Neurodevelopment of HIV-Exposed and HIV-Unexposed Uninfected Children at 24 Months

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abstract

BACKGROUND: We sought to determine if HIV-exposed uninfected (HEU) children had worse neurodevelopmental outcomes at 24 months compared with HIV-unexposed uninfected (HUU) children in Botswana.

METHODS: HIV-infected and uninfected mothers enrolled in a prospective observational study ("Tshipidi") in Botswana from May 2010 to July 2012. Child neurodevelopment was assessed at 24 months with the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III: cognitive, gross motor, fine motor, expressive language, and receptive language domains) and the Development Milestones Checklist (DMC), a caregiver-completed questionnaire (locomotor, fine motor, language and personal-social domains). We used linear regression models to estimate the association of in-utero HIV exposure with neurodevelopment, adjusting for socioeconomic and maternal health characteristics.

RESULTS: We evaluated 670 children (313 HEU, 357 HUU) with ≥1 valid Bayley-III domain assessed and 723 children (337 HEU, 386 HUU) with a DMC. Among the 337 HEU children with either assessment, 122 (36%) were exposed in utero to maternal 3-drug antiretroviral treatment and 214 (64%) to zidovudine. Almost all HUU children (99.5%) breastfed, compared with only 9% of HEU children. No domain score was significantly lower among HEU children in adjusted analyses. Bayley-III cognitive and DMC personal-social domain scores were significantly higher in HEU children than in HUU children, but differences were small.

CONCLUSIONS: HEU children performed equally well on neurodevelopmental assessments at 24 months of age compared with HUU children. Given the global expansion of the HEU population, results suggesting no adverse impact of in-utero HIV and antiretroviral exposure on early neurodevelopment are reassuring.

WHAT'S KNOWN ON THIS SUBJECT: The effect of in-utero HIV exposure on the neurodevelopment of HIV-exposed uninfected (HEU) children is uncertain. Previous studies have been limited by a lack of comparison with HIV-unexposed controls, small sample size, and inadequate assessment of potential confounders.

WHAT THIS STUDY ADDS: We evaluated the association of in-utero HIV exposure with neurodevelopmental functioning among 337 HEU and 387 HIV-unexposed uninfected (HUU) 24-month-old children in Botswana. We found no differences in neurodevelopment between HEU and HUU children.

ARTICLE

Approximately 1.5 million HIV-infected women globally deliver babies each year,[1] with the vast majority of their HIV-exposed children now HIV-uninfected because of the successful expansion of programs to prevent mother-to-child HIV transmission.[1–5] The authors of studies investigating the effects of in-utero exposure to HIV and antiretrovirals (ARVs) on neurodevelopment in HIV-exposed uninfected (HEU) children in Western settings have provided reassurance[6–10] but have highlighted language delay associated with atazanavir.[11,12] Although a growing number of studies have now been conducted in low-resource settings, the authors of previous studies in Africa have focused on neurodevelopment in children with HIV infection.[13–15] Thus, the authors of studies in sub-Saharan Africa, where the burden of maternal HIV infection is highest, offer limited examination of HEU child neurodevelopment.[14–19] African studies have been further limited by a lack of HIV-unexposed uninfected (HUU) controls who share similar key environmental characteristics to HEU children,[16] by inclusion of only small numbers of HEU children (n = 35–136),[17–19] or by lack of examination of confounders relevant to the neurodevelopment of HEU children in low-income settings.[16–20]

Differences observed in neurodevelopment between HEU and HUU children may be at least partially attributable to environmental and socioeconomic differences, rather than being solely attributable to the biological consequences of HIV exposure.[16] This has been found to be particularly relevant in US settings, where HIV-infected women have historically had lower socioeconomic status and higher rates of substance use than the general population,[9,11] potentially confounding the estimated effects of exposure to HIV on child

neurodevelopment. The generalized HIV epidemic in Botswana allows better control of these potential confounders, as HIV affects all socioeconomic strata and maternal substance use is low.[21] Additionally, children in Botswana are more likely to have similar exposure to potentially important environmental confounders of interest irrespective of their HIV-exposure status.[16]

To address these knowledge gaps, we compared neurodevelopmental outcomes between HEU and HUU children in Botswana.

