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In-utero triple antiretroviral exposure associated with decreased growth among HIV-exposed uninfected infants in Botswana

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Abstract

Objective—To assess associations between in-utero triple antiretrovirals (cART) versus zidovudine (ZDV) monotherapy exposure and growth among HIV-uninfected children of HIV-infected women in Botswana.

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ClinicalTrials.gov Registration Number: NCT00197587 (Mashi) and NCT00270296 (Mma Bana).

Design—Secondary retrospective data analysis from two randomized intervention trials of mother-to-child HIV transmission prevention.

Methods—The Mashi and Mma Bana studies enrolled HIV-infected pregnant women, following their children through 24 months of age. This analysis includes singleton, full-term, HIV-exposed uninfected children. Mothers received cART or ZDV at least 2 weeks predelivery, and breastfed up to 6 months. Weight-for-age (WAZ), length-for-age (LAZ) and weight-for-length (WLZ) z-scores were derived. Mean z-scores were compared by exposure group at 24 months (*t*-test, linear regression).

Results—Of 819 children, 303 were ZDV- and 516 cART-exposed *in utero*. Maternal median enrolment $CD4^+$ was higher among ZDV versus cART-treated mothers (393 versus 324 cells/μl; $P < 0.0001$). Median duration of antepartum antiretroviral use was shorter among ZDV-treated women (5.7 versus 12.0 weeks; $P < 0.0001$). Median months breastfed were similar (5.9 and 6.0; $P = 0.43$). At 24 months, mean LAZ and WAZ were significantly lower among cART-exposed children (LAZ −1.01 versus −0.74; $P = 0.003$) (WAZ −0.53 versus −0.30; $P = 0.002$) in unadjusted analyses. Adjusting for maternal $CD4^+$, viral load, enrolment site and maternal anthropometric measures, cART-exposed children had significantly lower LAZ and WAZ at 24 months ($P = 0.0004$ for both).

Conclusion—At 24 months, in-utero cART-exposed children had significantly lower LAZ and WAZ. Poor growth impacts childhood and adult mortality. These findings raise concerns for potential lasting health impacts among HIV-exposed uninfected children with in-utero cART exposure.

Keywords

growth; HIV-exposed uninfected infants; in-utero triple antiretrovirals

Introduction

Maternal triple antiretrovirals (cART) during pregnancy and breastfeeding are highly effective in preventing of mother-to-child HIV transmission (PMTCT) [1–4]. As a result, WHO guidelines recommend universal maternal use of triple ART (cART) during pregnancy and breastfeeding as one PMTCToption [5]. Implementation of these recommendations is exposing an ever increasing number of children to cART *in utero*. In countries such as Botswana with high maternal HIV-prevalence, approximately one-quarter of all children are born HIV-exposed uninfected (HEU) [6,7].

Over 90% of HEUs reside in resource limited settings where growth faltering in infancy and childhood is associated with increased morbidity and mortality [8–10]. Growth faltering following in-utero ART exposure has not been well studied. In the pre-ART era, an observational study in the Democratic Republic of Congo found length-for-age (LAZ), weight-for-age (WAZ) and weight-for-length (WLZ) z-scores among HEU children did not vary significantly beyond the first year of life when compared with HIV-unexposed children [11]. Understanding any adverse growth effects of in-utero exposure to cART compared with monotherapy will inform maternal cART regimen selection and highlight specialized

healthcare services required by HEUs to offset potential adverse growth effects associated with cART exposure.

Using data from two completed large randomized clinical trials in Botswana, we previously compared infant growth outcomes through the first 6 months of life for breastfed HEU infants based on in-utero exposure to maternal zidovudine (ZDV) monotherapy or cART [12]. In-utero exposure to cART was associated with significantly lower LAZ and WAZ at birth compared with ZDV exposure. By 3 months of age, WAZ was no longer significantly different between the two exposure groups. However, cART-exposed infants had a persistently lower mean LAZ throughout the first 6 months of life compared with ZDV-exposed infants. Availability of complete data from both cohorts now allows us to present growth outcomes from 6 through 24 months of life.

Methods

Study population and monitoring

The Mashi and Mma Bana PMTCT studies were conducted at the same four study sites in southern Botswana. Both studies enrolled HIV-1 infected pregnant women regardless of baseline $CD4^+$ cell count. The Botswana Health Research Development Committee and the Harvard School of Public Health Human Subjects Committee approved both studies, and independent Data and Safety Monitoring Boards reviewed study safety and efficacy data approximately every 6 months. Participants in both studies provided written informed consent.

The Mashi Study, described previously [13,14], enrolled 1200 HIV-1 infected pregnant women between March 2001 and October 2003. Women received ZDV 300 mg twice daily initiated at 34 weeks gestation and continued through labour. Dosing frequency was increased to every 3 h during labour. By randomized design, half of the women participating in the Mashi study also received a single dose of nevirapine (NVP), as did more than half of infants (all infants received NVP following a design modification 17 months into the study). The feeding intervention randomized half of the mother–infant pairs to 6 months of exclusive breast feeding with 6 months of prophylactic ZDV for the infant, versus formula feeding with 4 weeks of prophylactic ZDV to the infant. Combination ART was offered as part of the Botswana National PMTCT programme midway through the study. A total of 71 women started cART antenatally in the Mashi Study and timing of cART initiation was not restricted to a specific gestational age. Infants born to Mashi study participants who took cART during pregnancy were included in the cART exposure group of this longitudinal growth analysis.

