Neurodevelopmental and Neurobehavioral Outcomes in Early Antiretroviral Treated Young Children with Perinatally-Acquired HIV Infection (PHIV) compared to Age-matched Perinatally HIV-Exposed Uninfected Children (PHEU)

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การศึกษาพัฒนาการ และพฤติกรรมในเด็กติดเชื้อเอชไอวีที่ได้รับการรักษาโดยเร็วเทียบกับเด็กที่เกิดจากมารดาติดเชื้อเอชไอวีแต่ไม่ติดเชื้อ

น.ส.วรรษมน จันทรเบญจกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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วรรษมน จันทรเบญจกุล : การศึกษาพัฒนาการ และพฤติกรรมในเด็กติดเชื้อเอชไอวีที่ได้รับการรักษาโดยเร็วเทียบกับเด็กที่เกิดจากมารดาติดเชื้อเอชไอวีแต่ไม่ติดเชื้อ. (Neurodevelopmental and Neurobehavioral Outcomes in Early Antiretroviral Treated Young Children with Perinatally-Acquired HIV Infection (PHIV) compared to Age-matched Perinatally HIV-Exposed Uninfected Children (PHEU)) อ.ที่ปรึกษาหลัก : รศ. นพ.ชิษณุ พันธุ์เจริญ

แม้ว่าการเริ่มยาต้านไวรัสอย่างเร็วที่สุดในเด็กทารกที่ติดเชื้อเอชไอวีจากมารดาจะลดอัตราการเจ็บป่วยและอัตราการเสียชีวิตอย่างชัดเจน แต่ผลกระทบด้านพัฒนาการและพฤติกรรมยังต้องเฝ้าติดตาม ดังนั้นการศึกษานี้มีจุดประสงค์หลักเพื่อศึกษาผลทางด้านพัฒนาการและพฤติกรรมในเด็กติดเชื้อเอชไอวีที่ได้รับการรักษาภายในอายุ 12 เดือน เทียบกับเด็กที่เกิดจากมารดาที่ติดเชื้อเอชไอวีแต่ไม่ติดเชื้อ และจุดประสงค์รองเพื่อประเมินผลทางพัฒนาการและพฤติกรรมตามเวลาในการเริ่มให้ยาต้านไวรัส รวมทั้งศึกษาปัจจัยที่มีผลต่อพัฒนาการและพฤติกรรม การศึกษานี้เป็นแบบเชิงวิเคราะห์แบบไปข้างหน้าในเด็กอายุ 12 ถึง 56 เดือนที่เกิดจากมารดาติดเชื้อเอชไอวี ประเมินพัฒนาการด้วยวิธี Mullen Scales of Early Learning โดยกุมารแพทย์ผู้เชี่ยวชาญด้านพัฒนาการและพฤติกรรม และประเมินพฤติกรรมด้วยแบบสำรวจพฤติกรรมเด็ก (Child Behavioral Checklist) เมื่อเข้าร่วมโครงการและ 12 เดือนหลังจากเข้าโครงการ ซึ่งจะวินิจฉัยว่ามีปัญหาพัฒนาการล่าช้าจากผลคะแนน Early Learning Composite น้อยกว่าเท่ากับ 70 คะแนน และวินิจฉัยว่ามีปัญหาพฤติกรรม เมื่อประเมินคะแนนปัญหาภายใน (internalizing behavior) ปัญหาภายนอก (externalizing behavior) และปัญหาภาพรวม (total behavior) มากกว่าเท่ากับ 64 คะแนน ในการวิเคราะห์ใช้การวิเคราะห์แบบถดถอยในการเปรียบเทียบอัตราการเกิดปัญหาพัฒนาการล่าช้าระหว่างกลุ่ม และวิเคราะห์ปัจจัยเสี่ยงโดยใช้สมการการประมาณค่านัยทั่วไป (Generalized estimating equations) ผลการศึกษาระหว่างปี 2559 ถึง 2560 มีเด็กติดเชื้อเอชไอวี 50 คน และเด็กไม่ติดเชื้อที่เกิดจากมารดาติดเชื้อ 100 คนเข้าร่วมการศึกษา มีค่ามัธยฐานของอายุคือ 28 เดือนในการประเมินครั้งแรก และค่ามัธยฐานของอายุที่เริ่มการรักษาเอชไอวี 2.9 เดือน พบว่าเด็กติดเชื้อเอชไอวีมีน้ำหนัก ส่วนสูง เส้นรอบศีรษะเทียบตามอายุ น้อยกว่าเด็กไม่ติดเชื้อที่เกิดจากมารดาติดเชื้ออย่างมีนัยสำคัญ ($p < 0.05$) พบอัตราการเกิดพัฒนาการล่าช้า ร้อยละ 32 (95% CI 20 - 47) ในกลุ่มติดเชื้อเอชไอวีที่รับการรักษาภายในอายุ 12 เดือน และ ร้อยละ 18 (95% CI 11 - 27) ในกลุ่มไม่ติดเชื้อเอชไอวีแต่เกิดมารดาติดเชื้อ โดยไม่แตกต่างกันตามนัยสำคัญทางสถิติ (OR 2.14; 95%CI 0.97 – 4.70, $p = 0.06$) แต่พบว่าเด็กเอชไอวีที่รับการรักษาหลังอายุ 3 เดือนนั้นมีอัตราการเกิดพัฒนาการล่าช้าสูงกว่าเด็กไม่ติดเชื้อที่เกิดจากมารดาติดเชื้ออย่างมีนัยสำคัญทางสถิติ ($p = 0.01$) ปัจจัยที่เกี่ยวข้องกับการเกิดพัฒนาการล่าช้าคือ เพศชาย (aOR 4.65, 95% CI 1.09 - 19.85, $p = 0.04$) และปัจจัยที่ทำให้คะแนนด้านพัฒนาการลดลงคือ การไม่ได้เข้าร่วมโรงเรียนก่อนวัยเรียน (adjusted coefficient -2.83, 95% CI -5.05 ถึง -0.60) และรายได้ต่อครอบครัวน้อยกว่า 10,000 บาทต่อเดือน (adjusted coefficient -3.16; 95% CI -5.89 to 0.44) ส่วนอัตราการเกิดปัญหาพฤติกรรมภายใน (internalizing behavior) ภายนอก (externalizing behavior) และภาพรวม (total behavior) ไม่มีความแตกต่างกันระหว่างเด็กติดเชื้อเอชไอวีและเด็กไม่ติดเชื้อเอชไอวี ($p > 0.05$) ปัจจัยที่มีผลต่อการเกิดปัญหาพฤติกรรมคือ ภาวะซึมเศร้าของผู้เลี้ยงดู และแนวโน้มการเลี้ยงดู โดยสรุปแม้ว่าเด็กติดเชื้อเอชไอวีก่อนวัยเรียนที่ได้รับการรักษาก่อนอายุ 12 เดือนมีปัญหาพัฒนาการล่าช้าและปัญหาพฤติกรรมไม่แตกต่างกับเด็กไม่ติดเชื้อเอชไอวีที่เกิดจากมารดาติดเชื้อเอชไอวี แต่เด็กติดเชื้อที่ได้รับการรักษาหลังอายุ 3 เดือนมีแนวโน้มที่มีปัญหาพัฒนาการล่าช้ามากกว่า โดยปัจจัยทางสังคมเป็นปัจจัยหลักที่ส่งผลต่อพัฒนาการและพฤติกรรม ดังนั้นเด็กติดเชื้อเอชไอวีควรได้รับการเริ่มการรักษาอย่างเร็วที่สุด และเด็กกลุ่มนี้ควรได้รับการติดตามและกระตุ้นพัฒนาการอย่างเพื่อให้เติบโตอย่างเหมาะสม

สาขาวิชา	เวชศาสตร์คลินิก	ลายมือชื่อนิสิต .....
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Watsamon Jantarabenjakul : Neurodevelopmental and Neurobehavioral Outcomes in Early Antiretroviral Treated Young Children with Perinatally-Acquired HIV Infection (PHIV) compared to Age-matched Perinatally HIV-Exposed Uninfected Children (PHEU). Advisor: Assoc. Prof. CHITSANU PANCHAROEN, M.D.

Introduction: Although early initiation of antiretroviral therapy (ART) in perinatally HIV infected (PHIV) infants significantly reduces morbidity and mortality, neurodevelopmental and neurobehavioral problems are still issues of concern. *Objectives:* This study aims primarily to compare neurodevelopmental outcomes and neurobehavioral outcomes between PHIV children who initiated ART within 12 months of life and perinatally HIV-exposed uninfected (PHEU) children. The secondary aims are to assess the outcomes by timing of ART initiation and to delineate factors and predictors associated with neurodevelopmental and neurobehavioral outcomes. *Methods:* This study was a prospective observational study which enrolled PHIV and PHEU children aged 12-56 months. Neurodevelopmental outcomes were assessed with the Mullen Scales of Early Learning (MSEL) and neurobehavioral outcomes were assessed with Child Behavioral Checklist (CBCL) at enrollment and at 12-month follow up visit. Global Developmental Impairment (GDI) was defined as Early Learning Composite (ELC) $\leq$ 70 on the MSEL. Logistic regression was used to compare prevalence of GDI. Clinical range behavioral problems was defined as T-score of internalizing, externalizing and total problems $\geq$ 64. Factor associated with GDI and behavioral problems were analyzed with generalized estimating equations (GEE) logistic regression model whiles predictors of changing ELC scores and behavioral scores were analyzed with GEE linear regression model. *Results:* From 2016 to 2017, 50 PHIV and 100 PHEU children were enrolled. Median (IQR) age at first assessment was 28 (19-41) months. Median (IQR) age of ART initiation was 2.9 (1.0 -5.1) months old. PHIV children had lower age-relevant Z scores for weight, height, and head circumference compared to the PHEU group (p <0.05). The prevalence of overall GDI was 32% (95% CI 20 - 47) in PHIV children and 18% (95% CI 11 - 27) in PHEU with OR 2.14 (95%CI 0.97 – 4.70, p = 0.06). There was significantly higher rate of GDI in PHIV children initiated ART after 3 month-old when compared to PHEU children (p = 0.01). Only factor associated with GDI was boy (adjusted odd ratio 4.65, 95%CI 1.09 to 19.85; p = 0.04). Predictors of changing ELC scores included no nursery school attendance (adjusted coefficient -2.83, 95% CI -5.05 to -0.60) and income less than 10,000 Baht/month (adjusted coefficient -3.16; 95% CI -5.89 to 0.44). The prevalence of internalizing, externalizing and total problem were not different between PHIV and PHEU children (p > 0.05). Caregiver depression and parenting style were risk factors for behavioral problems. *Conclusion:* Even the rate of GDI in preschool PHIV children who initiated ART within 12 months old was not different when compare to PHEU children, PHIV children who initiated ART after 3 months old tend to had higher rate of GDI. The behavioral problems were not different between groups. Psychosocial factors mainly contributed to these outcomes. Therefore, early ART initiation should be emphasized and these children should have appropriated monitoring and early stimulation to survive and thrive.

Field of Study:	Clinical Sciences	Student's Signature .....
Academic Year:	2018	Advisor's Signature .....