METHODS

The "Tshipidi" study is a prospective cohort study that enrolled HIV-infected and HIV-uninfected mothers between May 2010 and July 2012 at 2 sites in Botswana: Gaborone (the capital city) and Mochudi (a nearby village). Mothers aged ≥18 years were either pregnant (any gestational age) or recruited within 7 days of live-born delivery. All participants (regardless of HIV status) were recruited from local government health clinics at the time of routine antenatal care visits (or from maternity wards at the time of delivery, in a small number). Mothers and their infants were followed to evaluate effects of in-utero exposure to maternal HIV or to ARV drugs on neurodevelopment and child health outcomes at ~24 months of age. HIV-infected mothers received either the ARV zidovudine (ZDV) prophylaxis or 3-drug ARV treatment (ART), in accordance with Botswana program guidelines. Most women with a cluster of differentiation 4 (CD4) count >350 cells/mm^3 received ZDV during pregnancy, and women with a CD4 count ≤350 cells/mm^3 or World Health Organization stage 3 or 4 HIV received ART. Combivir plus nevirapine (NVP) was the most commonly prescribed regimen (for just under two-thirds of the HIV-infected mothers who were receiving

combination ART in this study). Less-frequently prescribed ARVs included atazanavir, NVP, tenofovir/emtricitabine, and lopinavir/ritonavir. HEU children received prophylaxis with a single dose of NVP and 1 month of ZDV. Infants were fed according to maternal choice in keeping with Botswana national program guidelines. HIV-infected mothers received infant feeding counseling in accordance with the Botswana HIV program guidelines during the period of the study (which promoted formula feeding with free infant formula provision for infants of HIV-infected mothers). The Botswana Health Research Development Committee and the Office of Human Research Administration at the Harvard T.H. Chan School of Public Health granted ethics approval, and all mothers provided written informed consent.

All women underwent HIV testing at enrollment (and HIV-infected women had CD4-count and HIV-1 RNA testing). Negative maternal HIV status was documented by negative rapid HIV test or licensed enzyme-linked immunosorbent assay (ELISA). Maternal HIV-1 infection was defined as positive rapid HIV or licensed ELISA test, followed by confirmation by 1 of these 2 tests: a Western blot test, or detectable HIV-1 RNA test. Discordant confirmatory results were followed by plasma HIV-1 RNA testing. Infant HIV DNA polymerase chain reaction (PCR) testing was performed in HEU children. A DNA PCR test with positive results was confirmed promptly with a second DNA PCR test or HIV-1 RNA to determine if a child was HIV-infected. All children underwent an 18-month HIV-1 ELISA (unless they were previously documented to be HIV-infected), regardless of maternal HIV status or feeding method.

Research nurses who were trained in questionnaire administration collected all maternal data via direct interview. We collected data on

socioeconomic status, demographics, maternal health history (including ARV use for HIV-infected women), infant feeding method, and food insecurity.[22] Questionnaire items included the Beck depression screen[23] and social support[24] scores, with higher scores reflecting worse depression and better social support, respectively. Additionally, a maternal substance use screen was used to capture use of tobacco and alcohol. Data on the primary child care provider were also collected via interview at 1, 6, 12, 18, and 24 months of child age. Birth outcomes and complications and child health outcomes were collected via interview and child examination, in conjunction with review of medical records. Child height and weight were measured, and all children underwent HIV testing through the study. Questionnaire items and neurodevelopment instruments were translated into Setswana. Between the ages of 22 and 29 months, children completed 2 types of neurodevelopmental assessment. The first was the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III).[25] Five raw domain scores were recorded for each child: cognitive, gross motor, fine motor, expressive language, and receptive language. The Bayley-III was adapted to be more culturally appropriate through focus groups, item modification, piloting, modifying, and repiloting. The second measure was the Development Milestones Checklist (DMC), developed for use within a sub-Saharan African context.[26,27] The DMC is a parent report questionnaire with 4 domains: locomotor, fine motor, language and personal-social. This measure has been shown to have good internal consistency, with a test-retest reliability correlation coefficient of 0.85.[26] Trained research nurses administered both the DMC and Bayley-II neurodevelopmental testing and recorded raw scores. Testing of both HEU and HUU

children was performed in quiet study clinic rooms that had been prepared for this purpose. Neurodevelopmental assessors were observed during training until competency was confirmed. An invalid score was assigned to children who were unable to complete neurodevelopmental testing or whose scores did not appear to be an accurate reflection of ability, because of physical or behavioral problems suggestive of potential clinical impairment. Invalid scores were noted by clinical assessors and confirmed by the lead neuropsychologist (B.K.). Assessor performance was monitored periodically through video and/or direct observation by study coordinators and lead neuropsychologist (B.K.). Regular meetings were held to address any concerns raised during the quality control procedures for the assessments.

