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Neruodevelopmental Outcomes in Pre-School Children Living with HIV-1 Subtypes A and D in Uganda

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Abstract

Background—HIV is a neuropathogenic virus that may result in detrimental neurodevelopmental (ND) outcomes early in life. This is the first study to evaluate the effect of HIV-1 subtype on neurodevelopment of Ugandan pre-school children.

Methods—Neurodevelopment of 87 HIV-1 infected and 221 HIV exposed uninfected (HEU) Ugandan children aged 1.8 to 4.9 years was assessed using four scales of the Mullen Scales of Early Learning (MSEL), two scales of the Color Object Association Test (COAT), and one score of the Early Childhood Vigilance Test (ECVT). HIV-1 subtype was defined by phylogenetic analyses. General linear models were used to relate test scores to HIV-1 subtype (A versus D) while adjusting for relevant covariates. The scores were benchmarked against HEU group to facilitate the interpretation.

Results—Seventy-one percent of children infected with subtype A vs. 60% of children with subtype D were currently on antiretroviral therapy (ART) ($p=0.49$). Children with HIV-1 subtype A infection were older when compared to subtype D (3.29 vs. 2.76 years, respectively, $p=0.03$), but similar regarding sex, socioeconomic status, weight-for-age z-score, CD4+ and CD8+ (% and total), viral load. No statistically significant differences by HIV-1 subtype were observed in the MSEL, COAT, and ECVT. Differences 0.33 of the standard deviation were observed for the MSEL Composite Score, Receptive Language (MSEL), and Total Memory (COAT).

Conclusions—In contrast to previously reported differences in ND outcomes of school-aged children by HIV-1 subtype, ND scores among pre-school children were similar for subtypes A and D, with few potential differences on language production and memory outcomes that favored subtype A. Further investigation with larger sample sizes and longitudinal follow-up is needed.

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Keywords

HIV subtype; children; neurodevelopment

HIV's entry into the central nervous system (CNS) occurs early in the course of infection^[1]. Being neuropathogenic, HIV can cross the blood brain barrier inside target cells, can infect target cells within this system, and may have a severe negative effect on neurodevelopment^[2]. HIV has been shown to have a detrimental effect on cognition and motor functions of perinatally infected children^[3], even among children who have a CD4 cell count > 350 cells/μl^[4]. In conjunction with environmental factors, poverty, and nutrition, HIV has emerged as an additional potential cause of neurodevelopmental (ND) delay among children in countries that have high disease prevalence^[5-8]. The virus has been shown to have a direct effect on overall ND, including motor deficits, visual and learning disorders among children with and without antiretroviral therapy (ART)^[3, 9]. ART scale-up in low-income countries contributes to neurodevelopmental improvement in infected children; however, social and environmental factors also have an important role on a child's ND^[8, 10]. The association between HIV infection and ND in resource-constrained settings is complex and intertwined with multiple proximal and distal factors, including nutrition, access to early ART, and the child's learning environment^[11]. Thus, it is fundamental to identify neurocognitive deficits early in life and ensure access to interventions that aim to enhance neurocognition.

HIV-1 is divided into three groups: M (major), O (outlier) and N (new, non-M, and non-O)^[12]. Group M is subdivided into nine different subtypes (A-D, F-H, J, K), and multiple recombinants exist with different geographic distributions^[13]. In East Africa, for example, subtypes A, C, and D are the most prevalent^[14], and, specifically in Uganda, HIV subtypes A and D are the most common^[14]. HIV subtypes are classified phylogenetically on the basis of differences in their envelope nucleotide sequences^[15].

To the best of our knowledge, only a handful of studies have reported on the neurocognitive performance of Ugandan adults and children based on HIV subtype, immunological, and ART status^[16-18]. Among school aged Ugandan HIV- infected children who had not yet received ART, HIV subtype A infection was associated with children having poorer neurocognitive performance when compared to subtype D^[16]. However, Bangirana et al., reported no differences in neurocognitive outcomes when comparing Ugandan children on ART infected by HIV subtype A or D^[19].

Given that findings reported in the literature have been inconsistent and mainly focused on school-age children, we aimed to evaluate differences in neurocognitive outcomes among young Ugandan HIV-infected children on the basis of HIV subtype and to compare their outcomes to those of HIV exposed uninfected (HEU) children.