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3TC	Lamivudine
ADHD	Attention deficit hyperactivity disorder
AIDS	Acquired immunodeficiency syndrome
ANOVA	Analysis of variance
ART	Antiretroviral therapy
ARV	Antiretroiviral medicines
AZT	Zidovidine
BASC-2	Behavior Assessment System for Children
BINS	Bayley Infant Neurodevelopmental Screener
BSID	Bayley Scales of Infant and Toddler Development
CASI-4R	Child and Adolescent Symptoms Inventory-4R
CBCL	Child Behavior Checklist
CDC	Center for Disease Control
CES-DC	Center for Epidemiologic Studies Depression Scale for Children
CHER	Children with HIV Early Antiretroviral Therapy Trial
CNS	Central nervous system
coef	Coefficient
CPRS	Conners' Parenting Rating Scale
CRFs	Case record forms
CT	Computer tomography
d4T	Stavudine
ddI	Didanosine
DDST	Denver Developmental Screening Tool
DISC-IV	Diagnostic Interview Schedule for Children
DSM	Diagnostic and Statistical Manual of Mental Disorders
DTI	Diffusion Tensor Imaging
ECD	Early childhood development
ELC	Early Learning Composite
GA	Gestational age
GDI	Global developmental impairment
GEE	Generalized estimating equations
GMDQ	Gross motor developmental quotient
GMDS	Griffiths Mental Development Scales
HAZ	Height for age Z-score
Hb	Hemoglobin
HCAZ	Head circumference for age Z-score
HIV	Human immunodeficiency virus
HUU	HIV-unexposed uninfected
IQ	Intelligent quotient
IQR	Interquartile range
LPV/r	Lopinavir/ritonavir
MDI	Mental development index
MRI	Magnetic resonance imaging

MSCA	McCarthy Scale of Childhoods Abilities
MSEL	Mullen Scales of Early Learning test
MUACZ	Mid upper arm circumference for age Z-score
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
PCR	Polymerase chain reaction
PDI	Psychomotor development index
PHEU	Perinatally HIV exposed uninfected
PHIV	Perinatally-acquired HIV infected
PHQ-9	Patient Healthy Questionnaire-9
PI	Protease inhibitors
PMTCT	Prevention mother to child transmission
PREDICT	Pediatric Randomized to Early vs Deferred ART Initiation in Cambodia and Thailand
PSDQ	Parenting Styles and Dimensions Questionnaire
PVL	Periventricular leukomalacia
SB-5	Stanford Binet Intelligence Scales
SD	Standard deviation
TDF	Tenofovir disproxil fumarate
UNAIDS	The Joint United Nations Programme on HIV and AIDS
USA	United States of America
WAZ	Weight for age Z-score
WHO	World Health Organization
WISC	Wechsler Intelligence Scale for Children
WPPSI	Wechsler Preschool and Primary Scale of Intelligence
YI-4	Youth self-report Inventory

CHAPTER 1

Introduction

Thailand is one of the highest HIV prevalence in Asia and the Pacific, accounting for 9% of the region's total population of people living with HIV. In 2017, there were estimated 440,000 people living with HIV which 3400 were children and 94,000 were HIV-exposed uninfected children in Thailand [1]. However, Thailand is the first country in Asian to effectively eliminate mother to child transmission with a transmission rate of less than 2% in 2015 [2]. In addition, all newly diagnosed perinatally-acquired HIV infected (PHIV) children were recommended to receive an antiretroviral therapy (ART) regardless of symptoms and CD4+ T cell count since 2010 [3].

PHIV children had significantly higher of morbidity and mortality compared to perinatally HIV-exposed uninfected (PHEU) children [4, 5]. These PHIV children confront with the variety of physical and psychological effects from HIV infection which may alter by ART [6]. The Children with HIV Early Antiretroviral Therapy (CHER) Trial in South Africa demonstrated a mortality rate reduced by 75% if ART is initiated in infancy period [7]. In addition, those treated early had better neurodevelopmental profile than deferred ART infants [8]. Conversely, the Pediatric Randomized to Early vs. Deferred ART Initiation in Cambodia and Thailand (PREDICT) study which evaluated in older children treated after 1 year old, discovered that the a mortality rate was not different between early and deferred group as well as neurodevelopmental scores were significantly lower in PHIV children than PHEU children regardless of timing of ART initiation after 1 year old [9]. Therefore, only early ART initiation in infancy period will be the great opportunity to prevent poor neurodevelopmental outcome. The pathogenesis of global developmental impairment (GDI) in PHIV children may include direct effects of HIV on the central nervous system (CNS) and indirect effects from systemic illness, nutritional status, and psychosocial factors [10-14]. In resource-rich settings, early treated PHIV children without history of AIDS-defined symptoms often demonstrate near normal developmental functioning in all domains and are comparable to PHEU children [15-18]. However, some studies reveal subtle but significant differences in executive function, language skills, and memory in PHIV children as they develop [18-20]. There is likely a limited window of opportunity to mitigate HIV insults to the brain. Nonetheless, ART initiated before the age of 1 year may prevent or minimize neurological and neurodevelopmental impairment in PHIV individuals [9]. Besides ART, several interventions

could be used to improve neurodevelopmental and neurobehavioral outcomes in children such as physical therapy, occupational therapy, speech-language therapy, and computerized cognitive rehabilitation therapy [21, 22]. Therefore, it is important to early assess neurodevelopmental and neurobehavioral outcomes in young children and monitor the changes over time.

Problem statement

Neurodevelopmental outcome and neurobehavioral outcomes of PHIV children who initiated ART within 12 months old have not been studied in Thailand. As the Thailand's health policy recommends ART in all infants in order to minimize morbidity and mortality in PHIV children, these additional data about neurodevelopmental outcome and neurobehavioral outcome will be support the benefit about quality of life for those children.

Aim of this study

This study primarily aims to compare neurodevelopmental outcomes and neurobehavioral outcomes between PHIV children and PHEU children.

Primary Objective:

- To compare age-matched PHIV who initiated ART within 12 months of life and PHEU young children for neurodevelopmental and neurobehavioral outcomes

Secondary Objectives:

- To assess the neurodevelopmental and neurobehavioral outcomes by timing of ART initiation before and after 3 months of age
- To assess factors associated with neurodevelopmental and neurobehavioral outcomes
- To evaluate neuroimaging signatures in PHIV children

We hypothesized that the GDI rate and total behavioral problem rate would not be significantly different between PHIV children initiated by 12 months of age and PHEU children. Moreover, the GDI rate would be lower in PHIV children initiated by 3 months than those initiated later.

Significance of the study

Being developmental impairment or behavioral problems hugely affects the quality of life of the children themselves, and also their families for example their academic opportunity and achievement as well as their future employments and earnings. Early identification and treatment will maximize their developmental potentials and some children may catch up to other over time. In the past, the primary goal for treating PHIV children was to increase their survival rate, however, in recent day, the opportunity to thrive has been include. Thus, the

information from this study which aim to evaluate the impact of early ART therapy on the children's neurodevelopmental and neurobehavioral outcomes, it will be emphasize the importance of early ART therapy. In addition, it will be to determine strategies to prevent and mitigate neurodevelopmental and neurobehavioral impairment. There has been limited researches in resource-limiting countries particularly in the middle-income countries as Thailand. There are several factors that contribute to neurodevelopmental and neurobehavioral outcomes in PHIV and PHEU children. The results from other resource-limiting countries and resource-rich countries might not be fully explain in our circumference. Besides, there is limited available research that collect the confounding factors that affect neurodevelopmental and neurobehavioral outcomes. PHEU is selected as the comparison groups to control for socioeconomic confounders as PHEU children typically live in similar demographic and socioeconomic circumstances to children with PHIV. Besides, this study will assess neuroanatomical outcome in PHIV children. Magnetic resonance imaging (MRI) brain imaging comprehensively evaluates neurodevelopmental stages and trajectories but alterations on MRI in early-treated children is limited.

This study will fill the knowledge gap on the neurodevelopmental, neurobehavioral and neuroanatomical outcomes in early treated ART young children in resource limiting setting as Thailand as model of care for comprehensive care of PHIV and PHEU children.

CHAPTER 2

Literature Review

This chapter will review epidemiology of children born to HIV-infected mothers, children's development and early childhood development. Then this chapter will focus on neurodevelopment and neurobehavioral outcomes in PHIV and PHEU children as well as the effect of ART. The review includes the studies in resource rich countries and resource limited countries. The last part will review on neuroanatomical outcomes.

2.1 Epidemiology of children born to HIV-infected mothers

Global data

From the Joint United Nations Programme on HIV and AIDS (UNAIDS) Fact Sheet in July 2018, 36.9 million (31.1 – 43.9 million) people globally were living with HIV in 2017, of which 1.8 million (1.3 – 2.4 million) were children under 15 years old. Since 2010, new HIV infections among children have declined by 35% from 270,000 (170,000 – 400,000) in 2010 to 180,000 (110,000-260,000) in 2017 (Figure 1). Fifty two percent of children living with HIV had accessed ART and 80% of pregnant women living with HIV had accessed to ART to prevent transmission of HIV to their babies. PHEU have increased by 103% from 7.0 million in 2000 to 14.8 million in 2017 [23].

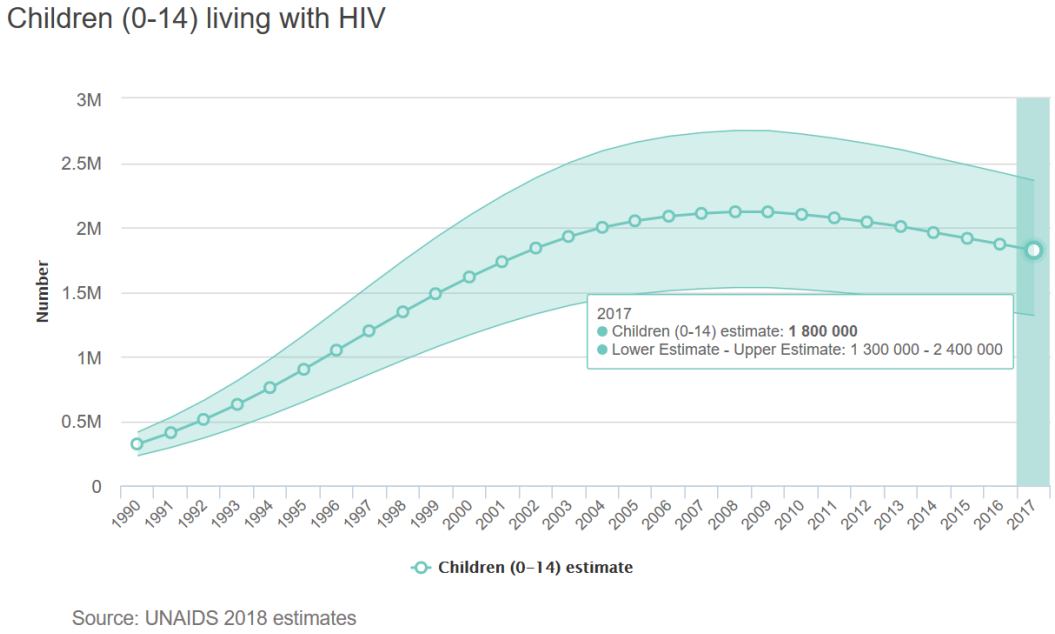

Figure 1. Global data of estimated children aged 0 to 14 years living with HIV

Thailand data

Thailand is one of the highest HIV prevalence in Asia and the Pacific, accounting for 9% of the region's total population of people living with HIV. In past two decades, the new HIV infection in Thailand has reduced by successful efforts from multiple collaborations (Figure 2). In 2017, there were estimated 440,000 people (390,000-510,000) living with HIV and 3400 (2,900-4,100) were children. Eighty percent of infants born to HIV-positive women were tested for HIV within 2 months of age. Early diagnosis and early treatment in children are implemented from the prevention mother to child transmission (PMTCT) program. Infant testing is depend on the risk of transmission. Children in high risk group whose mother had on ART less than 12 weeks or known HIV RNA > 1000 copies/ml before delivery were tested by polymerase chain reaction (PCR) at birth, 1, 2 and 4 months old. While children in low risk group whose mother had on ART more than 12 weeks or suppressive status before delivery were tested by PCR at 1 month and 4 months olds [24]. Free infant formula also provided by national PMTCT program. Thus, Thailand is the first country in Asian to effectively eliminate mother to child transmission with a transmission rate of less than 2% in 2015 [2]. The Thai government provides ART for free as part of the country's universal health insurance scheme, thus 84% (72 - >95%) children were on antiretroviral treatment in 2017 [1].

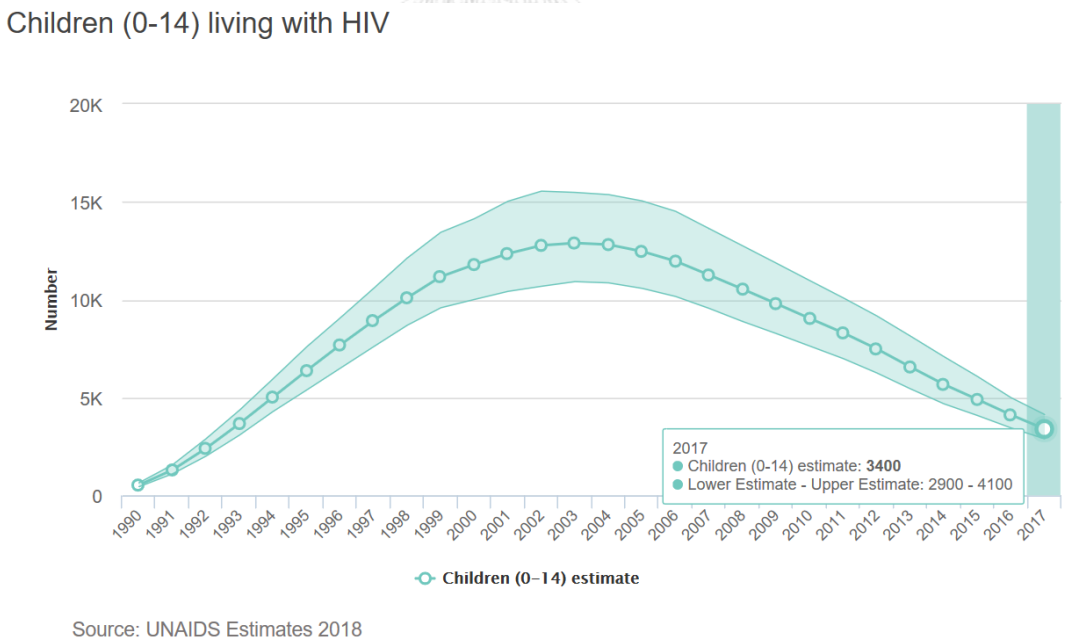

Figure 2. Estimated children aged 0 to 14 years living with HIV in Thailand

2.2 Child development and early childhood development

Child development contributes by genetic inheritance, biological factors and psychosocial factors [11]. The first few year of life are particularly importance because vital development occurs in all domains (Figure 3) [25]. The brain develops rapidly through neurogenesis, axonal and dendritic growth, synaptogenesis, cell death, synaptic pruning, myelination and gliogenesis. These events happen at difference time and build on each other. In addition, brain development is modified by the environment and can modified by earlier interventions that possible to remarkable recovery.

