Original article

Changes in brain structural volume and white matter abnormalities in young perinatally-acquired HIV infected children treated with antiretroviral therapy

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Background: Several studies have reported human immunodeficiency virus (HIV) effects on brain volume and found white matter signal abnormality (WMSA) on brain magnetic resonance imaging (MRI).
Objective: To evaluate brain volume and WMSA of antiretroviral therapy (ART)-treated perinatally-acquired HIV infected (PHIV) young children.
Methods: From November 2016 to March 2018, MRI of 19 ART-treated PHIV young children, aged 12 - 56 months, were analyzed for structural brain volume using FreeSurfer software with manual correction. WMSA were classified into 4 grades. Comparison between the brain volumes and WMSA between early and late ART-treated groups as well as changes of the brain volumes after 1-year follow-up were investigated. The correlations between MRI data and neurodevelopment were explored.
Results: Mean differences of total intracranial volume (TICV), total brain volume (TBV), and cerebral WM volume were significantly increased ($P < 0.05$) in the early ART-treated group after 1-year follow-up. WMSA was seen in most patients (n = 16, 79.0%). The positive correlations of higher severity of WMSA with very early age at start of ART and with lower early learning composite (ELC) scores were found.
Conclusion: Brain volume in the early ART-treated PHIV group tends to grow more after 1-year follow-up. A higher severity degree of WMSA was significantly associated with very early ART treatment and poorer neurodevelopment.

Keywords: Antiretroviral therapy, brain volume, children, HIV, MRI.

Human immunodeficiency virus (HIV) encephalopathy has been reported as the most common HIV-related central nervous system (CNS) disease in children and is believed to result in pediatric delayed developmental milestones. [1 - 3]

Since 2010, the World Health Organization (WHO) guidelines on HIV/AIDS treatment and prevention have recommended antiretroviral therapy (ART) for all newly diagnosed perinatally-acquired HIV infected (PHIV) children regardless of age, symptoms, and CD4 count.[4] With the widespread use of ART, the prevalence of progressive HIV encephalopathy in HIV-infected children in the United States has markedly declined from 21 - 35% to less than 2.0%.[5]

Although the incidence of HIV encephalopathy has decreased in the ART era, neurodevelopmental deficits have continued to be found in ART-treated HIV children. [6] Several studies have reported ART-treated PHIV-infected children and adolescents performed poorer neurodevelopmental outcomes than uninfected children. [7 - 9]

Moreover, a prior report investigating neuro-development in the CHER trial found that early ART-treated HIV-infected infants, for which treatment was initiated before three months of age, had better neurodevelopmental status than deferred ART infants, which provides evidence of the neurodevelopmental benefits of early ART. [10]

Imaging findings of the brain in HIV-infected children include brain atrophy, calcification, and white matter hyperintense signal. [11 - 13] A meta-analysis of

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Received: September 19, 2021
Revised: March 18, 2022
Accepted: April 20, 2022

brain structural changes following HIV infection in older children and adults (aged 5 - 85 years) has supported the findings that widespread use of ART resulted in a decline in macroscopic neurostructural changes. [6] The previous study, used 1.5T magnetic resonance imaging (MRI) scans to investigated brain abnormality and cognitive performance of ART-treated PHIV children and adolescents (aged 8 - 18 years) and compared to a healthy control group, revealed that the ART-treated PHIV group had significantly lower brain volumes and greater white matter hyperintensity compared to the control group. [14]

Our study aimed to clarify the data on brain structural volume change and white matter signal abnormality in young ART-treated PHIV children.

Materials and methods

Study population

The study population was young ART-treated PHIV children aged between 12 - 56 months, formerly enrolled in a prior research prospective study performed at King Chulalongkorn Memorial Hospital (KCMH), known as the 'DOET' study, from November 2016 to March 2018. All children were born to HIV-positive mothers, GA > 34 weeks, with no major congenital anomalies, genetic disorders, current neurologic, and no presence of active opportunistic infection.