Linear regression models were used to compare 24-month neurodevelopmental outcomes between HEU and HUU children, both unadjusted and adjusted for confounders. Raw Bayley-III scores were assessed and a similar age distribution noted among HEU and HUU children. A separate model was fit for each of the 5 Bayley-III and 4 DMC neurodevelopmental domain scores. Unadjusted and adjusted mean Bayley-III and DMC domain scores were assessed by HIV exposure status, along with differences in means (with corresponding confidence intervals) between HEU and HUU children. Standardized effect sizes for mean differences are also reported by using Cohen's d.[28] Children with invalid scores for specific neurodevelopmental domains were excluded from primary analyses for that outcome but were included in any analyses of outcomes for which they had valid scores.

Potential confounders were selected by using a priori knowledge and directed acyclic graphs to delineate assumptions regarding the causal pathway of interest.

Multiple socioeconomic (maternal age, education, and income), environmental (electricity, housing, water, access to sanitation, and study site), and maternal mental health characteristics (depression, alcohol, and tobacco use during pregnancy) were evaluated as potential confounders of the association of interest. Among these potential confounders, all child and maternal characteristics with unadjusted P values < .20 for association with a specific neurodevelopmental domain score were included in adjusted models. Covariates with P values > .20 in multivariable models were subsequently excluded.

Low birth weight (<2500 g) and preterm birth (<37 weeks' gestation) have previously been associated with both in-utero ARV exposure and impaired infant neurodevelopment.[29–31] Hence, neither low birth weight nor preterm delivery was considered within primary analyses because they were deemed to be possible mediators on the causal pathway between ARV exposure and neurodevelopmental outcomes. Sensitivity analyses to examine these potential mediators were performed by including the suspect variable within adjusted models to look for changes in the specific domain effect estimate.

Further analyses were conducted by using logistic regression to compare HEU with HUU children for occurrence of a dichotomous "adverse neurodevelopmental outcome" defined as the presence of either a low score (<1 SD below the Bayley-III domain-specific mean) or an invalid score. Other demographic and home environment predictors of an adverse neurodevelopment outcome in each domain were also explored.

All statistical analyses were conducted with SAS Version 9.4 (SAS Institute Inc, Cary, NC). Two-sided *P* values < .05 were considered statistically significant.

RESULTS

Enrollment, Neurodevelopmental Assessment Completeness, and Baseline Characteristics

A total of 949 eligible mothers enrolled; 1 died before delivery, 15 moved out of the study area, and 21 refused further contact. Thus, 912 women (454 HIV-infected, 458 HIV-uninfected) were followed, and delivered 910 live-born infants (453 HEU and 457 HUU infants, Fig 1). Vital status at 24 months was ascertained for 905 (99.5%) of children; 412 (90%) of the HEU children and 422 (92%) of the HUU children attended a 24-month study visit. Among those attending a 24-month visit, 313 HEU children (76%) and 357 HUU children (85%) completed at least 1 valid Bayley-III domain score, and 337 HEU children (82%) and 386 HUU children (92%) had a caregiver-completed DMC. Of the mothers who were on ART during pregnancy, 48% started before conception, whereas 52% started during pregnancy. The median duration of ART was 9.5 months (interquartile range: 3–48), and the median duration of ZDV was 2.5 months (interquartile range: 2–2.9).