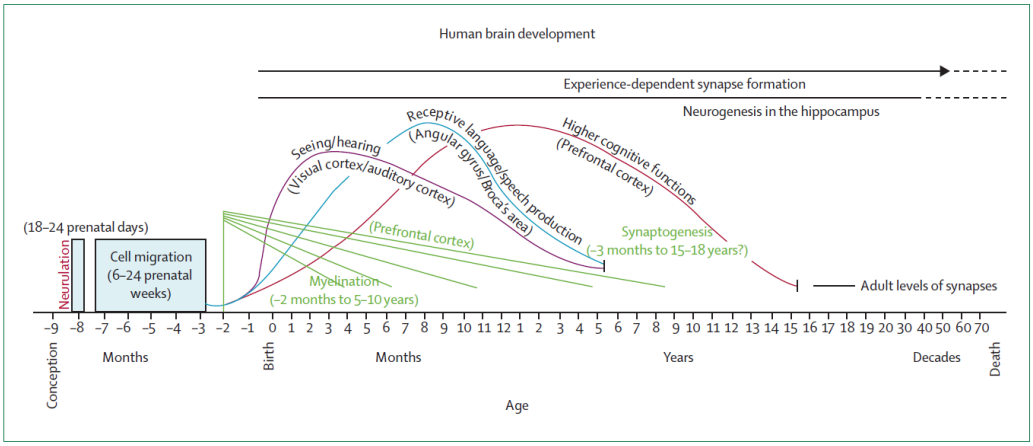

Figure 3. Human brain development
(Adapted from Thompson RA, Nelson DA. Developmental science and the media 2001;1:5-15[25])

Early childhood development (ECD) is defined that children's cognitive, physical, language, motor and social and emotional development between conception and age 8 [26]. However, the period from pregnancy to age 3 is the scientifically proven that is a very sensitive period for brain development and the most susceptible to environment influence [14, 27, 28]. This period lays the foundation for health, well-being, learning and productivity throughout a person's whole life. World Health Organization (WHO) and partners have developed the nurturing care framework to provide a roadmap for ensuring that children can survive and thrive [26].

Risk factor for child development

The factors threatens child development consists with biological risk factors and psychosocial risk factors [13].

1. Biological risk factors

Biological risk factors include nutrition, infectious diseases and environmental exposures. A comprehensive review indicated that nutrition deficiency particularly intrauterine growth restriction, stunting, wasting, iodine deficiency, iron deficiency and other nutritional factors associated with poor developmental outcomes. Besides, infectious diseases can affect development through direct and indirect pathways. CNS infection had direct effect to neurological impairment. However, other infections (e.g. diarrhea, otitis media and malaria) could effect on nutritional status and decreased physical activity and play. Environment exposure consists of lead exposure, contaminated water have been reported in negative outcomes.

2. Psychosocial risk factors

Psychosocial risk factors that related to children development and behavioral outcome are parenting factors and contextual risk factors. All studies involved children who were given additional cognitive stimulation or learning opportunities reported higher developmental than non-stimulated controls. In addition, caregivers who have high sensitivity and responsivity were associated with higher cognitive outcome and less behavior problem. Other contextual risk factors are caregiver depression, exposure to violence and socioeconomic setting especially poverty (Figure 4).

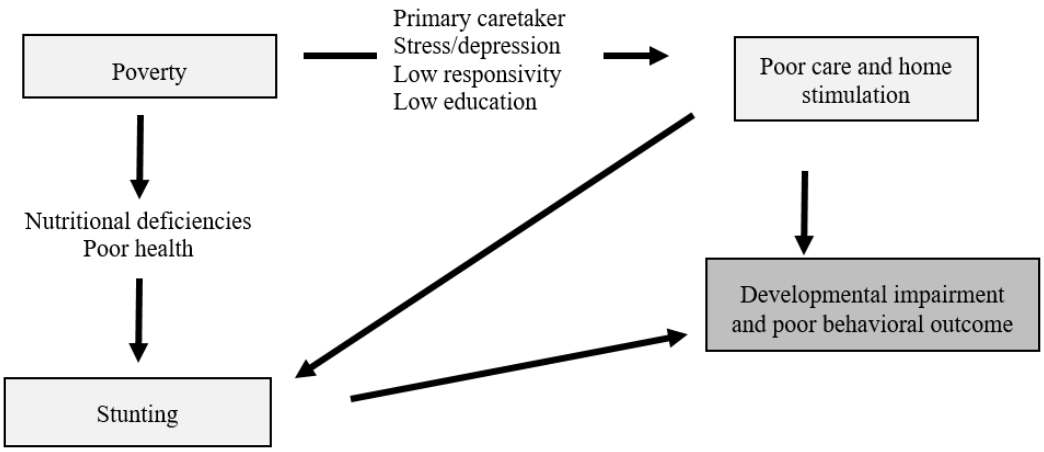

Figure 4. Hypothesis of factors associated developmental impairment and poor behavioral outcomes

(Adapted from Grantham-McGregor et al. Development potential in the first 5 years for children in developing countries. Lancet 2007; 369:60-70[11])

2.3 HIV infection and impacts on neurodevelopmental and neurobehavioral outcomes in perinatally HIV-infected children

HIV infection has the impact on physical and psychological outcome in PHIV children [6]. ART dramatically changed the course of HIV, significantly decrease HIV-associated morbidity and mortality, improve growth parameters and reduce the incidence of opportunistic infection and hospital admission [29]. Nevertheless, there are still some concern in neurodevelopmental and neurobehavioral outcomes in PHIV children.

HIV is a neurotropic and neurotoxic virus that enters the central nervous system early after infection via infected monocyte, macrophage and CD4+ T lymphocytes as the direct effect. The HIV-1 infected macrophages, microglia and astrocytes lead to a cascade of neurotoxic events and may be associated with neuronal damage as the indirect effect [12, 30, 31]. This damage creates a variety of CNS abnormalities which are known as HIV encephalopathy. In the developing brains of young children with PHIV, these effects may be more pronounced compared to infection in adults due to more susceptibility to perturbations in astrocyte function [31]. Recognition of the impact of PHIV during sensitive periods of brain development provides an opportunity to intervene early to prevent later neurological and ND impairment.

Before ART era, neurodevelopmental complication of HIV infection have been well-known and cause of significant morbidity and mortality. According to the Center for Disease Control (CDC), HIV encephalopathy must include criteria in at least one of the following areas for at least 2 months in the absence of a concurrent illness: a) failure to attain or loss of developmental milestones or loss of intellectual ability, b) impaired brain growth or acquired microcephaly and/or c) acquired symmetric motor deficit [32]. The neuroradiological hallmarks are cortical atrophy and basal ganglia calcification on computer tomography (CT) scans, as well as white matter lesions and central atrophy on MRI. After ART era, rate of HIV encephalopathy has significantly declined [33-35]. In US-based Pediatric AIDS Clinical Trial Group cohort, incidence of HIV encephalopathy decreased 10-fold beginning in 1996 with stable incidence rates since 2002 at around 2 cases per 1000 person-years [35]. In South Africa, HIV encephalopathy has been reported even in children who commence ART before age 12 months [36].

PHIV on ART may experience less severe neurodevelopmental complications, including deficits in global development, gross motor, fine motor, language and speech. However, the effect of ART on neurodevelopmental outcomes have been reported in various result due to depend on age range of children, method to access, setting of co-morbidity and

socioeconomic status. As we mentioned above, multiple risks which including poverty, malnutrition, poor health and unstimulating home environment affect neurodevelopmental and neurobehavioral outcomes. Several potential mechanism for developmental impairment have been proposed, including (1) irreversible pre-ART neuronal injury, (2) neuronal injury from inflammatory response and neurotoxic viral proteins; (3) poor CNS penetration of ART resulting in ongoing CNS viral replication and (4) neurotoxic effects of ART [37-39].

2.4 Clinical studies on neurodevelopmental, neurobehavioral and neuroanatomical outcomes

This section will review the clinical studies on neurodevelopmental and neurobehavioral outcomes in PHIV and PHEU children from resource rich and resource limiting settings as well as neuroanatomical outcome in PHIV children. Due to various developmental and behavior assessment and cut-off range, this section will also review assessment tools to improve understanding about the results.

2.4.1 Neurodevelopmental outcomes in PHIV and PHEU children

Studies of neurodevelopment in PHIV children evaluated by various standardized tool. There are some frequently used standardized tests for young children (Table 1). [40]

Table 1. Frequently used standardized tests of development and cognition in young children

Test	Age	Assessment
Bayley Infant Neurodevelopmental Screener (BINS)	3 to 24 months	Basic neurological functions/intactness, receptive function, expressive functions, cognitive process
Bayley Scales of Infant and Toddler Development, (BSID)	1 to 42 months	5 subscales: motor, language, cognitive, social-emotional, adaptive behavior Overall: mental development index (MDI) and psychomotor development index (PDI)
Mullen Scales of Early Learning (MSEL)	Birth to 68 months	5 subscales: gross Motor, visual reception, fine motor, expressive language, and receptive language Overall: early learning composite (ELC) score

Test	Age	Assessment
Wechsler Preschool and Primary Scale of Intelligence, 3rd edition (WPPSI)	2 years 6 months to 7 years 3 months	4 composite scores: full scale intelligent quotient (IQ), verbal IQ, performance IQ, processing speed quotient
Wechsler Intelligence Scale for Children (WISC)	6 to 17 years	Full scale IQ, Index scores, subtest scaled scores
Griffiths Mental Development Scales (GMDS)	Birth to 2 years	5 subscales: locomotor, personal-social, hearing and language, eye & hand coordination and performance Overall as general score
Griffiths Mental Development Scales, Extended Revised (GMDS, Extended Rev)	2 to 8 years	6 subscales: locomotor, personal-social, language, eye and hand co-ordination, performance and practical reasoning Overall as general score
Stanford Binet Intelligence Scales (SB)	2 to 85 years	5 subscales: knowledge, quantitative reasoning, visual-spatial processing, working memory and fluid reasoning Overall as intelligence quotient
McCarthy Scale of Childhood Abilities (MSCA)	2.5 to 8.5 years	6 subscales: verbal, perceptual-performance, quantitative, composite (general cognitive), memory and motor
Denver Developmental Screening Tool (DDST)	Birth to 6 years	4 domains: gross motor, find motor and adaptive, language and personal-social
Berry-Buktenica Development Test of Visual-Motor Integration (Berry VMI)	2 to 18 years	Visual-motor

Neurodevelopmental outcomes of PHIV and PHEU children in resource rich settings and resource limiting settings are shown in Table 2 and Table 3. The neurodevelopment of PHIV tended to poorer than PHEU children [18]. The rate of severe GDI varied across studies. Most studies reported rate of GDI was between 21 to 35% [41]. There are few studies that documented time of initiation ART. However, CHER study reported that PHIV infants initiated ART within 3 months have improved neurodevelopmental outcome [8].

Abnormalities in the development of motor and language skills are prominent in PHIV children. Delay in language skills has been shown in PHEU children in some but not all studies [18, 42, 43]. The factors affected neurodevelopment independent of HIV status were prematurity, low birth weight, low weight for height score and low maternal education [18].