The Mma Bana Study enrolled 730 HIV-1 infected pregnant women between July 2006 and May 2008 and has been described in detail previously [1,15]. A total of 560 women with $CD4^+$ cell counts $\geq$200 cells/μl were randomized to receive either abacavir/ZDV/lamivudine co-formulated as Trizivir (GlaxoSmithKline) (TZV) twice daily or lopinavir/ritonavir with ZDV/lamivudine co-formulated as Kaletra (Abbott)/Combivir (GlaxoSmithKline) (KAL/CBV) twice daily. A total of 170 women with $CD4^+$ cell counts <200 cells/μl or with an AIDS-defining illness were enrolled in an observational arm and received NVP/ZDV/

lamivudine (NVP/CBV) twice daily (following 2 weeks of 200 mg once-daily NVP) in accordance with Botswana National PMTCT Guidelines. During labour, Mma Bana participants received ZDV every 3 h, regardless of their assigned treatment arm. Women initiated cART between 18 and 34 weeks gestation and continued through scheduled weaning by 6 months postpartum; cART was continued after weaning for maternal health if indicated. Infants received single-dose NVP at birth and ZDV from birth through 4 weeks of age in keeping with the Botswana National PMTCT Guidelines.

Children enrolling in both studies were evaluated within 72 h of birth, monthly for the first 7 months and once every 3 to 6 months from 9 months through 24 months. During the visit, study staff trained in acquisition of anthropometric measures used calibrated scales and length boards to ascertain a child's recumbent length and weight. A physical examination was performed at each visit, and interim illnesses, hospitalizations and any medications prescribed by nonstudy clinicians were documented. HIV-1 testing by means of qualitative PCR DNA assay (Amplicor HIV-1, Roche Diagnostic Systems, New Jersey, USA) was performed on infant samples collected at birth and routine schedules throughout both studies. An enzyme-linked immunosorbent assay (ELISA)was performed on samples obtained at 18 months from surviving children previously documented as HIV-uninfected.

Statistical methods

We performed a retrospective analysis comparing growth data at the 24-month visit for all qualifying children in the Mashi and Mma Bana studies who attended this visit. We restricted this analysis to singleton infants carried to term (≥37 weeks gestational age) who were breast fed. Gestational age was calculated from an algorithm using maternal reported last menstrual period and a second trimester ultrasound. Infants born to mothers who took any ART, monotherapy or cART, for less than 2 weeks prior to delivery were excluded from the analysis. Only children with negative HIV-1 DNA PCR and ELISA tests were included in the analysis.

Anthropometric data obtained during study visits, from birth through the 24 months were used to calculate a child's z-scores for WAZ, LAZ and WLZ using the 2006 WHO's Child Growth Standards [16]. Two key measures of infant growth that correlate with increased infant mortality include wasting and stunting [17–19]. WHO guidelines define wasting as a WLZ of more than 2 standard deviations below the median for the reference population, and stunting as a LAZ more than 2 standard deviations below the median for the reference population. Using derived z-scores, children were assessed for the presence of wasting and stunting.

Statistical analyses were performed using SAS, version 9.3 (SAS Institute, Cary, North Carolina, USA). Maternal and infant characteristics where compared by attendance at the 24-month visit and exposure groups. *P*-values for comparison of all anthropometric measures were derived from a two-sided Student's *t*-test. Wilcoxon rank-sum test was employed for the remaining continuous variables. *P*-values for comparisons of categorical variables were derived from Fisher's exact testing. To provide a descriptive analysis of longitudinal growth outcomes over time, mean WAZ, LAZ and WLZ, along with confidence intervals by exposure group for all study visits were calculated from birth through 24

months. A Student's *t*-test was used to compare mean WAZ, LAZ and WLZ at 24 months. Univariate linear regression models were developed to identify covariates significantly associated with WAZ and LAZ at 24 months. In addition to adjusting for in-utero exposure to either ZDV or cART, maternal enrolment viral load and $CD4^+$ cell count and sex of the child, covariates with a *P*-value ≤0.10 in univariate analysis in either the WAZ or LAZ models were included in the multivariate linear regression model. We opted to use maternal BMI 1-month postpartum as a surrogate marker for maternal nutritional status early in pregnancy as a covariate in the univariate analysis. All testing used a significance level of 0.05, with two-sided hypothesis testing and there were no corrections for multiple testing.

Results

There were 1877 live births among 1930 HIV-infected pregnant women enrolled in the Mashi and Mma Bana studies (Fig. 1). Of liveborn children, 1856 were the product of a singleton birth. Two hundred twenty-seven children were excluded, as delivery took place prior to 37 weeks gestational age [204 (11%)] or gestational age was lacking [23 (1%)]. Another 517 (28%) Mashi children randomized to formula feeding were excluded from the current growth analysis. Additional exclusions included 50 children with positive HIV-1 DNA PCR results [45 (10%) ZDV-exposed and 5 (0.8%) cART-exposed], six children born less than 14 days after maternal cART was initiated, and 237 children [134 (30.7%) ZDV-exposed children and 103 (16.6%) cART-exposed children (*P*-value for Fisher's exact test <0.0001)] who did not attend the 24-month visit.