Among children with at least 1 valid Bayley-III domain score, those missing 1 or more other domain scores did not differ according to their baseline characteristics (compared with children not missing any assessed domains), with the exception of children born preterm (Supplemental Table 5): preterm infants were significantly more likely to have missing Bayley-III assessments. Among children eligible for neurodevelopmental testing but not tested (Supplemental Table 6), higher percentages of children
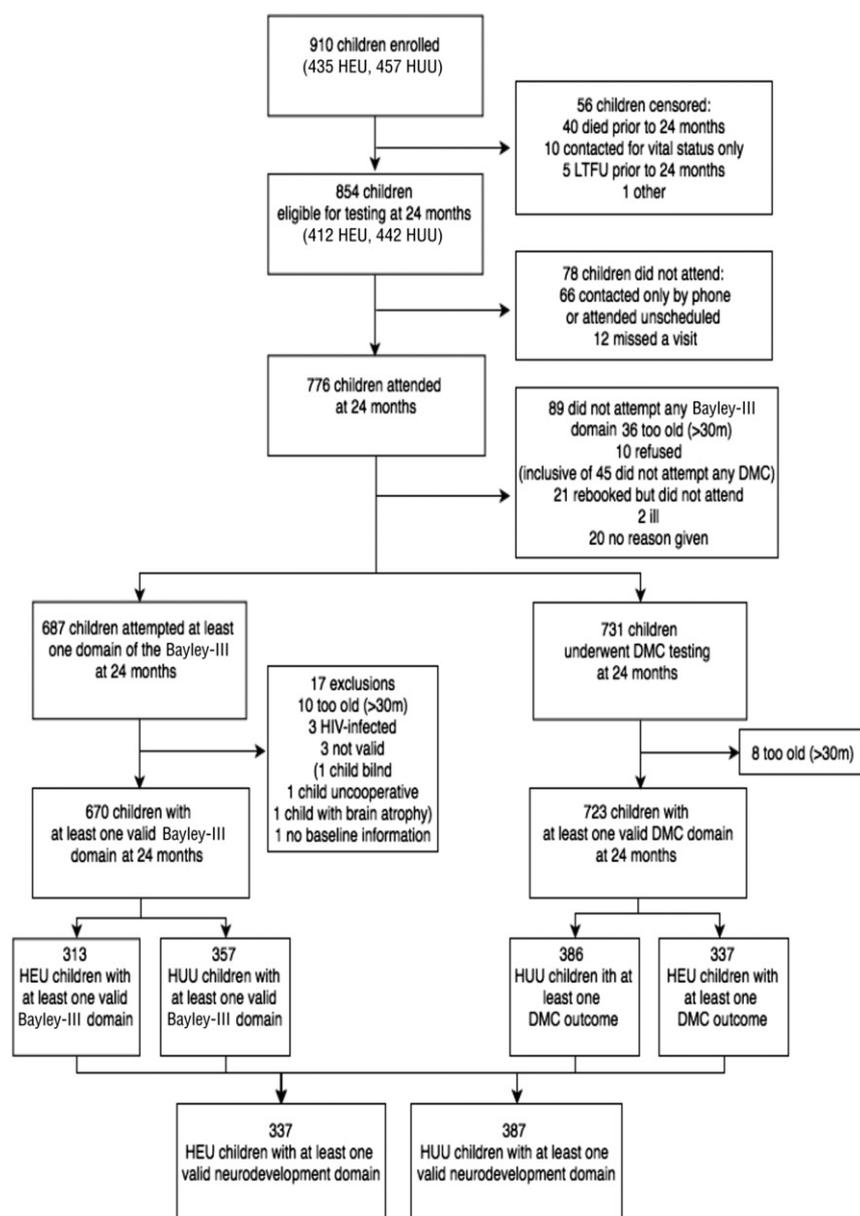

FIGURE 1

Flow diagram of HEU and HUU infants undergoing neurodevelopment assessment. LTFU, loss to follow-up.

were exposed to HIV (67% vs 46%), formula fed (57% vs 35%), of low birth weight (<2500 g, 22% vs 13%), and born preterm (gestational age <37 weeks, 22% vs 13%), compared with children who were tested.

Maternal and infant clinical and sociodemographic characteristics according to maternal HIV exposure status (among children with at least 1 valid neurodevelopmental domain score) are presented in

Table 1. Although only 9% of HEU children were ever breastfed, nearly all HUU children were breastfed. A greater proportion of HEU children were born with low birth weight compared with HUU children (17% vs 9%). HIV-infected women were generally older than HIV-uninfected women, with more than half being over the age of 25 at delivery (53% vs 26%, respectively). Approximately one-third of the HIV-infected

mothers took 3-drug ART during pregnancy (n = 122, 36%) and 214 (64%) took ZDV monotherapy (ARV regimens are summarized in Table 1). HIV-infected mothers reported lower levels of education and had less access to electricity and water within their households and greater food insecurity than HIV-uninfected mothers (although higher proportions of HIV-infected women reported working and having some personal earnings). HIV-infected mothers scored worse on the depression screen but reported similar levels of social support compared with HIV-uninfected mothers. Maternal self-report of illicit substance use was low (<1%); 6% reported ever having used alcohol, with a slightly higher proportion among HIV-infected women. The most common primary caregivers were the infant's mother or father, followed by the infant's grandparents (with increasing frequency of care by grandparents or other individuals with increasing child age). The type of primary caregiver was similar among HEU versus HUU children (data not shown).

Bayley-III Outcomes

Each neurodevelopmental domain was found to be significantly associated with at least 1 previously described maternal socioeconomic or environmental predictor of worse neurodevelopment in univariable linear regression models (Supplemental Tables 7 and 8). For example, increasing maternal age, income, and educational levels were associated with higher Bayley-III scores in several domains (as was breastfeeding). Lower-quality housing; worse food insecurity; and maternal depression, higher stigma, low social support, and alcohol use were each associated with lower Bayley-III score in at least 1 domain.

