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Neurodevelopmental outcome of HIV-exposed but uninfected infants in the Mother and Infants Health Study, Cape Town, South Africa

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Abstract

OBJECTIVES To compare neurodevelopmental outcomes of HIV-exposed uninfected (HEU) and HIV-unexposed uninfected (HUU) infants in a peri-urban South African population. HEU infants living in Africa face unique biological and environmental risks, but uncertainty remains regarding their neurodevelopmental outcome. This is partly due to lack of well-matched HUU comparison groups needed to adjust for confounding factors.

METHODS This was a prospective cohort study of infants enrolled at birth from a low-risk midwife obstetric facility. At 12 months of age, HEU and HUU infant growth and neurodevelopmental outcomes were compared. Growth was evaluated as WHO weight-for-age, length-for-age, weight-for-length and head-circumference-for-age Z-scores. Neurodevelopmental outcomes were evaluated using the Bayley scales of Infant Development III (BSID) and Alarm Distress Baby Scale (ADBB).

RESULTS Fifty-eight HEU and 38 HUU infants were evaluated at 11–14 months of age. Performance on the BSID did not differ in any of the domains between HEU and HUU infants. The cognitive, language and motor scores were within the average range (US standardised norms). Seven (12%) HEU and 1 (2.6%) HUU infant showed social withdrawal on the ADBB ($P = 0.10$), while 15 (26%) HEU and 4 (11%) HUU infants showed decreased vocalisation ($P = 0.06$). There were no growth differences. Three HEU and one HUU infant had minor neurological signs, while eight HEU and two HUU infants had macrocephaly.

CONCLUSIONS Although findings on the early neurodevelopmental outcome of HEU infants are reassuring, minor differences in vocalisation and on neurological examination indicate a need for reassessment at a later age.

keywords HIV-exposed uninfected infants, neurodevelopmental outcome, infant growth, South Africa, low- and middle-income countries

Introduction

HIV-exposed but uninfected (HEU) infants face biological and environmental risk factors that could potentially affect cognitive development [1–4]. Neurodevelopmental outcome of this vulnerable group in low- and middle-income countries remains uncertain [5–7]. Sher, reviewing studies prior to 2009, found that research methodologies differed and studies generally lacked HIV-unexposed uninfected (HUU) child comparison groups [8]. The few African studies with HUU comparison groups have shown mixed findings. In Zaire, cognitive delay was observed in HEU *vs*. HUU children [9]; in the Democratic Republic of Congo motor, expressive language delays were observed [10], and in Zambia, differences in scholastic performance were reported [11]. However, numerous other studies in Africa have found no differences in cognitive, motor and language development between HEU and HUU children [12–16].

In South Africa, the antenatal HIV prevalence is one of the highest in the world (29.5% in 2011) [17]. Prevention

of mother-to-child transmission (PMTCT) programmes have successfully reduced vertical transmission rates to less than 5% [18], resulting in an estimated 290 000 HEU newborns annually in South Africa [19]. Early detection of neurodevelopmental delays and targeted intervention during the pre-school years improves long-term developmental outcome in all children irrespective of HIV exposure [20]. South African policymakers require information about the neurodevelopmental status of the large population of HEU infants and children to provide future surveillance and support.

The HUU comparison group is crucial as there are many additional factors that can affect neurodevelopmental outcome including maternal education, mental health, alcohol or recreational drug use in pregnancy and poverty [21]. Most developmental tools are not validated locally or contextually appropriate for African infants, so comparison with USA standardised norms may be insufficient. This study therefore aimed to compare cognitive, motor and language development of HEU and HUU South African infants.

