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Structural brain changes in perinatally HIV infected young adolescents in South Africa

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Abstract

Objective—To describe the structural brain changes, neurocognitive and mental health associations in adolescents perinatally infected with HIV-1 infection.

Design—Cross-sectional

Methods—204 adolescents with perinatally acquired HIV and 44 uninfected frequency matched controls aged 9 to 11 years were enrolled within the Cape Town Adolescent Antiretroviral Cohort. Diffusion tensor imaging and structural brain magnetic resonance imaging (MRI) was done to determine fractional anisotropy (FA), mean diffusivity (MD), gray and white matter volumes, cortical thickness and cortical surface area. Correlation coefficients were calculated between total gray and white matter volume, cortical surface area, cortical thickness, whole brain FA and whole brain MD and clinical and laboratory parameters including general intellectual functioning, Becks Youth Inventory, Child Motivation Scale and Child Behavior Checklist.

Results—HIV infected adolescents performed worse than controls on the Wechsler Abbreviated Scale of Intelligence (WASI)($p < 0.01$). HIV infected adolescents had significant FA decreases, MD increases, and decreases in cerebral gray matter volumes, cortical surface area and decreased

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Authors contributions:

Prof Hoare was the principal investigator of this study. She conceptualized and designed the study, drafted the initial manuscript, and approved the final manuscript as submitted.

Mr Fouche carried out the initial imaging analyses, reviewed the manuscript, and approved the final manuscript as submitted. Ms Phillips designed the data collection instruments, and coordinated and supervised data collection and entry, critically reviewed the manuscript, and approved the final manuscript as submitted.

Prof Zar is principal investigator on CTAAC, she critically reviewed the manuscript, and approved the final manuscript as submitted. Prof Stein, Prof Joska and Prof Myers assisted in study conceptualization, advised on statistical analysis, critically reviewed the manuscript, and approved the final manuscript as submitted.

gyrification. Whole brain mean FA was significantly reduced in the HIV infected group ($p=0.031$). There were significant correlation coefficients between greater total gray ($p=0.008$) and white matter volume ($p=0.004$) with the WASI and the Becks self-concept subscale ($p=0.038$). Lower whole brain FA was associated with higher scores on the Becks anger ($p=0.018$) and disruptive behavior subscales ($p=0.031$). Higher whole brain MD was associated with apathy ($p=0.046$).

Conclusion—The pattern of increased risk of white matter microstructure alterations, smaller gray matter volumes, reduced cortical surface area and decreased gyrification, suggests abnormal neurodevelopment in perinatally infected younger adolescents.

Keywords

HIV; adolescence; neuroimaging; DTI; MRI; neurocognitive; South Africa

Introduction

Globally, an estimated 3.4 million children are living with HIV-1(HIV), yet little is known about the effects of HIV and antiretroviral treatment (ART) on the developing brain, and the cognitive and mental health outcomes of perinatally HIV-infected adolescents are poorly understood [1] Early initiation of ART has been shown to positively impact neurodevelopment, however, children continue to be diagnosed with neurocognitive disorders despite treatment [2]. Data on adolescents is especially lacking in sub-Saharan Africa (SSA), which has the highest HIV prevalence [1]. The effects on neurocognitive function and brain development in HIV infected adolescents commenced on ART from an early age in resource-constrained settings remains unclear [3]

The most frequent macro-structural brain abnormalities reported in perinatally infected HIV are ventricular enlargement, cortical and subcortical atrophy, involvement of the basal ganglia, frontal white and frontal grey matter abnormalities, calcifications and damage to the corpus callosum [4]. Most studies have used computed tomography (CT) which is more accessible than Magnetic Resonance Imaging (MRI) in resource limited settings [4]. MRI neuroimaging studies have reported lower brain volumes compared to matched controls [5], while others have reported grey matter volume increases in several regions [6]. In addition to macro- structural changes noted on CT and MRI, earlier work conducted by our team on a smaller, cross-sectional, perinatally infected, mixed treatment cohort (6–16yrs), found microstructural white matter alterations using Diffusion tensor imaging (DTI)[7]. Common DTI metrics include fractional anisotropy (FA) and mean diffusivity (MD). Axial diffusivity (AD) and radial diffusivity (RD) are additional DTI-derived metrics corresponding to diffusion parallel and perpendicular to the direction of the white matter tract, respectively [8]. Higher MD and lower FA are traditionally representative of poorer directional diffusion suggesting alterations in neuronal microstructure. Myelin loss (measured in DTI by an index of RD) and axonal damage (measured in DTI by an index of AD) are both observed in white matter injuries, such as have been previously described in adults with HIV [9]. HIV infected youth in our earlier study displayed decreased FA and AD, and increased MD and RD, suggesting HIV related changes to white matter development. [7].