HEU children had lower unadjusted and adjusted Bayley-III expressive language scores than HUU children

(adjusted mean difference = −0.58, P = .09). Bayley-III unadjusted and adjusted receptive language and DMC language scores were no different in HEU versus HUU children. HEU children had higher unadjusted and adjusted scores in Bayley-III cognitive (adjusted mean difference = 0.59, P = .03) and DMC personal-social domains (adjusted mean difference = 0.71, P = .03) than HUU children (Tables 2 through 4). No further significant differences were identified between HEU and HUU children across all other Bayley-III and DMC domains. Sensitivity analyses further incorporating potential mediators (birth weight and preterm delivery) into multivariable models resulted in no substantial changes in differences between HEU and HUU children in adjusted Bayley-III and DMC domain effect estimates (Supplemental Table 9).

Predictors of Composite Adverse Neurodevelopmental Outcome

When evaluating the dichotomous measure of an adverse neurodevelopmental outcome (low score or invalid test), we observed few differences by HIV exposure status. However, HEU children were more likely to have an adverse expressive language outcome than HUU (53% vs 44%, Supplemental Tables 10 and 11), which persisted after adjustment for confounders (adjusted odds ratio 1.44 [95% confidence interval: 1.01 to 2.06], Supplemental Table 12). We also observed a higher risk of adverse cognitive outcomes for children born to younger mothers or residing in low-income households. Low birth weight and preterm birth were fairly consistently associated with adverse outcomes across all 5 Bayley-III domains.

DISCUSSION

We found no clinically important differences in neurodevelopment at 24 months of age among children

exposed to HIV in utero, with small effect sizes observed (ranging from −0.19 to +0.22). The HEU group scored lower then HUU group in expressive language, as did the HEU adverse outcome group, and the HEU group scored higher in the cognitive domain on the Bayley-III and personal-social scales. However, the effect sizes are small and unlikely to be of clinical significance. Our overall findings provide reassurance that in-utero HIV exposure (and associated ARV exposures) does not adversely affect neurodevelopment in young children in Botswana.

We explored potential environmental and socioeconomic confounders of relevance. Many of the domain-specific predictors we identified have been described previously, although a number are highlighted here for the first time. For instance, it has been well described previously that maternal education, age, and income are associated with Bayley-III cognitive and language neurodevelopmental domains, as was further confirmed in our study.[6–11,16–18] In our study, we highlight the potential importance of maternal depression in influencing a number of domain-specific neurodevelopment outcomes, such as Bayley-III cognitive and DMC locomotor, language, and personal-social scores. Various socioeconomic factors, such as access to food, housing, water, sanitation and electricity, and cooking method within the household, were found to be associated with a number of outcomes across adjusted domain-specific models. Approximately 6% of the mothers in this Botswana study sample reported alcohol use, although this may have been underreported because of social-desirability bias. Maternal alcohol use was significantly associated with worse scores in both Bayley-III cognitive and expressive language domains in univariable linear

TABLE 1 Baseline Characteristics of Mothers and Their Uninfected Children According to HIV Exposure in the Tshipidi Study, Botswana, 2010–2012