Table 2. Clinical studies on neurodevelopmental outcomes in resource rich settings

Study	Tools	Developmental outcomes	Additional outcome measure
Pollack et al[44], 1996, USA 18 PHIV, 29 PHEU, 18 HUU Age 0 to 2 years ART: AZT monotherapy	BSID	Mean MDI and PDI scores of PHIV was comparable at 4 month but lower versus PHEU and PHUU at 12, 18, 24 month. PHIV had MDI score < 1 SD x 10.5 times, PDI score < 1 SD x 4.4 times below population mean	Growth failure predict of MDI and PDI
Raskino et al[45], 1999, USA 831 PHIV Age 2 months to 18 years ART: monotherapy or combination of AZT and ddI therapy	BSID MSCA WISC	23% PHIV had cognitive score < 70 (2SD) at entry and overall developmental results improved in combination AZT and ddI therapy	
Chase et al[46], 2000, USA 114 PHIV and 481 PHEU Age birth to 30 months ART: not documented	BSID	PHIV had significantly lower MDI and PDI score than PHEU	Prematurity and maternal education were associated with poor outcomes
Smith et al[47], 2000, USA, 114 PHIV Age 0 to 3 years ART: 33 infants received ART treatment	BSID	Early infection children (within 48 hours documented HIV culture positive) were significantly lower score than late infection children.	

Study	Tools	Developmental outcomes	Additional outcome measure
Blanchette et al[48], 2001, Canada, 25 PHIV, 25 PHEU, Age 6 to 37 months ART: 3 PI-based regimen and other combination NRTI	BSID	Mean MDI and PDI lower in PHIV infected group when compare to PHEU ($p<0.001$).	
Blanchette et al[49], 2002, Canada, 14 PHIV, 11 PHEU, Age 5 to 12 years ART: on ARV with/without PI	WISC	PHIV school age had normal cognitive development and subtle motor impairment. No different outcome between PHIV and PHEU was reported.	No significant association existed between CD4+ T cell and outcome.
Llorente et al[50], 2003, USA, 157 PHIV Age 0 to 36 months ART: Either AZT monotherapy, dual therapy without PI or triple drug	BSID	Children with worse diseases had score > 2 SDs below population mean. Increased risk of mortality per 10 point decrement in initial MDI and PDI scores versus population mean, even after adjusted for treatment	Low birth weight and prematurity were associated with poor outcomes
Jeremy et al[19],2005, USA 489 PHIV Age 4 months to 18 years ART: dual or triple drug	BSID WPPSI	PHIV had significant poorer neurodevelopmental outcome at baseline as compare with established norm for age.	PHIV with higher viral load had poorer cognitive. After 48 weeks of treatment, there was significant improvement in only vocabulary score.

Study	Tools	Developmental outcomes	Additional outcome measure
Foster et al[51], 2006, UK 62 PHIV Age 7 to 33 months 54 infants received HAART	BSID, GMDS	PHIV had lower MDI and PDI score than population mean especially in expressive and motor score	PHIV with severe disease and immune compromised had significantly more abnormal neurological signs and developmental delays than children presenting with milder symptom.
Nozyce et al[52],2006, USA 274 PHIV Age 3 to17 years with ART	BSID WPPSI WISC	PHIV had lower score than established norms.	
Smith R et al[16], 2006, USA 117 PHIV, 422 PHEU Age 3 to 7 years ART: 33% monotherapy, 17% HAART, 10% other multidrug	MSCA	Only children with class C diseases performed poorly. All other scores comparable with norms	Lower mean scores associate with viral load, primary language and maternal education
Lindsey et al[53], 2007, USA 145 PHIV 1059 PHEU Age 0 to 2 years ART: 133 PHIV on ART (dual or triple therapy +/- PI)	BSID	PHIV children had lower MDI and PDI score than PHEU. Limited improvement in MDI and PDI with additional PI-based ART)	Low birth weight and prematurity were associated with poor outcomes

Study	Tools	Developmental outcomes	Additional outcome measure
Caplo et al[54], 2008, Italy 15 PHIV, 14 PHEU, Age 2 weeks to 36 months ART: on ART	DDST	62.5% PHIV vs 14 % PHEU had abnormal score	Treatment before 12 weeks of age improved scores versus those treated later
Cohen et al[55], 2015, The Netherland 35 PHIV and 37 healthy children Age 8 to 18 years ART: on ART	WISC	PHIV scored lower than the healthy controls on all cognitive domain.	
Crowell CS et al[56], 2015, USA 396 PHIV Age 1 to 5 years ART: on ART	WISC	Virological suppression during infancy or early childhood is associated with improved neurocognitive outcome in school age PHIV.	

Table 3. Clinical studies on neurodevelopmental outcomes in resource limiting settings

Study	Tools	Developmental outcomes	Additional outcome measure
Louthrenoo O et al[57], 2004 Thailand 10 PHIV, 29 PHEU Age 12 months ART: not documented	BSID	PHIV had significant lower mental development index (MDI) and psychomotor development index (PDI) than PHEU.	Symptomatic HIV infection had lower scores than asymptomatic ones.
Van Rie et al[58], 2008 Congo 35 PHIV, 35 PHEU, 90 HUU Age 18 to 72 months ART: on ART	BSID	PHIV had significant higher rate of impairment than control. 60% PHIV vs 40% PHEU had cognitive impairment, 29% PHIV vs 14% PHEU had motor impairment. 85% PHIV vs 47% PHEU had language expression delay. 77% PHIV vs 11% PHEU had language comprehension delay.	Stunting and wasting higher in PHIV group.
Leartvanangkul et al[59], 2009 Thailand 25 PHIV, 279 PHEU Age 0 to 5 years ART: no ART	DDST	36% PHIV and 27% PHEU had developmental impairment Gross motor and language impairment were reported in PHIV while fine motor and language impairment were reported in PHEU	Stunting and wasting had higher prevalence in PHIV children
Ferguson et al[60], 2009 South Africa 51 PHIV, 35 PHEU Age 1 to 33 months ART: 67% on ART	BSID	67% PHIV vs 6% PHEU had motor delay	

Study	Tools	Developmental outcomes	Additional outcome measure
Potterton et al[22], 2010 South Africa 122 PHIV Age < 2.5 years ART: 18 PHIV on ART	BSID	52% had severe cognitive impairment and 72% had severe motor impairment	Stunting and wasting common associated with poor outcomes Home stimulation program taught to the caregiver significantly improve cognitive and motor development in PHIV
Kandawasvika et al[61], 2011 Zimbabwe 65 PHIV, 183 PHEU, 287 HUU, 58 unknown status No ART	BINS	PHIV had higher risk of neurodevelopmental impairment when compare to PHEU.	Head circumference and family financial were risk factors for neurodevelopmental impairment
Laughton B et al[8], 2012 South Africa 64 early ART PHIV (ART initiated within 3 months old), 26 deferred ART (ART initiated until clinical or immunological progress), 28 PHEU, 34 HUU Age 10 to 12 months	GMDs	Deferred ART PHIV had lower GMDS general score and locomotor score than early ART children. Early ART PHIV performed similar outcome to PHEU children except locomotor score. Both infected and uninfected mean score were within the average range	Mean age of initiated ART was 8.4 weeks in early ART and 31.4 weeks in deferred ART PHIV children ($p < 0.01$)

Study	Tools	Developmental outcomes	Additional outcome measure
Puthanakit et al[9], 2013 Thailand and Cambodia 284 PHIV, 155 PHEU 164 HUU Aged 2 to17 year (early ART PHIV initiated ART at CD4 + T cell 15-24%, deferred ART PHIV initiated ART when CD4+ T cell was < 15% or CDC category C event)	WISC WPPSI SB Beery VMI	Neurodevelopmental scores did not differ by early and deferred ART PHIV. PHIV performed worse than PHEU and HUU on IQ and Berry VMI memory	Cognitive and development deficits in HIV-infected children occur earlier than one year of life and they do not improve with initiation of ART
Whitehead et al[43], 2014 South Africa 27 PHIV with mean (SD) initiated ART 4.9 (2.8) months 29 PHEU aged < 12 months	BSID	PHIV had lower motor and language score than HEU at baseline (before initiated ART), 3 months, 6 months after initiated ART. No significant improved occurred overtime, yet, did not decrease	Significant increases of weight, height and head circumference after ART initiation and also received nutritional advice at HIV clinic.
Hutchings et al[62], 2014 Zimbabwe 28 PHIV, 32 PHEU Age 6 weeks to 12 months ART: not documented	BSID	64% PHIV had cognitive delay 61% PHIV had language delay 54% PHIV had motor delay All was significantly different from PHEU (p< 0.001)	64% PHIV on ART PHIV infants had malnutrition, stunting and smaller head circumference than PHEU.

Study	Tools	Developmental outcomes	Additional outcome measure
Brahmbhatt et al[63], 2014 Uganda 116 PHIV, 105 PHEU, 108 HUU ART:44% PHIV on ART before 24 months, only 5 PHIV initiated age < 12 months	MSEL (15% cutoff)	24.1% PHIV have global developmental impairment while only 7.6% PHEU and 5.6% HUU were, $p < 0.001$. A significantly higher proportion of children in PHIV were impaired in all domains (except gross motor) when compare to PHEU and HUU.	Longer duration of ART was associated with decreased impairment
Benki-Nugent et al[64]. 2017 Kenya 73 PHIV with ART initiated < 5 months, 92 HUU	Developmental milestones	Early ART PHIV had delay developmental milestones than HUU in the first 2 years of life	Median age (IQR) of initiating ART was 3.7 month (3.1-4.0) PHIV with viral suppression on ART had better recovery of developmental milestones than those without suppression
Laughton B et al[65]. 2018 South Africa, 28 deferred ART, 35 early ART with 40 weeks interrupted, 33 early ART with 96 weeks interrupted, 34 PHEU, 39 HUU, age 10 – 60 months	GMDs	Early locomotor delay in the deferred ART PHIV resolved by 5 years. PHIV and PHEU were similar neurodevelopmental outcomes at 5 years, except visual perception where lower in PHIV.	Weak correlations between neurodevelopmental outcomes at 5 years and age initiating ART, baseline CD4+ T cell count, time on ART and time to first viral load suppression.

2.4.2 Neurobehavioral outcomes in PHIV and PHEU children

Most neurobehavioral outcomes data were in youth participants and not documented about ART. A variety of neurobehavioral assessment tools and different cutoff range were used such as Conners' Parenting Rating Scale (CPRS), Child Behavior Checklist (CBCL), Child and Adolescent Symptoms Inventory-4R (CASI-4R), Diagnostic Interview Schedule for Children (DISC-IV), Behavior Assessment System for Children, 2nd edition (BASC-2), Youth self-report Inventory-4 (YI-4) and Center for Epidemiologic Studies Depression Scale for Children (CES-DC) (Table 4).

Several studies from resource rich setting and resource limited setting demonstrated that PHIV and PHEU had high prevalence of neurobehavioral problems without significant difference between groups (Table 5-6). However, these reports were inconsistent e.g. Mellin et al [66] reported higher prevalence in PHIV children and Malee et al [67] reported higher prevalence in PHEU children. Most frequent problems were psychosomatic, depression, anxiety and hyperactivity/attention deficit. Factors associated neurobehavioral outcomes included child factor (e.g. gender, cognitive function, CD4+ T cell status and HIV viral load status) and family-social context (e.g. caregiver education, caregiver mental health, family communication and parenting). The etiologies of behavioral problems in children with PHIV and PHEU are not fully understood. Some comorbid risk factors make it difficult to establish causal relationships between HIV and behavioral outcome. Besides, the study in young children especially in younger than 5 years with early ART initiation is still limited.