Methods

This prospective cohort study was nested in the Mother Infant Health Study (MIHS), a longitudinal study with the primary objective of comparing risk for infectious morbidity in HEU and HUU infants [22]. From July 2012 to June 2013, HIV-infected and HIV-uninfected women were enrolled within 72 h of delivery from a single, community, low-risk, midwife obstetric unit serving a peri-urban community on the outskirts of Cape Town, South Africa. HIV-infected and HIV-uninfected mothers were frequency matched on race–ethnicity to reduce heterogeneity in the primary language and maternal social characteristics between HEU and HUU infants which were identified as confounders to neurodevelopmental outcome in a preceding pilot study [23].

Maternal HIV infection status was confirmed on presentation in labour using standard South African national HIV testing algorithms and confirmed again 2 weeks postnatally [24]. The HIV-infected mothers received routine PMTCT interventions during the study according to the Western Cape Provincial guidelines at the time (WHO Option A) [25]. This included combination antiretroviral therapy (cART) for all pregnant women with CD4 < 350 cells/μl or WHO stage 3 or 4 disease, or zidovudine (ZDV) monotherapy if criteria for cART were not met. Infant eligibility criteria included birthweight >2000 g and ≥34 weeks' gestation. Only confirmed HIV-uninfected infants aged 12 ± 2 months were included in the neurodevelopmental assessment. All infants underwent HIV testing (HIV-ELISA and HIV DNA-PCR if HIV-ELISA was positive) to exclude HIV infection at the assessment visit. Neurodevelopmental assessments were rescheduled if infants were physically unwell on the day.

The assessments took place at the Tygerberg Hospital (TBH) paediatric outpatient department. Each mother–infant pair was allocated a study number to anonymise data. A research assistant took consent and administered the Centre for Epidemiological Studies Depression Scale (CES-D) [26] to the primary caregiver in their language of choice. One of two developmental paediatricians blinded to the HIV-exposure status assessed the infant using Bayley Scales of Infant and Toddler Development III (BSID III) [27] followed by a full neurological examination. The hearing was screened with a high-frequency rattle, and if there were concerns, infants were referred for audiological evaluation. The paediatricians initially assessed five infants together using the BSID III and reached consensus on discrepant pass or fail items. The principal investigator (PS) then assessed a further 86 infants, while the second assessor (HS) assessed five infants independently. An interpreter assisted the paediatricians in completing the scales with Xhosa-speaking participants. At least 30 min of each assessment was videotaped to allow for review of the behavioural features. Infant anthropometry and baseline maternal, socioeconomic and infant feeding data collected at study visits prior to the 12-month neurodevelopmental assessment were accessed from the MIHS database.

The BSID III is an internationally recognised standardised, norm-referenced tool that has been used in South Africa but not adapted or standardised for South African children [28]. Infants were tested using the cognitive, language and motor scales. Children with developmental delay (any BSID III composite score below 80) were referred to the TBH neurodevelopmental service.

The Alarm Distress Baby Scale (ADBB) is a screening tool that detects social withdrawal by observing an infant's behaviour with a stranger while in the presence of the mother [29]. It has previously been used in South African research [30]. The principal investigator obtained distance-training accreditation using videotaped examples and performed all of the ADBB assessments. The assessor rates behaviours including infant's facial expressivity, eye contact, vocalisation, activity and ability to form a relationship with an observer to generate a total ADBB score. A threshold score of 5 has shown optimal sensitivity and specificity to detect infants at risk [29]. A vocal score of above zero indicates reduced vocalisation and rates the quantity of vocalisation ranging from brief spontaneous vocalisation (score 1) to a complete silence (score 4). Videotaping each

assessment allowed for review of the ADBB scoring. Two accredited scorers reviewed 15 of the more difficult assessments with the principal investigator for final consensus. Infants rated as having social withdrawal were rebooked over a fortnight to confirm behavioural features. The CES-D was used to screen the primary caregiver's mental health [26]. It is a 20-item self-rating scale previously used in South Africa with a threshold of 16 indicating significant depressive symptomatology. Primary caregivers with significant depressive symptomatology were referred to the community psychiatric outpatient service and those who reported thoughts of self-harm were referred to the TBH emergency psychiatric service.