Characteristic	Overall (N = 724), N (%)	HIV-Infected Mothers/HIV-Exposed Children (N = 337), N (%)	HIV-Uninfected Mothers/HIV-Unexposed Children (N = 387), N (%)	P (χ² or t test)
Breastfed	415 (57.3%)	30 (8.9%)	385 (99.5%)	<.01
Female	367 (50.7%)	168 (49.9%)	199 (51.4%)	.67
Preterm (<37 wk)	89 (12.8%)	46 (13.9%)	43 (11.7%)	.37
Low birth weight	92 (12.8%)	58 (17.2%)	34 (8.9%)	<.01
3-drug ART	122 (16.9%)	122 (36.2%)	—	—
CBV + NVP	68 (9.4%)	68 (20.2%)	—	—
NVP + TRV	11 (1.5%)	11 (3.3%)	—	—
ATR	10 (1.4%)	10 (3.0%)	—	—
ALU + CBV	9 (1.2%)	9 (2.7%)	—	—
Other	24 (3.3%)	24 (7.1%)	—	—
ZDV monotherapy	214 (29.6%)	214 (63.5%)	—	—
No ARVs	1 (0.1%)	1 (0.3%)	—	—
Mean CD4 cells/mm³ (SD)	—	449 (190)	—	—
Mean log viral copies/mL (SD)	—	4.2 (2.6)	—	—
Site of enrollment	—	—	—	<.01
Gaborone	369 (51.0%)	203 (60.2%)	166 (42.9%)	—
Mochudi	355 (49.0%)	134 (39.8%)	221 (57.1%)	—
Mean maternal age (SD)	27.2 (6.1)	28.9 (5.8)	25.7 (6.0)	<.01
Education				
None	10 (1.4%)	7 (2.1%)	3 (0.8%)	<.01
Primary	60 (8.3%)	43 (12.8%)	17 (4.4%)	—
Secondary junior	362 (50.1%)	204 (60.7%)	158 (40.9%)	—
>Secondary junior	290 (40.2%)	82 (24.4%)	208 (53.9%)	—
Maternal income				
None	466 (64.5%)	187 (55.8%)	279 (72.1%)	<.01
<P500	37 (5.1%)	26 (7.8%)	11 (2.8%)	—
P501–P1000	96 (13.3%)	65 (19.4%)	31 (8.0%)	—
>P1000	123 (17.0%)	57 (17.0%)	66 (17.1%)	—
Access to sanitation				
Indoor	155 (21.6%)	57 (17.0%)	98 (25.6%)	<.01
Private	481 (66.9%)	229 (68.2%)	252 (65.8%)	—
Shared	83 (11.5%)	50 (14.8%)	33 (8.6%)	—
Electricity in home	412 (57.0%)	164 (48.8%)	248 (64.1%)	<.01
Access to water				
Home	143 (19.8%)	55 (16.4%)	88 (22.7%)	<.01
Yard	495 (68.5%)	226 (67.3%)	269 (69.5%)	—
Communal	74 (10.2%)	48 (14.3%)	26 (6.7%)	—
Other	11 (1.5%)	7 (2.1%)	4 (2.1%)	—
Main cooking method				
Gas, electric	525 (72.6%)	240 (71.4%)	285 (73.6%)	.12
Wood	181 (25.0%)	84 (25.0%)	97 (25.1%)	—
Kerosene	8 (1.1%)	7 (2.1%)	1 (0.3%)	—
None	9 (1.2%)	5 (1.5%)	4 (1.0%)	—
Housing type				
Formal	715 (98.9%)	331 (98.5%)	384 (99.2%)	.61
Informal	6 (0.8%)	4 (1.2%)	2 (0.5%)	—
None	2 (0.3%)	1 (0.3%)	1 (0.3%)	—
Food insecurity mean (SD)	—	2.5 (1.1)	2.1 (1.1)	—
None	252 (34.9%)	95 (28.2%)	157 (40.7%)	<.01
Mild	164 (22.7%)	63 (18.7%)	101 (26.2%)	—
Moderate	176 (24.3%)	97 (28.8%)	79 (20.5%)	—
Severe	131 (18.1%)	82 (24.3%)	49 (12.7%)	—
Depression mean score (0–20) (SD)[a]	2.5 (2.7)	2.8 (2.8)	2.1 (2.6)	<.01
Social support mean score (0–40) (SD)[b]	33.9 (6.0)	34.4 (6.0)	33.5 (6.0)	.05
Alcohol use (ever)	46 (6.4%)	24 (7.1%)	22 (5.7%)	<.01
Tobacco use (ever)	10 (1.4%)	5 (1.5%)	5 (1.3%)	.25

Percentage of participants missing values for variables: preterm: 3.6%, low birth weight: 0.7%, maternal education: 0.1%, maternal income: 0.3%, sanitation: 0.7%, electricity in home: 0.1%, source of water: 0.1%, cooking method: 0.1%, housing: 0.1%, food insecurity[22]: 0.1%, depression: 0%, social support: 5.5%, alcohol use (ever): 55.6%, tobacco use (ever): 58.8%. ATR, Atripla, ALU, Aluvia; CBV, Combivir; P, pula; TRV, Truvada; —, not applicable.

[a] Highest score for a given mother on the Beck Depression Score,[23] from antepartum and postpartum assessments (high scores indicating worse depression).

[b] Highest score for a given mother's assessment of her social support[24] (high scores indicating better social support).

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TABLE 2 Unadjusted Mean and Adjusted Bayley-III and DMC Neurodevelopment Domain Scores According to HIV Exposure in the Tshipidi Study, Botswana, 2010–2012

