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White Matter Microstructural Integrity and Neurobehavioral Outcome of HIV-Exposed Uninfected Neonates

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Abstract: The successful implementation of prevention programs for mother-to-child human immunodeficiency virus (HIV) transmission has dramatically reduced the prevalence of infants infected with HIV while increasing that of HIV-exposed uninfected (HEU) children. Neuropsychological assessments indicate that HEU children may exhibit differences in neurodevelopment compared to unexposed children (HUU). Pathological mechanisms leading to such neurodevelopmental delays are not clear. In this observational birth cohort study we explored the integrity of regional white matter microstructure in HEU infants, shortly after birth.

Microstructural changes in white matter associated with prenatal HIV exposure were evaluated in HEU infants (n = 15) and matched controls (n = 22) using diffusion tensor imaging and tract-based spatial statistics. Additionally, diffusion values were extracted and compared for white matter tracts of interest, and associations with clinical outcomes from the Dubowitz neonatal neurobehavioral tool were investigated.

Higher fractional anisotropy in the middle cerebellar peduncles of HEU compared to HUU neonates was found after correction for age and gender. Scores on the Dubowitz abnormal neurological signs subscale were positively correlated with FA ($r = 0.58$, $P = 0.038$) in the left uncinate fasciculus in HEU infants.

This is the first study to present data suggesting that prenatal HIV exposure without infection is associated with altered white matter microstructural integrity in the neonatal period. Longitudinal studies of HEU infants as their brains mature are necessary to understand further the significance of prenatal HIV and antiretroviral treatment exposure on white matter integrity and neurodevelopmental outcomes.

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Abbreviations: DCHS = Drakenstein Child Health Study, DTI = diffusion tensor imaging, FA = fractional anisotropy, HEU = HIV-exposed uninfected, HIV = human immunodeficiency virus, HUU = HIV-unexposed uninfected, M-/A-/RD = mean/axial/radial diffusivity, TBSS = tract-based spatial statistics.

outcomes from the Dubowitz neonatal neurobehavioral tool were investigated.

INTRODUCTION

The human immunodeficiency virus (HIV) remains one of the most severe global public health issues, with an estimated 35 million people living with HIV in 2013.[1–3] Sub-Saharan Africa, especially southern Africa, remains the global epicenter of the HIV epidemic, accounting for 70% of the people living with HIV worldwide.[3] The successful implementation of prevention of mother-to-child programs has dramatically reduced the rate of vertical transmission while increasing the number of HIV-exposed uninfected (HEU) children being born.[4–6] Subtle deficits in cognition, motor function, language, and behavior have been observed among HEU children compared to HIV-unexposed uninfected (HUU) controls;[7,8] however, there remains lack of consistency in outcomes of studies exploring the neurodevelopmental outcome of HEU children.[9,10]

Neuroimaging studies using quantitative magnetic resonance imaging techniques are contributing to the improved understanding of the underlying biology of the primary effects of HIV infection on the developing brain. White matter hyperintensities, subtle white matter microstructural and neurochemical abnormalities, and reduced white matter volume have been reported in older children and adults with HIV infection.[11–13] In addition, white matter microstructural alterations have been observed in prenatally infected adolescents in brain areas including the superior longitudinal fasciculus, cingulum, corpus callosum, external capsule, middle cerebral peduncles, and basal pons.[11,14] However, the effect of prenatal HIV exposure on white matter microstructure

utilizing diffusion tensor imaging (DTI) in HEU infants has not been previously documented.

To address these gaps in the understanding of the effects of prenatal HIV exposure on white matter microstructural integrity and neurobehavioral outcome of HEU infants, we conducted this study in South African neonates.[15] Using a cohort from a peri-urban community in the Western Cape, the present study investigated whether the impact of prenatal HIV exposure on early white matter microstructural integrity may be discernible in neonates and whether associations with early neurobehavioral measures are present. Based on the literature in children and adults with HIV infection, we hypothesized that disruptions in white matter integrity would occur (even if more subtly) in similar areas including association and projection pathways and in the cerebellum and that these changes would be associated with infant neurobehavior.