In total, 516 children with in-utero cART exposure and 303 children with ZDV exposure were included in the study. Comparing characteristics of the 237 women and children who were included in the initial analysis of growth through 6 month of life but who did not attend the 24-month visit with the 819 children included in the current analysis, mothers of nonattending children were younger [median age 26.0 (interquartile range (IQR) 23.0–29.8) versus 27.0 (IQR 23.6–32.0) $P = 0.002$] with fewer pregnancies. Median duration of breastfeeding among 24-month nonattenders was 5.9 months (IQR 3.1–6.0) versus 5.9 (IQR 4.9–6.0) for attenders ($P = 0.01$). However, there were no significant differences in maternal enrolment viral load, $CD4^+$ cell count or other maternal or child socio-demographic characteristics between mother–child pairs who failed to attend the 24-month study visit and those who attended (data not shown). There was no significant evidence that any difference in maternal–child characteristics between nonattenders and attenders varied according to in-utero exposure to cART versus ZDV. Furthermore mean WAZ, LAZ and WLZ at the 6-month visit did not vary significantly between nonattenders and attenders. Twenty eight (20.9%) of the children in the AZT exposure group and 22 (21.4%) children in the triple ART group had died prior to the 24 month of life visit ($P = 1.0$).

Baseline characteristics of the 819 mother–child pairs included in this analysis are presented in Table 1. Median enrolment $CD4^+$ cell count among women taking cART was lower compared with women taking ZDV [324 cells/μl (IQR 202–469) versus 393 cells/μl (IQR 273–547); $P < 0.0001$], but there was no significant difference in viral load between the maternal groups [4.28 $\log_{10}$copies/ml (IQR 3.68–4.90) versus 4.34 $\log_{10}$copies/ml (IQR 3.85–4.87) respectively; $P = 0.24$]. The median weeks of in-utero ART exposure was higher

among cART-exposed children compared with ZDV-exposed children [12 weeks (IQR 9.5–13.7) versus 5.7 weeks (IQR 4.6–6.9); $P < 0.0001$]. More women taking cART resided in a city compared with women taking ZDV, a higher proportion of whom resided in a village or town ($P < 0.0001$). Additionally, a higher proportion of women taking cART reported the presence of electricity in their household [35.5% versus 19.9% respectively; $P < 0.0001$]. Children exposed to cART in-utero had significantly lower mean birth weights compared with those exposed to ZDV *in utero*. Birth length did not differ significantly by exposure groups.

Mean anthropometric z-scores at each study visit and the 95% confidence intervals are presented in Fig. 2. Mean WAZ at birth was significantly lower among cART-exposed children, consistent with the original analysis [10]. At study visit time points from birth to 24 months, cART-exposed children experienced lower mean LAZ compared with ZDV-exposed children. Mean LAZ for both groups declined after 6 months, the time at which both study protocols called for breastfeeding cessation, with rate of decline in both exposure groups appearing similar from 6 to 24 months. Mean WLZ for cART-exposed children increased from 4 through 9 months, and plateaued thereafter through 24 months upon approaching a normal z-score value of 0. Mean WLZ from 6 to 24 months for the two exposure groups demonstrated overlapping confidence intervals. Mean WAZ for both groups increased from 6 through 9 months. However, from 12 through 24 months, both groups experienced declining mean WAZ. Although the rate of decline appears to be similar, cART-exposed children experienced lower mean WAZ compared with ZDV-exposed children in this 12-month period.

Children from both exposure groups experienced mean LAZ and WAZ below a z-score of zero at the 24-month visit (Table 2). However, children exposed to cART *in utero* had significantly lower mean LAZ and WAZ at 24 months compared with the ZDV-exposed group. Mean LAZ for the cART-exposed group was −1.01 compared with −0.74 [$P = 0.003$] for the ZDV-exposed group. Mean WAZ for the cART-exposed group was −0.53 compared with −0.30 [$P = 0.002$] for the ZDV-exposed group. Among children of mothers enrolled in the Mma Bana study with an enrolment $CD4^+$ cell count ≥200 cells/μl, for which mothers were randomized to TZV or KAL/CBV, there was no significant difference in mean LAZ [$P = 0.29$] or WAZ [$P = 0.81$] at 24 months based on TZV or KAL/CBV in-utero exposure. There was also no significant difference in LAZ [$P = 0.18$] or WAZ [$P = 0.48$] when comparing Mma Bana children with in-utero exposure to NVP/CBV, to children exposed to TZV or KAL/CBV, even though the NVP/CBV group were born to women with more advanced HIV-disease (enrolment $CD4^+$ count <200 cells/μl or with an AIDS-defining illness).

The prevalence of wasting or stunting did not differ significantly at 24 months between the cART and ZDV exposure groups. Wasting prevalence was 4.0% among cART-exposed children versus 2.8% among ZDV exposure children ($P = 0.43$). The prevalence of stunting was 17.5% among the cART exposure group versus 14.3% among the ZDV exposure group ($P = 0.28$).