Twenty children in the DOET study underwent MRI scans of the brain in 2 visits: the first visit when they were initially recruited for the study and the second visit about one year later.

MRI data from both visits were retrospectively analyzed for the present study. This study's exclusion criteria were poor image quality of the brain MRI and incomplete imaging data that could not process brain volume by using FreeSurfer software version 6.0.0 (http://surfer.nmr.mgh.harvard.edu).

The study protocol has been approved by our Institutional Review Board (IRB no. 604/60. Written informed consented was obtained from all subjects prior to partaking in the study.

For the informed consent process, here we use the prior informed consent from the previously mentioned research IRB no. 428/59 studied in King Chulalongkorn Memorial Hospital, which included the extension using data for further evaluated the prior performed MRI scan.

MR imaging data

1. MR imaging acquisition

The images were taken under general anesthesia using the dStream HeadSpine coil on 3.0T MRI scanner (Ingenia; Philips Healthcare, Best, the Netherlands). The average acquisition time was 51 minutes (range 40 - 60 minutes). MRI sequences acquired in this study are as follows:

3D T1-weighted images (3D T1WI): isometric with SENSE, sagittal plane, T1-weighted 3D turbo field echo (T1W 3D TFE), repetition time (TR)/echo time (TE) = 8.1 ms/3.7 ms, flip angle (FA) = 8°, voxel size = 1.00 x 1.00 x 1.00 mm^3, 160 slices with no gap. FOV = 22.4 x 27.1 cm^2. These images were reconstructed to sagittal, axial and coronal planes using 3-mm slice thickness with no slice gap.

T2-weighted images (T2WI): axial plane, TR/TE = 4000 ms/90 ms, FA = 90°, voxel size = 0.75 x 0.88 x 5.0 mm^3, 24 slices, slice thickness = 5 mm, slice gap = 0.5 mm, FOV = 22 x 26.7 cm^2.

FLAIR images: axial plane, TR/TE = 11000 ms/ 125 ms, TI = 2800ms, FA = 90°, voxel size = 0.75 x 0.88 x 5.0 mm^3, 24 slices, slice thickness = 5 mm, slice gap = 0.5 mm, FOV = 22 x 26.7 cm^2.

2. Automated data processing and volume measurement

The public domain algorithm FreeSurfer (v.6.0.0; http://surfer.nmr.mgh.harvard.edu) was used for automatic brain segmentation, including regional structures and calculated volume from the 3D T1WI.

Since the FreeSurfer software was designed for adults and children over 5 years of age, possible limitations of the software when applied to brain imaging of younger children should be considered.[15]

Despite the potential limitations, prior reports have shown that the FreeSurfer software provided an accurate method for measuring intracranial volume and total brain volume in young children aged 2 to 3 years, even in the presence of structural brain abnormalities. [16]

The brain segmentation quality was inspected visually for accuracy on each slice, and manual corrections were performed wherever a suboptimal segmentation was observed. The manual correction consisted of checking the Talairach transformation and the skull stripping, followed by inspection of the white matter and pial surfaces and the brain segmentations. Errors on the pial surface were observed mostly in parietal regions. These were corrected manually in

TKMEDIT, a tool integrated into the FreeSurfer software. In order to assess the impact of the manual corrections on the accuracy of the calculated brain volumes, the complete FreeSurfer data were compared with and without the manual correction.

3. Visual image assessment

Two reviewers evaluated the MRI images of the brains; one was a neuroradiologist with 8 years of experience, and the other one was a 2nd -year fellowship trainee in the diagnostic neuroimaging program-both were blinded to the clinical data. The imaging findings of white matter signal abnormalities (WMSA) on the MRIs were agreed upon by consensus.

WMSA was defined as any lesion that showed low signal intensity (SI) on T1WI and high SI on T2WI and FLAIR. Details of WMSA grading according to severity are as follows: Grade 1) no white matter abnormality; Grade 2) less than or equal to 5 punctate lesions; Grade 3) more than 5 punctate lesions; and Grade 4) patchy confluent lesions or extensive white matter signal change.

