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Early antiretroviral therapy in HIV-infected children is associated with diffuse white matter structural abnormality and corpus callosum sparing

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Abstract

Background, purpose—Fractional anisotropy in the frontal white matter, corpus callosum and internal capsule are abnormal in HIV+ adults. We describe the distribution, nature of white matter abnormalities in a cohort of children who started ART within the first year of life - and benefit of early treatment using DTI measures (fractional anisotropy, mean, axial and radial diffusion).

Materials, methods—DTI was performed on children in a neurodevelopmental sub study from the Children with HIV Early Antiretroviral (CHER) trial. Voxel-based group comparisons were performed to determine regions where fractional anisotropy and mean diffusion differed between HIV+ and uninfected children. Associations of DTI parameters with timing of ART initiation were examined.

Results—39 HIV+ children (15 male, mean age 5.4 years) and 13 controls (5 male, mean age 5.7 years) were imaged. 2 Clusters with lower fractional anisotropy and 7 clusters with increased mean diffusion were identified in the HIV+ group with symmetrical distribution predominantly due to increased radial diffusion, suggestive of decreased myelination. Corticospinal tracts rather

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than the corpus callosum were predominantly involved. Children on early interrupted ART had lower fractional anisotropy compared to those receiving continuous treatment.

Conclusion—HIV+ children at 5 years have white matter abnormalities measured by fractional anisotropy, despite early ART, suggesting that early ART does not fully protect the white matter either from peripartum or in utero infection. In contrast to adults, the corticospinal tracts are predominantly involved rather than the corpus callosum, possibly due to early ART. Continuous early ART can limit white matter damage.

Introduction

White matter (WM) structural abnormalities can be assessed using quantitative parameters determined from DTI[1]. Fractional anisotropy (FA) provides information about the microstructural integrity of highly oriented microstructures, but is not specific to the type of injury. Mean diffusivity (MD) is a measure of average molecular motion independent of any tissue directionality.

Loss of axonal integrity decreases FA and increases MD, however increased FA may also indicate loss of complexity in the underlying axonal matrix due to loss of crossing and other nonparallel fibres. Increased radial diffusivity (RD), a marker of excessive axonal packing density and/or poor myelination[2], and decreased axial diffusivity (AD), an index of axonal damage, occur in HIV-associated WM injury.[3, 4, 5] FA in the frontal subcortical WM, corpus callosum (CC) and internal capsule are abnormal in HIV-infected (HIV+) adults. [6, 7, 8] Those with the most advanced HIV disease have the highest diffusion constant elevations and largest anisotropy reductions, specifically in the CC and frontal WM.[6] Most early studies used *a priori* ROI analyses. Subsequently, more widespread WM damage has been shown using voxelwise and whole-brain analyses.[1, 3, 7, 9, 10] Animal neuro-AIDS models also show WM damage - macaques show reduced FA in the CC genu [11] and mice have reduced FA (mainly due to increased RD) and increased MD in the CC.[12]

Few studies have used DTI to examine HIV-associated alterations in WM in children. Lower FA, higher MD and RD in the CC and higher MD in the superior longitudinal fasciculus have been demonstrated in ART-naïve children (8-12 yrs) compared to age-matched controls [13], while ART failure was associated with decreased FA in the left superior and right posterior corona radiata and decreased AD in the left inferior cerebellar peduncle in 50 children on first line ART (6-15 yrs).[14] Regional and whole brain decreases in FA, and increased MD and RD, compared to controls, have been reported in HIV+ children and adolescents (6-20 years)[15,16] irrespective of treatment status.[15] Regional alterations were related to past disease severity, measured by nadir CD4% and peak viral loads.[16] ART-naïve children (6-11 yrs) showed reduced myelin compared to children on ART (6-16 yrs), but were also younger.[15] These studies did not document ART history.

Adolescents stable on ART (n=15, 13-17 yrs, mean age at ART initiation 9.5 yrs) had lower FA in the CC, superior and posterior corona radiata, frontal and parietal WM, pre-and post-central gyrus and superior longitudinal fasciculus (mainly due to increased RD) than controls (n=26).[17]

Despite consistent evidence of HIV-related WM alterations, studies have included wide age ranges over developmental phases when both WM volume and FA demostrates notable increase.[18, 19, 20] Few studies have controlled adequately for age or ART regimens. To date, no DTI studies have been performed in younger children, and none in children receiving standardised early ART (within the first year of life).

