AGA (87% vs 88%) and SGA (13% vs 12%), comparing those born to HIV-infected and HIV-uninfected women. Likewise among preterm infants. the proportions who were AGA (87% vs 84%) and SGA (13% vs 16%) were similar in the HIV-infected and HIV-uninfected women.

Subgroups of antiretroviral agents

Because the associations between ART and birth outcomes may depend on choice of ARV, we carried out the same comparisons restricted to subgroups by antiretroviral agents. The incidences of PTD, LBW and SGA were not appreciably different among women on EFV-based regimens compared with the total HIV-infected sample (Supplementary Table 1, available as Supplementary data

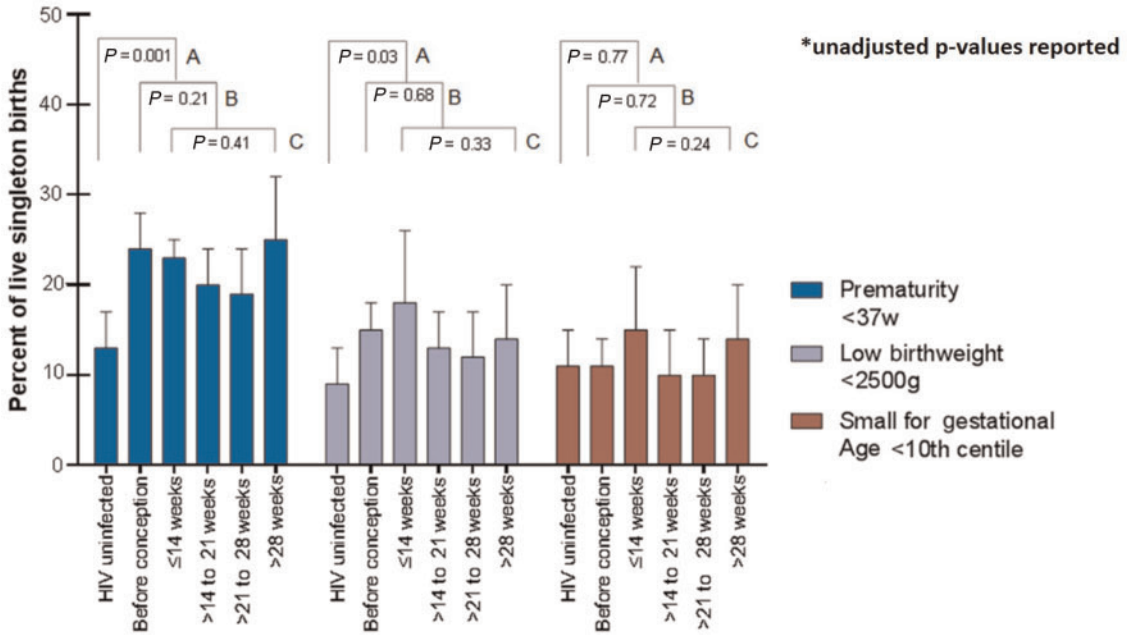

Figure 2. Incidence of preterm, low birthweight and small for gestational age deliveries by HIV status and timing of ART initiation before and during pregnancy among 1554 women who had live singleton deliveries; unadjusted *P*-values reported.

at *IJE* online). Following adjustment for age, parity, height and previous PTD, HIV infection was associated with an increased odds of PTD (AOR 1.97, 95% CI: 1.27, 3.04) but not LBW (AOR 1.51, 95% CI: 0.91, 2.48). Among HIV-infected women (Comparison B), a higher incidence of PTD was observed among women conceiving on EFV-containing regimens (29%) compared with those initiating EFV-containing regimens during pregnancy (21%). This difference in PTD persisted following adjustment for confounders (AOR 0.60, 95% CI: 0.39, 0.94) (Supplementary Table 2, available as Supplementary data at *IJE* online). When comparisons were restricted to women initiating ART in pregnancy (Comparison C), no differences were observed.

When analysis was restricted to women on PI-based regimens, a higher incidence of PTD (34% vs 13%) was observed in the HIV-infected ($n = 29$) compared with HIV-uninfected women ($n = 278$) (Supplementary Table 3, available as Supplementary data at *IJE* online). This incidence in women on PI-based regimens (34%) was higher than that observed in HIV-infected women in the unrestricted (22%) and EFV-based analyses (23%). In multivariable analysis, HIV infection was associated with an increased odds of PTD (AOR 4.46, 95% CI: 1.55, 12.83) but not LBW or SGA (Supplementary Table 4, available as Supplementary data at *IJE* online). When women on PI-based regimens were compared with women on any NNRTI regimens, a higher incidence of PTD in women on PI-based regimens was noted (36% vs 24%) (Supplementary Table 5, available as Supplementary data at *IJE* online).