Methods

Participants

This is a secondary data analysis of baseline data from 87 HIV infected children and 221 HEU controls who participated in a randomized controlled trial of a parenting intervention called Mediational Intervention for Sensitizing Caregivers (MISC). During enrollment, all children underwent physical examination by trained staff, which included auditory and visual testing. HIV infected children aged 2 to 5 years with no history of non-HIV illness or injury that could have caused a CNS insult, including HIV encephalopathy, were recruited from The AIDS Support Organization (TASO) treatment center in Tororo, along with TASO satellite clinics from the surrounding area of Busia. After consent, 120 HIV-infected children were enrolled and evaluated between March 2012 and April 2013. HIV status was confirmed by blood test upon enrollment into the MISC trial. Dried blood spots (DBS) were collected for all participants and stored for analysis. Out of the 120 HIV infected children who were enrolled into MISC, sufficient viral material to conduct subtype assignment was available for 87 children (72.5%). No statistically significant differences in socioeconomic or biological characteristics were observed between HIV infected children with subtype (n=87) assignment and those without (n=33). HEU children were recruited from rosters of a completed malaria chemoprevention study ^[20] (n=175) or by referral from a trial of prevention of mother to child transmission of HIV (n=46) at the Infectious Disease Research Collaboration at Tororo District Hospital, Uganda ^[21].

Study procedures and ethical considerations

Child neurocognitive measures were administered in the project office by trained testers in one of three local languages (Japhadola, Ateso, or Luganda) between March and September of 2012. Study testers were trained on site by the PI and co-author (MJB). On site test supervision was provided by the following authors: HRE, IF, RO, and MJB. Quality assurance and re-training was provided by data managers on-site and authors: IF, MJB, and HRE and by following standardized operating procedures developed for each test.

The Institutional Review Boards of Michigan State University, the School of Medicine Research Ethics Committee at Makerere University, and the Ugandan National Council for Science and Technology approved this study. The trial was registered on ClinicalTrials.gov (NCT01640561).

Measures

Child demographics included age, sex, and parent report of current ART (yes/no). Weight-for-age z-scores were computed using the macro created by the World Health Organization ^[22].

An economic wealth index was constructed from a checklist of eight material possessions (shoes, radio, mattress, blanket, bicycle, motorcycle, cows, and goats) and six housing quality items (type of roof, availability of water supply, type of fuel used, frequency of meat in diet, and food security). Respondents reported presence/absence of each asset at their

homes. Top 20th, middle 60th, and bottom 20th percentiles were defined based on factor scores derived from principal component analysis of the 14 items.

The MSEL is a comprehensive test measuring specific developmental domains. Items are combined into four cognitive scales (Visual Reception, Fine Motor, Receptive Language and Expressive Language) that can be summarized to yield an overall score (MSEL Composite score) as well as domain-specific sub-scores. The Composite score serves as a general measure of fluid intelligence reflective of general cognitive ability [23]. Age-adjusted T-scores were obtained from standardized samples from the U.S., and lower scores indicate diminished cognitive abilities. The Mullen test has been extensively used by our group with young children in rural Uganda showing high sensitivity [24-26].

The COAT was used to assess visual memory independent of language competency [27]. The principal outcomes are immediate recall and total recall. Lower scores indicate poorer performance. The COAT has been previously used as an experimental measure of memory for Ugandan children [25].

The ECVT is a measure of vigilance used in preschool children to evaluate sustained attention [28, 29]. Children are required to monitor a colorful computer screen for the sudden appearance of active and engaging cartoon. The principal outcome is the proportion of time looking at the animation as scored from a video recorded from a webcam mounted on the computer.

Home Observation for the Measurement of the Environment (HOME) [30] was used to measure quality of child-caregiver interactions in the home environment. This measure has been validated previously in Uganda [31]. For this analysis we use the Infant-Toddler version, which includes 45 yes/no items. A total HOME score was generated by summing the number of 'yes' responses to each item, with higher scores indicating higher quality.

HIV subtype assignment for either A or D was done using a modification of a previously published method [32]. PCR-based assay with sequencing of a 324 bp of gp41 ENV gene. Subtype was defined by phylogenetic analyses with subtype references. HIV subtype was classified as being subtype A or D on the basis of ENV sequencing. For all children, baseline viral load testing, CD4 and CD8 cell counts were completed using a 5 ml blood draw at each visit, with samples evaluated by the Joint Clinical Research Center Mbale District Hospital. HIV subtype assignment was performed at Johns Hopkins Medical Institutions, Baltimore, Maryland.