A range of factors could contribute to altered brain macro and microstructure in perinatally infected adolescents living with HIV. These may include environmental/ socio-demographic factors, nutritional-hematological status, HIV-relevant clinical variables, and ART. Being on second line ART, increased viral load, low CD4 counts, low hemoglobin and poor cognitive function have been associated with poor white matter integrity in HIV infected youth in Cape Town [10]. There could be many potential influences of ART treatment on cognition and brain macro and microstructure, including age of initiation of ART, duration of ART use and potential ART neurotoxicity. Strategies to enhance penetration of ART into the CNS could help to control HIV replication in this reservoir but could also carry an increased risk of neurotoxicity [11]. Efavirenz (EFV), is a common component of first-line combination ART in South African children with good CNS penetration. A metabolite of EFV, identified as a potent neurotoxin in primary neuronal culture, can damage dendritic spines, suggesting a potential role of EFV neurotoxicity in the neuronal injury that may contribute to poor neurocognitive functioning [12,13]. Given the multiple potential treatment and clinical factors that could contribute to neurodevelopment in adolescents living with HIV, it would be valuable to examine the associations of CD4 cell counts, viral load, age of initiation of ART, duration of ART use and duration of efavirenz use with intellectual functioning and brain macro and microstructure.

The overall goal of Cape Town Adolescent Antiretroviral Cohort (CTAAC) is to investigate markers of chronic disease processes and progression in perinatally HIV-infected adolescents including neurocognitive function and neuroimaging longitudinally over a three-year period. This study investigated baseline neuroimaging analysis in early adolescence using DTI and structural MRI. Our primary analysis involved testing the hypothesis that perinatal infection with HIV is associated with altered neuro-developmental trajectories in HIV infected adolescents, and that this will be detectable at the macro and microstructure levels of brain anatomy. We hypothesize that adolescents living with HIV will have higher MD, lower FA, and reduced brain volumes at both the whole brain and regional structure level. Our secondary analysis related mental health status, clinical variables, ART variables and general intellectual function to whole brain volumetrics and microstructure.

Methods

A study of DTI and structural brain magnetic resonance imaging (MRI) measures was conducted in a subgroup of perinatally infected adolescents enrolled in CTAAC. Routine care providers told all adolescent/caregiver dyads attending one of the 4 recruitment sites, who were in the target age range, about the study. Interested adolescents/caregivers were formally screened, and if eligible and willing to participate, provided with an appointment to attend an enrollment visit 1–2 weeks later at Red Cross War Memorial Children's Hospital. Adolescents were recruited primarily from primary care sector health care service from across Cape Town; inclusion criteria were aged 9–11 years, perinatally infected, had been on ART for at least 6 months, knew their HIV status and able to provide informed parental consent and participant assent. Adolescents were sampled consecutively and were not recruited based on disease complexity or virological suppression/unsuppression. Eleven HIV infected adolescents in the CTAAC cohort were included in a previous work conducted by our team on a smaller, cross-sectional, mixed treatment cohort. Controls were HIV negative

and frequency matched for age and sex. Controls were excluded if they had known pre-existing disease or if informed consent and assent was not obtainable. All youth screened for the control cohort underwent rapid HIV testing prior to enrolment to confirm negative status. HIV exposure in the control group is unknown, however the adolescents selected have similar ethnicity, home language, years of education and annual household income.

Exclusion criteria for both groups were: an uncontrolled medical condition, such as poorly controlled diabetes mellitus, epilepsy, or active tuberculosis requiring admission; an identified CNS condition, such as past or current TB meningitis or bacterial meningitis, documented cerebrovascular accident, lymphoma; a history of head injury with or loss of consciousness, or any radiological evidence of skull fracture; a history of perinatal complications such as hypoxic ischemic encephalopathy or neonatal jaundice, or neurodevelopment disorder not attributed to HIV. Participants were enrolled from August 2013 to April 2015. Baseline health and sociodemographic questionnaires were administered to obtain general health information, past history and data on ancestry, language, education and treatment. Recent CD4 and viral load and ART data were abstracted from care records. Ethical approval was obtained from the University of Cape Town's Faculty of Health Sciences research ethics committee.