METHODS

Study Population

This is a nested observational substudy of infants enrolled in a larger population-based birth cohort study, the Drakenstein Child Health Study (DCHS).[16,17] The DCHS is located in the Drakenstein area in Paarl, a peri-urban area in the Western Cape province of South Africa. The local community of ~200,000 people is of low socioeconomic status, live in informal housing or crowded conditions, and have high levels of unemployment. Infectious diseases including pneumonia, HIV, and tuberculosis are common. The area has a well-established free healthcare system, where ~90% of women access public sector antenatal care and child health services.

Participants

The DCHS recruited >1000 pregnant women in their second trimester attending 2 primary health clinics serving different populations—TC Newman (serving a mixed race population) and Mbekweni (serving a black African population).[16,17]

We recruited pregnant women at 20 to 24 weeks gestation, obtained written informed consent, and collected background data as per the DCHS protocol.[16,17] We confirmed the HIV status of mothers antenatally utilizing standard HIV testing algorithms and available laboratory tests.[18] We confirmed the HIV status of the infants by testing postnatally using the qualitative polymerase chain reaction (PCR) technique. Infants who tested positive on the PCR testing had their HIV status confirmed using quantitative HIV viral load testing.

The study collected measures of potential confounding factors. The Alcohol, Smoking and Substance Screening Test (ASSIST) was used to assess maternal substance abuse.[17–20] Following this initial screen, prenatal alcohol exposure was further defined by a history of moderate-severe alcohol use in any of the pregnancy trimesters. Maternal cigarette smoking status was further documented using the Fagerström Test for Nicotine Dependence.[21]

Objective measures of maternal substance use were also included.[16,17] Maternal urine cotinine was measured antenatally and at the time of birth to detect and quantify current smoking status. In addition, maternal urine samples were tested antenatally with rapid urine dipstick testing for recent use of common illicit substances, including methamphetamines, cocaine, cannabis, methaqualone, and opiates.

Following birth, mother–child pairs identified through the HIV-testing approach were included for study unless mothers had a positive urine screen for illicit drugs of abuse (any group), the infants were premature (<36 weeks), or had low APGAR scores (<7 at 5 minutes), and/or history of neonatal ICU admission for hypoxic ischemic encephalopathy or other significant neonatal complications. Infants were also excluded from this study if they had an identified genetic syndrome or congenital abnormality. In this nested substudy, 39 infants were assessed: 15 HEU infants and 24 matched HUU controls. No infants who were identified in the antenatal visit for inclusion were lost by the time of scanning and none refused consent for scanning.

In this study we imaged 2 to 4-week-old infants during natural (ie unsedated) sleep. Earplugs and mini-muffs were used for ear protection, a pulse oximeter was used to monitor pulse and oxygenation, and a qualified neonatal nurse or pediatrician was present with the infant during the imaging session. At the time of scanning, basic infant anthropometry was acquired, including length, weight, and occipito-frontal head circumference. The Dubowitz neurobehavioral scale, a measure of neonatal neuromotor and neurobehavior status, was used to study early neurological and neurobehavioral changes and to identify potential associations with neuroimaging findings.[22] The score is based on the distribution of the scores for each item in the population of low-risk term infants, and the optimality score is obtained by summating the optimality scores of individual items. Together, the examination can be used to detect abnormal neurological signs associated with specific patterns of lesions observed on brain imaging. For this study, the "behavior" cluster, which includes items scoring irritability, cry, consolability, alertness, visual and auditory orientation and eye movements, and the "abnormal signs" cluster, which focuses on posture, tremor, and startle items, were of particular interest.

The DCHS was approved by the Faculty of Health Sciences human research ethics committees of the University of Cape Town and Stellenbosch University in South Africa, as well as by the Western Cape Department of Health Provincial Research Committee. This substudy was independently reviewed and approved as HREC 525/2012. As above, mothers provided written informed consent for participation in the study.