Statistical Analysis—Distribution of demographics and socio-economic characteristics, as well as immunological and neurodevelopmental scores were summarized according to HIV status and subtype classification. Two sets of general linear models (GLM) were built. First, unadjusted models related the COAT, MSEL, and ECVT (one at a time) to a 3-level variable reflecting membership in the uninfected reference group, HIV subtype A or HIV D subtype group. Second, age, sex, weight-for-age, socio-economic status, and HOME score were included in our first adjusted model. The p-values for the differences among three groups (subtype A, subtype D, and HEU) were reported, as well as the p-values for the tests

comparing HIV subtypes A versus D. Third, ART status and viral load were added to the adjusted model to estimate the effects of HIV subtype on neurodevelopment over and above these factors. Given that key variables, such as viral load and ART status are only relevant among those infected, the adjusted models did not include the HEU group. The least square (LS) means by HIV subtype were output from the adjusted models, and differences between them were tested. All statistical tests were two-sided.

The available sample sizes for the HIV subtypes allowed to detect mean differences of at least 0.75 of the standard deviation with power of 0.80 or greater in two-sided tests conducted at 0.05 level of significance. Differences of smaller magnitude may not have been detected as statistically significant, therefore the p-values in tables are accompanied by the estimates of the effect sizes. The effect sizes for the comparisons by subtype are expressed as Cohen's d, difference between means in the standard deviation units in the unadjusted analyses, and differences between the LS means divided by the square root of the mean squared error in the adjusted analyses. Analyses were performed in SAS 9.4 and STATA 12.

Results

Group A children were significantly older than group D (3.29 vs. 2.76 years, $p<.01$) (Table 1), with no other pairwise group differences. Three groups also differed with respect to WAZ ($p=.04$), with HEU children having lower WAZ (-1.20) than subtype A group (-0.80), $p=.02$. Mean WAZ for group D (-0.72) was not different from HEU ($p=.12$) due to the sample size of group D. The proportion of boys differed across three groups ($p<.01$), with subtype D having the lowest point estimate for the percentage of boys (22%), however test of pairwise differences in proportions with HEU and A groups resulted in p-values of 0.09 and 0.08, respectively.

Statistically significant differences in the adjusted means among three groups were observed in ECVT scores in the unadjusted model (HEU mean=0.69 vs. group A mean=0.62 vs. group D mean=0.55, $p<0.01$) (Table 2). Effect sizes of subtype A vs. D were near $\frac{1}{2}$ of the standard deviation for COAT Total Memory ($d=0.47$, difference of 4.45 points) and ECVT scores ($d=0.53$, difference of 0.07). Figure 1 shows the unadjusted mean differences for the five MSEL domains and the Composite score. In the adjusted analysis that include HEU effect sizes over $\frac{1}{3}$ of the standard deviation were observed for Gross Motor ($d=0.34$, difference of 1.05 points) Visual Reception ($d=0.39$, difference of 1.68 points) and the Composite score ($d=0.38$, difference of 5.3 points) of the MSEL (Table 2).

In adjusted analyses, children with HIV subtype A had higher LS mean (LSM) scores in the MSEL Composite (LSM=72.97 vs. LSM=66.16, $d=0.39$), Receptive Language (LSM=38.17 vs. LSM=33.76, $d=0.41$), and Total Memory of the COAT (LSM=9.30 vs. LSM=5.40, $d=0.42$) when compared to children with HIV subtype D (Table 3).

Discussion

This work represents the first study evaluating ND in pre-school children by HIV subtype in sub-Saharan Africa using a homogeneous sample of children in terms of socio-demographic, immunological, and treatment status. Results presented here suggest that even though the

statistical significance for the differences in infant neurodevelopment outcomes for subtypes A versus D was not reached, some of the outcomes, such as language production and memory, may be better among those with subtype A based on the standardized effect sizes. After adjusting for potential confounding factors, other outcomes were similar for the two subtype groups and the control group of HEU children.

Research on the effects of HIV subtype on ND in children is limited and supports the hypothesis that HIV subtype A has a greater impact on cognitive ability and visual attention than subtype D [16]. On the other hand, studies describing the impact of HIV on adult ND suggest that subtype D has a greater negative impact, however, results are difficult to extrapolate to children living with HIV [17, 18, 33-35]. In our results, adjusted models showed higher scores with differences near 0.4 of the standard deviation among infants infected with subtype A when compared to subtype D in Receptive Language and Composite scores of the MSEL, and for the Total Memory score of the COAT. Findings presented in this analysis can be viewed as hypothesis-generating. The hypothesis that general cognitive ability and Receptive Language might be affected early on among children infected with HIV subtype D will need future testing with larger samples. Our findings and this hypothesis are consistent with what has been reported among adults infected with HIV subtype D in Uganda [18].