The aim of the present study was to determine the spatial distribution and nature of WM abnormalities at age 5 years in a cohort of HIV+ children beginning ART well within the first year of life. An additional aim was to explore associations of timing of ART initiation and DTI-derived parameters (FA, AD, RD), to interrogate potential protection of early ART on WM microstructure.

We hypothesized poorer WM integrity when starting ART after 12 weeks of age.

Methods

Subjects

We present data for fifty-two of 62 children enrolled in a neurodevelopmental substudy of the Children with HIV Early Antiretroviral (CHER) trial [21, 22] in Cape Town, South Africa. The group comprised HIV+ children on ART and age-matched controls from a parallel vaccine study, with informed consent from parents or caregivers.[23]

Exclusions were: six with mixed ancestry, one HIV+ child whose structural image was motion corrupted, one control child with incidental periventricular leucoencephalopathy and two HIV+ children with data inter-slice instabilities.

The CHER trial was a two-centre study in which HIV+ infants between 6 and 12 weeks of age and CD4 ≥25% were randomized to one of three treatment strategies: ART-Deferred (ART-Def) until indicated; early limited ART for 40 weeks (ART-40W); or early limited ART for 96 weeks (ART-96W). Infants with a CD4% < 25% were enrolled into a separate group (part B), initially to be randomised into ART-40W and ART-96W, but then retained on early continuous ART. The entire cohort comprised 451 HIV-infected infants below 12 weeks of age. Four hundred and eleven infants had baseline CD4 ≥ 25%, of whom 377 were reported in the main trial.[22]

Continuous ART was initiated in ART-Def when the CD4 declined below 25% in the first year of life and 20% thereafter or for Centres for Disease Control severe stage B or C disease. These criteria also applied to restarting ART in ART-40W and ART-96W. Since some children in ART-Def began ART early, we stratified children into those starting ART after (Late ART) or before 12 weeks (Early ART), irrespective of treatment arm. Also, as some in ART-40W and ART-96W arms met endpoint during primary therapy, the early ART group was sub-divided into those with or without treatment interruption.

First-line ART was lopinavir-ritonavir, lamivudine and zidovudine. Most mothers participated in the prevention of mother to child transmission program, which included zidovudine antenatally from 32 weeks and single dose nevirapine at delivery. Mothers with

CD4 count below 250 cells per mm^3 received ART antenatally. Newborn infants received a single dose of nevirapine at birth and zidovudine for 7 days.

Children were in regular follow-up with three-monthly clinical assessments.

Baseline laboratory and clinical data at enrolment and within 6 months of MRI scan, including CD4, CD8 parameters and viral load (VL) were obtained from participant medical records and the CHER database. VL >750 000 copies/mL were assigned as 750 001 and those <400 copies/mL as 399 (viral suppression).

Ethics approval for the study was obtained from ethics boards of all institutions involved.

MRI Data Acquisition

The children were imaged on a 3T MRI using structural T1 imaging followed by 2 DTI acquisitions with opposite phase encoding directions using a twice-refocused spin echo sequence.[24] The 3D echo EPI-navigated [25] multiecho MPRAGE [26] (MEMPR) sequence was acquired in a sagittal orientation with the following parameters: FOV 224×224 mm, 144 slices, TR 2530 ms, TE 1.53/3.19/4.86/6.53 ms, TI 1160 ms, flip angle 7°, voxel size 1.3×1.0×1.0 mm^3. DWI was performed in 30 directions with b-value 1000 s/mm^2, voxel size 2×2×2 mm^3, TR/TE 9500/86 ms, and 4 volumes with b = 0 s/mm^2.

Data analysis

Pre-processing

Diffusion weighted volumes with signal dropout or motion corrupted slices were removed,[27] and diffusion encoding scheme adjusted, with a constraint that the same volumes be removed in both DTI acquisitions. Co-registration and susceptibility correction were performed.[28, 29] Briefly, co-registration of individual volumes to the first unweighted image was performed using linear affine (12 degrees of freedom) transformation (FLIRT) in FSL (Oxford Centre for Functional Magnetic Resonance Imaging of the Brain, Oxford, UK). Subsequently, these images were imported to MATLAB (Mathworks, Natick, MA) for susceptibility correction and outlier rejection.[29] Outliers of each acquisition were examined by first calculating z-scores based on 25 and 75 percentile limits; data points above 3 standard deviations beyond the mean were discarded. The two acquisitions were combined into a single corrected image; FA, MD and eigenvalue (e_1,e_2, and e_3) images were generated. The first eigenvalue (e_1) was AD; the remaining two were used to compute RD ($e_{23} = [e_2 + e_3]/2$).