Table 4. Frequently used standardized test of neurobehavioral assessment tools in children and adolescent

Test	Age	Assessment
Conners' Parent Rating Scale 48 (CPRS)	3 to 17 years	Conduct problems, learning problems, psychosomatic, impulsive-hyperactivity and anxiety problems
Child behavior checklist (CBCL) Preschool age	1 year and 6 months to 5 years	Diagnostic and Statistical Manual of Mental Disorders (DSM)-oriented scales include affective problems, anxiety problems, pervasive developmental problems, attention deficit/hyperactivity problems and oppositional defiant problems Syndrome scales include emotionally reactive, anxious/depressed, somatic complaints, withdrawn, sleep problems, attention problems, aggressive behavior and other problems Grouping scales as internalizing, externalizing and total problems
Child behavior checklist (CBCL) School age	6 to 18 years	DSM-oriented scales include depressive problems, anxiety problems, somatic problems, attention deficit problems, oppositional defiant problems and conduct problems Syndrome scales include anxious/depressed, depressed, somatic complaints, social problems, attention problems, thought problems, rue-breaking behavior, aggressive behavior Grouping scales as internalizing, externalizing and total problems
Child and Adolescent Symptoms Inventory-4R (CASI-4R)	5 to 18 years	DSM-IV threshold criteria of attention-deficit/hyperactivity, oppositional defiant disorder, conduct disorder, generalized anxiety disorder, social phobia, separation anxiety disorder, major depressive episode, manic episode, dysthymic disorder, schizophrenia, autistic/Asperger's disorder, anorexia, and bulimia

Test	Age	Assessment
Diagnostic interview schedule for children (DISC-IV)	Parent of children aged 6 to 17 years and youth aged 9 to 17 years	Anxiety disorders, mood disorders, disruptive disorders, alcohol/substance use disorders, miscellaneous disorders
Behavior assessment system for children, 2nd edition (BASC-2) Self-report of personality Parent rating scale	2 to 21 years 11 months	Behavioral symptoms index includes scales measuring hyperactivity, aggression, depression, attention problems, atypicality and withdrawal Emotional symptoms index includes scales measuring social stress, anxiety, depression, sense of inadequacy, self-esteem and self-reliance
Youth self-report inventory-4 (YI-4)	12 to 18 years	Attention deficit hyperactivity disorder (ADHD), Oppositional defiant disorder, conduct disorder, generalized anxiety disorder, social phobia, separation anxiety disorder, obsessive-compulsive disorder, specific phobia, panic attacks, major depressive disorder, dysthymic disorder, bipolar disorder, schizophrenia, motor tics, vocal tics, schizoid personality disorder, somatization disorder, anorexia vervosa, bulimia and drug use
Center for Epidemiologic Studies Depression Scale for Children (CES-DC)	6 to 17 years	Depression symptoms

Table 5. Clinical studies on neurobehavioral outcomes in resource rich settings

Study	Assessment	Behavior problems	Additional outcome measures
Mellin et al[68], 2003,USA 96 PHIV, 211 PHEU Age > 3 years ART: no documented	CPRS	52% at least 1 abnormal score 29% at least 2 abnormal score No difference rate of behavior outcome between PHIV and PHEU	Factors associated with conduct score were prenatal drug exposure, ethnicity, primary caregiver and number of changes in living situation Factor associated with hyperactivity score were gender, ethnicity and maternal education Factors associated with impulsivity were gender and maternal education Factors associated with anxiety scores were gender, age, maternal education, ethnicity and primary caregiver
Jeremy et al[19], 2005, USA 489 PHIV Age 4 months to 17 years ART: dual or triple drug	CPRS	PHIV was more problematic than established norms on all scales except anxiety scale	Poor neuropsychological functioning was worse for children with higher viral loads.
Nozyce et al[52], 2006, USA 274 PHIV Age 3 to17 years with ART	CPRS	25% learning problem 28% psychosomatic problem 20% hyperactive 19% impulsive-hyperactive 16% conduct problem 8% anxiety problem 52% at least 1 behavioral problem	Children > 9 years of age were more likely to have anxiety problem than younger (16% vs 5%, p =0.006) Children with a CD4+ T cell count of < 660 cells/mm3 were more likely to be identified as having conduct problem than those with higher CD4+

Study	Assessment	Behavior problems	Additional outcome measures
			T cell count (22% vs 11% p= 0.04) No statistically significant association between behavioral problems and race/ethnicity, gender, weight/height adjusted for age and gender, median HIV-1 RNA at baseline. Children with a higher WISC-III IQ were significantly less likely to have a learning problem or behaviors associated with ADHD. Hyperactivity was more frequent in children with a WISC-III performance IQ of < 90 (31% vs 13%, p= 0.006)
Chernoff et al[69],2009, USA 319 PHIV, 174 PHEU, 82 uninfected children living in household with HIV-positive member Aged 6 to 17 years ART: no documented	CASI-4R	PHIV and control group had a similar prevalence of psychiatric symptoms (61%) and impairment (14-15%)	More PHIV than control received psychotropic medication and behavioral treatment. Caregiver-reported symptoms or impairment were associated with higher odds of intervention than reports by children alone.

Study	Assessment	Behavior problems	Additional outcome measures
Gadow et al[70], 2010, USA 319 PHIV, 168 PHEU, 86 uninfected children living with an infected family member Age 6 to 17 years ART: no documented	CASI-4R	69% PHIV and 70% peer comparisons met DSM-IV symptom cutoff criteria for at least 1 targeted psychiatric disorder.	Youth with greater HIV disease severity (entry CD4+ T cell < 25% vs 25% or more) had higher probability of depression symptoms (19% vs 8% respectively)
Mellin et al[66], 2009, USA 196 PHIV, 129 PHEU Age 9 to16 years ART: no documented	DISC-IV	61% PHIV and 49% PHEU met criteria for non-substance use psychiatric disorder (OR 1.59, CI 1.03,2.47, $p <$ 0.05) 46% anxiety disorder 25% behavioral disorder with ADHD being most prevalent (PHIV had higher rate of ADHD when compare to PHEU) 7% mood disorder 4% substance abuse disorder	HIV status and caregiver type were associated with mental health outcomes
Elkington et al[71], 2011, USA, 196 PHIV, 249 PHEU, 100 Healthy Age 9 to 16 years ART: no documented	CBCL Clinical range cutoff	14% had clinical range of total behavioral problems, 12% of internalizing problems and 16% of externalizing problems Youth HIV status was not associated with youth mental health after adjusting for the effects of other key contextual	Caregiver mental health particularly anxiety and depression were associated with youth mental health Family interaction variables including caregiver child communication and caregiver involvement were also associated with better CBCL scores

Study	Assessment	Behavior problems	Additional outcome measures
		or social factors.	
Malee K et al[67], 2011, USA 295 PHIV, 121 PHEU Ages 7 to < 16 years with ART	BASC-2	29% participants had mental health problem at entry 38% PHEU vs 25% PHIV had mental health problems, $p < 0.01$	Factor associated with higher odds of mental health problems at $p < 0.10$ included caregiver characteristic (psychiatric disorder, limit-setting problems, health-related functional limitations) and child characteristics (younger age and lower IQ)
Mellins et al[72], 2012, USA, 166 PHIV and 114 HIV negative children Aged 9 to16 year ART: no documented	DISC-IV	68.8% PHIV+ and 69.3% PHIV – youth met criteria for any psychiatric disorder at either time point Anxiety and behavioral disorders were the most frequent co-morbidity	Among PHIV+ youth, there was a significant decrease in prevalence of any psychiatric disorder and anxiety disorder CD4+ T cell count and HIV RNA viral load were not associated with presence or absence of disorder

Table 6. Literature review of neurobehavioral outcomes in resource limiting settings studies

Study	Assessment	Behavior problems	Additional outcome measures
Sanmaneechai et al[73], 2005, Thailand, 30 PHIV and 35 healthy Age 3 to 5 years ART: no documented	CBCL Clinical range cutoff scale	17% PHIV vs 30% healthy had clinical range of internalizing problem; 7% PHIV and 14% healthy had clinical range of externalizing problem and 13% PHIV and 14% healthy had clinical range of total behavior problem without significant statically difference.	
Mendoza et al[74], 2007 Dominican Republic 43 PHIV Age 2-8 years (32 PHIV age <5 years 11 PHIV age > 5 years)	CBCL preschool and school age Cut-off borderline/clinical range	Preschool group 31.25% somatic complaints 25% anxiety problem 6.26% aggressive behavior 3.13% attention problem 40% internalizing problems 20% externalizing problems 30% Other problems including sleep problems School age group 46% withdrawn/depressed 27% somatic symptoms 46% internalizing problems 36% externalizing problems 36% total problems	

Study	Assessment	Behavior problems	Additional outcome measures
Puthanakit et al[9], 2013 Thailand and Cambodia 284 PHIV, 155 PHEU 164 HUU, aged 2 to17 year ART: early ART PHIV initiated ART at CD4+ T cell 15-24%, deferred ART PHIV initiated ART when CD4+ T cell was < 15% or CDC category C event	CBCL preschool and school age borderline-clinical range	Early and deferred PHIV children had similar mean T scores on the CBCL but they had higher than PHEU. 16% early PHIV, 20% deferred PHIV, 16% PHEU and 15% HUU had borderline-clinical range behavioral problem for total problem (p= 0.6); 22% early PHIV, 19% deferred PHIV, 13% PHEU and 11% HUU had externalized behavior (p = 0.04; 22% early PHIV, 19% deferred PHIV, 16% PHEU and 17% HUU had internalized behavior problem (p = 0.6)	No significant differences in full scale IQ were noted in the younger children with borderline/clinical problem syndrome T scores. Older children with borderline/clinical problem T scores in several syndrome scales had significantly lower full scale IQs in several domain (anxious/depressed, aggressive behavior, thought problems and attention problems)
Betancourt et al[75], 2014 Rawanda 218 PHIV, 218 PHEU, 237 healthy Age 10 to17 years ART: not documented	CES-DC YI	PHIV and PHEU children demonstrated higher level of depression, anxiety, conduct problems and functional impairment compared with healthy while PHIV demonstrated not statistically different from PHEU children	
Monico et al[76]. 2014 Portugal 15 PHIV Age 8 to 17 years	CBCL for school age, YI	26.7% behavioral problem, particularly in opposition/immaturity Lowest score in anxiety domain	

Study	Assessment	Behavior problems	Additional outcome measures
Louthrenoo et al[77]. 2014 Thailand, 50 PHIV (on ART), 56 controls Age 11 to 18 years	YI-4, CBCL for school age	Internalizing problem score from YI were significantly higher in PHIV than control (p = 0.02) Total competence scores from both self-report and caregiver repot in PHIV were significantly lower than control (p = 0.005 and .001)	
Ruisenor-escudero et al[78]. 2015 Uganda, 144 PHIV (82 on ART) Age 5 to12 years ART: not documented	CBCL for school age BRIEF	Mean (SD) internalizing problem T score = 60.9 (9.0) Mean (SD) externalizing problem T score = 58.9 (7.9) Mean (SD) total problem T score = 58.5 (8.5)	Poorer behavioral outcomes were associated with higher viral loads
Louw et al[79]. 2016 South Africa 78 PHIV, 30 healthy control Age 6 to 16 years ART: not documented	CBCL for school age Clinical range and borderline cut-off range	No significant differences in between group comparisons for the prevalence of internalizing, externalizing and total problems 12% PHIV vs 10% healthy – internalizing problems 8% PHIV vs 20% healthy – externalizing problems 14% PHIV vs 17% healthy- total problem	Caregiver depression was significant predictor of greater total problems scores

2.4.3 Neuroanatomical outcomes in PHIV

Neuroimaging is an important tool to diagnose HIV encephalopathy and other comorbidities of PHIV such as meningitis and malignancy [80, 81]. Since ART became widely available, the prevalence of HIV-encephalopathy has decreased. However, milder and stable forms of HIV-associated neurodevelopmental problems continue to exist. The previous studies showed that MRI changes correlate with neurodevelopment in older children and adults [82, 83]. Thinning of cerebral cortex and brain atrophy correlated with cognitive deficit [83]. Lower fractional anisotropy, higher mean diffusivity and radial diffusivity in diffusion tensor imaging (DTI) had been detected in untreated PHIV children with slow progressing disease [84]. However, neuroimaging studies in early treated, young PHIV children are limited. The CHER study of 44 children with early ART found 50% had HIV-related neurological disease, but white matter signal abnormalities from T2/FLAIR were not associated with neurodevelopmental scores and ART onset [85].

In the young children, brain myelination is a crucial component of neurodevelopment. Normal myelination starts in utero and continues to reach maturity until 2 years of age or so. T1-weighted and T2-weighted images continue to provide the most important information regarding cerebral myelination and correlate very closely to developmental milestone. T1-weight images achieve adult appearance at 12 months old. T2-weight images and T2-weight FLAIR images usually have a relatively mature appearance by 2 years old. In contrast to T1- and T2-weighted imaging, the majority of the major white matter tract in the brain is visible at birth on fractional anisotropy maps DTI. However, it achieves adult appearance by 4 years old [86].