However, our findings regarding infant neurodevelopment differ from those reported in a previous study by our group in Uganda among a sample of ART naive children 6-12 years of age, where poorer neurocognitive performance in children infected with HIV subtype A was reported [16]. That study used a different set of neurocognitive tests that were appropriate for school-age children (Kaufman Assessment Battery for Children Second Edition, KABC-II; Bruininks-Oseretsky Test for Motor Proficiency second edition, BOT-2; and the Test of Variables of Attention, TOVA), and viral loads among children infected with HIV subtype A were higher than in those infected with subtype D [16]. In the present study, while older age of the subtype A group compared to subtype D can explain some of the differences in the unadjusted means, the means adjusted for age and other potential confounders still favored subtype A. Also, a similar proportion of children infected with subtype A and D were currently on ART. This could result in less neuropathogenic effects among these children, highlighting the importance of providing treatment to all children living with HIV [19, 36]. However, caution is warranted when interpreting the effects of HIV subtype on children's ND scores given the small sample size and the lack of statistical significance observed when comparing subtype groups. Nonetheless, these studies add to the knowledge on the effect of HIV subtypes on specific areas of the developing brain at different developmental stages.

One mechanism that has been hypothesized for HIV subtype A's higher degree of encephalopathogenicity relative to subtype D is on the basis of chemokine receptor tropism (CCR5 vs. CXCR4) [37]. CCR5 may facilitate infection of macrophages, HIV's main target cells for transport across the blood brain barrier into the CNS, resulting in infection of microglia and other target cells in the CNS [38]. However, the association between HIV and neuropathogenesis is likely to be more complex than the association between HIV subtype and chemokine receptors, involving other factors such as age, socio-economic and

ART status, time of ART initiation, type of ART being received, ART adherence, viral load, and nutritional status.

Results presented here should be viewed in light of certain limitations. First, our results are based on the associations using cross-sectional data, hindering our capacity to establish causality. Children included in this study were evaluated at a young age prior to development of cognitive skills that can potentially be measured later in life. Second, standard interpretation of T-scores for the MSEL proposes that any scores at or below 7th percentile or -1.5 to -2 standard deviations below the average indicate significant delays. However, without specific norms for this Ugandan population, it is difficult to establish appropriate cut-off scores to gauge clinical significance of any differences. Therefore this study relied upon the standardized effect size to address the magnitude of the differences. Also, sample size was too small to analyze the interaction between ART status and HIV subtype in relation to the neurocognitive outcomes. Finally, only 18 children with HIV subtype D were included in the analysis, which may result in wide confidence intervals and thus, should be interpreted with caution.

Conclusions

ND in young children (2- 5 years old) infected with HIV subtype A compared to subtype D was similar, with some differences favoring subtype A found in language development, overall cognition, and memory. The estimates of the effect sizes provided in this report can be used to formally power future prospective studies, including longitudinal studies that would test the association between HIV subtype and neurodevelopment and capture the complex pathological interaction between HIV and CNS, while accounting for other important factors.

Acknowledgments

Dr. Ruiseñor-Escudero conceptualized and designed the study, drafted the initial manuscript and approved the final manuscript as submitted.

Dr. Sikorskii conducted data analysis and critically reviewed and approved the final manuscript.

Drs. Familiar Lopez and Boivin critically reviewed all sections of the manuscript providing substantial input.

Drs. Persaud and Ziemniack did HIV subtype characterization and provided input to the manuscript.

Drs. Nakasujja and Opoka coordinated and supervised data collection and reviewed the manuscript before final submission.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Figure 1. Boxplot of Mullen Scales of Early Learning Scores by HIV-1 subtype

Demographic characteristics and immunological parameters of 87 children living with HIV and 221 uninfected controls in Tororo, Uganda

Table 1

Characteristics	HIV-1 Subtype A N=69 (79.3%)	D N=18 (20.7%)	P-value for subtype comparison	HEU group Uninfected reference group N=221	P-value for comparison of A, D, and reference groups
Age (yrs), Mean (SD)	3.29 (0.93)	2.76 (0.74)	<.01	2.87 (0.42)	<0.01
Sex, N (%)			0.08		<0.01
Boys	31 (45)	4 (22)		125 (57)	
Girls	38 (55)	14 (78)		96 (43)	
Wealth Group			0.99		0.72
Lowest 20%	12 (17)	3 (17)		46 (21)	
Middle 60%	46 (67)	12 (67)		124 (58)	
Upper 20%	11 (16)	3 (16)		45 (21)	
WAZ, Mean (SD)	-0.80 (1.65)	-0.72 (1.40)	0.84	-1.20 (1.12)	0.04
CD4 ⁺ %, Mean (SD)	45.63 (13.81)	43.47 (14.40)	0.57	N/A	
CD8 ⁺ %, Mean (SD)	47.57 (13.88)	45.00 (15.66)	0.51	N/A	
CD4 ⁺ lymphocyte count (copies/ml), Mean (SD)	1439.6 (801.5)	1362.9 (744.5)	0.72	N/A	
CD8 ⁺ lymphocyte count (copies/ml), Mean (SD)	1544.3 (947.7)	1270.5 (641.5)	0.26	N/A	
Log plasma HIV RNA level (copies/ml), Mean (SD)	8.58 (2.90)	10.01 (2.92)	0.09	N/A	
Currently on ART, N (%)			0.49		
No	21 (30)	7 (39)		N/A	
Yes	48 (70)	11 (61)		N/A	