Co-registration

The FA images were first co-registered to corresponding structural images to achieve intrasubject alignment. Structural images of all subjects were then co-registered to a ‘most representative’ control image, then subsequently co-registered to the National Institutes of Health paediatric MRI Data repository T1-template image for children aged 4.5 – 8.5 years with isotropic resolution 1.0 × 1.0 × 1.0 mm^3 using linear (FLIRT) and non-linear (FNIRT) co-registration algorithms in FSL.[30] FA images were warped using the same transforms for inter-subject alignment. The same transforms were applied to MD, AD and RD images. A

WM binary mask was generated for each subject by applying a FA threshold of 0.2. Individual masks were multiplied to generate a final binary image representing WM regions where FA ≥ 0.2 in all subjects. The binary image was multiplied with the co-registered FA and MD images of each subject to localise statistical analyses, explained below, to the same WM regions.

Statistical analysis

Voxel-based group comparisons were performed in FSL to determine regions where FA and MD differed significantly between HIV+ and control children, and between HIV+ children starting ART late or early, and those with and without interruption. To account for multiple comparisons when determining significant clusters, AFNI's AlphaSim command was used with overall significance level $\alpha = 0.05$ and individual voxel-wise significance level $p = 0.01$. FWHM values ranged between 3.8-5.2 mm across the masked thresholded WM masks and we performed 5000 Monte Carlo simulations. [31] Clusters of at least 258mm^3 were significant at these levels.

Locations of clusters showing group differences were identified using the Harvard-Oxford cortical and subcortical and John Hopkins University WM tractography atlases provided in FSL and an MRI atlas of human WM anatomy.[32, 33] For each cluster, average FA and MD, and corresponding AD and RD values, were extracted.

Categorical variables were summarised using frequency and percentage frequency distributions overall and by group. Continuous measurements were summarised using means and standard deviation. Variables were compared between the groups using ANOVA and Chi-square tests.

Results

After exclusions, we present data for 13 healthy controls (mean age 5.7 ± 0.5 yrs., 5 male) and 39 HIV+ children (5.4 ± 0.3 yrs., 15 male). Demographic and clinical data of HIV+ children are presented in table 1.

Ten children receiving early ART fulfilled criteria for continuous ART. Sixteen children interrupted after primary therapy, and 3 had not re-started ART by the time of MRI scan. Parents of one child randomized to ART-96W initially withheld ART without knowledge of the investigators. This child was included in the late treatment group.

Four children started ART under part B, 2 were interrupted and 2 were on continuous ART.

Cumulative period on ART was longest for those receiving early continuous ART.

Eighty-seven percent (n=34) had VL suppression (< 400 copies/ml). Of the 13% (n=5) unsuppressed at MRI scan, four were in the late ART group (with VL 3590, 5980, 8870 and >750 000 HIV RNA copies/ml) and one in the early ART interrupted group (204 000 HIV RNA copies/ml).

Imaging

On the T1W MR sequences structural abnormalities were identified in three HIV+ children: mild cerebellar atrophy, mild generalised atrophy, pineal multilobed cyst and none in controls.

Two clusters were identified in the right corticospinal tract (CST) where FA was lower in HIV+ children than controls - mean FA ± standard deviation: 0.42 ± 0.03 versus 0.46 ± 0.03; and 0.43 ± 0.04 versus 0.49 ± 0.04. Differences in FA were attributable to increased RD ($p<0.01$, table 2). Left-sided similar clusters were seen, however not surviving cluster size correction. The FA and RD values in these clusters for the child with VL > 750 000 HIV RNA copies/ml at scan were below the group average (excluding this child) but not the lowest overall. FA values did not differ significantly between the other 4 unsuppressed children and the remaining group.

Seven clusters showed higher MD at $p<0.01$, in infected children than controls, the largest being 7503 mm^3 which included several tracts. Both AD and RD contributed to the increased MD (table 3).

Comparison of FA between children starting ART before and after 12 weeks of age

Against our hypothesis, children starting ART later did not demonstrate poorer white matter integrity as measured by FA. Rather, one cluster was identified in the brainstem in the left CST where FA was lower in early compared to late ART initiation. When comparing early continuous and early interrupted ART individually against late ART, we found lower FA *only* in the early interrupted ART group, suggesting that interruption is harmful to WM. No regions showed FA differences between early continuous and late ART. The reduced FA in the children on early interrupted ART was attributable to increased RD and AD.