Measures

Questionnaires were administered by study staff to adolescent/parent or guardian dyads at enrolment. Interviews were conducted in the participants' home language, in private rooms by trained counsellors with extensive experience working with HIV infected children. We have used each of these measures in the local population, with evidence of good reliability in isi-Xhosa speaking populations [2]. General intellectual functioning was measured using the *Wechsler Abbreviated Scale of Intelligence (WASI)* [14]. *Beck Youth Inventories* [15], a self-report scale to assess an adolescent's experience of depression, anxiety, anger, disruptive behaviour, and self-concept. The *Children's Motivation Scale (CMS)*, a 16-item instrument [16], was used to measure the adolescent's motivation levels, or tendency toward apathy. The *Child Behaviour Checklist (CBCL)* [17] is one of the most widely used and psychometrically sound measures for assessing adolescent behavioural and emotional problems and psychopathology.

Image acquisition

Structural and Diffusion weighted imaging was performed at the Cape Universities Brain Imaging Centre on a 3T Siemens Allegra scanner within 7 days of neuropsychological assessment. For the diffusion-weighted imaging a single-channel transmit-receive head coil was used with the following parameters: TR = 8800ms, TE = 88ms, field-of-view of 220mm, $1.8 \times 1.8 \times 2.0 \text{ mm}^3$ image resolution, 65 slices, 0% distance factor and 2x Generalized Autocalibrating Partial Parallel Acquisition (GRAPPA) acceleration. Images were acquired in an axial orientation with 30 gradient directions at $b = 1000 \text{ mm/s}^2$, and 3 directions with $b = 0 \text{ mm/s}^2$. The acquisition was repeated 3 times to allow for redundancy in data. A multi-echo Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) T1-weighted image was acquired with the following parameters: FOV = $256 \times 256 \text{ mm}$, TR

= 2530ms, TE = 1.53/3.2/4.89/6.57ms, TI = 1100ms, flip angle = 7°, 144 slices, in-plane resolution = $1.3 \times 1.0\text{mm}^2$ and slice thickness of 1.0mm.

DTI Preprocessing

Diffusion weighted images from individual participants were co-registered to their corresponding b=0 image in order to correct for eddy current distortions and movement artifacts within FMRIB Software Library (FSL) 5.0.1 and imported into MATLAB R2013b for processing. This entailed the affine registration to the average b= 0mm/s² image of the first acquisition. For each of the acquisitions, outlier data points were determined by calculating the Z-values at the 25th and 75th percentile of the registered diffusion image. Any data points that were 3 SD from the mean were excluded. The corrected images were exported to FSL 5.0.1 after correction. In FSL 5.0.1 images underwent brain extraction in the Brain Extraction Toolbox (BET) to remove any non-brain tissue and fit a linear tensor model to produce fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD) maps.

Fractional anisotropy images were analysed with the Tract-based Spatial Statistics (TBSS) pipeline [18]. Each participant's FA was registered to a study-specific target. This target was determined by registering each participant to every other participant. The mean square displacement coefficient of each image was calculated and the participant with the lowest mean displacement was chosen as a representative target for the group. After registration to the study-specific target, each image was then up-sampled to Montreal Neurological Institute (MNI) space, taking into account the previous transformation parameters. An average FA was created and thinned to produce a mean FA skeleton with a threshold of 0.2. This skeleton is representative of the centres of white matter tracts common to the group. Registration and skeleton projection were also applied to the MD, AD and RD images as described above. For the calculation of total MD and FA, the 48 white matter regions, defined by the JHU-atlas [19] were extracted from the average FA and MD of every subject's registered TBSS skeleton. The means of these regions were then averaged and exported to SPSS 24.0 as total FA and MD for each participant.

Freesurfer preprocessing

T1-weighted images were processed with Freesurfer V5.3 on the Lengau cluster at the Centre for High Performance Computing (CHPC), Rosebank, Cape Town, South Africa. The pipeline has been described previously by Fischl et al. [20]. T1-weighted images were normalized, bias-field corrected for intracranial volume and skull-stripped. Inner and outer cortical surfaces were modeled as triangular tessellation. Cortical thickness measurements were obtained by calculating the distance (in mm) between pial and grey-white matter surfaces at each vertex location. Cortical surface area was calculated as the average of the grey matter vertices over regions. The vertex data was normalized to the "fsaverage" template included with Freesurfer by utilizing a curvature matching technique. Cortical volume was calculated as the product of surface area and cortical thickness and corrected for intracranial volume by utilizing the proportion method of correction [21]

For subcortical region volumes the brain was segmented into volume-based labels utilizing probabilistic methods to determine volumes of the caudate, thalamus, putamen, pallidum, hippocampus, amygdala and accumbens [22]. In addition, total gray matter volume, surface area and cortical thickness were exported to SPSS 24.0 for correlation analyses.