Table 7. Clinical studies of neuroanatomical outcome in PHIV children

Study	Imaging	Neuroanatomical outcomes
Raskino et al[45], 1999, USA 831 PHIV Age 2 months to 18 years ART: monotherapy or combination of AZT and ddI therapy	CT or MRI	87% had no atrophy identified 10% had mild atrophy 3% had moderate or marked atrophy
Blanchette et al[48], 2001, Canada, 20 PHIV Age 6 to 37 months ART: 3 PI-based regimen and other combination NRTI	CT	9 children had abnormal scan, brain atrophy in 4 PHIV, calcification in 7 PHIV, ventricular enlargement in 4 PHIV and white matter low attenuation in 2 PHIV Children with evidence of CT abnormalities performed significantly worse on the PDI than children with normal CT scan.
Blanchette et al[49], 2002, Canada, 14 PHIV Age 5 to 12 years ART: on ARV with/without PI	CT	5 PHIV had abnormal scans: brain atrophy was observed in 1, calcification in 1, ventricular enlargement in 4 and white matter abnormalities in 5
Nozyce et al[52], 2006, USA 258 PHIV Age 3 to17 years with ART	CT or MRI	7% PHIV had basal ganglia/subcortical calcification, 3% had white matter abnormalities, and 1% had focal mass lesion No significant associations between CT/MRI finding and any behavioral problems

Study	Imaging	Neuroanatomical outcomes
Ackermann et al[85], 2014 South Africa 44 PHIV Age 8-54 months ART: 34 early ART and 10 deferred ART from CHER study	MRI	Multiple high signal intensity lesion on T2/Flair were documented in 22 patients (50%), predominantly in frontal (91%), parietal (82%) white matter No differences in neurodevelopmental scores comparing children with and without white matter signal abnormality No correlation with score Trend for associated of white matter signal abnormality and longer time on ART and nadir CD4+ T cell
Jahanshad et al[87], 2015 Thailand and Cambodia 30 HEU and 30 control Age 10 years	MRI	No difference in brain volume or diffusion tensor imaging metric was detected between PHEU and control Higher fractional anisotropy and lower mean diffusivity were each associated with higher IQ score
Ackermann C et al[88], 2016 South Africa 38 PHIV and 13 control from CHER study Age 5 years	MRI	White matter abnormalities measured by fractional anisotropy was observe in PHIV with early ART at 5 years. The corticospinal tracts are predominantly involved rather than the corpus callosum. Continuous early ART can limit white matter damage.
Cohen S et al[89], 2016 The Netherlands 35 PHIV and 38 PHEU Age 8-18 years ART: on ART	MRI	PHIV had lower brain volumes, more white matter hyperintensities, poorer brain structural integrity and worse cognition compare to PHEU.

Study	Imaging	Neuroanatomical outcomes
Jankiewicz et al[90], 2017 South Africa 65 PHIV, 19 PHEU and 27 HU from CHER study Age 7 years old	MRI	Lower fractional anisotropy and higher mean diffusivity were observed in inferior fronto-occipital fascicular in PHIV compared to controls.
Nwosu et al[91], 2018 South Africa 60 PHIV, 40 PHEU from CHER study Age 7 years	MRI	PHIV children showed reduced gyrification compare to controls in bilateral medial parietal regions, as well as reduced volumes of the right putamen, left hippocampus and global white and gray matter and thicker cortex in small lateral occipital region. Earlier ART initiation was associated with lower gyrification and thicker cortex in medial frontal regions and early ART appears to preserve cortical thickness and volumes of certain brain structure.

CHAPTER 3
Methodology

This chapter will report the study design, ethical consideration, study procedures, study endpoint and data analysis.

3.1 Study design

Study design: a prospective, observational study

Study participants

The study population will be children who born to HIV-positive mothers and aged 12-56 months old

Inclusion criteria

1. Age 12-56 months old
2. Born to HIV-positive mothers
3. Caregiver signed written informed consent
4. Categorized in 2 groups by HIV status as follows:
    - Group PHIV: HIV-infected children
        - The children had documented HIV infection by positive HIV DNA PCR.
        - The children must have initiated ART ≤ age 12 months and have had ≥ 12 months of ART
            - In substudy, early ART PHIV group initiated ART at age ≤ 3 months. Standard ART PHIV group initiated ART age ages > 3 to ≤ 12 months.
    - Group PHEU: age matched HIV-exposed uninfected children
        - The children had documented negative HIV DNA PCR test at age ≥ 4 months or non-reactive anti-HIV antibody age ≥ 12 months.

Exclusion criteria

1. Gestational age < 34 weeks
2. Major congenital anomalies and genetic disorders

3. Current neurologic diseases such as CNS infection ad neoplasm
4. Head injury with a loss of consciousness of greater than one hour or known long-term cognitive sequelae
5. Persistent and active AIDS-defining opportunistic infection within 30 days prior to enrollment (stable and treated opportunistic infections on maintenance therapy, minor infections such as oral thrush will be allowed)

Group-wise matching for age was performed every 6 months stratified group e.g. 12-18, 19-24, 25-30, 31-36, 37-42, 43-48 and 49-56 months. Gender does not match as the neurodevelopmental assessment in young children did not categorized in gender.

PHIV children for MRI sub-study group were sampling by exploratory with caregiver permission. However, the priorities of enrollment are children who are more than 2 years old and might be at least 5 children who do not viral suppression.

3.2 Ethical considerations

This study was approved by the Research Ethics Committee, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. Inform consent was obtained from the primary caregiver prior to assessment. A copy of the signed Informed Consent Form will be given to the caregiver to keep.

Respect for person

The caregiver will be informed of the objective, the procedure and any risks or benefits associated with the study before children's parents decide to participate in the study. The caregivers will be given enough time to study the Informed Consent Form and have a chance to ask questions about the study. They must understand that taking part in the study is of their own choice. They may decide not to take part in the study or stop being the study at any time without it making any difference to the medical care they receives now or in the future.

Beneficence/Non-maleficence

Participants will have the neurodevelopmental and neurobehavioral assessment, which may lead to early detection of neurodevelopment impairment and an opportunity to seek further guidance.

Participants and their caregivers may experience stress with neurodevelopmental testing, MRI imaging, light general anesthesia, intravenous line placement and phlebotomy. If there is harm from participation this study, the participants will be treated appropriately and immediately. Participants will receive medical treatment at no cost to them for injuries or medical problems that have resulted from participation in the study.

Clinical and laboratory information generated by study procedures will be identified only with a serial identification number, which will be assigned at the time of enrollment. The name of the participant will only appear on source documents for enrollment (e.g., consent form) and potentially from clinical data obtained during the course of their clinical care separate from participation in the study. Records will be kept at the Infectious Diseases Unit in double-locked storage (locked cabinets in a locked room). Only investigation team will have access to these records. The investigators will keep confidential the patients' information.

Justice

In this research all participants who are qualified on the basis of research, are eligible to be selected to join the project equally. We will enroll both male and female participants who meet the eligibility criteria for the study without any sex and gender discrimination.

3.3 Study procedures

After the primary caregiver signed the informed consent form, children and caregivers will be asked to participate as follows (Table 8)

1. History taking and physical examination (Appendix A)
    a. Information regarding antenatal history, perinatal history, illness and developmental history will be obtained.
    b. Physical examination, including measurement of weight, height, and head circumference, were performed at each visit; raw scores were converted to Z-scores, using the WHO child growth standard reference population which adjustment for prematurity (gestational age 34-37 weeks) until the age of 24 months (WHO anthropometry). Underweight, stunting, and microcephaly were defined as Z-scores < -2 for weight for age Z-score (WAZ), height for age Z-score (HAZ), and head circumference for age Z-score (HCAZ), respectively.
2. The primary caregiver will be interviewed for relevant medical and social history as well as child rearing history by answering the Thai version of Parenting Styles and Dimensions Questionnaire (PSDQ) to assess parenting styles and the specific parenting practices with their children (Appendix B) and they will be interviewed for mental health status and asked to answer the Thai version of Patient Health Questionnaire-9 (PHQ-9) (Appendix C). The questionnaires will be completed by the primary caregiver. However, if the primary caregiver could not read the questionnaire, the trained staff will read it out and if

the primary caregiver does not understand the questions, the trained staff will explain according to the protocol.

3. Neurodevelopmental assessments using the MSEL will be performed at enrollment and 1 year later. The assessments will be done by well-trained examiners who will be blinded to the study group (Appendix D).
4. Neurobehavioral assessment with the Thai version of the CBCL will be completed by the primary caregiver (Appendix E). However, if the primary caregiver could not read the questionnaire, our trained staff will read it out and if the primary caregiver does not understand the question, our trained staff will explain according to the protocol.
5. The children will be checked complete blood count and reticulocyte count. Only PHIV children will be checked CD4+ T cell and HIV RNA.
6. Twenty PHIV will be asked to undergo MRI at enrollment and 1 year later (Appendix F).

Table 8. Study procedure

Procedures	**Month 0**	**Month 12 (+/- 3 month)**
Review eligibility and Consent	X	
Medical history, Physical Examination	X	X
Primary caregiver interview about family history, child rearing history (PSDQ), mental health status (PHQ -9)	X	X
The Mullen Scales of Early Learning	X	X
Child Behavior Checklist	X	X
CBC, Reticulocyte count	X[1]	X
CD4+ T cell/HIV RNA (Only PHIV children)	X[1]	X
MRI brain[2] (20 PHIV children)	X	X

[1]Laboratory result within 3 month before visit are allowed

[2]MRI brain should be performed within 3 month after neurodevelopmental and neurobehavioral assessment

Parenting Style and Dimensions Questionnaire (Appendix B)

Parents' attitude to children, manners and behaviors directly affect the children's personality and temperament shaping as well as mental health development [92, 93]. Parenting Style and Dimensions Questionnaire short version (PSDQ-short version) was internationally recognized as one of the scales with parents as the respondents to evaluate the parenting style and is demonstrated to have good reliability and validity [92, 94]. PSDQ is with 32 self-report items and measuring continuous scales of authoritative (15 items), authoritarian (12 items) and permissive parenting (5 items). To obtain an overall authoritative, authoritarian, and permissive parenting style score, an average of those items relevant to each parenting style was then computed. The PSDQ-Thai version was translated by Dr.Weerasak Chonchaiya and used in King Chulalongkorn Memorial Hospital Longitudinal Cohort.

The primary caregiver is asked to describe their parenting style using a 5-point scale [ranging from "never" to "always" (code 1 to 5)] in the PSDQ-Thai version. The primary caregiver is defined as the caregiver with the most responsibility for caring for the child. It will take time around 10 minutes.

Patient Health Questionnaire-9 (PHQ-9) (Appendix C)

Patient health questionnaire-9 (PHQ-9) is an instrument for screening, diagnosing, monitoring and measuring the severity of depression and incorporates DSM-V depression diagnostic criteria. The primary caregiver is asked to complete the questionnaire about feeling of depression, using a 4-point scale (ranging from "not at all" to "nearly every day") in PHQ-Thai version. The validated PHQ-9 Thai version was translated by Dr.Manote Lotrakul and widely used in Thailand [95]. Depression was characterized by a total score of ≥ 9. It will take time around 5 minutes.

Mullen Scales of Early Learning test (Appendix D)

The Mullen Scales of Early Learning (MSEL) test is a measure of cognitive function for infants and preschool-age children from birth through age 68 months. The children will be in stable mood and with their caregiver before performing the test. The testing environment is set up to be fun for the child and includes room to play/interact pleasantly with well-trained developmental behavioral pediatricians at King Chulalongkorn Memorial Hospital.

The children will be test in 5 distinct areas:

1. Gross motor to measure central control and mobility in supine, prone, sitting, and fully upright positions;

2. Visual reception to measure a child's performance in processing visual pattern, visual discrimination, memory, organization, sequencing and spatial awareness;
3. Fine motor to measure visual and motor ability which reflects the expressive side of visual organization;
4. Receptive language to measure a child's ability to process linguistic input, auditory comprehension, memory, organization, sequencing and use of spatial concepts;
5. Expressive language to measure a child's ability to use language productively, speaking ability and language formation

The test performs around 15-60 minutes depend on age. T-scores are derived from raw scores, with mean of 50 and standard deviation of 10. An early learning composite (ELC) score is calculated from total scores of all subscales, with the exception of gross motor domain, with a mean of 100 and standard deviation of 15. ELC scores of ≤ 70 indicate global developmental impairment; each domain T scores ≤ 30 indicates significant impairment. According to the MSEL, gross motor skills were assessed for children from birth to 34 months old. The gross motor developmental quotient was calculated by age equivalent divided by actual age, multiplied by 100.

MSEL was administered at the enrollment and the 12-month follow-up visits by well-trained developmental behavioral pediatricians who were blinded to children's HIV status. Each primary caregiver received advice with respect to the child's developmental outcomes and developmental promotion specific to each participant's context. Children with significant developmental problems were referred for appropriate diagnostic and therapeutic services.

Child Behavioral Checklist (Appendix E)

Childhood behavioral checklist (CBCL) test is a well-standardized and widely used 100 item rating scale for the identification of behavior problem in children aged 1 year 6 months – 5 years old [96]. The primary caregiver is asked to describe how much a particular behavior describes their children within the past 2 months, using a 3-point scale (ranging from "not true" to "very true or often true") in CBCL-Thai version. The validated CBCL-Thai version was translated by Dr.Orawan Louthrenoo and widely used in Thailand by our group and others in Thailand [9]. It will take time around 15 minutes.