Bayley-III Domain	N	Mean (SD)		Unadjusted Difference		Adjusted Difference		Effect Size
		HEU	HUU	Estimate (95% CI)	P	Estimate (95% CI)	P	Cohen's D (95% CI)
Cognitive	657	53.8 (3.4)	53.0 (3.3)	0.7 (0.2 to 1.2)	<.01	0.6 (0.1 to 1.1)	.03	0.22 (−0.04 to 0.47)
Gross motor	642	52.6 (2.8)	52.9 (2.7)	−0.3 (−0.7 to 0.2)	.21	−0.1 (−0.5 to 0.3)	.63	−0.10 (−0.31 to 0.11)
Fine motor	668	37.3 (1.8)	37.4 (1.8)	−0.1 (−0.4 to 0.2)	.47	0.0 (−0.3 to 0.3)	.98	−0.06 (−0.19 to 0.08)
Expressive language	652	25.0 (4.4)	25.8 (4.1)	−0.8 (−1.4 to −0.2)	.02	−0.6 (−1.3 to 0.1)	.09	−0.19 (−0.51 to 0.13)
Receptive language	652	21.1 (3.5)	20.7 (3.3)	0.3 (−0.2 to 0.9)	.21	0.3 (−0.3 to 0.8)	.35	0.10 (−0.16 to 0.36)
DMC domain								
Locomotor	723	32.2 (1.8)	32.2 (1.8)	−0.1 (−0.3 to 0.2)	.58	−0.1 (−0.3 to 0.2)	.66	−0.04 (−0.18 to 0.09)
Fine motor	718	19.1 (2.3)	19.4 (1.9)	−0.3 (−0.6 to 0.0)	.05	−0.1 (−0.4 to 0.2)	.63	−0.15 (−0.30 to 0.01)
Language	723	16.5 (2.6)	16.5 (2.7)	0.0 (−0.4 to 0.4)	.92	0.2 (−0.2 to 0.6)	.43	−0.01 (−0.20 to 0.19)
Personal-social	720	45.1 (3.9)	44.5 (4.5)	0.6 (0.0 to 1.2)	.06	0.7 (0.1 to 1.3)	.02	0.14 (−0.17 to 0.45)

Bayley-III: cognitive adjusted for maternal income and depression; gross motor adjusted for household access to water and food insecurity; fine motor adjusted for access to sanitation, maternal education, and housing type; expressive language adjusted for maternal education and household access to electricity; receptive language adjusted for maternal age. DMC: locomotor adjusted for maternal income and depression, fine motor adjusted for maternal education and social score, language adjusted for maternal education and maternal depression, personal-social adjusted for cooking method and maternal depression. CI, confidence interval.

TABLE 3 Adjusted Mean Differences in Bayley-III Scores According to Significant Predictors in the Tshipidi Study, Botswana, 2010–2012

Covariate	Bayley-III Neurodevelopment Domain Estimates									
	Cognitive Estimate (SE)	P	Gross Motor Estimate (SE)	P	Fine Motor Estimate (SE)	P	Expressive Language Estimate (SE)	P	Receptive Language Estimate (SE)	P
In-utero exposure to HIV	0.59 (0.26)	.03	—		—		−0.58 (0.35)	.09	—	
Maternal factors										
Older age	—		—		—		—		0.14 (0.11)	.20
Depression	0.09 (0.05)	.07	—		—		—		—	
Socioeconomic factors										
Higher education	—		—		0.18 (0.11)	.10	0.77 (0.26)	<.01	—	
Higher income	0.33 (0.11)	<.01	—		—		—		—	
Less access to water	—		−0.30 (0.10)	<.01	—		—		—	
Food insecurity	—		−0.18 (0.10)	.06	—		—		—	
Less access to sanitation	—		—		−0.28 (0.13)	.02	—		—	
Lower quality housing	—		—		−0.89 (0.48)	.06	—		—	
Access to electricity	—		—		—		0.58 (0.34)	.09	—	
Preterm (<37 wk)[a]	−0.64 (0.41)	.12	−0.91 (0.33)	<.01	−0.37 (0.22)	.09	−0.06 (0.52)	.90	−0.82 (0.42)	.05
Low birth weight[a]	−1.24 (0.39)	<.01	−1.19 (0.33)	<.01	−0.31 (0.21)	.15	−0.94 (0.50)	.06	−0.63 (0.40)	.12

[a] Bivariate associations for these covariates are shown in models fitted with the main exposure of interest (in-utero exposure to HIV). —, not applicable.

regression but was not significant in any of the adjusted models.

The strengths of our study included its prospective nature, its large sample size, and our ability to test and control for a large number of previously unexplored potential environmental and socioeconomic confounders. Additionally, the context of investigation within a large generalized HIV epidemic (in which HIV-infected and uninfected mothers were similar in many key ways) facilitated assessment of the specific effect of HIV exposure on neurodevelopment (with less unmeasured confounding). The large sample size provided 80% power to detect small but clinically significant differences (0.18 SDs) in mean neurodevelopmental scores between HEU and HUU children. Although a multitude of studies have included examinations of neurodevelopment among HIV-infected children[14,15] by using HEU children only as controls,[15] our study is one of few studies to date in which neurodevelopment in HEU and HUU children in sub-Saharan Africa were compared.[16–19]