The sample size for comparison between the two groups was calculated using information from interim analysis of Bayley scores. A sample size of at least 50 HEU and 50 HUU infants was deemed necessary to show a clinically meaningful 5-point difference in the composite scores. Expected mean general quotients were estimated to be in the region of 100 with a standard deviation of 15 [27]. US norms classify scores between 70 and 85 as moderately impaired and <70 as severe, and thus, BSID III composite scores below 85 were classified as 'poorer neurodevelopmental outcome' [28]. World Health Organization (WHO) child growth standards of head circumference, weight and length of infants were converted into standardised Z-score anthropometric values utilising WHOAnthro (WHO 2011) and included weight for age (WAZ), length for age (HAZ), weight for length (WHZ) and head circumference for age (HCZ). Definitions included the following: underweight (WAZ < −2 Z-score), stunted (HAZ < −2 Z-score) wasted (WHZ < −2 Z-score), macrocephaly (HCZ > +2 Z-score) and microcephaly HCZ < −2 Z-score). Categorical data were analysed using chi-square analysis or Fisher's exact test. Numeric data were analysed using a t-test or Wilcoxon rank-sum test. A *P*-value less than 0.05 was considered significant. Stata version 13.1 (StataCorp, Texas, USA) was used for analysis.

Ethics approval was granted (N13/03/028) for the neurodevelopmental substudy of the Mother and Infant Health Study (S12/0/009) by Human Research Ethics committees of Stellenbosch University and the University of British Columbia. Caregivers gave separate consent for participation in the neurodevelopmental study and maternal mental health questionnaire and received verbal feedback and a report.

Results

Of the 176 mother–infant pairs enrolled at 2 weeks of age, 103 (58.5%) returned for the 12-month visit and 96 (54.5%) infants underwent neurodevelopmental assessments, 58 HEU and 38 HUU infants (Figure 1). Significantly more HIV-infected than HIV-uninfected mothers were retained ($P = 0.03$); a higher proportion of those retained had completed secondary education ($P = 0.04$), while their infants had a lower mean birthweight ($P = 0.05$). There were no other major differences between mother–infant pairs retained in the study and those that were not, with regard to baseline maternal data (age, ethnicity, home language, primiparity, pregnancy ARV regime and delivery CD4 count) and infant characteristics (gestational age, length and low birthweight) (Table S5).

Ninety-four (98%) infants were accompanied by their biological mother and two (2%) by maternal grandmothers, one of whom was the primary caregiver as the mother resided in another province and the other the daytime caregiver while the mother worked. All infants lived with their biological mother except the one HEU infant who was in the grandmother's care. Mothers of HEU infants were older, less educated and fewer reported having planned their pregnancy than mothers of HUU infants (Table 1). The mean gestation at booking was 21.8 weeks for both HEU (SD 6.2) and HUU (SD 6.8) groups ($P = 0.99$).

Two mothers, one HIV-infected and one HIV-uninfected, were treated antenatally for tuberculosis, and one HIV-uninfected mother was treated for syphilis. Two HIV-infected mothers admitted to using illicit drugs during pregnancy. Twenty-nine (50%) of the HEU infants were exposed *in utero* to combination antiretroviral therapy (cART) and the remaining 50% to ZDV monotherapy (24). Ninety-four mothers and one grandmother who was the primary caregiver completed the CES-D questionnaire, while one mother (HUU group) declined. Scores indicated depressive symptomatology in more than half of mothers, with no difference between HIV-infected and HIV-uninfected participants (HIV-infected 30/58 (52%) *vs*. HIV-uninfected 20/37 (54%)) (Table 1).

All infants had uncomplicated perinatal histories, and maternal mean body mass index (BMI) at 2 weeks postpartum did not differ ($P = 0.85$) in the two groups. There was a significant difference in the proportion of infants breastfeeding at 2 weeks (39% HEU *vs*. 100% HUU infants) ($P < 0.001$) and at 6 months (12% HEU *vs*. 87% HUU infants) ($P < 0.001$) but no group difference in 12-month anthropometry (Table 2). However, four (6.7%) HEU and six (15.7%) HUU infants were stunted, two (3.4%) HEU infants were stunted and underweight, and one (1.7%) HEU infant was underweight, stunted and wasted.