DTI Acquisition

White matter microstructure can be characterized in vivo with diffusion tensor imaging (DTI), a noninvasive technique that utilizes the intrinsic directionality of water diffusion along fiber pathways to provide highly specific anatomical information.[23] The most widely used index is fractional anisotropy (FA). This represents orientation-dependent variation in the diffusivity of water and reflects a number of microstructural properties such as degree of myelination, axon diameter, fiber coherence, and fiber tracking density.[24,25] Other reported indices include mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD). MD represents a measure of average diffusivity. Alterations in MD may indicate a decrease in cellular density, myelin degradation, or an increase in the extra- and/or intracellular volumes.[24] Organized neural microstructure associated with improved cognition and behavior are typically associated with higher FA values and lower MD values. White matter microstructural pathology, however, is more generally associated with lower FA and higher MD values.[23,24] AD represents diffusivity along the axonal structure, typically reflecting axonal membrane integrity and fiber coherence.[26] AD may be higher when there is damage to the neurofilaments or axons.[27,28] RD typically reflects average

perpendicular diffusion and indicates degree of myelination; RD is generally higher with myelin damage or reduced myelination.[28,29]

Diffusion weighted images were acquired in the transverse plane on a Siemens Magnetom 3T Allegra MRI system using a spin-echo, echo-planar sequence along 45 noncollinear diffusion directions (b-values 0 s/mm^2 and 1000 s/mm^2, TR/TE = 7900/90 ms, slice thickness 1.6 mm, FOV 160 mm, voxel size $1.3 \times 1.3 \times 1.6 \text{ mm}^3$, 2 averages in anterior-posterior and posterior-anterior orientation, scanning time 6.27 min per average).

Data Processing

Diffusion imaging techniques are highly sensitive to the motion of subjects during scan acquisition. As a result, acquiring diffusion imaging data in infants offers particular logistical and technical challenges. Initially, manual quality control of individual subject data was applied. Only subjects with a minimum of 12 acquisition volumes that were artifact-free were allowed through the data preprocessing step. Subsequently, FMRIB's Diffusion Toolbox and processing streams from Tract-Based Spatial Statistics (TBSS) were used to perform a whole-brain analysis.

Diffusion weighted images from individual subjects were registered to a corresponding $b = 0$ image. This step was performed in order to correct for distortions resulting from eddy currents as well as movement. Estimation of susceptibility-induced off-resonance field was performed using the FSL top-up tool. Subsequently, a single corrected image was created using the combination of the 2 images. The FSL Brain Extraction Tool was then used for the brain-extraction of images and following this the calculation of diffusion tensors was performed at each voxel. Values for each subject for FA, MD, AD, and RD were then obtained for between group analyses. Diffusion values by regions of interest (ROIs) were extracted, exported, and compared by group using standard SPSS statistical packages as below. Statistical analyses controlled for gender and postnatal age at the time of the scan. Age (in days) was considered particularly critical due to the rapid pace at which white matter maturation evolves in early neonatal life.[30]

Whole-Brain Tract-Based Spatial Statistics

The standard pipeline for TBSS analysis was applied for statistical analysis. In this study, the FMRIB FA template for adults, provided by FSL was not considered appropriate for neonatal DTI analysis. Thus, each subject was registered to a representative target that was preselected from the control cohort. The subject with the lowest mean warp coefficient from the control cohort was chosen as the target image.

Each FA image was aligned into a standard space and upsampled to $1 \times 1 \times 1 \text{ mm}^3$ voxel size. Next, the average FA image was created and thinned to create a skeletonized mean FA image, which represents the center of all white matter tracts common to the study group. An FA value of 0.2 was used as a threshold for the skeleton. This study was explorative and so we applied a more stringent threshold compared to that of some previous studies of this age group that used a threshold FA value of 0.15. Subsequently, we projected diffusion data onto this skeleton for the statistical analysis.

The FSL's Randomize tool was used to assess voxelwise differences in DTI metrics among the study groups. Specifically, between group variations were investigated with unpaired t tests and correlational analyses, and statistical analyses were corrected for multiple comparisons with threshold-free cluster enhancement. We considered results with a P value of < 0.05 as statistically significant.