Subgroups by gestation at first ANC visit

When analysis was restricted to women who entered ANC before 20 weeks of gestation, in whom gestational estimation is likely to be most accurate, results for each comparison mirrored those of the main analysis. A higher incidence of PTD (22% vs 9%) and LBW (15% vs 7%) was observed in HIV-infected ($n = 582$) compared with HIV-uninfected women ($n = 128$), whereas the frequency of SGA deliveries (12% vs 11%) was similar (Supplementary Table 6, available as Supplementary data at *IJE* online). In multivariable analysis, HIV infection was associated with an increased odds of PTD (AOR 2.75, 95% CI: 1.38, 5.48) (Supplementary Table 7, available as Supplementary data at *IJE* online); however, the association with LBW (AOR 2.19, 95% CI: 0.97, 4.94) did not persist. Among HIV-infected women there were no differences observed between women initiating ART before pregnancy compared with those initiating during pregnancy (Comparison B). Similarly when comparisons were restricted to women initiating ART in pregnancy (Comparison C), no differences were observed between groups.

Discussion

In this cohort of HIV-infected and -uninfected pregnant women seeking ANC at a large South African public sector primary care facility, PTD appeared consistently associated with HIV infection and ART use, with HIV-infected women receiving ART being approximately twice as likely

Table 2. Birth outcomes by HIV/ART status among women with live singleton births ($N = 1554$)

	HIV-uninfected $N = 278$	HIV-infected $N = 1276$	HIV-infected vs uninfected P-value	HIV-infected				ART initiation before vs during[a] P-value
				Initiation before pregnancy $N = 477$	ART initiation during pregnancy $N = 799$			
					First trimester $N = 146$	First half of second trimester $N = 246$	Second half of second trimester $N = 230$	Third trimester $N = 177$	
Gestational age (weeks)			<0.0001						0.001
Term (≥37)	242 (87)	986 (78)		358 (75)	111 (76)	197 (80)	187 (81)	133 (75)	
Any preterm (<37)	36 (13)	285 (22)		115 (24)	34 (23)	49 (20)	43 (19)	44 (25)	
Late preterm (34–37)	21 (8)	167 (13)		69 (14)	19 (13)	29 (12)	24 (10)	26 (15)	
Moderately preterm (32–34)	13 (5)	91 (7)		37 (8)	9 (6)	16 (7)	11 (5)	18 (10)	
Very preterm (28–32)	2 (0.7)	27 (2)		9 (2)	6 (4)	4 (2)	8 (3)	0	
Birthweight (grams)			0.03						0.68
Normal (≥)	252 (91)	1085 (85)		402 (84)	118 (81)	214 (87)	199 (87)	152 (86)	
Any LBW (<2500)	26 (9)	181 (14)		70 (15)	27 (18)	31 (13)	28 (12)	25 (14)	
LBW (2500–1500)	23 (8)	157 (12)		61 (13)	23 (16)	27 (11)	21 (9)	25 (14)	
Very LBW (<1500)	3 (1)	24 (2)		9 (2)	4 (3)	4 (2)	7 (3)	0	
Mean (SD)	3199 (548)	3052 (580)	0.003	3090 (602)	3080 (641)	3120 (533)	3130 (574)	3150 (531)	0.63
Size for gestational age (centile)			0.013						0.011
LGA (>90th)	35 (13)	112 (9)		56 (12)	13 (9)	13 (5)	11 (5)	19 (11)	
AGA (10th–90th)	211 (76)	984 (77)		356 (75)	105 (72)	202 (82)	190 (83)	131 (74)	
SGA (<10th)	31 (11)	112 (9)		53 (11)	22 (15)	25 (10)	22 (10)	25 (14)	

AGA, appropriate for gestational age.

[a]P-values refer to comparisons between women who initiated ART before pregnancy vs during pregnancy (not expanded into the four time periods). All the variables had <4% missing data, with similar proportions of missing data across the comparison groups.