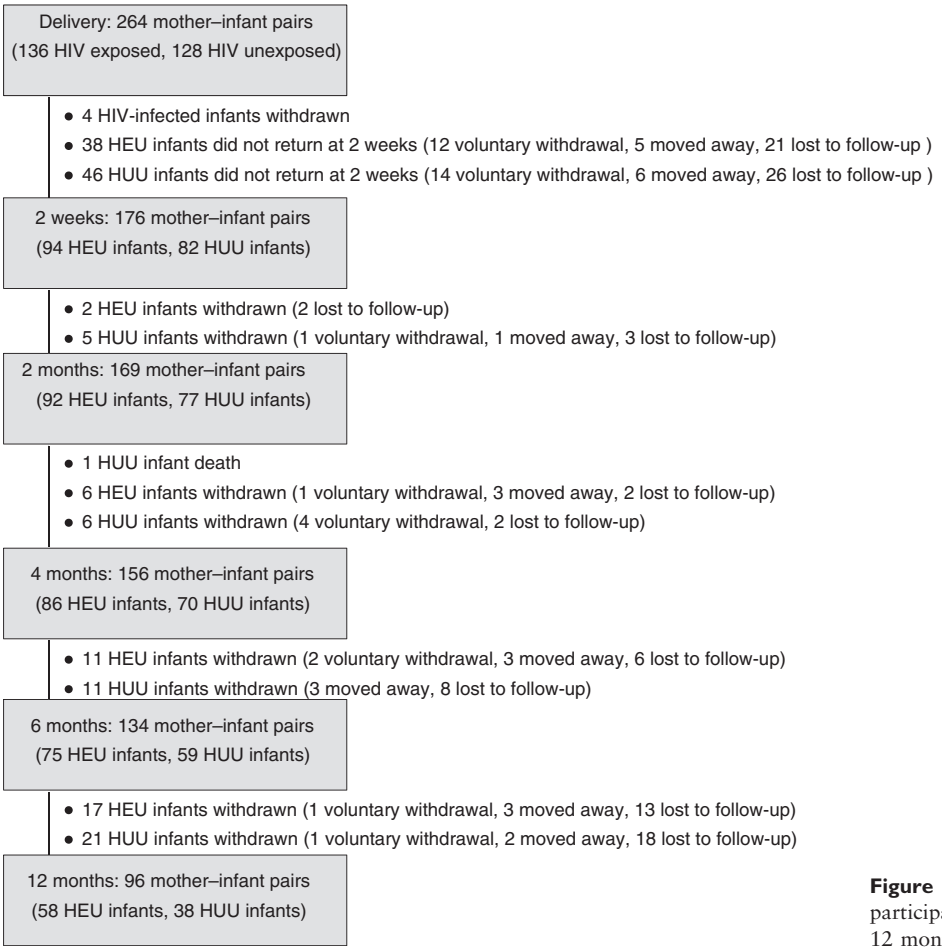

Figure 1 Disposition of study participants between enrolment and 12 months of age.

Fourteen (24%) of the HEU infants and seven (18%) of the HUU infants required hospitalisation in the first year of life with four (6.9%) HEU infants and two (5.2%) HUU infants requiring two admissions and one HEU infant requiring three admissions. No complications related to hospitalisation resulted in neurological compromise.

The infant requiring three hospitalisations had congenital macrocephaly and global developmental delay and presented with torticollis and history of a head injury but no loss of consciousness. The torticollis resolved and neuroimaging of brain and spine was normal. The infant was exposed to efavirenz from the first trimester of pregnancy.