TABLE 1. Demographic, Anthropometric, and Dubowitz Data of Exposed and Unexposed Infants

	HIV-Exposed (n = 15)	HIV-Unexposed (n = 24)	Statistics
Gestation (weeks, SD)	37.80 (2.11)	38.42 (1.74)	$t = -0.95, P = 0.35$
Age (days, SD)	23.20 (7.38)	19.87 (4.31)	$t = 1.59, P = 0.13$
Gender (male/female)	7/8	14/10	$t = 0.69, P = 0.49$
Weight (kg, SD)	3.91 (0.55)	3.78 (0.70)	$t = 0.64, P = 0.53$
Head circumference (cm, SD)	36.38 (1.79)	35.73 (1.69)	$t = 1.12, P = 0.27$
Length (cm, SD)	49.93 (3.55)	49.82 (4.62)	$t = 0.092, P = 0.93$
Dubowitz optimality scores (mean [SD])			
Behavior (/7)	4.43 (1.39)	3.52 (1.28)	$t = 2.06, P = 0.049*$
Abnormal signs (/3)	2.53 (1.06)	2.67 (0.82)	$t = -0.42, P = 0.68$
Maternal education[†] (mean [SD])	−0.54 (0.74)	0.15 (1.00)	$t = -2.31, P = 0.027^*$
Primary	2	2	–
Some secondary	11	9	–
Completed secondary	2	12	–
Any tertiary	0	1	–
Socio-economic status[†] (mean [SD])	−0.63 (1.46)	0.34 (2.00)	$t = -1.63, P = 0.11$
Body mass index (mean [SD])	28.54 (5.04)	24.97 (6.45)	$t = 1.82, P = 0.08$
Maternal smoking (cotinine) (yes/no)	3/12	8/16	$t = -0.92, P = 0.38$
Maternal alcohol (yes/no)	3/12	3/21	$t = 1.13, P = 0.24$
Maternal CD4 count (cells/mm³, SD)	484.87 (173.31)	–	–

HIV = human immunodeficiency virus, SD = standard deviation.
* $P < 0.05$.
† Standardized score.

Data Analysis by Regions of Interest

Following FSL preprocessing, we examined group main effects using extracted data on different diffusion indices by ROI. We applied affine-registration to each brain and a standard FMRIB58_FA template. The white-matter atlas from Johns Hopkins University was used for the extraction of MD values for each subject for the ROIs. ROIs included associative bundles, commissural bundles, projection bundles, and large white matter tracts of the brainstem and cerebellum. Generalized linear models were used to evaluate group differences in diffusion parameters, with neonatal postnatal age (days) at scan time as well as gender as covariates. Results were Bonferroni corrected.

RESULTS

Demographic Characteristics and Anthropometric Measurements

The sample for the present analysis included 15 HEU infants and 24 matched HUU controls (Table 1). The mean (SD, range) age of all infants at scanning was 21.1 (5.83, 11–34) days and 46% were women. There were no significant differences in the mean values for infant age at scanning, gestational age at birth, weight, length, and head circumference for HEU infants compared to HUU infants. Although maternal smoking and alcohol misuse in pregnancy were present in this cohort, there were no significant differences in these prenatal exposures between HEU and HUU groups (Table 1). All HIV-infected mothers were on triple therapy before delivery and had CD4 T lymphocyte counts between 200 and 800 cells/mm^3. Undetectable or low levels of viral loads were observed in 80% of HIV-infected mothers, with 2 mothers having viral loads >50,000 copies/mL.

Whole-Brain Group Comparisons of Diffusion Parameters

There were no group differences that reached significance in any diffusion parameters on whole-brain analysis. Infant age at scanning was positively correlated with FA and negatively correlated with MD and RD across groups in several white matter tracts, including the internal and external capsule, and the anterior corona radiata.

Comparisons by Regions of Interest

General linear models by ROIs showed significant difference in diffusion parameters by group in major white matter fiber tracts that interconnect cerebellar and brainstem regions. There was significantly higher FA in the middle cerebellar peduncles of HEU infants compared to HUU [$F(3,38)=6.15$,

TABLE 2. Results of Group Differences in Diffusion Parameters by Regions of Interest. Fractional Anisotropy was Significantly Higher in the Middle Cerebellar Peduncles of Exposed Infants Compared to Controls