In univariate linear regression, in-utero cART exposure was significantly associated with a lower 24-month LAZ [−0.27 (95% confidence interval (CI) −0.44, −0.09); $P = 0.003$] compared with ZDV exposure (Table 3). After adjusting for maternal screening viral load, $CD4^+$ cell count, site of enrolment, maternal BMI 1-month postpartum, maternal height, maternal education, employment status, presence of electricity in the child's household, sex of the child and duration of breastfeeding, cART remained significantly associated with lower LAZ [−0.34 (95% CI −0.53, −0.15); $P = 0.0004$]. Additional significant risk factors for lower 24-month LAZ included increasing number of pregnancies ($P = 0.02$), lower maternal BMI 1-month postpartum ($P = 0.0004$), lower maternal height ($P < 0.0001$), mother being unemployed ($P = 0.04$) and male child ($P = 0.04$).

In-utero exposure to cART was also significantly associated with a lower WAZ [−0.23 (95% CI −0.38, −0.09); $P = 0.002$] at 24 months compared with the ZDV (Table 4) in univariate analysis. After adjusting for the same maternal and infant covariates employed in the LAZ multivariate regression model, cART exposure remained significantly associated with lower WAZ [−0.29 (95% CI −0.44, −0.13) $P = 0.0004$]. Additional significant risk factors for lower 24-month WAZ in adjusted analyses were increasing number of pregnancies ($P = 0.04$), lower maternal BMI 1-month postpartum ($P < 0.0001$), lower maternal height ($P < 0.0001$) and male child ($P = 0.04$).

Discussion

Breastfed HEU children in Botswana exposed *in utero* to cART were found to have significantly lower LAZ and WAZ at 24 months compared with breastfed HEU children exposed *in utero* to ZDV. The lower mean LAZ among cART-exposed children persisted from birth through 24 months of life, suggesting an *in utero* insult to linear growth. Initial improvement in WAZ was observed from birth through 9 months of life, the period at which mean WLZ approached a near normal z-score value of 0 (Fig. 2). After 9 months of life, while mean WAZ was declining in both groups, WLZ remained plateaued at a near normal value, suggesting that the declining WAZ for both exposure groups is a function of poor linear growth, as both groups had mean weights that were appropriate for their length (i.e. mean WLZ approaching 0). Although mean LAZ, and as a result mean WAZ were significantly lower at 24 months among cART-exposed infants, it is reassuring that actual stunting and wasting, extreme forms of growth faltering, did not differ significantly between the two exposure groups.

The long-term clinical significance of lower LAZ associated with in-utero exposure to cART is uncertain. Particularly for women, lower LAZ may have longer-term health implications. For example, maternal short stature is associated with foetal growth restriction, neonatal death and in some cases need for caesarean delivery [18–20]. In resource-limited settings without routine access to surgical obstetric options, short maternal stature puts both the mother and infant at risk for morbidity and mortality.

The Mma Bana and Mashi study protocols encouraged breastfeeding cessation at 6 months of life, in keeping with prevailing WHO recommendations at that time. The decline in LAZ was temporally associated with this period of early weaning, and may have been mitigated

by a longer period of breastfeeding. WHO guidelines currently recommend at least 12 months of breastfeeding with appropriate ART prophylaxis for HEU children, and breastfeeding should only stop once a nutritionally adequate and safe diet without breast milk can be provided [21]. This recommendation is consensus, not evidenced-based. The results of our study suggest that shorter periods of breastfeeding may be deleterious to child growth. Further research is needed to quantify the optimal length of breastfeeding with ARV prophylaxis in resource-limited settings.

Our study had several strengths, including the prospective nature of the Mashi and Mma Bana studies, complete assessment of baseline predictors including maternal ARV drugs taken during pregnancy, both studies were conducted at the same four sites, common timing of study visits between Mashi and Mma Bana studies that allowed for longitudinal ascertainment and comparisons of child anthropometric measures, identical protocol breastfeeding durations (6 months) between Mashi and Mma Bana, and repeated HIV testing of infants throughout breastfeeding and at 18 months, allowing for definitive identification of our cohort of HEUs. However, we recognize that our study has several limitations. Twenty-two per cent of the children evaluated in the original analysis through 6 months of life did not attend the 24-month visit, yet the proportion of nonattenders among AZT-exposed children at 30.7% was significantly higher than the proportion of cART-exposed children at 16.6% ($P < 0.0001$). Comparison of mother–child characteristics between attending and nonattending dyads did not reveal differences in maternal HIV-disease progression or other socioeconomic differences between the groups. We conclude from this that it is unlikely that AZT-exposed children with poorer growth were disproportionately unrepresented in Mashi cohort at the 24-month visit. However, this conclusion cannot be definitively confirmed. Breastfed ZDV-exposed children, by protocol, took prophylactic ZDV throughout breastfeeding for up to 6 months, whereas cART-exposed children only took 1 month of ZDV immediately after delivery, as mothers of these children remained on cART throughout breastfeeding. This was in keeping with Botswana national treatment guidelines at the time each study was conducted. Direct ingestion of ZDV by children may have provided protection against bacterial pathogens responsible for diarrhoeal disease and pneumonia, as ZDV has antibacterial properties [22–24] and illnesses in infancy and childhood have been associated with poor growth [25]. However, analyses of hospitalizations for respiratory disease and diarrhoeal illness between the two groups did not reveal any significant differences (data not shown) and mortality was not significantly different between the two groups. We also recognize the limitation of a temporal difference between Mashi and Mma Bana, although we do not believe significant changes in healthcare occurred between the two periods that might have introduced bias to our findings. Given increased access to electricity in the Mma Bana households as a surrogate marker for improved living conditions, we would have expected improved growth outcomes in the ART exposure group. Between the Mashi and Mma Bana studies, there were no national policy changes calling for nutritional, vitamin, or mineral supplementation. Although inherent socioeconomic confounders cannot be excluded, a strength of this study includes the fact that both cohorts enrolled women based on nadir $CD4^+$ cell count and prior to any treatment or prophylactic use of antiretrovirals and both studies enrolled women across all $CD4^+$ cell count strata, minimizing indication bias. Lastly, although, in secondary analysis, we