Discussion

We demonstrated WM areas with significantly reduced FA in HIV+ children initiating ART at a median age of 4 months compared to uninfected controls.

No frontal or parietal white matter predilection for abnormal findings

Our findings confirm the presence of FA abnormalities found in HIV+ adults and adolescents but differ in volume and distribution. Young children on early ART had very few regions with abnormal FA. The predilection for frontal lobe involvement described in adults [34, 35, 36, 37, 38] was not seen, possibly due to the small sample size, specifically of the control group (N = 13). We previously reported multi-focal WM signal abnormalities on standard T2W MRI sequences in frontal (91%) and parietal WM (82%) of HIV+ children at mean age 31.9 months.[39] Twenty of these children are also included in the present study. Ten had WM signal abnormality on FLAIR. [39] Unfortunately, a limitation in the present study was an absence of FLAIR, thus an inability to assess interval WM signal change. However, absence of frontal and parietal involvement on FA does suggest interval improvement on ART.

Although clusters showing left-sided FA differences did not survive cluster size correction, FA reductions were bilateral in the CST. The MD differences were more widely distributed and included the inferior longitudinal fasciculus (bilateral), CST, inferior fronto-occipital fasciculus, forceps minor and uncinate fasciculus.

Because frontal WM myelination continues into adulthood, children demonstrate inherently lower frontal FA values than adults. [40] To exclude frontal predominance of WM abnormality being maturational [41] we determined areas of significant FA difference between HIV+ children and age-matched controls by using voxelwise group comparisons. The predominant contribution to decreased FA was RD, while the increased MD was due to both RD and AD, indicating both reduced myelin and loss of axonal integrity.[42]

Age difference between the HIV+ and control group was only a few months, not considered clinically significant. Our study has a much narrower age range than previous studies, facilitating improved comparison to controls representing the age-related normal developing brain.

Children's age and ART relevance

The higher FA values in those beginning ART after 12 weeks was surprising as we expected this in those beginning ART before 12 weeks. However, the difference was attributable to ART interruption, possibly negating the benefits of early ART, rather than neurotoxicity due to longer ART exposure. [43, 44, 45]

The timing of interruption may be important with reference to WM maturation. There are 3 phases of maturation observed by FA: rapid change in first 3-6 months, slower change until 24 months and relative stability thereafter. Most WM tracts are formed at birth then increase in size together with FA over 24 months.[46] Deep WM structures such as the CC and internal capsule have high FA at birth which rapidly rises. In contrast, frontal WM has low FA, increasing to intermediate values around 24 months. In neonates, the CST is present within the brainstem but size and intensity are much lower than in the older brain.[47] Autopsy studies however, show that the CST and the superior cerebellar peduncles mature early.[48, 49] ART was interrupted at 40 weeks (around 10 months of age) which may have coincided with a critical stage of CST maturation.

Notably, we found no CC involvement in HIV+ children. HIV-associated FA abnormalities in the CC are reported in adults and in children.[6, 7, 8, 13] In contrast, our children started ART early compared to other studies. Of interest, CC volume and thickness were similar to controls in a study by Andronikou et al. which included the 20 HIV+ children previously reported.[39, 50] The CC genu demonstrates a variable growth spurt at 2 months of age, followed by similar growth in the splenium by 4-6 months, with myelination being visible on T1W MRI from 4-6 months.[51] Our data support early ART being neuroprotective for the CC.

That no FA differences were noted between the late and early continuous groups may have been a ‘survivor effect’. Eight children in ART-Def died in the first year of life and were not studied. In addition, those on early continuous rather than early limited ART, were more

severely affected by HIV, having already reached a trial endpoint during primary ART, thus ineligible for interruption. All participants on continuous therapy had suppressed VLs at the time of scanning. Notably, those from Part B had baseline CD4 below 25% and therefore had more advanced HIV. Nevertheless, the early interrupted children had the most WM damage, suggesting that WM is more vulnerable at the time that ART interruption occurred.

Our data strongly suggests that WM damage, although not prevented by early ART, can be ameliorated or reversed, possibly through reduced neuroinflammation.[52] The children in this study are enrolled in a longitudinal neuroimaging study that includes DTI at age 7 and 9 years, which will provide vital information on the continuous effect of ART and/or HIV as well as answer questions relating to the influence of white matter maturation.