Clinical data

Baseline characteristics such as age, gender, date of starting treatment with ART, as well as laboratory results such as CD4 cells, CD4 percentage (CD4%), and viral load, were collected from the medical records.

Neurodevelopmental assessment

Neurodevelopmental assessments were previously performed in the DOET study using the Mullen scale of early learning (MSLE). The MSLE test assesses pediatric neurodevelopment in 5 distinct areas: gross motor, visual reception, fine motor, receptive language, and expressive language. This tool provided global early learning composite (ELC) scores that were divided into either low scores (scores < 70) or normal to high scores (scores 70 - 100).

Statistical analysis

Demographic information, neurodevelopmental scores, laboratory results, structural brain volumes, and degree of WMSA between early and late ART-treatment PHIV groups were analyzed using unpaired *t* - test. Comparison between the 1st and 2nd visits were analyzed using paired *t* - test. Pearson correlation coefficient (*R*) was used to evaluate any correlation among the imaging data, neurodevelopmental status, and baseline characteristics.

Unpaired Student *t* - test was applied to calculate the differences between FreeSurfer with and without manual corrections. Statistical analysis was performed with the SPSS, version 22.0 (IBM, Armonk, New York) computer software program. Statistical significance was defined as *P* - value less than 0.05.

Results

Data baseline characteristics and laboratory results

After excluding one child from the study, 19 young ART-treated PHIV children were recruited, including 9 males and 10 females.

We divided the 19 PHIV children into 2 groups, namely: 1) an early ART-treated PHIV group, meaning young PHIV children who initially received ART before 3 months of age, and 2) a late ART-treated PHIV group, meaning PHIV children who initially received ART after 3 months of age.

Ten patients were in the early ART-treated PHIV group, and 9 patients were in the late ART-treated PHIV group, with a significant difference in mean age of starting ART of 2 months (range 1.4 - 2.6) and 5.6 months (range 3 - 8.2), respectively ($P = 0.003$).

There was no significant difference regarding sex among early and late ART-treated PHIV groups, with 5 males (50.0%), 5 females (50.0%) and 4 males (44.4%), 5 females (55.6%) in each group, respectively ($P = 0.809$).

The mean age at the taking of MRI scans and duration of time from the beginning of ART treatment to the date of MRI for both 1st and 2nd visits were not significantly different between the early and the late ART-treated PHIV groups.

There was no significant difference in laboratory results for CD4%, CD4 cells, and HIV viral load at times close to the MRI scans between the two groups.

The details of baseline characteristics and laboratory results are described in Table 1.

Neurodevelopmental data

ELC scores from MSLE were significantly higher in the early ART-treated PHIV group than in the late ART-treated PHIV group for both visits: 82.2 (range 71.7 - 92.7) *versus* 68.9 (range 54.4 - 83.4) for the first visit ($P = 0.034$) and 88.3 (range from 76.3 - 100.3) *versus* 73.9 (range from 59.2 - 88.6) for the second visit ($P = 0.031$).

Table 1. Demographic and clinical data of early and late ART-treated PHIV children.

Demographic and clinical data	Visit 1				Visit 2			
	Total (n = 19)	Early ART (n = 10)	Late ART (n = 9)	*P*	Total (n = 19)	Early ART (n = 10)	Late ART (n = 9)	*P*
Age at MRI (mo)	30.6	29.1	32.3	0.385	41.4	39.8	43.3	0.382
Duration of time since start of ART treatment to MRI (mo)	27.0	27.1	26.8	0.933	37.7	37.8	37.7	0.991
CD4%	31.3	31.3	31.2	0.983	28.7	29.4	27.9	0.653
CD4 Cells	1980.5	2003.5	1955.0	0.903	1659.8	1656.5	1663.6	0.981
Patients with HIV viral load < or = 200 copies, n (%)	15 (78.9%)	8 (80.0%)	7 (77.8%)	0.906	15 (78.9%)	9 (90.0%)	6 (66.7%)	0.213
Patients with HIV viral load > 200 copies, n (%)	4 (21.1%)	2 (20.0%)	2 (22.2%)		4 (21.1%)	1 (10.0%)	3 (33.3%)	

The percentage of patients with low ELC and normal to high ELC scores in each group for both visits is shown in Figure 1.