explored differences between cART regimens to which Mma Bana enrolled children were exposed, lack of differences by regimen may represent inadequate power to detect a small difference.

The efficacy of maternal use of cART in pregnancy for PMTCT is indisputable [1–4]. However, to maximize health outcomes among HEU children we need to understand the long-term implications of in-utero cART exposure and mitigate any identified adverse consequences to the greatest extent possible. Further research is needed to confirm whether or not in-utero exposure to cART is adversely impacting linear growth of HEU infants, and if so, to identify the mechanism. Registries of HEU children are urgently needed in resource-limited settings, so that long-term health implications of in-utero exposure to cART can be fully understood.

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K.M.P. was a study physician on the Mma Bana study, performed the analyses in this manuscript and was the primary author of the manuscript. L.S., M.D.H. and K.W. assisted K.M.P. with statistical analyses. M.D.H. was the senior statistician and co-Investigator on the Mma Bana study. E.A.T. and S.S. were Mma Bana Study physicians, and J.M. provided oversight to the conduct of the Mma Bana study. M.E. was the Principal Investigator of the Mashi study. S.L. and R.L.S. were study physicians and Co-Investigators on the Mashi study and R.L.S. was the Principal Investigator of the Mma Bana Study. J.J. and W.F., with expertise in child growth studies, assisted with manuscript drafting and all authors reviewed and approved the final version of this manuscript.

Conflicts of interest

Conflicts of Interests and Sources of Funding: M.H. has served as a paid Data Safety and Monitoring Board member for Boehringer Ingelheim, Pfizer, Tibotec and Medicines Development. L.S. served as a paid Data Monitoring Committee member for Pfizer.

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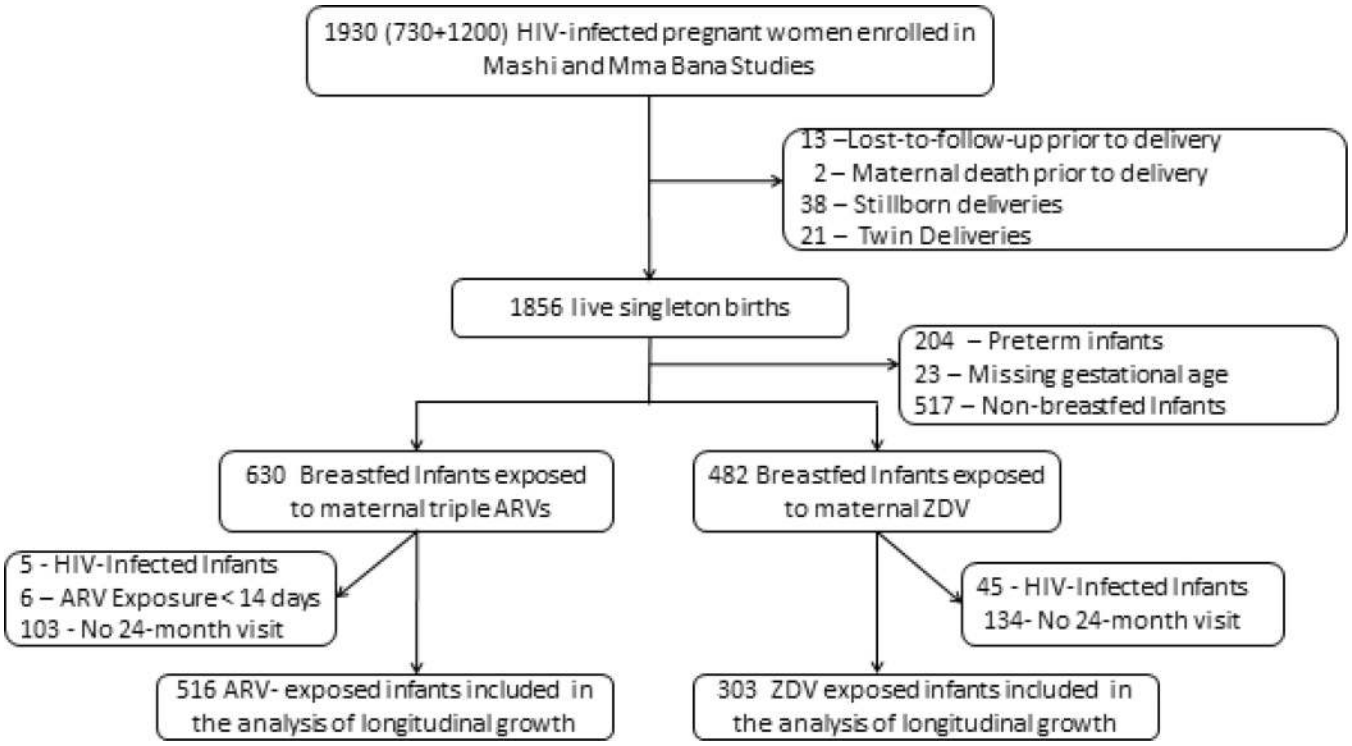