Behavioral profiles converted as DSM-oriented scales and syndrome scales. DSM-oriented scales include affective problems, anxiety problems, pervasive developmental problems, attention deficit/hyperactivity problems and oppositional defiant problems. Syndrome scales consist with emotionally reactive, anxious/depressed, somatic complaints, withdrawn, sleep problems, attention problems, aggressive behavior and other problems. This syndrome scales can be scored in term of three broad grouping of syndromes as internalizing, externalizing and total problem scores. Internalizing consists of 4 syndromes (emotionally reactive, anxious/depressed, somatic complaints and withdrawn). Externalizing consists of 2 syndromes (attentions problems and aggressive behavior) and total problem score is the sum of internalizing, externalizing, sleep problems and other problems. T-scores are derived from raw scores. DSM-oriented scales and syndrome scales T score $\geq$ 70 were considered as behavioral problems. The borderline clinical range of internalizing, externalizing and total problems was set at T score of 60-63 and the clinical range at T score $\geq$ 64 [96].

MRI brain

The MRI scan will be performed with 3D T1-weight sequence (time ~ 8 minutes) then DTI (time ~ 12 minutes). Therefore the typical total acquisition time is around 20 minutes. If MRI includes optionally FLAIR (T2-weighted) (time ~ 5 minutes) and repeated some series if needed, the acquisition time is around 35 minutes. The MRI prescriptions may be modified as appropriate as long as the maximum acquisition time remains unchanged.

The children will be evaluated health conditions by pediatricians and pediatric anesthesiologists. For safety, an intravenous line will be put in place for the MRI in case of emergency and need for administration of intravenous medications. We will perform scans when children are sleepy and more likely to fall asleep in the scanner. Caregivers will be allowed to be with children in the MRI suite to provide support and give children reminders not to move. The pediatric anesthesiologists will be available to monitor children. If children are uncomfortable in lying in the MRI scanner, with caregivers' consent, the pediatric anesthesiologists will provide light general anesthesia by laryngeal mask airway. Children who receive light general anesthesia will be monitored at least 6 hours after MRI.

If children cannot tolerate the MRI or the caregivers feel uncomfortable, they may ask for the MRI to be stopped at any time without this affecting his/her medical care in the future. However, they can continue in the developmental and behavioral tests. The investigators will use any results that are available. The MRI results were reported by neuro-radiologists.

3.4 Endpoints

Primary Endpoint

1. Global development impairment in the PHIV children compared to PHEU children.

Secondary Endpoints

1. Domain scores and impairment of the MSEL in PHIV and PHEU children
2. CBCL scores and behavior problem in PHIV and PHEU children
3. Neurodevelopmental and neurobehavioral outcome among PHEU, early ART PHIV and standard ART PHIV
4. Brain imaging results in PHIV children

3.5 Sample size calculation

Sample size for primary objective:

Power calculations are based on test of two-independent proportions. Prior data indicated that the prevalence of delayed development by MSEL is 0.076 in PHEU group [63]. This will have 80% power with 0.05 alpha and proportion between PHIV and PHEU will 1:2.

For testing two independents (two-tailed test)

Proportion in group 1(P1) = 0.28, Proportion in group 2 (P2) = 0.076

Alpha (α) = 0.05, Beta (β) = 0.20, ration (r) = 2.00

$$n_1 = \left[\frac{z_{1-\frac{\alpha}{2}} \sqrt{\bar{p}\bar{q}\left(1+\frac{1}{r}\right)} + z_{1-\beta} \sqrt{p_1 q_1 + \frac{p_2 q_2}{r}}}{\Delta} \right]^2$$

$$r = \frac{n_2}{n_1}, q_1 = 1 - p_1, q_2 = 1 - p_2$$

$$\bar{p} = \frac{p_1 + p_2 r}{1+r}, \bar{q} = 1 - \bar{p}$$

The sample will be at least 50 children of PHIV and at least 100 children of PHEU with potential 10% of loss follow up for MSEL and CBCL to be able to reject the null hypothesis. Under these assumptions, a sample size of 150 subjects would give 80% power to detect a change in discordant proportion of 20% from baseline values at a two sided significance level of 5%.

Sample size for secondary objective:

The MRI assessment is exploratory and for feasibility, it will include 20 PHIV children. We anticipate to over enroll in this group in order to obtain the expected 20 children. This study will concentrate an effort in performing MRI in the PHIV group only because of the lack of clinically significant differences in TBM/DTI between PHIV and PHEU thus far in the PREDICT study [87].

3.6 Data collection

Case record forms (CRFs) and protocol: Case report or data collection forms will be provided for each subject and used in accordance with Good Clinical practices. The following CRFs and protocol will be used: Personal Data Form (Appendix A), Parenting Style and Dimensions Questionnaire (Appendix B), Patient Health Questionnaire (PHQ-9) (Appendix C), Mullen Scales of Early Learning protocol (Appendix D), Child behavioral checklist protocol (Appendix E), and MRI brain protocol (Appendix F).

Data storage and compilation: Data, both electronic and hard copy (CRFs) will be stored in locked facilities at Infectious Disease Unit. Data form CRFs will be entered and complied in an electronic database and it will be backed up. The database is password protected and maintained in a locked room. In addition, personal identifying information such as names will not be stored in the electronic database.

3.7 Data analysis and statistical analysis

Characteristics were reported as median and interquartile ranges for continuous variables and percentage for categorical variables. The chi-squared test or the Fisher's exact test were used to compare categorical variables. The Wilcoxon rank sum test were used to compare median between two group and the Kruskal Wallis test for three group. Neurodevelopmental scores by MSEL and neurobehavioral scores by CBCL were presented as mean (standard deviation). Comparison mean scores between PHEU and PHIV children was used independent two sample t-tests and for three group using analysis of variance (ANOVA). Rate of GDI and their 95% confidence interval (CI) were estimated based on the binomial distribution. The overall rate of GDI included children who had GDI at enrollment and/or follow-up visit. Odds ratio (OR) and 95% CI for comparing the prevalence of any GDI between PHEU and PHIV (early and standard ART) children was estimated by simple logistic regression.

Generalized estimating equations (GEE) for logistic regression were used to analyze factors which were associated with GDI and GEE for linear regression were used to analyze

predictors of changing in ELC scores over time. Multivariate models were developed including covariates with $p < 0.1$ from univariate model and backward stepwise regression was used for the final model selection. Covariates were demographics, including the children's age, sex, gestational age, birth weight, and exposure to antiretroviral prophylaxis for prevention of mother-to-child transmission (PMTCT); family history, including parents' and caregiver's age, education, maternal history of substance use, child-rearing history, and income; HIV characteristics, including the child's age at ART start, duration of ART, CD4+ T-cell counts, and HIV-RNA. Statistical significance was defined as $p < 0.05$.

The STATA software, version 13.1 (Stata Corp., College Station, Texas, USA) was used for analysis.

CHAPTER 4

Result

From 2016 - 2018, 150 children were enrolled in this study. The baseline characteristic data for children will be presented, followed by the family and primary caregiver data. After that, the result about neurodevelopmental outcomes, neurobehavioral outcomes and neuroanatomical outcomes will be presented. The neurodevelopmental outcomes will be present as developmental score and rate of developmental impairment. The data of 2 groups (PHIV and PHEU) will be presented first and sub-analysis in 3 groups (early ART PHIV group initiated ART at age ≤ 3 months, standard ART PHIV group initiated ART age ages > 3 to ≤ 12 months and PHEU) will be presented later. The neurobehavioral outcomes will be analyzed in children age ≥ 18 months at time of assessment. Result as DSM-oriented scales and syndrome scales will be presented first, then overall scales as internalizing, externalizing and total problems scales will be presented later. The data will be presented as raw score/T score and rate of behavioral problem. Finally, the neuroanatomical outcome in 20 PHIV children will be reported in the aspect of baseline characteristic and MRI result.

4.1 Children data

4.1.1 Baseline characteristics of children

Baseline characteristics for the PHIV and PHEU are presented in Table 9. At enrollment, 50 PHIV and 100 PHEU were enrolled. Three PHEU children, who loss to follow up at 12-month visit due to relocation, were in normal health status by telephone call. Twenty eight (56%) PHIV and 45 (45%) PHEU children were male with the median (IQR) age were 29 (22 -36) month and 27 (19-42) month in PHIV and PHEU, respectively. There was no significant difference for gender, age at the first assessment, gestation age and birth weight. In addition, there was no difference for rate of prematurity (GA 34 - < 37 Month s) and low birth weight (birth weight < 2500 g).

Table 9. Baseline characteristics of PHIV and PHEU children

Variable	Month 0			Month 12		
	PHIV (n=50)	**PHEU (n=100)**	***p***	**PHIV (n=50)**	**PHEU (n=97)**	***p***
Male sex, n (%)	28 (56%)	45 (45%)	0.20	28 (56%)	44 (45%)	0.22
Age, month, median (IQR)	29 (22-36)	27 (19-42)	0.80	39 (32-47)	39 (29-54)	0.7
Gestational age week, median (IQR)	38 (37-39)	38 (37-39)	0.35			
Prematurity (GA 34-<37 weeks), n (%)	19 (38%)	32 (32%)	0.49			
Birth weight, gram, median (IQR)	2,723 (2,430 – 2,850)	2,845 (2,550 – 3,050)	0.06			
Low birth weight (< 2,500 g), n (%)	15 (30%)	18 (18%)	0.14			

GA; Gestational age

4.1.2 History of prevention mother to child transmission

For prevention mother to child transmission (PMTCT) program, 27 mothers did not received ART which was significant higher in PHIV group than PHEU (42% *vs* 6%) with unsurprisingly (Table 10). Most common maternal ART regimens were lopinavir/ritonavir based regimen and follow-by efavirenz based regimen. According to PMTCT program in Thailand, children with low risk for transmission (mother on ART more than 12 weeks or viral suppression (HIV-RNA < 50 copies/mL at delivery) will received only AZT for prophylaxis and children with high risk for transmission (mother on ART less than 12 weeks or detectable viral load at delivery) will received combination ART (AZT/3TC/NVP) for prophylaxis). Child ART prophylaxis regimen were significant different between group as 91% PHEU was received AZT monotherapy and 74% in PHIV was received combination therapy for PMTCT.

Table 10. History of prevention mother to child transmission

Variable	PHIV (n=50)	PHEU (n=100)	*p*
Maternal ART regimen, n (%)			<0.001
• No ART	21 (42)	6 (6)	
• NNRTI-based regimen	11 (22)	46 (46)	
• PIs-based regimen	18 (36)	48 (48)	
Child ART prophylaxis regimen, n (%)			<0.001
• Combination regimen	37 (74)	9 (9)	
• AZT monotherapy	16 (32)	91 (91)	
• No prophylaxis	4 (8)	0	
• Unknown data	3 (6)	0	

ART; antiretroviral therapy, NNRTI; Non-nucleotide reverse transcriptase inhibitor, PIs; protease inhibitors, AZT;zidovudine

4.1.3 Baseline characteristics of PHIV children

Baseline characteristics of PHIV children are shown in Table 11. Median (IQR) age of ART initiation was 2.9 (1.9-5.1) month old. Eight PHIV children were intrauterine infection which identified by positive HIV DNA PCR at birth and 7 PHIV children were peripartum infection which were negative HIV DNA PCR at birth but positive later. The other PHIV children were unknown in mode of infection due to no HIV DNA PCR result at birth. Most of PHIV (84%) was on LPV/r based regimen which was the first line therapy as the national recommendation in Thailand. Median (IQR) duration of ART at first assessment was 25 months (19-31). Median (IQR) CD4+ T cell was 1824 (1139-2188) cell/mm3 at enrolment and 1555 (1220-1818) at 12-month visit. Only 4 children at the enrolment and 8 children at 12-month visit had CD4+ T cell <1000 cell/mm3. Thirty-seven (74%) and 35 (70%) PHIV children had HIV RNA ≤ 200 copies/mL at enrolment and 12-month visit. Five PHIV had virological failure at 12-month visit (HIV RNA was undetectable at enrolment and then rebound > 200 copies/mL at 12-month visit) and 3 PHIV had new-onset of viral suppression at 12-month visit.