To calculate gyrification, the localGI flag was used in Freesurfer [23]. This toolbox computes the local gyrification index (LGI) across the whole surface area of the cortex. After reconstruction, each individual scan was checked for any major errors in segmentation, corrected and rerun if needed.

Some of the corrections were: 1) fixing skull strip or brain extraction i.e too much dura and/or skull were included, 2) adding control points to adjust the intensity normalization of white matter regions which were incorrectly labeled as gray matter, 3) correcting topological defects in the pial/gray matter surface area which can cause certain regions such as the temporal poles and posterior occipital lobe to be missed during segmentation.

Statistical analysis

DTI Statistical analysis—DTI Statistical analysis was performed with FSL's randomise. Randomise is a toolbox within FSL that allows the user to perform permutation methods for thresholding on statistic maps when the null distribution is unknown as in the case of imaging data [24]. HIV infected children were compared to the control group for each DTI measurement utilizing a general linear model with age, sex and ethnicity included as covariates of no interest. Although the 2 groups were well matched for age, sex and ethnicity we also adjusted for these variables to improve the robustness of the results, given that these factors are known to affect brain macro and microstructure. Analysis was performed at 5000 permutations per contrast with threshold-free cluster enhancement (TFCE). This method utilizes a raw statistic image such as a T-test image map to produce an output image in which voxelwise values represent the amount of local spatial support. In this way arbitrary selection of cluster thresholds are avoided, while automating the thresholding process [25]. Due to the large number of permutations possible with imaging data, it is not feasible to run a permutation on each voxel or cluster. Therefore Conditional Monte Carlo permutation tests are utilized to account for the large number of multiple comparisons. These are simulations of a random sample of permutations on the dataset. Usually the number of permutations are set at $n = 10000$ which translates to a confidence limit or margin of error of ± 0.0044 at $p = 0.05$.

Freesurfer vertex-wise statistical analysis—Vertex-wise statistical analysis was performed with the Freesurfer Query, Design, Estimate, Contrast (QDEC) toolbox. HIV infected adolescents were compared to the control group utilizing a general linear model (GLM) to examine group differences. Sex, age and ethnicity were controlled for in the model. Gray matter volume, cortical surface area, cortical thickness and local gyrification index were compared between groups. For each measure, data was smoothed at a full-width half-maximum (FWHM) of 10mm to minimize noise and account for residual mis-registration. To correct for multiple comparisons, 10 000 Monte-Carlo simulations or permutations were performed at $p = 0.05$ as discussed above.

Correlation analysis: Correlation analyses were performed in SPSS 24.0 (Amarok, IBM). For the correlation analysis clinical variables were correlated with whole brain measures only to avoid a large number of comparisons (5 whole brain measures). Pearson correlation coefficients were calculated to evaluate the association between imaging measures of whole brain gray matter volume, whole brain white matter volume, whole brain cortical thickness, mean whole brain FA, mean whole brain MD and immunological measures of current CD4 cell counts, age of initiation of ART, duration of ART use and current efavirenz treatment. The same procedure was followed for variables WASI, Becks youth inventories, CMS and CBCL total competence for both healthy controls and HIV-infected adolescents. Log-transformed viral load was not normally distributed, and Spearman-rank correlation coefficients were calculated for the association with imaging measures. Both bivariate Pearson and Spearman-rank correlations were performed at $p < 0.05$. A Pearson's point-biserial correlation test was used to correlate current efavirenz use and viral suppression/unsuppression, with correlations performed at $p < 0.05$. Correlation coefficients were adjusted for sex, ethnicity and age.

The proposed sample size of 200 HIV infected adolescents was based on the correlation of imaging findings (e.g. white matter fiber bundles in the frontal brain region) with tests of cognitive function (eg, executive function); the proposed sample size would allow $>90\%$ power to detect correlations of >0.2

Results

204 HIV infected adolescents and 44 matched controls were enrolled. Demographic characteristics were similar between cases and controls (Table 1). HIV infected adolescents were more likely to have had repeated grades at school and perform poorly on tests of general intellectual functioning ($p < 0.01$). Most HIV-infected adolescents were on first line ART (79.9%), 106 (52%) were currently taking EFV, with a mean CD4 count of 955 cells/mm³, median viral load of $< \text{LOD}$ (level of detection) copies/mL and mean age of initiation of ART of 3.41 years. A Pearson's point-biserial correlation test revealed no significant association between IQ and whether or not the participant was virally suppressed or unsuppressed ($r = -.04$, $p = .65$).