Fig. 1. Study consort diagram

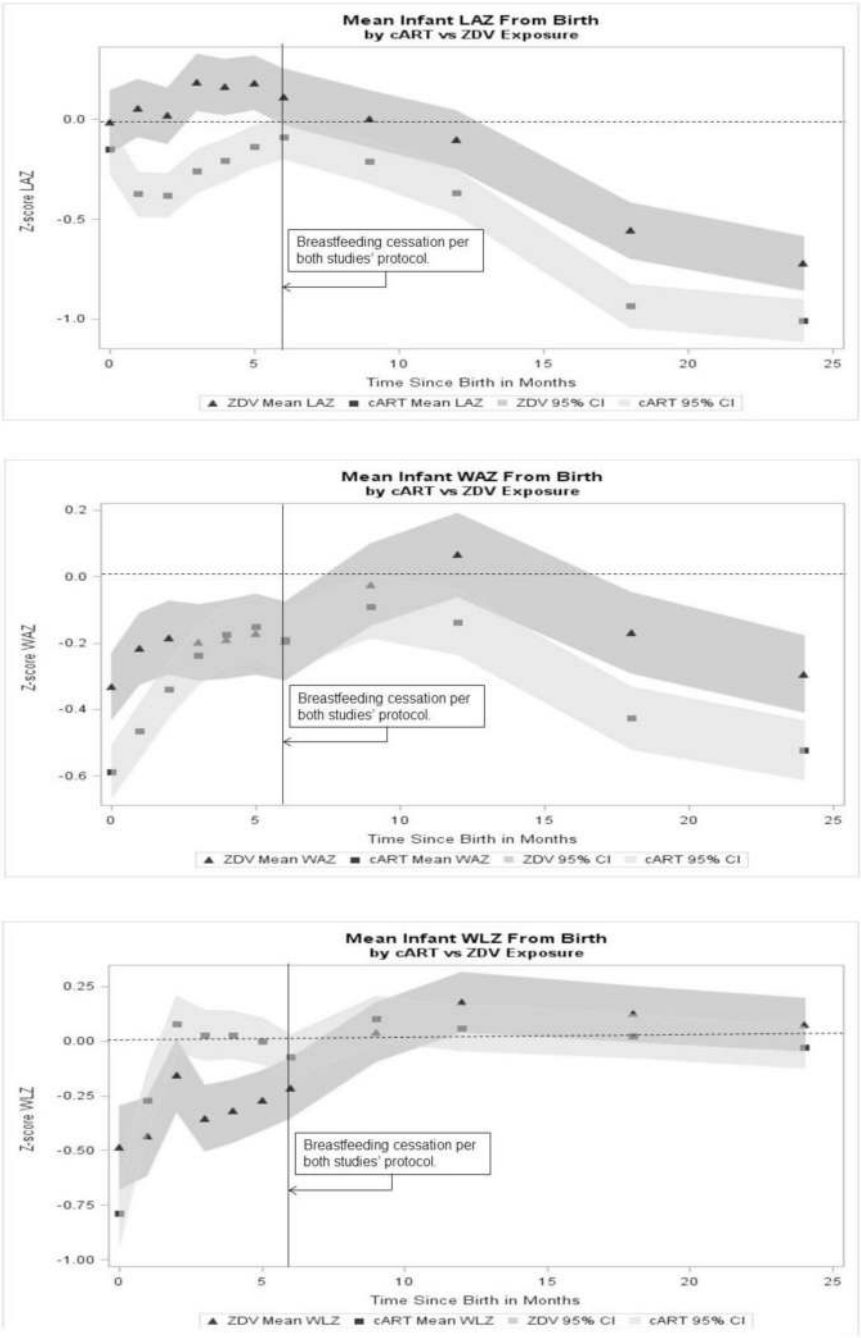

Fig. 2. Mean anthropometric measures over time by exposure group ZDV exposure group *n* = 303; cART exposure group *n* = 516

Horizontal dotted value line represents a z-score value of zero.

Table 1

Comparison of maternal-infant characteristic by in-utero exposure group.