Eight HEU (13.8%) and two HUU (5.5%) infants had macrocephaly at the 12-month visit ($P = 0.19$), whereas none were microcephalic. Three of the eight macrocephalic HEU infants had been exposed antenatally to cART including two infants to tenofovir disoproxil fumarate (TDF), lamivudine (3TC) and efavirenz and one infant to TDF, 3TC and lopinavir/ritonavir. Four (6%) HEU infants had abnormal neurological signs including generalised hypotonia ($N = 1$), unilateral dystonia ($N = 1$), convergent squint ($N = 1$) and congenital unilateral ptosis ($N = 1$). One HUU infant had generalised hypotonia.

There were no significant differences in the cognitive, language (receptive and expressive) or motor (gross motor and fine motor) composite scores between the HEU and HUU infants (Table 3).

The group mean (standard deviation (SD)) language composite scores were 91.75 (11.3), which was lower than the cognitive (100.46 (10.23)) and motor scores (96.5 (9.42)), but still within the average range. Cognitive and motor composite scores for all HEU and HUU infants were within two standard deviations of the mean (≥70), but one HUU infant had a language composite score in the severely impaired range (<70) despite normal

Table 1 Maternal, infant and socio-economic characteristics compared between HIV-exposed uninfected (HEU) and HIV-unexposed uninfected (HUU) infants

Characteristic	HEU (*N* = 58)	HUU (*N* = 38)	*P*-value
Maternal characteristics			
Age at delivery (in years) – mean (SD)	27.8 (5.15)	24.8 (4.8)	0.005
Ethnicity			
African† (%)	51 (87.9%)	34 (89.5%)	0.54
Mixed ancestry	7 (12.1%)	4 (10.5%)	
Marital status (%)			
Never married	40 (69.0%)	26 (68.4%)	0.69
Married	16 (27.6%)	12 (31.6%)	
Widowed/Divorced/separated	2 (3.4%)	0 (0%)	
Highest level of education (%)			
Primary school education only	5 (8.6%)	0 (0%)	0.02
Attended Secondary school	35 (60.3%)	17 (44.7%)	
Completed secondary education	18 (31.0%)	21 (55.3%)	
Primiparous (%)	8 (13.8%)	11 (29.0%)	0.07
Planned pregnancy (%)	11 (19.0%)	14 (36.8%)	0.05
Any tobacco use during pregnancy (%)	9 (15.5%)	1 (2.6%)	0.08
Any alcohol use during pregnancy (%)	11 (19.0%)	4 (10.5%)	0.39
Depressive symptoms	30/58	20/37	0.68
CES-D score ≥16 *n*/*N* (%)	(51.7%)	(54.0%)	
Infant characteristics			
Male	28 (48.3%)	16 (42.1%)	0.55
Gestational age in weeks mean (SD)	38.5 (1.56)	39.0 (1.64)	0.16
Birthweight in grams – mean (SD) (95% CI)	3068 (392) (2965–3172)	3157 (449) (3010–3304)	0.31
Low birthweight <2500 g	5 (8.6%)	1 (2.6%)	0.40
Breastfeeding: at age 2 weeks	23 (39.6%)	38 (100%)	<0.001
Still breastfeeding at age 6 months	7 (12.1%)	25 (65.8%)	<0.001
Socio-economic characteristics			
Mother's mean (SD) monthly income at 6 months postpartum (ZAR)	1055.8 (992.3)	1068.3 (1024.5)	0.98
Receiving Child Support Grant for study infant	34 (63.0%)	23 (67.6%)	0.65
Type of Housing: Stand-alone house	21 (36.2%)	17 (44.7%)	0.32
Informal stand-alone	23 (39.7%)	8 (21%)	
Other (apartment)	14 (24.1%)	13 (34.3%)	

†African includes infants of Xhosa-speaking South African (*n* = 76), Ndebele-speaking South Africans (*n* = 2) and Zimbabwean (*n* = 7) descent; CES-D, Centre for Epidemiological Studies Depression Scale; HEU, HIV exposed uninfected; HUU, HIV unexposed uninfected; SD, standard deviation.

hearing. Five HEU infants demonstrated cognitive scores <85 *vs*. no HUU infants ($P = 0.15$). A similar proportion of HEU (6.9%) *vs*. HUU (5.2%) demonstrated poorer neurodevelopmental outcome on motor domain (score <85) ($P = 0.74$); however, a higher proportion of HEU infants compared to HUU infants had 'poorer neurodevelopmental outcome' (<85) on the language domain; 28% HEU *vs*. 18% HUU ($P = 0.23$).