For the 106 participants currently taking EFV, only 17 (16%) of these were virally unsuppressed. The participants currently on EFV had a mean CD4 count of 949.21 and SD of 508.71. A Pearson's point-biserial correlation test was calculated to evaluate the association between IQ and current EFV treatment. Lower IQ was associated with being on EFV ($r = -.18$, $p = .01$).

FA and MD comparison between healthy controls and HIV infected adolescents ($p < .05$ corrected for multiple comparisons, Table 2 and figure 1)

The mean whole brain FA and MD of the healthy controls were $0.304 (\pm 0.022)$ and $8.5 \times 10^{-4} (\pm 4.7 \times 10^{-5})$ respectively. The mean FA and MD of the HIV infected adolescents were $0.291 (\pm 0.031)$ and $8.7 \times 10^{-4} (\pm 5.3 \times 10^{-5})$.

The HIV infected adolescents had significantly FA lower in regions of the left superior cerebellar peduncle, bilateral anterior limb of the internal capsule, left posterior limb of internal capsule, right retrolenticular part of the internal capsule, right superior corona radiata, left sagittal stratum, left superior longitudinal fasciculus and right superior fronto-occipital fasciculus. In addition there was higher MD in HIV infected adolescents when compared to healthy controls in regions of the left anterior limb of the internal capsule and the left posterior limb of the internal capsule. There were no significant differences for AD and RD between the groups. Whole brain mean FA was significantly lower in the HIV infected group ($p=.031$), no significant difference was found for whole brain mean MD.

Gray matter, cortical surface area and gyrification differences between healthy controls and HIV infected adolescents ($p < 0.05$, corrected for multiple comparisons, Table 3 and figure 2,3)

There was significantly lower gray matter volume in the left postcentral gyrus of HIV infected adolescents compared to controls. The left precentral gyrus and the left pericalcarine gyrus cortical surface areas were lower in HIV infected adolescents. In addition there was lower gyrification in the left inferior temporal and right lateral occipital gyri in the HIV infected group. There were no significant differences in cortical thickness or white matter volumes between the two groups. No significant differences were found for total brain white matter or gray matter volume.

Association of brain structure measurements with clinical and treatment variables, WASI IQ and mental health measures ($p < 0.05$, Table 4)

Within both the HIV infected and healthy controls groups, there were significant positive associations of gray matter and white matter volume with general intellectual functioning as measured by the WASI, as well as a positive association of the Beck Self-Concept Inventory with white matter volume. Fractional anisotropy was negatively associated with the Anger and Disruptive Behavior subscales of the Beck Inventory. Mean diffusivity was negatively associated with the Children Motivation's scale. There were no associations of any of the whole brain imaging variables with immunological, treatment or clinical variables within the HIV infected group.

Discussion

These neuroimaging findings provide novel, important data and correlations with WASI IQ and mental health in young HIV infected adolescents who have been on ART for several years. HIV infected adolescents performed poorly on a test of general intellectual functioning and were more likely to repeat grades at school. HIV infected adolescents had significantly lower FA; suggesting altered neuronal microstructure; and higher MD, suggestive of inflammation. In addition significantly lower gray matter volume, cortical surface area and gyrification was found using FreeSurfer software analysis. These adolescents were exposed to HIV during a period of rapid brain development, have lifelong infection and long term ART exposure. These findings suggest that HIV infected adolescents remain at high risk of alterations in neuronal microstructure compared to typically

developing adolescents, and certain alterations are related to lower average IQ and mental health.

HIV infected adolescents performed worse than controls on the WASI. HIV-infected children and adolescents have performed poorly in the WASI compared to negative controls in prior studies conducted in Cape Town[2]. In addition asymptomatic slow progressors performed poorly compared to controls on Performance and Verbal tasks of the WASI[26]. A more detailed assessment of cognitive functioning of this cohort has recently been published with impairment reported in the following domains: executive functioning, attention, working memory, verbal memory, visual memory, visual spatial ability, language and processing speed [27]. It is possible that impairment across multiple domains has affected school performance and increased the likelihood of repeating grades at school in adolescents living with HIV. For the participants currently taking EFV, very few were virally unsuppressed, however lower average IQ was associated with being on EFV. Results here suggest a potential role of EFV neurotoxicity that may contribute to lower average IQ, however no significant associations of current EFV treatment were found with whole brain macro and microstructure.