Maternal/infant characteristics	Children with ZDV in-utero exposure (n = 303)	Children with ART in-utero exposure (n = 516)	P-value
Median maternal age (years) [IQR]	27.1 [23.5–32.0]	27.0 [24.0–32.0]	0.95[a]
Gravida including current pregnancy (#, %)			0.30[b]
1	70 (23.1%)	107 (20.7%)	
2	95 (31.3%)	162 (31.4%)	
3	52 (17.2%)	116 (22.5%)	
4 or more	86 (28.4%)	131 (25.4%)	
Enrolment screening labs			
Median HIV-1 RNA (log_{10}copies/ml) [IQR]	4.34 [3.85–4.87]	4.28 [3.68–4.90]	0.24[a]
Median $CD4^+$ count (cells/μl) [IQR]	393 [273–547]	324 [202–469]	<0.0001[a]
Maternal mean anthropometric measures			
BMI 1-month postpartum (kg/m^2) [±SD]	23.9 [±4.1]	23.7 [±4.1]	0.62[c]
Height (cm) [±SD]	160.2 [±6.2]	160.2 [±6.3]	0.88[c]
Enrolment site (#, %)			<0.0001[b]
Molepolole (village)	93 (30.7%)	143 (27.7%)	
Mochudi (village)	72 (23.8%)	94 (18.2%)	
Lobatse (town)	72 (23.8%)	95 (18.4%)	
Gaborone (city)	66 (21.7%)	184 (35.7%)	
Marital status (#, %)			0.61[b]
Single	247 (82.1%)	413 (80.0%)	
Married/cohabitating	50 (16.6%)	98 (19.0%)	
Widowed/divorced/other	4 (1.3%)	5 (1.0%)	
Education (#, %)			0.38[b]
None or primary	83 (27.6%)	120 (23.2%)	
Secondary	205 (68.1%)	374 (72.5%)	
University	13 (4.3%)	22 (4.3%)	
Employment (#, %)			0.90[b]
Employed	178 (35.5%)	181 (35.1%)	
Unemployed	189 (62.8%)	328 (63.6%)	
Student	5 (1.7%)	7 (1.3%)	
Electricity present in home (#, %)	60 (19.9%)	183 (35.5%)	<0.0001[b]
Infant sex (#,%)			0.15[b]
Male	164 (54.1%)	251 (48.6%)	
Female	139 (45.9%)	265 (51.4%)	
Median gestational age at delivery [IQR]	40.0 [39.0, 41.0]	40.0 [39.0, 41.0]	0.74[c]
Median weeks of ART in-utero exposure [IQR]	5.7 [4.6, 6.9]	12.0 [9.5, 13.7]	<0.0001[a]
Mean anthropometric measures			

Maternal/infant characteristics	Children with ZDV in-utero exposure (*n* = 303)	Children with ART in-utero exposure (*n* = 516)	*P*-value
Birth weight (kg) [±SD]			
Male infants	3.20 [±0.44]	3.09 [±0.40]	0.009[c]
Female infants	3.11 [±40]	2.98 [±40]	0.004[c]
Mean length (cm) [±SD]			
Male infants	49.7 [±3.0]	49.5 [±3.2]	0.35[c]
Female infants	49.2 [±2.7]	48.7 [±3.0]	0.11[c]
Low birth weight (<2.5 kg) (#,%)	15 (5.0%)	42 (8.2%)	0.09[b]
Median months of breastfeeding [95% CI]	5.9 [4.8, 6.1]	6.0 [5.0, 6.0]	0.43[a]

CI, confidence interval; cm, centimetres; IQR, interquartile range; kg, kilograms; m, metres. Highlighted *P*-values are statistically significant.

[a] *P*-value from Wilcoxon rank-sum test.

[b] *P*-value from a Fisher's exact test.

[c] *P*-value from a Student's *t*-test.

Table 2

Mean anthropometric z-scores at 24 months of life by exposure group.

Anthropometric z-score	ZDV-exposed group; mean z-score (95% CI) (*n* = 303)	cART-exposed group; mean z-score (95% CI) (*n* = 516)	*P*-value
Length-for-age	−0.75 (−0.89 to −0.60)	−1.01 (−1.12 to −0.91)	0.003
Weight-for-age	−0.30 (−0.41 to −0.18)	−0.53 (−0.62 to −0.44)	0.002
Weight-for-length	+0.06 (−0.06 to 0.19)	−0.04 (−0.13 to 0.05)	0.19

cART, combined antiretroviral treatment consisting of three antiretrovirals; CI, confidence interval; ZDV, zidovudine.

Table 3

Length-for-age z-score univariate and multivariate linear regression results.

Maternal/Infant characteristics	Effect estimate	Univariate analysis 95% confidence interval	*P*-value	Effect estimate	Multivariate analysis 95% confidence interval	*P*-value
In-utero exposure			0.003			0.0004
ZDV cART (triple antiretroviral regimen)	REF-0.27	REF-0.44, −0.09		REF-0.34	REF-0.53, −0.15	
Maternal age (years) [95% CI]	0.001	−0.01, 0.02	0.92			
Gravida including current pregnancy			0.01			0.02
1	REF	REF		REF	REF	
2	−0.004	−0.24, 0.23		−0.07	−0.31 0.17	
3	−0.20	−0.45, 0.06		−0.27	−0.54, 0.01	
4 or more	−0.33	−0.58, −0.09		−0.41	−0.69, −0.12	
Enrolment screening labs						
Log_{10} viral load (per 1 log_{10} copies/ml increase)	−0.03	−0.13, 0.07	0.54	0.01	−0.10, 0.11	0.93
$CD4^+$ (per 100 cells/μl increase)	0.02	−0.02, 0.06	0.39	0.00	−0.04, 0.05	0.87
Maternal anthropometric measures						
BMI 1-month postpartum	0.03	0.01, 0.05	0.02	0.04	0.02, 0.06	0.0004
Maternal height	0.05	0.04, 0.06	<0.0001	0.05	0.04, 0.07	<0.0001
Enrolment site (#,%)			0.02			0.13
Molepolole (village)	−0.18	−0.40, 0.04		−0.18	−0.41, 0.05	
Mochudi (village)	−0.22	−0.46, 0.02		−0.17	−0.43, 0.08	
Lobatse (town)	0.14	−0.10, 0.38		0.08	−0.16, 0.32	
Gaborone (city)	REF	REF		REF	REF	
Marital status (#, %)			0.37			
Single/widowed/divorced	−0.10	−0.32, 0.20				
Married/cohabitating	REF	REF				
Education (#, %)			0.08			0.82
None or primary	−0.50	−0.93, −0.06		−0.07	−0.56, 0.42 −0.56, 0.33	
Secondary	−0.38	−0.79, 0.04		−0.11	REF	
University	REF	REF		REF		
Employment (#, %)			0.001			0.04