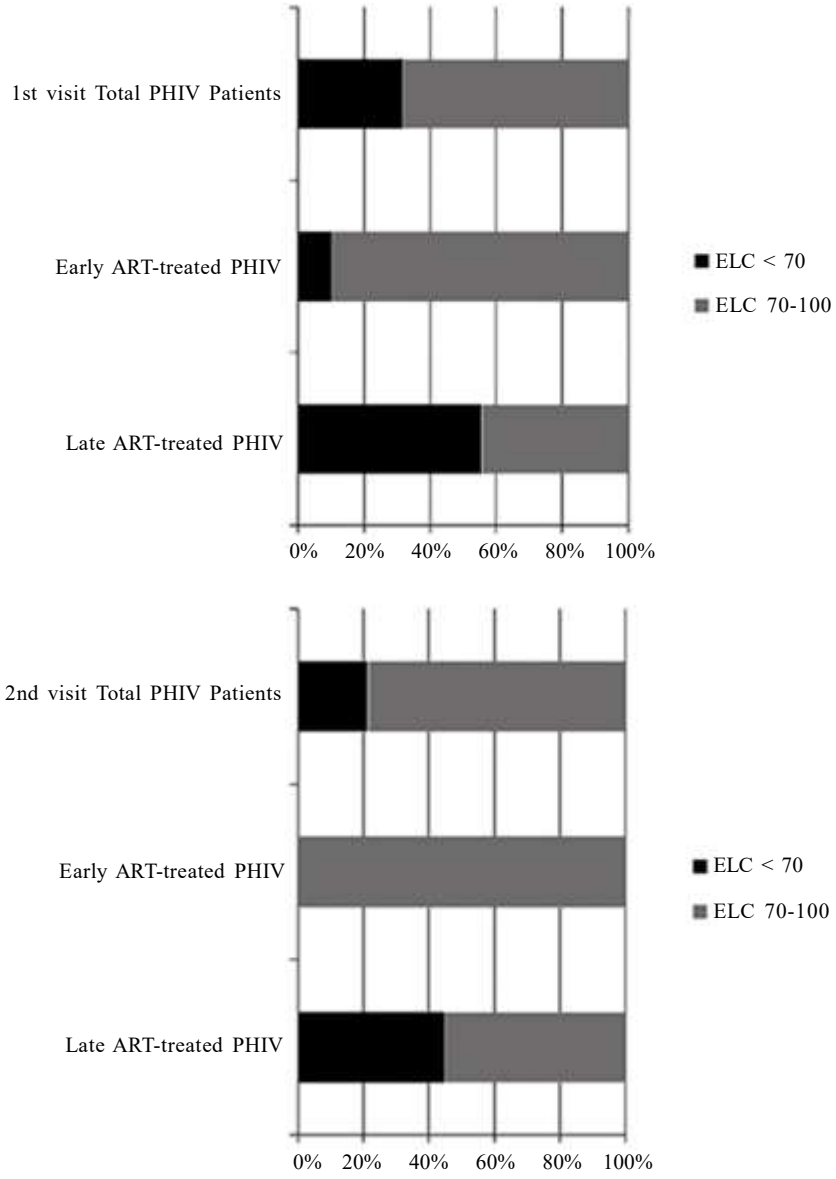

Figure 1. Percentage of ART-treated PHIV children with low ELC scores (< 70) and normal to high ELC scores (70 - 100) on 1st and 2nd visits.

Brain volume assessment

There was no significant difference in TICV, TBV, TBV/TICV, the volume of cortical gray matter, cerebral white matter, caudate nuclei, putamina, thalami, and hippocampi between the two groups on each visit.