Conclusions

HIV+ children at 5 years of age have WM fibre abnormalities measured by FA despite early ART, suggesting that early ART does not fully protect the WM either from peripartum or in utero infection. In contrast to adults, the CSTs are predominantly involved rather than the CC, possibly due to early ART. Continuous ART can limit WM damage.

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Abbreviation list

WM	white matter
FA	fractional anisotropy
MD	mean diffusivity
RD	radial diffusivity
AD	axial diffusivity
CC	corpus callosum
CHER	children with HIV early antiretroviral trial
ART-Def	ART Deferred
ART-40W	ART at 40 weeks
ART-96W	ART at 96 weeks
CST	corticospinal tract

Table 1
Sample characteristics of HIV infected children

	Late ART (>12 weeks)	Early ART (<12 weeks) interrupted	Early ART (<12 weeks) not interrupted	p
N	13	16	10	
Gender	4M/9F	5M/11F	6M/4F	0.30
Age at scan (yrs.)	5.3 (0.30)	5.4 (0.24)	5.6 (0.43)	0.20
Age ART started (weeks)	36 (17)	8 (2)	8 (2)	**<0.01**
Time on ART (weeks)	241 (22)	203 (59)	285 (22)	**<0.01**
Time interrupted (weeks) *	n/a	85 (90)	n/a	
Clinical measures at baseline				
CD4 count	2064 (711)	1969 (1118)	1720 (978)	0.57
CD4%	37 (7)	35 (10)	30 (13)	0.21
CD8	1751 (1109)	1460 (675)	1978 (945)	0.34
VL>750 000 %, (n)	69 (9)	56 (9)	40 (4)	0.37
400<VL<750 000 %, (n)	31 (4)	44 (7)	60 (6)	
Clinical measures within 6 months of scan				
CD4 count	1027 (392)	1110 (460)	1289 (592)	0.58
CD4%	37 (8)	34 (7)	36 (10)	0.49
CD8 count	902 (450)	1083 (544)	1087 (625)	0.57
VL>750 000 %, (n)	8 (1)	0	0	0.14
400<VL< 750 000 %, (n)	23 (3)	6 (1)	0	
Suppressed VL %, (n)	69 (9)	94 (15)	100 (10)	

* calculated up to time of scan in 3 children who had not restarted ART.

Values: mean (Standard Deviation)

Table 2

Clusters where FA was lower in HIV+ children compared to controls

Cluster Location	Size mm^3	Coordinates	AD			RD		
			HIV+	Control	*p*	HIV+	Control	*p*
CST								
Right internal capsule	365	27,-23,-1	1.18 (0.03)	1.20 (0.03)	0.10	0.60 (0.10)	0.56 (0.02)	<0.001
CST								
Right parietal lobe	294	19,-24,42	1.18 (0.06)	1.22 (0.07)	0.08	0.60 (0.04)	0.56 (0.03)	<0.001

Values: mean (Standard deviation)

Table 3

Clusters where HIV+ children had significantly greater MD compared to controls

Cluster Location	Size mm^3	Coordinates	AD			RD		
			Control	HIV+	*p*	Control	HIV+	*p*
ILF/SLF								
Right temporal	7503	32,0,-24	1.19 (0.03)	1.23 (0.03)	0.001	0.62 (0.02)	0.65 (0.03)	<0.001
Left putamen	6916	-29,-26,-2	1.20 (0.03)	1.25 (0.03)	0.001	0.59 (0.02)	0.62 (0.03)	<0.001
CST								
Right brainstem	916	21,-15,-9	1.25 (0.04)	1.29 (0.03)	0.01	0.58 (0.02)	0.63 (0.02)	<0.001
IFOF								
Left temporal	555	-37,-10,-17	1.19 (0.05)	1.25 (0.04)	0.003	0.66 (0.02)	0.70 (0.04)	<0.001
Forceps minor								
Left frontal	336	-19,43,14	1.20 (0.06)	1.24 (0.06)	0.04	0.63 (0.03)	0.67 (0.05)	0.0030
Left frontal	266	-17,42,-1	1.22 (0.06)	1.26 (0.06)	0.05	0.62 (0.03)	0.65 (0.05)	0.0040
UF								
Right frontal	330	15,38,-12	1.19 (0.05)	1.25 (0.06)	0.003	0.64 (0.03)	0.67 (0.04)	0.0046

Values: mean (Standard Deviation).

ILF /SLF (inferior/superior longitudinal fasciculus), IFOF (inferior fronto-occipital fasciculus), UF (uncinate fasciculus)