	Hemisphere	FA		MD		AD		RD	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Superior longitudinal fasciculus	R	0.15	0.929	0.45	0.721	0.53	0.664	0.35	0.791
	L	0.77	0.520	0.98	0.413	0.41	0.747	1.19	0.340
Superior fronto-occipital fasciculus	R	1.27	0.301	0.54	0.657	1.16	0.338	0.26	0.857
	L	0.10	0.958	0.29	0.836	0.40	0.752	0.22	0.885
Uncinate fasciculus	R	0.89	0.458	0.05	0.987	0.24	0.869	0.03	0.991
	L	3.31	0.031*	0.89	0.472	0.90	0.451	0.86	0.470
Cerebellar peduncle									
Inferior	R	2.77	0.056	0.69	0.571	0.44	0.725	0.85	0.476
	L	0.54	0.657	0.56	0.643	0.49	0.692	0.51	0.681
Middle		6.15	0.002†	4.04	0.014*	1.68	0.189	5.77	0.003*
Superior	R	0.07	0.976	2.26	0.099	2.02	0.129	1.24	0.311
	L	0.23	0.874	1.14	0.347	0.86	0.469	0.78	0.514
Corticospinal tract	R	0.61	0.611	0.28	0.839	0.06	0.982	0.46	0.714
	L	0.71	0.555	0.81	0.494	0.39	0.758	0.88	0.462
Cerebral peduncle	R	2.16	0.110	1.16	0.337	1.15	0.344	1.25	0.307
	L	1.59	0.208	1.31	0.288	1.22	0.318	1.36	0.272
Posterior thalamic radiation	R	1.26	0.304	1.28	0.298	0.97	0.416	1.42	0.255
	L	1.04	0.387	2.56	0.070	1.39	0.264	2.39	0.085
Fornix		2.40	0.084	0.97	0.418	0.49	0.689	1.33	0.281
Cingulum	R	1.95	0.140	2.64	0.064	2.51	0.075	2.66	0.064
	L	3.40	0.028*	1.30	0.291	0.75	0.528	1.67	0.191
Hippocampal cingulum	R	0.66	0.584	0.39	0.760	0.39	0.761	0.48	0.695
	L	1.96	0.138	0.55	0.651	0.89	0.458	0.86	0.470
Corpus callosum									
Genu		4.24	0.012*	1.54	0.222	0.55	0.655	2.27	0.098
Body		2.92	0.048*	0.68	0.570	0.23	0.872	1.01	0.401
Splenium		2.06	0.124	0.87	0.467	0.36	0.784	1.51	0.230

AD = axial diffusivity, FA = fractional anisotropy, MD = mean diffusivity, RD = radial diffusivity.
* Post-hoc investigation of univariate tests of significance revealed that age contributed significantly to the model.
† Significant group differences at $P < 0.05$ after controlling for age and gender.
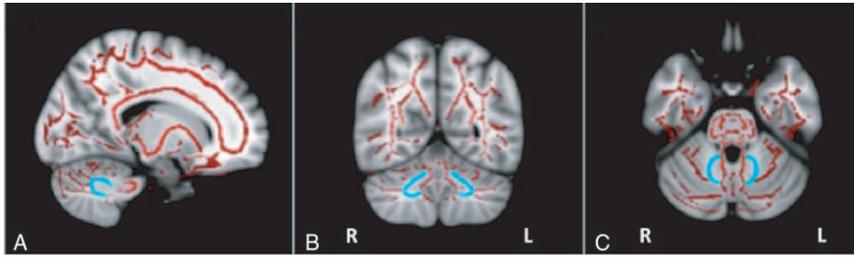
FIGURE 1. White matter tracts superimposed on a 3D brain template: analysis by regions of interest. Diffusion anisotropy was higher in the middle cerebellar peduncles (blue) of HIV-exposed uninfected infants compared to controls as seen in sagittal (A), coronal (B), and axial (C) views. HIV = human immunodeficiency virus.

$P = 0.002$] after correction for age and gender (Table 2, Figures 1 and 2).

Neurobehavioral Measures and Diffusion Parameters

There was a significant difference between the scores on the abnormal neurological signs subscale of the Dubowitz for HEU infants compared to HUU neonates.

Scores on the Dubowitz abnormal neurological signs subscale were positively correlated with FA ($r = 0.58$, $P = 0.038$) in the left uncinate fasciculus; negatively correlated with MD ($r = -0.58$, $P = 0.048$) and AD ($r = -0.61$, $P = 0.026$) in the right inferior cerebellar peduncle; negatively correlated with MD ($r = -0.65$, $P = 0.017$) and AD ($r = -0.75$, $P = 0.003$) in the left hippocampal cingulum; and negatively correlated with MD ($r = -0.66$, $P = 0.014$) in the right hippocampal cingulum of HEU infants (Figure 3).