Kandawasvika et al[16] enrolled 188 HEU children and 287 HUU children, but did not present a comparison of their neurodevelopmental outcomes. Msellati et al[17] followed 91 HEU and 84 HUU children to 24 months of age and did not find any differences in their neurodevelopmental outcomes, following both groups largely to serve as controls for HIV-infected children. In this study, we are the first to offer adequate assessment of many potential confounders relevant to this setting, as previous efforts have provided unadjusted comparisons of HEU

TABLE 4 Adjusted Mean Differences in DMC Neurodevelopment Scores According to Significant Predictors in the Tshipidi Study, Botswana, 2010–2012

Covariate	DMC Neurodevelopment Domains Estimates							
	Locomotor Estimate (SE)	P	Fine Motor Estimate (SE)	P	Language Estimate (SE)	P	Personal-Social Estimate (SE)	P
In-utero exposure to HIV	—		—		—		0.74 (0.32)	.02
Maternal								
Income	0.12 (0.06)	.04	—		—		—	
Depression	−0.08 (0.03)	<.01	—		−0.06 (0.04)	.10	−0.11 (0.06)	.08
Education	—		0.46 (0.12)	<.01	0.35 (0.15)	.02	—	
Social score	—		0.02 (0.01)	.06	—		—	
Household								
Cooking method (gas or electric vs wood burning)	—		—		—		0.69 (0.37)	.06
Cooking method (kerosene vs wood burning)	—		—		—		−2.76 (1.55)	.08
Cooking method (none vs wood-burning)	—		—		—		1.01 (1.44)	.48
Preterm (<37 wk)[a]	−0.50 (0.21)	.02	−0.41 (0.24)	.09	−0.28 (0.31)	.35	−0.63 (0.49)	.19
Low birth weight[a]	−0.40 (0.21)	.05	−0.05 (0.24)	.84	−0.39 (0.30)	.19	−0.13 (0.48)	.78

[a] Bivariate associations for these covariates are shown in models fitted with the main exposure of interest (in-utero exposure to HIV) in the Tshipidi study, Botswana, 2010–2012. —, not applicable.

versus HUU child neurodevelopment only.[17–19] Van Rie et al[19] compared 19 HEU children with 31 HUU children in the Democratic Republic of Congo and found a possible expressive language delay among HEU children when compared with HUU children, although overall sample size was too small to control for potential confounders. HEU and HUU children within their study differed significantly across a range of socioeconomic factors with inadequate opportunity to control for these differences.[19] With our findings, we further confirm the importance of socioeconomic and environmental influences on child neurodevelopment in sub-Saharan settings, underscoring the importance of adjusted comparisons of HEU versus HUU children's neurodevelopmental outcomes.

With these findings, we also confirm the likely safety of in-utero exposures to maternal HIV and associated ARVs with respect to early child development.[6,7,9–11,17–19] Atazanavir has been implicated in possible language domain deficits in a number of studies in North America.[7,12,31] Only a small number of women were taking atazanavir-containing regimens during pregnancy in our study; hence, we did not have sufficient power to assess for

expressive language deficits among our atazanavir exposed children.

A major limitation of our study was our inability to adjust for the effect of breastfeeding because 99.5% of the HUU children were breastfed. Breastfeeding is a well-established determinant of improved neurodevelopment.[31–34] Despite this, the largely breastfed HUU children in this study did not have higher neurodevelopmental outcomes across all domains than formula-fed HEU children. Current World Health Organization guidelines support exclusive breastfeeding by mothers in resource-limited settings who are taking virologically suppressive ARVs.[35] Another limitation is the difference in some baseline characteristics between children who were tested versus those not tested. Sensitivity analyses identified that variables that were imbalanced between comparison groups, such as low birth weight and preterm delivery, did not impact Bayley-III and DMC domain effect estimates. Most baseline covariates (other than feeding method) were evenly distributed between HEU and HUU groups, and differences were accounted for in adjusted estimates. Differences in HEU and

HUU neurodevelopmental functioning may be subtle in early life, and microstructural alterations have been described.[36] Further differences may emerge with age; hence, our findings may not be applicable to older children, nor to HEU children exposed in utero to other ARV regimens.

CONCLUSIONS

Our findings provide reassurance that in-utero exposure to HIV and associated ARVs does not significantly adversely affect neurodevelopment in young children.

ABBREVIATIONS

ART: antiretroviral treatment
ARV: antiretroviral
Bayley-III: Bayley Scales of Infant and Toddler Development, Third Edition
CD4: cluster of differentiation 4
DMC: Development Milestones Checklist
ELISA: enzyme-linked immuno-sorbent assay
HEU: HIV-exposed uninfected
HUU: HIV-unexposed uninfected
NVP: nevirapine
PCR: polymerase chain reaction
ZDV: zidovudine

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