Table 3. Adjusted associations between HIV/ART status and adverse birth outcomes

Outcome measure	Comparison A[a] (Ref. category: HIV-uninfected)			Comparison B[b] (Ref. category: Before pregnancy)			Comparison C[b] (Ref. category: P1: <14weeks)		
		AOR (95% CI)	*P*-value		AOR (95% CI)	*P*-value		AOR (95% CI)	*P*-value
Preterm delivery (<37 weeks)	HIV-infected	2.03 (1.33–3.10)	0.001	During pregnancy	0.70 (0.45–1.07)	0.102	P2	0.91 (0.52–1.60)	0.75
							P3	0.79 (0.44–1.42)	0.442
							P4	1.41 (0.79–2.51)	0.244
Low birthweight (<2500 g)	HIV-infected	1.47 (0.90–2.40)	0.124	During pregnancy	0.72 (0.43–1.21)	0.217	P2	0.70 (0.36–1.35)	0.283
							P3	0.68 (0.34–1.34)	0.264
							P4	1.15 (0.59–2.28)	0.669
Small for gestational age (<10th centile)	HIV-infected	0.91 (0.58–1.43)	0.695	During pregnancy	1.05 (0.58–1.91)	0.861	P2	0.77 (0.38–1.56)	0.461
							P3	0.74 (0.35–1.54)	0.414
							P4	1.62 (0.79–3.30)	0.184

Comparison A (HIV-infected vs HIV-uninfected), Comparison B (ART initiated before pregnancy vs ART initiated during pregnancy), Comparison C (ART initiated during pregnancy at four time points: P1 (<14 weeks), P2 (14–20 weeks), P3 (21–27 weeks), P4 (>28weeks).

[a]Adjusted for age, maternal height, parity and previous PTD.

[b]Adjusted for age, maternal height, parity and previous PTD, CD4 count and VL.

to deliver preterm compared with HIV-uninfected women. We found few appreciable differences in adverse birth outcomes between women initiating ART during pregnancy vs those initiating ART before pregnancy, though in subgroup analyses restricted to EFV-based regimens, PTD appeared to be more likely in women conceiving on ART compared with those initiating during pregnancy.

Our finding of a higher incidence of PTD in HIV-infected women regardless of timing of ART use is consistent with several previous studies from African populations as well as from high-income countries.[4,17,20] However, the PTD incidence among both HIV-infected (22%) and HIV-uninfected (13%) women observed here is higher than previous estimates for South Africa (approximately 10%).[31] These results raise concern, as PTD is the most common cause of neonatal morbidity and mortality globally,[32] particularly in LMICs.[4,33] Nonetheless, a larger proportion of our PTDs occurred later in gestation (>32weeks), which is somewhat reassuring.[24,34]

We did not observe any differences in the proportions of term or preterm SGA or any significant associations with SGA across any of the three major analytical comparisons. The lack of association between HIV status and SGA is different from a study in Botswana during the NVP-based ART era;[4] however, our findings were similar to those from another recent study in Botswana which evaluated TDF + FTC + EFV.[11] In LMIC, SGA is usually a result of intrauterine growth restriction (IUGR) which leads to LBW, as opposed to being normally grown but small because of PTD (constitutionally small).[35] IUGR in these settings tends to be caused by extrinsic factors and is late onset (>32 weeks).[36] Given our higher frequency of late PTD (34–37 weeks), our SGA findings could be a result of a reduction in the time for the effects of late onset growth restriction to take place because of earlier delivery. Consequently any reductions in birthweight would be insufficient to achieve the definition of SGA.

Overall among all HIV-infected women, we found that timing of initiation of widely used NNRTI-based regimens, before or during pregnancy, was not associated with adverse birth outcomes (PTD, LBW and SGA). A study in Cameroon came to similar conclusions in terms of PTD.[19] However, our overall results differ from a number of previous studies that have demonstrated an increased risk of PTD and/or LBW in women initiating ART before pregnancy[9,37] and in women initiating during pregnancy.[5] There are concerns that previous studies comparing timing of ART initiation and adverse birth outcomes did not take into account gestational age at ART initiation, as women initiating ART later in pregnancy do not have equal opportunity to experience different outcome compared with those who initiated earlier or before pregnancy. As part of

our subgroup analyses, we restricted the analysis to women who initiated before 26 weeks and those who experienced an outcome after 26 weeks; these results were similar to the results of the overall analysis.