After evaluating the difference in brain structural volumes after a 1-year follow-up, we found a significantly increased ($P < 0.05$) the mean difference in TICV, TBV, the volume of total cerebral white matter, bilateral thalami, right putamen, and left pallidum in the early ART-treated PHIV group, while the late ART-treated PHIV group showed increased volumes but no significant difference ($P > 0.05$) (Table 2).

The volumes of cortical gray matter, caudate nuclei, corpus callosum, and hippocampi showed no significance after a 1-year follow-up.

Both corrected and uncorrected values from FreeSurfer showed no statistically significant difference in brain volume of subsegmental analysis ($P > 0.05$).

White matter signal abnormality (WMSA)

In this study, WMSA were classified into 4 grades; examples of the cases are shown in Figure 2. The WMSA was found in most young ART-treated PHIV children (n = 16, 79.0%). The number of patients in each WMSA grade is described in Table 3. We found no significant difference in WMSA between the early and the late ART-treated PHIV groups. In all cases, a stable degree of WMSA in the first and second visits for MRI scans was found.

Correlations between MRI data and clinical data

There was a significant positive correlation between brain structural volumes, including TICV, TBV, total cerebral white matter volume, whole hippocampal volume, and age at MRI scans (Table 4). We also found a significant positive correlation between brain structural volumes and the time from beginning ART treatment to the time at MRI scan on the 1st visit, and a trend toward a statistically significant on the 2nd visit.

We found a significant negative correlation between WMSA severity and ELC scores, showing higher WMSA grades in lower ELC scores ($r = -0.30$, $P = 0.008$) when combined data from both visits ($r = -0.30$, $P < -0.008$).

A significant negative correlation between the severity of WMSA and age at the start of ART treatment ($r = -0.44$, $P < 0.001$), age at MRI on 1st visit and 2nd visit ($r = -0.48$, $P = 0.002$ and $r = -0.39$, $P = 0.016$, respectively), were also presented. There was no significant correlation between brain structural volume, and ELC scores, WMSA grade, CD4%, or viral load resulted close to the time of MRI scans.

Table 2. Difference in structural brain volumes after 1-year follow up.- Please use 1-digit decimal point for all data.

Structural volumes (mm^3)	Early ART treatment (n = 10)		Late ART treatment (n = 9)	
	Mean difference	*P*	Mean difference	*P*
TICV	58095.81	0.027*	60801.88	0.363
TBV	44029.02	0.021*	38588.11	0.506
Total Cortical GM	- 13613.54	0.601	9278.30	0.722
Total Cerebral WM	25098.49	0.002*	23039.92	0.369
Left Thalamus	359.29	0.020*	242.00	0.655
Right Thalamus	400.94	0.029*	283.60	0.598
Left Putamen	318.49	0.063	332.16	0.314
Right Putamen	338.31	0.041*	203.32	0.501
Left Pallidum	64.92	0.031*	- 6.18	0.958
Right Pallidum	58.71	0.081	57.81	0.634