There was no significant difference between the groups with regard to total ADBB scores (Table 3). Seven (12.1%) HEU infants and one (2.6%) HUU infant were classified as 'socially withdrawn' (Fisher exact=0.14). Seven (87%) of the eight socially withdrawn infants were female (Fisher exact $P = 0.07$). Fifteen (25.9%) HEU *vs*. four (10.5%) HUU infants showed decreased vocalisation on the ADBB vocal subscale (Fisher exact $P = 0.07$); however, there was no sex difference in these 19 infants with decreased vocalisation (Fisher exact $P = 1.00$). The 'socially withdrawn' infants scored lower on the BSID III language subscale ($P < 0.001$). The seven 'socially withdrawn' HEU infants had a mean language quotient of 81.1 (range 79–91), and the single HUU infant's quotient was 77. The 19 infants with decreased vocalisation on ADBB subscale also had decreased BSID III receptive ($P < 0.01$), expressive ($P < 0.01$) and composite language quotients (mean 80.3 $P < 0.01$); the mean composite

Table 2 Infant sociodemographic and anthropometric characteristics at age 12 months compared between HIV-exposed uninfected (HEU) and HIV-unexposed uninfected (HUU) infants

Characteristic	HEU	HUU	*P*-value
Sociodemographic characteristics	*N* = 58	*N* = 38	
Age at assessment in days – mean (SD)	364.3 (11.8)	365.1 (8.7)	0.60
Daycare attendance – *N* (%)	12 (20.7%)	3 (8.3%)	0.11
Living with mother – *N* (%)	57 (98%)	38 (100%)	
Anthropometric characteristics at age 12 months	*N* = 58	*N* = 38	
Head circumference Z-score mean (SD)	0.56 (1.23)	0.41 (1.03)*	0.55
Weight-for-age Z-score mean (SD)	0.12 (1.31)	0.28 (1.07)*	0.53
Length- for-age Z-score mean (SD)	−0.75 (0.95)	−0.59 (1.24)**	0.46
Weight-for-length Z-score mean (SD)	0.62 (1.39)	0.74 (0.93)**	0.65

HEU, HIV exposed uninfected; HUU, HIV unexposed uninfected; SD, standard deviation.
Data missing *Two participants **Three participants.

Table 3 Neurodevelopmental outcomes at 12 months of age in HIV-exposed uninfected (HEU) and HIV unexposed uninfected (HUU) infants according to the Bayley Scales of Infant and Toddler Development 3rd edition (BSID III) and the infant social withdrawal scale (ADBB)

	HEU (*N* = 58)	HUU (*N* = 38)	*P*-value
BSID III			
Composite scores – mean (SD)			
Cognitive	99.9 (7.8)	101.3 (11.6)	0.51
Motor	95.6 (9.1)	97.8 (9.8)	0.26
Language score	90.4 (9.4)	92.5 (11.0)	0.31
Scaled scores – mean (SD)			
Gross motor	9.0 (2.5)	9.6 (2.5)	0.17
Fine motor	9.5 (1.5)	9.6 (1.2)	0.86
Receptive language	8.1 (2.0)	8.2 (1.9)	0.72
Expressive language	8.7 (1.9)	9.2 (2.2)	0.28
ADBB			
Increased social withdrawal – *N* (%)	7 (12.1%)	1 (2.6%)	0.10
Decreased vocalisation – *N* (%)	15 (25.9%)	4 (10.5%)	0.06

ADBB, Alarm Distress Baby Scale; BSID III, Bayley Scales of Infant and Toddler Development 3rd edition; SD, standard deviation.

language score of the 15 HEU infants with decreased vocalisation on ADBB was 81.9 (range 71–91) and that of the four HUU infants was 75.5 (range 59–89).