DISCUSSION

To the best of our knowledge, this is the first time that DTI has been used to investigate white matter microstructural integrity in neonates exposed to HIV, but uninfected at birth. Our study found significantly higher FA in the middle cerebellar peduncles of HEU infants compared to unexposed neonates. In addition, correlational analyses of diffusion parameters with the Dubowitz neurobehavior scores of HEU infants showed abnormal neurological signs cluster was positively correlated with FA in the left uncinate fasciculus. These preliminary results are consistent with

and expand on previous studies that found subtle neurodevelopmental effects of prenatal HIV exposure in older children.[7,8]

Diffuse white matter alterations have been documented in cerebellar pathways of HIV-infected adults and older, specifically in the middle cerebellar peduncles.[31,32] These pathways have been implicated in a variety of intellectual and neuropsychological deficits, which are most pronounced in visuospatial, language and memory functions.[33] We described higher FA in the middle cerebellar peduncles neonates exposed to prenatal HIV, which suggests that the developing cerebellum may be particularly sensitive to the effects of HIV exposure. Although an elevated FA may indicate increased white matter connectivity,[24,25] it has also been postulated as possibly representing microscopic deficits in axonal structures or reductions in axonal diameter, density of axonal packing, and branching by some authors.[34,35]

Correlational analyses of diffusion parameters with the Dubowitz neurobehavior scores of HEU infants showed associations between the abnormal neurological signs cluster with DTI metrics in several white matter tracts including the uncinate fasciculus, inferior cerebellar peduncles, and hippocampal cingulum. Functions of these networks are believed to include cognition, higher level motor tasks, memory, learning, attention, language, and emotional processing.[36–39] These are areas that have been identified as being affected in children with prenatal HIV exposure.[7,8] A decrease in AD is usually indicative of axonal damage and might suggest thinner axons due to reduced axonal caliber, less well-ordered axons as a consequence of misguided cell migration during development, or a lower number of axons.[28,40,41] In addition, a few studies have documented decreased AD with dysmyelination or demyelination.[28,42] Increased MD is normally observed in conditions of reduced membrane density associated with increased volume of extracellular space or with decreased barriers to diffusion in white matter, whereas reduced MD may reflect increased neuronal activity and connectivity.[43] Although the clinical significance of the negative correlation between MD and abnormal neurological signs observed in our HEU infants is unknown, we hypothesize that it may relate to abnormal cell proliferation or aberrant pruning. Longitudinal studies of these infants by following up their developmental and behavioral outcomes as they mature may further define the functional or real-world significance of the associations between the abnormal neurological signs and DTI metrics in these tracts.

Infant postconception age at scanning was positively correlated with FA and negatively correlated with MD and RD across both groups in several white matter tracts, including the internal and external capsule, and the anterior corona radiata. These findings are consistent with previous research, which showed a basic pattern of the maturation process in pediatric DTI: an increase in FA and a decrease in MD as a
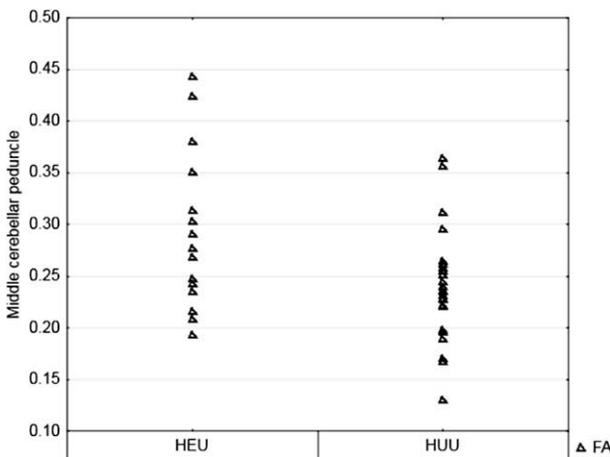