Evidence suggestive of altered neuronal microstructure in HIV infected adolescents observed in this study is consistent with neuroimaging results reported previously, however brain regions involved differ between studies [7]. The FA differences were widely distributed and included whole brain FA and the typically described fronto-striatal regions of HIV in adult studies[9]. Young South African HIV infected children have been found to have white matter abnormalities measured by FA, despite early ART initiation, suggesting that treatment does not fully protect the white matter from either peripartum or in utero infection[28]. Lower whole brain FA, with higher MD in older HIV infected adolescents (mean age 16 years) has also been found, with severe past HIV disease being associated with lower FA of the inferior fronto-occipital and uncinate tracts; higher MD of the inferior longitudinal fasciculus [29]. Increases in mean diffusivity have been observed in pediatric HIV [5] as seen in the internal capsule in our study. Abnormal signal intensity in the internal capsule has been reported to be a predictor of poor neurodevelopmental outcome in term infants suffering hypoxic encephalopathy [30]. In typically developing adolescents better working memory performance, attention and reading related skills would be associated with increased FA of the superior longitudinal fasciculus [31,32]. Cognitive correlates of anisotropic and diffusional change over time in adolescents are generally consistent with increased FA contributing to higher performances in the areas of complex attention and working memory and verbal fluency[33]. Prenatal HIV exposure without infection has been associated with altered white matter microstructural integrity in the neonatal period. Specifically the alterations were documented in the middle cerebellar peduncles [34]. These pathways have been implicated in a variety of intellectual and neuropsychological deficits, which are most pronounced in visuospatial, language and memory functions [35]. Alterations in the white matter integrity of the cerebellar peduncle were seen in the current study.

In neuroimaging studies focused on volumetrics, outcomes are not consistent. Similar to our findings, Cohen et al.[5] reported lower cerebral volumes in perinatally infected HIV

children aged 8–18, with no significant differences observed in subcortical gray matter volumes. Our study did not however find any difference in total gray matter volume or white matter volumes. Another study found HIV infected youth to have smaller total and regional gray matter volumes than HIV uninfected youth, with smallest volumes seen among HIV infected youth with higher past peak viral load and recent unsuppressed viral load [36]. One post ART pediatric study on cerebral volumes found a focal increase in gray matter, and attributed it to cell swelling as a result of ongoing infection or ART toxicity [6]. In addition to the volumetric changes observed in our study, reduced gyrification was found in the left inferior temporal gyrus and the right lateral occipital gyrus. FreeSurfer analysis of youth with HIV infection has been used to demonstrate subcortical shape deformation related to past HIV severity and cognition [37].

The inconsistency of neuroimaging signatures in pediatric HIV may reflect differences in patient characteristics, wide age range, imaging methods, and disease variables. We found no HIV-related effect for global MD, AD, or RD, and the only regional effect was for MD within the internal capsule. In addition, the lack of global gray and white matter volume or cortical thickness effects also differs from some previous work[5,29]. The current study has a larger sample size than previous studies, with a narrower age range examining early adolescence only, the study was conducted in South Africa where clade C is predominant, in addition the majority of participants were virally suppressed and all perinatally infected. These factors may contribute to the lack of global HIV-related effects as reported in some other previous studies.

There were significant associations of greater volumes with better intellectual functioning. Previous studies with HIV infected youth have found worse cognitive performance correlated with smaller volumes [36]. Higher MD has also been associated with lower intellectual functioning [5]. This pattern of smaller gray/white matter volumes and poor white matter integrity suggests that pediatric HIV infection may influence brain development and underlie the lower average IQ seen in this population.

This study is the first to investigate the associations with mental health and neuroimaging measures in HIV infected adolescents. Poor self-concept, anger, disruptive behavior and apathy were associated with higher MD and lower FA suggesting altered neuronal microstructure. Alterations in FA have previously been observed in depressed HIV infected adults [38]. Apathy in HIV infected adults has been associated with poor white matter integrity in the tracts that relay through the medial prefrontal cortex [39]. ART itself may cause damage to the CNS [40] lower IQ in this study was associated with being on EFV.