A high proportion of the caregivers (52.6%) scored above the CES-D threshold for depressive symptomatology; but there was no difference between HEU and HUU groups ($P = 0.55$). There was also no association between elevated CES-D score and poorer outcome on the BSID III language subscale ($P = 0.26$) or ADBB social withdrawal (Fisher exact $P = 0.29$) or vocal subscale (Fisher exact $P = 0.80$). However, the mother of the sole HUU infant with social withdrawal had the second highest CES-D score of all participants.

Accordingly, there was no association between poorer outcome on the BSID and maternal CD4 count < 350 cells/μl at delivery ($P = 0.53$), but four mothers (57%) of the seven HEU infants with social withdrawal (ADBB) had CD4 counts <350 cells/μl.

HEU infants born to mothers on cART had significantly poorer fine motor scaled scores than those on ZDV monotherapy ($P = 0.04$) although there were no significant differences in other domains of BSID and ADBB (Table 4). Stunting at 12 months was associated with social withdrawal ($P = 0.05$), but not reduced vocalisation on the ADBB ($P = 0.43$). There was no association between stunting and poorer outcome on the BSID cognitive ($P = 0.36$), language ($P = 0.66$) or motor ($P = 0.44$) composite scores and receptive ($P = 0.34$), expressive ($P = 0.32$), fine (0.42) or gross motor ($P = 0.5$) scaled scores. The numbers of underweight and wasted infants were too small for further comparisons.

Discussion

There was no difference in Bayley composite scores between HEU and HUU infants. This finding concurs with other African studies on children younger than 3 years [12–16]. Alimenti (Canada) reported no difference after adjusting for illicit drug exposure by the mothers of HEU infants [31].

The mean BSID III language scores of both groups, although still in the 'average' range, were lower than the cognitive and motor scores. A similar developmental

Table 4 Neurodevelopmental outcome according to antenatal antiretroviral exposure

Antenatal ARV regime	cART *N* = 29	Zidovudine monotherapy *N* = 29	None (HUU) *N* = 38	*P*-value
BSID III Composite score – mean (SD)				
Cognitive	99.1 (12.2)	100.7 (11.2)	101.3 (7.8)	0.52
Language	89.9 (10.4)	90.9 (8.7)	92.5 (11.0)	0.56
Motor	93.6 (9.5)	97.6 (8.5)	97.8 (9.8)	0.14
BSID III Scaled scores – mean (SD)				
Receptive language	7.9 (1.8)	8.3 (2.3)	8.2 (1.9)	0.54
Expressive language	8.9 (2.0)	8.6 (2.1)	9.2 (2.2)	0.49
Fine motor	9.1 (1.2)	10.0 (1.7)	9.6 (1.3)	0.04
Gross motor	8.8 (2.5)	9.2 (1.7)	9.6 (2.5)	0.31
ABBB social withdrawal (%)	3 (10.3)	4 (13.8)	1 (2.6)	0.11
ABBB reduced vocalisation (%)	6 (20.7)	9 (31)	4 (10.5)	0.19

profile was described in a US study involving HEU infants from a lower socio-economic group [32]. Language quotients may also have been influenced by cultural factors such as unfamiliarity with the pictures and nature of objects used in the BSID III. Although studies have reported language deficits or late language emergence in HEU *vs*. HUU children [7, 10, 33], our cohort showed no difference. However, language delay in HEU infants may not be evident as early as 12 months of age [34].