HEU: HIV Exposed Uninfected

Table 2
Unadjusted and adjusted least square means, and 95% confidence intervals for the MSEL, COAT and ECVT scores of 87 children living with HIV and 221 uninfected controls in Tororo, Uganda

Domains	Adjusted Means by HIV-1 subtype		D LS Mean (95% CI)	A v. D P-value (effect size)	Adjusted mean for reference group		P-value for comparison of A, D, and reference groups
	A LS Mean (95% CI)				HEU** LS Mean (95% CI)		
ECVT							
Proportion of time looking	0.59 (0.54, 0.64)		0.56 (0.49, 0.65)	0.58 (0.18)	0.70 (0.67, 0.72)		<0.01
COAT							
Immediate Memory	2.75 (0.24, 5.26)		4.25 (-0.34, 8.83)	0.56 (0.16)	2.11 (0.69, 3.53)		0.64
Total Memory	6.76 (3.49, 10.02)		5.07 (-0.91, 11.05)	0.62 (0.14)	5.97 (4.12, 7.82)		0.86
MSEL							
Gross Motor	25.34 (24.55, 26.14)		26.39 (24.93, 27.85)	0.21 (0.34)	26.99 (26.54, 27.44)		<0.01
Fine Motor	33.51 (30.80, 36.22)		29.44 (24.48, 34.40)	0.15 (0.18)	34.05 (32.52, 35.59)		0.21
Visual Reception	32.12 (29.63, 34.61)		30.44 (25.89, 34.99)	0.52 (0.39)	29.09 (27.68, 30.50)		0.10
Receptive Language	37.90 (35.47, 40.34)		35.21 (30.76, 39.66)	0.29 (0.29)	37.97 (36.59, 39.34)		0.49
Language	33.90 (31.13, 36.68)		32.05 (26.98, 37.13)	0.52 (0.17)	35.44 (33.87, 37.01)		0.33
Composite MSEL	72.31 (68.70, 75.93)		67.01 (60.40, 73.63)	0.16 (0.38)	70.74 (68.69, 72.79)		0.36

Table 3
Adjusted least square means, and 95% confidence intervals for the MSEL, COAT and ECVT scores of 87 children living with HIV living in Tororo, Uganda

	Adjusted Means by HIV-1 subtype		
Domains	A LS Mean (95% CI)	D LS Mean (95% CI)	P-value (effect size)
ECVT			
Proportion of time looking	0.62 (0.58, 0.65)	0.60 (0.53, 0.68)	0.75 (0.08)
COAT			
Immediate Memory	3.84 (2.83, 4.84)	3.75 (1.86, 5.63)	0.93 (0.03)
Total Memory	9.30 (6.49, 12.12)	5.40 (0.12, 10.69)	0.16 (0.42)
MSEL			
Gross Motor	26.31 (25.18, 27.46)	27.18 (25.22, 29.13)	0.42 (0.23)
Fine Motor	33.26 (29.28, 37.24)	29.36 (21.88, 36.83)	0.32 (0.30)
Visual Reception	32.10 (29.06, 35.15)	30.72 (25.00, 36.44)	0.65 (0.14)
Receptive Language	38.17 (34.89, 41.44)	33.76 (27.61, 39.91)	0.18 (0.41)
Expressive Language	35.74 (32.22, 39.27)	32.87 (26.25, 39.49)	0.41 (0.28)
Composite MSEL	72.97 (67.65, 78.29)	66.16 (56.18, 76.15)	0.20 (0.39)

* Adjusted for age, sex, HOME score, socio-economic status, weight-for-age z-score, HAART status and viral load

MSEL: Mullen Scales of Early Learning, COAT: Color Object Association Test; ECVT: Early Childhood Vigilance Test

** Bold type denotes statistically significant (P-value<0.05)