Limitations of this study include the cross sectional design of the study, but longitudinal follow-up is underway to better understand the impact of HIV on brain remodeling typically seen in later adolescence. The control group is small in comparison to the participant group, and HIV exposure in the control is unknown. However we feel that our controls represent adolescents from similar demographic backgrounds. There are limitations associated with DTI as a quantitative imaging technique. FA probably reflects some changes in some aspects of connectivity, although we cannot really say what precise aspect, and into which direction the change occurred [41]. Although other congenital infections and incidental CNS

abnormalities were excluded as far as possible on history, clinical examination and on clinical review of the MRI scans, it remains a possibility that there may be some overlapping effects of undiagnosed conditions such as congenital CMV.

Conclusion

This is the largest imaging cohort study to examine impact of HIV on brain structure in early adolescence. The study took place in South Africa, one of the countries most affected by HIV/AIDS with the highest rate of new HIV infections in the world. Despite a mean age of initiation of treatment of 3.41 years alterations in both brain macro and microstructure are observed here. Examination of our cohort longitudinally is needed to determine the impact of HIV on brain remodeling typically seen in later adolescence. Progressive myelination during adolescence implicates an increased vulnerability to HIV-related CNS damage [42]. Progressive cellular maturational events, such as increased myelination, may play as prominent a role during the adolescent years as regressive events, such as synaptic pruning, in determining the mature frontal lobe cortical gray matter [43]. Neuroadaptation may reflect additive and subtractive responses to HIV that are complicated by competing maturational processes[42].

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Conflicts of Interest and Source of Funding:

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Figure 1: Significant decreases in FA for HIV infected adolescents compared to controls
 Results displayed here at $p < 0.05$ corrected for multiple comparisons. Abbreviations:
LH_SCP: Left superior cerebellar peduncle; **RH_ALIC**: Right anterior limb of internal capsule; **LH_ALIC**: Left anterior limb of internal capsule; **LH_PLIC**: Left posterior limb of internal capsule; **RH_RLIC**: Retrolenticular part of the internal capsule; **RH_SCR**: Right superior corona radiata; **LH_SS**: Left sagittal stratum; **LH_SLF**: Left superior longitudinal fasciculus; **RH_SFOF**: Right superior fronto-occipital fasciculus, **FA**: Fractional anisotropy

Figure 2: Decreased cortical surface area for HIV-infected adolescents compared to controls
 Legend at the bottom indicates the extent (Z-value) of the difference in surface area between controls and patients. Abbreviations: **LH_PreCG**: Left precentral gyrus (image at the top); **LH_PC**: Left pericalcarine gyrus

Figure 3: Decreased gyrification in left inferior temporal and right lateral occipital gyrus of HIV infected adolescents compared to controls

Legend at the bottom indicates the extent (Z-value) of the difference between patients and controls. Top image and bottom image displays decreased gyrification in the right lateral occipital gyrus and the left inferior temporal gyrus, respectively.

Table 1:

Baseline demographic and clinical characteristics of the Neuro—study CTAAC cohort

Variable	HIV-infected (N = 204)	Controls (N = 44)	t	p
Age in years: Mean (SD)	10.38 (0.88)	10.38 (1.09)	-.01	.99
Age range in years:	9–11	9–11		
Gender: Male/Female	100/104	20/24	.43	.67
Ethnicity: Black African/Other	187/17	44/0	-1.99	.05
Home language: isiXhosa/Other	182/20	42/2	1.12	.26
Education in years: Mean (SD)	3.20 (1.13)	3.39 (1.35)	.96	.34
Repeated grades: YES (%)	121 (59.3)	18 (40.9)	-2.24	.02*
General intellectual function	80.26 (7.39)	85.09 (7.65)	3.90	.00*
Low household income: (%) ^a	199 (98.5)	44 (100)	-.72	.47
ART regimen ^b : First line/Second line/Third line/Missing	163/32/8/1	N/A	N/A	N/A
Duration of ART: mean years (SD)	7.18 (2.46)	N/A	N/A	N/A
Age of initiation of ART: mean years (SD)	3.41 (2.53)	N/A	N/A	N/A
Current EFV treatment: YES (%)	106 (52.0)	N/A	N/A	N/A
Viral load: median (IQR) (copies/mL)	<LOD (40.0)	N/A	N/A	N/A
CD4 count: mean (IQR) (cells/mm ³)	955.12 (587.75)	N/A	N/A	N/A
Virally unsuppressed ^c : YES (%)	30 (14.7)	N/A	N/A	N/A

NOTES:

^a: annual household income brackets in ZAR: R0 -R25000 p.a. = Low.