*Significant *P* – value

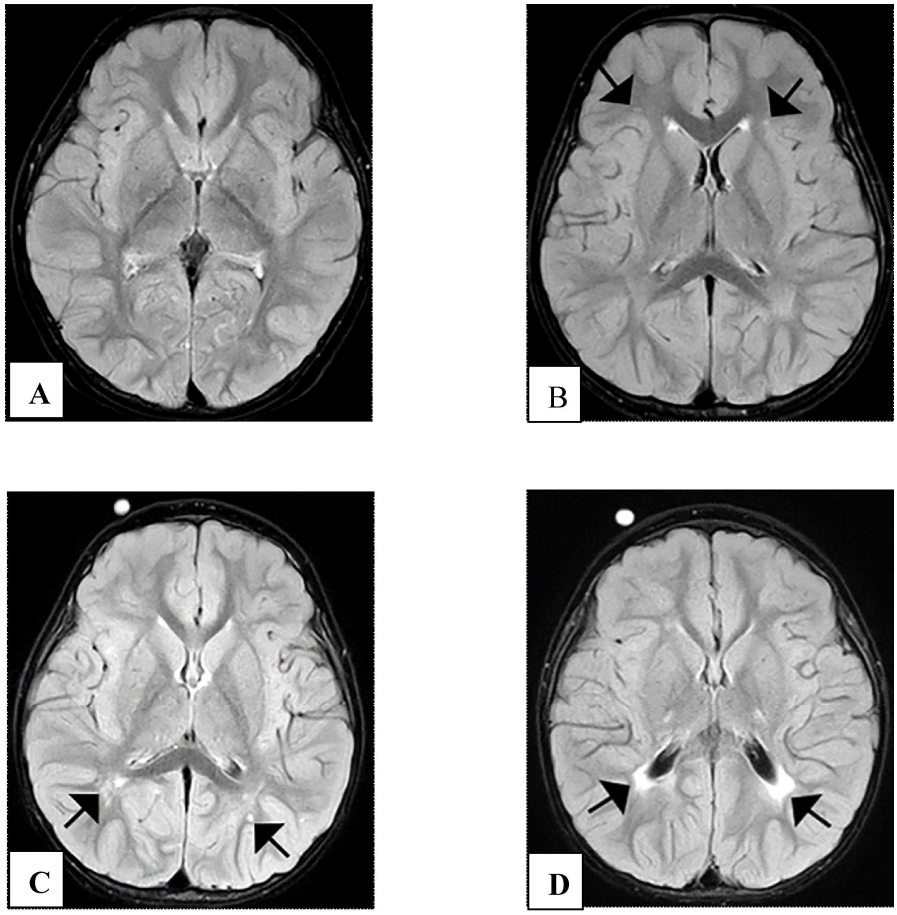

Figure 2. Grading of white matter signal abnormalities: WMSA (arrow) on T2 FLAIR, Grade 1: No WM abnormality **(A)**, Grade 2: Less than or equal to 5 punctate lesions **(B)**, Grade 3: More than 5 punctate lesions **(C)**, Grade 4: Patchy confluent lesions or extensive white matter signal change **(D)**.

Table 3. Grading of white matter signal abnormalities (WMSA) in ART-treated PHIV children. Note stable degree of lesions in all patients on 1st and 2nd visits for MRI.

Grading of WMSA	Total (n = 19)	Early treatment (n = 10)	Late treatment (n = 9)	*P*
Grade 1: Not seen	4 (21.1%)	1 (10.0%)	3 (33.3%)	0.349
Grade 2: ≤ 5 foci	10 (52.6%)	5 (50.0%)	5 (55.6%)	
Grade 3: > 5 foci	2 (10.5%)	2 (20.0%)	0 (0.0%)	
Grade 4: Patchy or extensive WM change	3 (15.8%)	2 (20.0%)	1 (11.1%)	

Table 4. Correlation of structural brain volumes, WMSA and clinical data.

Correlation	ELC scores		Age at start ART		Age at 1st MRI		Age at 2nd MRI		Time from start ART to 1st MRI		Time from start ART to 2nd MRI	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
TICV	0.07	0.662	0.17	0.312	0.55	0.014*	0.47	0.043*	0.50	0.028*	0.43	0.069
TBV	0.09	0.605	0.18	0.278	0.59	0.010*	0.53	0.020*	0.52	0.023*	0.49	0.032*
TBV/TICV	0.03	0.884	0.06	0.729	0.07	0.775	0.25	0.304	0.04	0.876	0.26	0.286
Total Cerebral WM volume	0.24	0.142	0.10	0.535	0.64	0.003*	0.56	0.012*	0.61	0.005*	0.55	0.015*
Whole hippocampal volume	-0.04	0.745	0.19	0.104	0.49	0.002*	0.37	0.022*	0.43	0.008*	0.33	0.041*
WMSA Severity	-0.30	0.008*	-0.44	<0.001*	-0.48	0.002*	-0.39	0.016*	-0.35	0.033*	-0.28	0.091

*Significant P – value < 0.05

Discussion

In this study, the neurodevelopmental status of the early ART-treated group was significantly higher than the late ART-treated group, similar to the results of an earlier report that suggested early initiated ART in PHIV infants (< 3 months old) leads to better neurodevelopmental outcomes than deferred treatment.[10] There was also provides evidence of the neurodevelopmental benefits of early ART.