One explanation for the lack of associations observed in our study could be the relatively high overall incidence of PTD (22%), possibly obscuring a weak signal for increased adverse birth outcomes among the women who initiated ART earlier during pregnancy. It should be noted that in subgroup analyses, when restricted to EFV-based regimens, women who initiated ART before pregnancy were at increased risk of PTD compared with those initiating during pregnancy, which is consistent with previous studies that demonstrated an increased risk of PTD and/or LBW in women on ART initiated before pregnancy.[9,37] Given missing regimen data, particularly among younger women, this subgroup analysis requires cautious interpretation, given that previous studies have shown that differences in birth outcomes between initiating ART before pregnancy compared with those initiating during pregnancy may be largely attributable to differences in other risk factors for adverse outcomes, such as gravidity[4] and maternal age.

Despite the global use of TDF + FTC + EFV, few studies to date have investigated the effect of this regimen in pregnancy on birth outcomes. Our results are consistent with a recent study of a national programme using this regimen in Botswana,[11] suggesting this regimen is unlikely to worsen rates of adverse birth outcomes. Despite observing no overall differences in adverse birth outcomes by timing of ART initiation or with NNRTI use, there is some suggestion in these results of increased risk linked to the use of PI-based regimens, consistent with previous studies.[14,38] PIs may cause increased adverse birth outcomes via mechanisms related to interference with the adrenal system, implicated in the spontaneous onset of labour,[15] and/or reductions in progesterone levels during pregnancy which could affect fetal growth.[39] To investigate this potential effect here, we compared women using PI-based to those using NNTRI-based regimens, and found a similarly increased risk of PTD among women using PI-based regimens, albeit with limited precision.

We found notable differences in miscarriages and stillbirths between HIV-infected and HIV-uninfected women. Stillbirths appeared more likely among HIV-infected women, consistent with a meta-analysis demonstrating a nearly 4-fold increase in stillbirths among HIV-exposed pregnancies.[3] Conversely, miscarriage appeared more likely in the HIV-uninfected women than HIV-infected women, a finding that is unexpected given that HIV status is often associated with early pregnancy loss.[3] In considering the latter finding, it is critical to note that these data–with enrolment of women as they present for routine care at a range of gestations–are not ideal for examining early pregnancy loss, and in turn, this finding should be approached with caution.

Interpretation of these data requires consideration of several strengths and limitations. This study of a public sector primary care population allowed examination of the impact of ART initiation across a range of gestations, compared with clinical trials where gestation at ART initiation is often fixed. Furthermore, the observational nature of our study provides good external validity of experiences in pregnancy. These results are also substantially strengthened by the use of high quality measures of gestation,[40] which contrasts with the reliance on SFH and/or LMP throughout previous analyses. Ultrasonography for gestational age determination has been shown to be highly reproducible up to the early second trimester.[40] Since we enrolled women entering ANC throughout pregnancy, we conducted subanalyses restricted to women entering ANC < 20 weeks, which did not affect our findings. A major limitation of our study is that our sample size is limited for certain subgroup analyses (including by ART regimen). We were also unable to directly measure birthweight and relied on data abstraction from routine records; although this approach is widely used in research, it may contribute to random measurement error, potentially attenuating findings for LBW and SGA outcomes. In addition, we had missing regimen data for women initiating ART before pregnancy; this is a result of the design of the parent study that collected less information on these women compared with those initiating during pregnancy.

This research focuses on widely used NNRTI-based regimens which include TDF + FTC + EFV, the first-line regimen currently recommended by the WHO and the most commonly used combination of antiretroviral drugs globally. However as new antiretroviral agents become more widely available, it will be critical to continue to evaluate birth and long-term outcomes associated with *in utero* ART exposure. This includes both ongoing epidemiological research and investigations of the pathophysiological mechanisms that may lead HIV infection and/or antiretroviral use to cause prematurity and/or growth restriction.

In summary, with the large and rapidly increasing numbers of HIV-infected women receivinging ART during pregnancy around the world, our study suggests that current NNRTI-based regimens are unlikely to further increase adverse birth outcomes. However, given the limited data on TDF + FTC + EFV, and the results of the EFV-based subgroup analyses, more studies investigating this regimen according to timing of ART initiation are required in representative cohorts. These data highlight the high incidence of PTD among HIV-infected women on ART,

pointing to a significant public health problem and an important consideration for the long-term health of HIV-exposed infants and children globally.

Supplementary Data

Supplementary data are available at *IJE* online.

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