FIGURE 2. Variability of FA values by the group in the middle cerebellar peduncle. FA = fractional anisotropy.
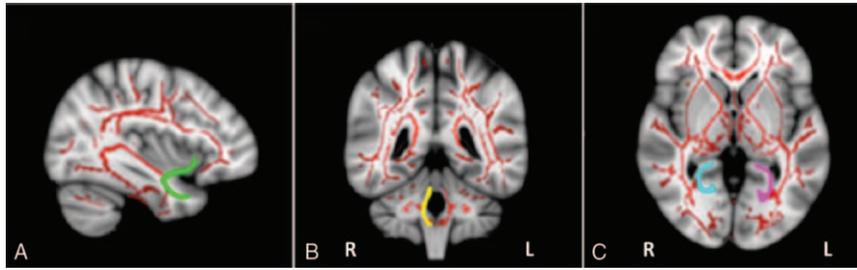
FIGURE 3. White matter tracts superimposed on a 3D brain template: tracts associated with neurobehavior. Scores on the Dubowitz abnormal neurological signs subscale was positively correlated with FA in the left uncinate fasciculus (green), negatively correlated with MD and AD in the right inferior cerebellar peduncle (yellow), negatively correlated with MD and AD in the left hippocampal cingulum (pink), and negatively correlated with MD in the right hippocampal cingulum (blue) of HIV-exposed uninfected infants, as seen in the sagittal (A), coronal (B), and axial (C) views. AD = axial diffusivity, FA = fractional anisotropy, HIV = human immunodeficiency virus, MD = mean diffusivity.

function of age.[44] This suggests that the expected increase in FA and decrease in MD should fail to appear in HEU infants in later years if white matter injury is present. A longitudinal follow-up of these infants as their brains mature will help to further understand the significance of prenatal HIV exposure on the development of these white matter tracts.

Although the precise mechanisms underlying potential neurodevelopmental delays in HEU children are not clear, the role of prenatal HIV exposure in neurodevelopment may potentially be understood in terms of the interactions between the immune and central nervous systems. HEU infants have been reported as having up-regulation of pro-inflammatory cytokines at birth when compared to unexposed controls.[45] The late prenatal and postnatal period is known to be a time when maximal brain growth as well as processes critical to effective CNS maturation occur. These processes include the maturation and elongation of both axons and dendritic trees and the sensitive migration of neuronal cells to their correct position in the layers of the cortex and may be particularly vulnerable to exposure to higher levels of proinflammatory cytokines and immune activation in general.[46,47]

A number of limitations to this study should be mentioned. First, our findings were based on a small sample size and so false negative findings cannot be excluded. Other demographic variables that were not controlled for may have influenced results and should be considered in larger samples. In addition, our study used a cross-sectional design and therefore causal inferences remain preliminary. Interpreting how neurobiological and clinical neurodevelopmental differences develop over time will necessitate robust longitudinal follow up and data.

Despite these limitations, we believe there are also some key strengths to our dataset documented here. These include the fact that these neonates were matched for age and gender as well as maternal alcohol use and cigarette smoking during pregnancy. This is the first study to report neurobiological changes in infants prenatally exposed to HIV, but who are uninfected. Cerebral changes are especially intense during the last trimester of gestation and the first several postnatal months[30] and structural abnormalities of the brain that manifest at this time may result in motor and cognitive deficits in later years. Imaging during early postnatal weeks may more accurately reflect the specific effects of antenatal HIV exposure on white matter microstructural integrity before postnatal risk factors known to have high prevalence in these high-risk communities additionally negatively affecting brain development.

In conclusion, the results documented in this study suggest that the effects of prenatal HIV exposure can be observed at a neurobiological level in neonates, with altered white matter microstructural integrity of major white matter tracts. The findings are consistent with those reported previously where changes in white matter microstructure in adults and older children with HIV infection were documented. Locating the altered white matter microstructural integrity in these brain regions so early in life may provide better understanding of the underlying mechanisms of neurodevelopmental delays observed in HEU children. Future studies with larger cohorts are more likely to be able to detect specific windows of vulnerability to prenatal HIV exposure on the developing brain. Furthermore, to evaluate the validity of these reported changes, longitudinal studies are needed to investigate the changes in diffusion metrics as these HEU infants age and their brains mature.

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