We found the brain structural volumes did not significantly differ between the early and the late ART-treated group in each MRI visit. However, when comparing the change in the volumes after 1-year follow-up, the early-ART treated group was a significant increase in TICV, TBV, and total cerebral white matter volumes. This again emphasizes the benefit of the early ART treatment, which the previous report mentioned the shorter illness duration before initiating treatment can decrease HIV-associated brain atrophy.[6] The changes in the brain structural volumes of two groups in each MRI visit were not significantly different; this might be due to the structural brain changes being too subtle to be detected by a single time point volumetric method.

Significant positive correlations between TICV, TBV, total cerebral WM volume and whole hippocampal volumes and age at the time of MRI on both visits were found, as well as with the duration of time since starting treatment to the time of MRI on both visits; there were significant correlations between TBV, total cerebral WM volume and whole hippocampal volume and duration of time since starting ART treatment to time MRI and a trend toward the significance of the TICV. These results present a case for the benefit of using ART to protect against brain atrophy in young PHIV children, which is concordant with a previous study that observed a significant decrease in the incidence of brain atrophy after the ART era in HIV-positive patients [6] and more recent studies failed to demonstrated HIV effects on the structural brain of ART-treated HIV patients. [6, 17]

For WMSA, we found a higher the prevalence of white matter hyperintensity in this study (n = 16, 79.0%) than in the prior report on ART-treated PHIV young children (50.0%) [13], which may be our study performed MRI scans under general anesthesia using 3.0T MRI scanner resulted increased sensitivity to detected subtle WMSA. In our study, lesions were demonstrable in both early and late ART-treated groups without a significant difference in severity between the two groups, with appeared stable in the severity after a 1-year follow-up.

A significant correlation between greater severity of WMSA and lower neurodevelopmental scores was also observed in this study. Contrasting a prior study in South Africa [13] investigating WMSA in early ART-treated PHIV infants showed a lack of correlation between WMSA and neurodevelopmental scores, which might be the difference in the prevalence of WMSA and the neurodevelopmental assessment tools.

A positive correlation of greater severity of WMSA with a younger age of starting ART was also presented in our study, consistent with the prior

mentioned study showed a trend for an association of WMSA and longer time on ART. [13] Therefore, we suggest that an abnormality in the white matter that occurred very early could represent greater disease severity or the effects of ART. However, ART toxicity is less possibly because these lesions had not progressed after a 1-year follow-up during continued ART treatment. To further explore this problem, we suggest a long-term follow-up.

Our study has some mentionable limitations. Firstly, our population was small. Further inclusion of more subjects is recommended. Secondly, there is no single standard software used for measuring brain volume. Differences in volumetric software may give slightly different results; however, the manual correction of volumetric data in the present study was meticulously performed without a statistically significant difference between corrected and uncorrected data. Thirdly, changes in brain volume may be confounded by other uncontrollable factors, and not only by starting the time to ART treatment. Fourthly, our subjects were treated with a standard ART regimen, and therefore the results may not apply to different ART regimens. Lastly, we conducted our research with a specific young age group; the inclusion of the wider age range of ART-treated children and the normal control group may indicate a clearer and the dynamic process of brain volume changes.

Conclusion

The results show significantly increased mean differences in total intracranial volume, total brain volume, and cerebral white matter volumes in early antiretroviral therapy-treated young perinatally-acquired HIV infected children. A positive correlation higher severity of white matter signal abnormality at a very early age at the start of the treatment and poorer neurodevelopment was found.

Acknowledgements

We greatly appreciate the support of Watsamon Jantarabenjakul, MD, of the Department of Pediatrics, King Chulalongkorn Memorial Hospital who provided the clinical data for our research.

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