Seven HEU infants (12%) and one (2.6%) HUU infant reached the ADBB threshold for social withdrawal behaviour. Puura reported a prevalence of 2.7% of social withdrawal in a study of 363 infants attending a well-baby clinic in Finland [35], while Guedeney reported 13% in a non-clinical community sample [36]. An earlier study on South African HIV-infected mother–infant dyads classified 31% of infants as socially withdrawn using the modified-ADBB; however, their study cohort included mothers with advanced HIV [37].

A higher proportion of HEU than HUU infants had abnormal vocalisation scores on the ADBB. Both HEU and HUU infants with decreased vocalisation also performed poorly on the BSID language subscales, suggesting subtle developmental language or emotional differences in these infants. A lower level of maternal education was not a confounder in this group. Previous studies have not explored the relationship between the ADBB vocal subscale and developmental outcomes. Molteno *et al.* found that infant emotional withdrawal on ADBB was a significant predictor of IQ at 9 years in children with foetal alcohol syndrome but did not report on individual subscales [30].

Although there was no significant group difference between caregiver CES-D scores, a high proportion of mothers scored above the threshold for psychiatric referral. South African studies have previously documented high prevalence rates of postnatal depression in mothers from lower socio-economic groups [38–41] as have other African countries [42, 43].

Parsons found an association between postnatal depression and adverse child developmental outcome in low- and middle-income countries [44], whereas we found no significant association between an elevated maternal CES-D score and abnormal infant ADBB ($P = 0.29$). However, the CES-D was administered at a single time-point and the sample size was small. Previous studies have associated prolonged depression with compromised infant development [44, 45].

HEU infants born to mothers on cART had lower fine motor scores (Table 4). There was no difference in other domains between cART-exposed and unexposed HEU infants, which concurs with previous findings regarding antenatal cART exposure [46, 47]. However, subgroups were small. Moreover, treatment with cART was also a marker of vulnerable maternal immunological health at the time of this study and we did not adjust for this in the analysis. This group of HEU mothers discontinued breastfeeding much earlier than would typically be the case in more resource-constrained sub-Saharan African settings, resulting in less exposure to cART or ZDV from breastfeeding but potentially increased the risk of infection.

The association of stunting and social withdrawal concurs with other sub-Saharan African studies relating growth measures to neurodevelopmental outcomes in young HIV-affected children [21]. However, macrocephaly in 13.8% of HEU infants was an unexplained finding not previously documented in research literature. Two of these children had language delay (composite scores 71 and 74) and were exposed antenatally to

efavirenz suggesting a need for further investigation. No other studies have reported similar findings [32,47] including drug event registries [48, 49].

A limitation of this study was the small sample size due to the high attrition rate, which is common in populations faced with socio-economic adversity [50]. In addition, seven of the eight infants assessed as socially withdrawn on the ADBB did not attend follow-up assessments to confirm the ADBB findings. Transient factors such as illness, anaemia or hunger may result in an elevated ADBB score, and follow-up is recommended for clinical confirmation. The ADBB tool cannot be used in isolation to diagnose infant social withdrawal [35]. Another limiting factor is that not all children attended an audiological evaluation to rule out hearing loss as a cause for decreased vocalisation and delayed BSID language scores. Although groups were matched socio-economically, there was no evaluation of the home environment to ascertain differences in the level of stimulation. There is also selection bias in that initial recruitment by MIHS would have excluded high-risk pregnancies and preterm delivery, which are known risk factors associated with maternal HIV infection. However, the main strength of this study is the well-matched control group with adjustment for confounders and use of comprehensive neurodevelopmental assessment tools.

In conclusion, in this low-risk group of HEU and HUU infants from a single community, there was no difference in neurodevelopmental outcome at 12 months of age. These findings are encouraging; however, language delay in particular may only emerge on longitudinal follow-up as environmental factors become more influential on child development. Therefore, it will be important to determine whether neurological and behavioural differences detected in the small subgroup of HEU infants have long-term sequelae.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S5. Differences between MIHS participants retained versus those lost: 12 months visit

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