Maternal/Infant characteristics	Univariate analysis 95% confidence interval Effect estimate		*P*-value	Multivariate analysis 95% confidence interval Effect estimate		*P*-value
Employed	REF	REF		REF	REF	
Unemployed /student	−0.29	−0.47, −0.12		−0.19	−0.39, −0.01	
Electricity present in home			0.05			0.38
No	−0.18	−0.37, 0.001		−0.09	−0.29, 0.11	
Yes	REF	REF		REF	REF	
Infant sex			0.02			0.04
Male	−0.20	−0.37, −0.03		−0.18	−0.34, −0.01	
Female	REF	REF		REF	REF	
Duration of breast feeding			0.24			0.62
≤2.0 months	REF	REF		REF	REF	
>2.0 months to ≤4.0 months	−0.04	−0.42, 0.35		−0.09	−0. 47, 0.31	
>4.0 months to ≤7.0 months	−0.21	−0.50, 0.08		−0.13	−0.43, 0.16	
>7.0 months	−0.61	−1.45, 0.23		−0.49	−1.29, 0.32	

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Table 4

Weight-for-age z-score: univariate and multivariate linear regression results.

Maternal/Infant characteristics	Univariate Analysis 95% confidence interval			Multivariate Analysis 95% confidence interval		
	Effect estimate		*P*-value	Effect estimate		*P*-value
In-utero exposure			0.002			0.0004
ZDV	REF	REF		REF	REF	
cART (triple antiretroviral regimen)	−0.23	−0.38, −0.09		−0.29	−0.44, −0.13	
Maternal age (years) [95% CI]	−0.00	−0.01, 0.01	0.80			
Gravida including current pregnancy			0.01			0.04
1	REF	REF		REF	REF	
2	−0.07	−0.20, 0.19		−0.04	−0.24, 0.17	
3	−0.12	−0.34, 0.10		−0.17	−0.40, 0.06	
4 or more	−0.28	−0.49, −0.08		−0.30	−0.54, −0.07	
Enrolment screening labs						
Log_{10} viral load (per 1log_{10} copies/ml increase)	−0.02	−0.11, 0.06	0.59	0.02	−0.07, +0.11	0.70
$CD4^+$ (per 100 cells/μl increase)	0.01	−0.03, 0.05	0.56	0.01	−0.03, +0.04	0.80
Maternal anthropometric measures						
BMI 1-month postpartum	0.0	0.02, 0.06	<0.0001	0.05	0.03, 0.07	<0.0001
Maternal height	0.04	0.02, 0.05	<0.0001	0.04	0.03, 0.05	<0.0001
Enrolment site (#,%)			0.12			0.31
Molepolole (village)	−0.16	−0.35, 0.02		−0.15	−0.35, 0.04	
Mochudi (village)	−0.10	−0.31, 0.10		−0.02	−0.23, 0.19	
Lobatse (town)	0.07	−0.35, 0.27		0.02	−0.19, 0.22	
Gaborone (city)	REF	REF		REF	REF	
Marital status (#, %)			0.54			
Single/widowed/divorced	−0.06	−0.25, 0.13				
Married/cohabitating	REF	REF				
Education (#, %)			0.003			0.46
None or Primary	−0.62	−0.99, −0.25		−0.26	−0.68, 0.16	
Secondary	−0.45	−0.80, −0.10		−0.24	−0.62, 0.15	
University	REF	REF		REF	REF	

Maternal/Infant characteristics	Univariate Analysis 95% confidence interval Effect estimate		*P*-value	Multivariate Analysis 95% confidence interval Effect estimate		*P*-value
Employment (#, %)			0.0007			0.11
Employed	REF	REF		REF	REF	
Unemployed /Student	−0.26	−0.40, −0.11		−0.13	−0.29, 0.03	
Electricity present in home			0.002			0.23
No	−0.24	−0.40, −0.09		−0.10	−0.27, 0.06	
Yes	REF	REF		REF	REF	
Infant sex			0.01			0.04
Male	−0.18	−0.33, −0.04		−0.15	−0.29, −0.01	
Female	REF	REF		REF	REF	
Duration of breast feeding			0.01			0.09
≤2.0 months	REF	REF		REF	REF	
>2.0 months to ≤4.0 months	0.00	−0.32, 0.31		−0.08	−0.41, 0.24	
>4.0 months to ≤7.0 months	−0.25	−0.49, −0.01		−0.23	−0.48, 0.02	
>7.0 months	−0.79	−1.49, −0.09		−0.69	−1.37, −0.02	

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