^b: The South African Department of Health first line antiretroviral (ART) regimen for children older than 3 years and more than 10kg is Abacavir (ABC) + lamivudine (3TC) + efavirenz (EFV). Failure of first line treatment would result in initiation of second line treatment etc. Missing: on ART but treatment regimen unknown.

^c: Virally unsuppressed participants were defined as having a VL>50 copies /mL. The range for initiation of ART is 0.25 – 10.98 years. The range for duration on ART is 0.42 – 11.06 years. Differences between groups tested by means of an independent t-test.

Table 2:

Comparison between HIV+ adolescents and controls on measures of FA and MD

Anatomy	MNI coordinates	Cluster size (mm ³)	Controls mean (SD)	HIV+ mean (SD)	P-value
<u>Decreased FA in HIV+ adolescents compared to controls</u>					
Left superior cerebellar peduncle	-6; -29; -19	659	0.343 (0.063)	0.330 (0.074)	0.043
Right anterior limb of internal capsule	15; 3; 7	690	0.323 (0.023)	0.303 (0.033)	0.026
Left anterior limb of internal capsule	-13; 3; 7	699	0.351 (0.025)	0.332 (0.029)	0.023
Left posterior limb of internal capsule	-18; -6; 15	992	0.431 (0.021)	0.420 (0.031)	0.035
Right retrolenticular part of internal capsule	29; -25; 6	992	0.392 (0.024)	0.369 (0.039)	0.018
Right superior corona radiata	27; -17; 24	3018	0.374 (0.020)	0.363 (0.028)	0.028
Left sagittal stratum	-41; -29; -12	7508	0.277 (0.025)	0.260 (0.035)	0.013
Left superior longitudinal fasciculus	-39; -21; 30	1236	0.256 (0.022)	0.249 (0.030)	0.025
Right superior fronto-occipital fasciculus	21; -5; 21	1155	0.362 (0.033)	0.341 (0.041)	0.028
<u>Increased MD in HIV+ adolescents compared to controls</u>					
Left anterior limb of internal capsule	-19; 7; 13	699	7.4E-4 (5.1E-5)	7.5E-4 (4.6E-5)	0.030
Left posterior limb of internal capsule	-22; -14; 17	992	7.1E-4 (5.3E-5)	7.3E-4 (4.9E-5)	0.040

Table 3:

Comparison between HIV+ adolescents and controls on Freesurfer morphometric measures

Gray matter region	Talairach coordinates (x,y,z)	Cluster size (mm ²)	Controls mean (SD)	HIV mean (SD)	Local maxima z-value	P-value
<u>Decreased gray matter volume (mm³) in HIV+ adolescents compared to controls</u>						
Left postcentral gyrus	−55.7, −9.9, 20.7	1310.77	10273 (1559)	10156 (1406)	−3.744	0.0002
<u>Decreased cortical surface area (mm²) in HIV+ adolescents compared to controls</u>						
Left precentral gyrus	−45.6, −7.3, 24.6	1193.17	4630 (640)	4504 (495)	−4.553	<0.0001
Left pericalcarine gyrus	−12.2, −65.2, 11.3	1282.49	1215 (296)	1136 (251)	3.121	0.0018
<u>Decreased gyrification (LGI)* in HIV+ adolescents compared to controls</u>						
Left inferior temporal gyrus	−49.4, −61.4, −1.6	1602.67	182	171	−2.567	0.0103
Right lateral occipital gyrus	31.8, −79.4, 13.0	3962.45	302	208	−3.016	0.0026

LGI: Local gyrification index, see reference [21] for detail on the calculation of LGI in the brain.

Table 4:

Clinical and treatment variables, WASI IQ and mental health measures that displayed a significant association with brain imaging measurements within HIV+ adolescents and healthy controls

Whole Brain structure measurement	Neurodevelopmental variables	Pearson correlation	P-value
Gray matter volume	WASI IQ	0.198	0.008
White matter volume	WASI IQ	0.212	0.004
	Beck Self-Concept Inventory for Youth	0.155	0.038
Fractional anisotropy	Beck Anger Inventory for Youth	−0.209	0.018
	Beck Disruptive Behavior Inventory for Youth	−0.192	0.031
Mean diffusivity	Children's Motivation Scale	−0.177	0.046