Table 11. Baseline characteristics of PHIV children

Variable	Month 0 (n=50)	Month 12 (n=50)
Age initiated ART, month, median (IQR)	2.9 (1.9-5.1)	
Mode of infection, n (%)		
• In utero	8 (16%)	
• Peripartum	7 (14%)	
• Unknown	35 (70%)	
Current ART regimen, n (%)		
• PIs-based regimen	42 (84%)	42 (84%)
• NPV-based regimen	8 (16%)	7 (14%)
• Integrase inhibitor-based regimen	0	1 (2%)
Duration on ART, month, median (IQR)	25 (19 -31)	34 (28-41)
CD4+ T cell, cell/mm^3, median (IQR)	1824 (1139- 2188)	1555 (1220-1818)
HIV RNA (copies/ml), n (%)		
• Undetectable < 200 copies/ml	37 (74%)	35 (70%)

ART; antiretroviral therapy, PI; protease inhibitor, NVP; nevirapine

4.1.4 Anthropometric data and nutritional status

Anthropometric data and anemic status are shown in Table 12. Median WAZ, HAZ, HCAZ, mid upper arm circumference for age Z-score (MUACZ) were significant lower in PHIV children when compare to PHEU children, $p < 0.05$. However, their WAZ, HAZ, HCAZ and MUACZ seem to be within normal limit (within -2 SD). Rate of underweight was 4% in both PHIV and PHEU at enrollment. However, prevalence of underweight was different at 12-month visit (8% in PHIV *vs* 1% in PHEU, $p = 0.046$). Prevalence of stunting was 14% in PHIV and 8% in PHEU children at enrollment, $p = 0.25$. However, prevalence of stunting was significantly different at 12-month visit (18% in PHIV *vs* 7% in PHEU, $p = 0.047$). Prevalence of microcephaly was 34% in PHIV and 13% in PHEU at enrollment, $p = 0.002$ as well as 15% in PHIV and 1% in PHEU at 12-month visit, $p= 0.003$.

Median of hemoglobin level was 12.1 g/dL in PHIV children and 12.2 g/dL in PHEU children. However, PHIV had a trend of higher rate of anemia (Hb <11 g/dL) than PHEU without statically significant (24% *vs* 14%, $p = 0.13$ at enrollment and 14% *vs* 12%, $p = 0.78$ at 12-month visit). Thirteen children were reported hemoglobinopathy (6 hemoglobin E trait, 1 homozygous hemoglobin E, 6 suspected for alpha thalassemia trait), 11 children have iron supplementation and 1 child had changed AZT to d4T due to AZT associated anemia.

Table 12. Anthropometric data and nutritional status of PHIV and PHEU children

	Month 0			Month 12		
Median (IQR)	**PHIV (n=50)**	**PHEU (n=100)**	***p***	**PHIV (n=50)**	**PHEU (n=97)**	***p***
Weight for age Z-score	-0.6 (-1.4 to 0.02)	-0.3 (-0.9 to 0.5)	0.02	-0.8 (-1.5 to -0.1)	-0.2 (-0.9 to 0.4)	0.001
Height for age Z-score	-1.1 (-1.6 to -0.4)	-0.6 (-1.3 to 0.06)	0.005	-1.0 (-1.7 to -0.4)	-0.8 (-1.3 to -0.2)	0.04
Head circumference for age Z-score	-1.3 (-2.4 to -0.2)	-0.8 (-1.5 to -0.05)	0.03	-0.8 (-1.5 to -0.5)	-0.3 (-1.0 to 0.5)	<0.001
Mid upper arm circumference for age Z-score	-0.6 (-1.7 to 0.2)	-0.3 (-1.0 to 0.9)	0.03	-0.3 (-0.9 to 0.4)	0.2 (-0.6 to 0.9)	0.02
Hb, g/dL	12.1 (11.1-13.0)	12.2 (11.5-12.7)	0.41	12.2 (11.7-12.5)	12.2 (11.5-12.6)	0.57
n (%)						
Underweight	2 (4%)	4 (4%)	0.99	4 (8%)	1 (1%)	0.046
Stunting	7 (14%)	8 (8%)	0.25	9 (18%)	7 (7%)	0.047
Microcephaly	17 (34%)	13 (13%)	0.002	7/47 (15%)*	1/89 (1%)*	0.003
Anemia	12 (24%)	14 (14%)	0.13	7 (14%)	12 (12%)	0.78

Underweight, stunting, and microcephaly were defined as Z-scores < -2 for weight for age Z-score, height for age Z-score, and head circumference for age Z-score, respectively while anemia was defined as Hb < 11 g/dL

*No reference data in children age > 5 years old

4.2 Parents and the primary caregiver data

4.2.1 Baseline characteristics of parents and family history

Baseline characteristics of parents and family history are shown in Table 13. There were significant difference parent characteristics between PHIV and PHEU including age, duration of education, employment status and marital status. Median (IQR) of mother age at birth of infants was 25 (20-32) years in PHIV and 31 (26-35) years in PHEU. Median (IQR) of father age at birth of infants was 29 (22-37) years in PHIV and 34 (28-39) years in PHEU. Mother age and father age of PHIV children are younger than PHEU, $p < 0.05$. Median duration of mother and father education was 9 years and 12 year in PHIV and PHEU, respectively. There were 3 and 2 maternal deaths in the PHIV and PHEU groups respectively. There was one paternal death each in the PHIV and PHEU groups. PHIV parents were employed less than PHEU parents, $p < 0.05$. For marital status, 28% PHIV families were in divorced or separated status while only 16% in PHEU families were. There was no different rate of family history of developmental or behavioral problem.

Table 13. Baseline characteristics of parents and family history

Variable	**PHIV (N=50)**	**PHEU (N=100)**	***p***
Mother age at birth of infants, years, median (IQR)	25 (20-32)	31 (26-35)	0.001
Duration of mother education, years, median (IQR)	9 (7-12)	12 (8-14)	0.07
Mother highest level of education, n (%)			0.03
• ≤ high school (Grade 12)	39 (78%)	70 (70%)	
• > high school	6 (12%)	30 (30%)	
• unknown	5 (10%)	0	
Father age at birth of infants, years, median (IQR)	29 (22-37)	34 (28-39)	0.003
Duration of father education, years, median (IQR)	9 (6-12)	12 (9-14)	0.08
Father highest level of education, n (%)			0.06
• ≤ high school (Grade 12)	32 (64%)	61 (61%)	
• > high school	6 (12%)	29 (29%)	

Variable	PHIV (N=50)	PHEU (N=100)	*p*
• unknown	12 (24%)	10 (10%)	
Marital status, n (%)			0.03
• married	29 (58%)	81 (81%)	
• divorced/separated	14 (28%)	16 (16%)	
• widowed	2 (4%)	1 (1%)	
• unknown	5 (10%)	2 (2%)	
Family history of developmental or behavioral disorder, n (%)	5 (10%)	8 (8%)	0.68

4.2.2 Baseline characteristics of the primary caregiver

Baseline characteristics of the primary caregiver are shown in Table 14. The primary caregiver is defined that the main person who take care children at the time of assessment. Seventeen (34%) PHIV primary caregivers and 31 (31%) PHEU primary caregivers were not their biological parents. Most of non-biological parents were their relatives as grandparents. Median age (IQR) of the primary caregiver was 36 (27-46) years in PHIV and 38 (33-44) years in PHEU. Median duration of the primary caregiver education was 9 years in both group. Twenty three (23%) primary caregivers in PHEU group and 5 (10%) PHIV group were graduated higher than high school (grade 12) level. Forty-two (84%) PHIV families and 62 (62%) PHEU families had income less than 25,000 baht which is the average Thai income per family. One PHIV child and 3 PHEU children had been changed the primary caregiver to non-biological parents at 12-month visit. Primary caregiver depression status by PHQ-9 is shown in Table 15. PHEU primary caregivers had higher rate of major depression than PHIV primary caregivers at enrolment (18% vs 14%, $p = 0.54$) but reversing at 12-month visit (8.3% vs 22%, $p = 0.07$).

Table 14. Baseline characteristics of primary caregivers

Variable	PHIV (n=50)	PHEU (n=100)	*p*
Primary caregiver, n (%)			0.29
• Mother	30 (60%)	67 (67%)	
• Father	3 (6%)	2 (2%)	
• Relatives	14 (28%)	29 (29%)	
• Non-relatives	3 (6%)	2 (2%)	
Primary caregiver age, years, median (IQR)	36 (27-46)	38 (33-44)	0.22
Duration of primary caregiver education, median (IQR)	9 (6-12)	9 (6-13)	0.22
Level of education, n (%)			0.05
• ≤ High school (Grade 12)	45 (90%)	77 (77%)	
• > High school	5 (10%)	23 (23%)	
Income per family, n (%)			0.001
• < 10,000	21 (42%)	16 (16%)	
• 10,000 – 25,000	21 (42%)	46 (46%)	
• >25,000	8 (16%)	38 (38%)	

Table 15. Primary caregiver depression by PHQ-9

Variable	Month 0			Month 12		
	PHIV (n=50)	PHEU (n=100)	*p*	PHIV (n=50)	PHEU (n=97)	*p*
Depression score, median (IQR)	3 (2-7)	4 (2-7)	0.51	4 (2-7)	4 (2-6)	0.54
Major depression status score ≥ 9, n (%)	7 (14%)	18 (18%)	0.54	11 (22%)	8 (8.3%)	0.07

PHQ-9; Patient health questionnaire 9

4.2.3 Child rearing history and parenting style

Child rearing history and parenting style are shown in Table 16 and 17, respectively. Rate of attending to nursery or pre-school was 32% for PHEU and 33% for PHIV at enrolment visit and the rate was increase at 12-month visit. Having children books at home was 72 % for PHEU and 58% for PHIV at enrolment visit and increasing at 12-month visit. Authoritative parenting style was more often reported in the PHEU group than PHIV group at both visit.

Table 16. Child rearing history

Variable	Month 0			Month 12		
	PHIV (n=50)	**PHEU (n=100)**	***p***	**PHIV (n=50)**	**PHEU (n=97)**	***p***
Nursery/school attendance, n (%)			0.90			0.81
• No attend	34 (68%)	67(67%)		27 (54%)	52 (54%)	
• Daycare/ nursery/ pre-school	16 (32%)	33(33%)		23 (46%)	45 (46%)	
Children books in home, n (%)			0.17			0.001
• None	20 (40%)	28 (28%)		11 (22%)	11 (11%)	
• 1-2 books	14 (28%)	24 (24%)		21 (42%)	22 (23%)	
• 3-5 books	9 (18%)	26 (26%)		14 (28%)	31 (32%)	
• >10 books	6 (12%)	22 (22%)		4 (8%)	33 (34%)	

Table 17. Parenting style by Parenting Style and Dimension Questionnaire (PSDQ)

Mean (SD)	**Month 0**			**Month 12**		
	PHIV (n=50)	**PHEU (n=100)**	***p*-value**	**PHIV (n=50)**	**PHEU (n=97)**	***p*-value**
Authoritative	3.6 (0.6)	3.8 (0.6)	0.046	3.3 (0.7)	3.8 (0.7)	<0.001
Authoritarian	2.1 (0.5)	2.1 (0.6)	0.89	2.1 (0.5)	2.0 (0.5)	0.42
Permissive	2.7 (0.7)	2.7 (0.7)	0.84	2.6 (0.7)	2.5 (0.6)	0.44

4.3 Neurodevelopmental outcomes

4.3.1 Global development

4.3.1.1 Early Learning Composite score

Early Learning Composite score (ELC) represents the overall of developmental skill including visual reception, fine motor, receptive language, and expressive language. Mean (SD) of ELC score was 82.1 (14.8) in PHIV and 89.8 (15.8) in PHEU, $p = 0.005$ at the first assessment and decline at 12-month visit, 82.1 (14.8) in PHIV *vs* 81.7 (15.1) in PHEU, $p = 0.05$ (Table 18). Mean difference (95% CI) of ELC score between at enrolment and 12-month visit was -0.42 (-4.7 to 3.8) in PHIV and -2.7 (-5.4 to 0.05) in PHEU, *p*-value = 0.67. Most PHIV and PHEU children had below average to average development outcome (Table 19). Eighteen to twenty-two percent of PHIV children had very low developmental outcome while only 9-16% of PHEU children had.

Table 18. Early Learning Composite score of PHIV and PHEU

Early learning composite score	**PHIV**	**PHEU**	***p***
Mean (SD)			
• month 0, n = 150	82.1 (14.8)	89.8 (15.8)	0.005
• month 12, n = 147	81.7 (15.1)	86.9 (15.4)	0.05
Mean difference between 12-month and at enrollment (95% CI), n = 147	-0.42 (-4.7 to 3.8)	-2.7 (-5.4 to 0.05)	0.67

Table 19. Frequency of Early Learning Composite (ELC) score according to descriptive category of PHIV and PHEU

Descriptive category	**ELC**	**Month 0**			**Month 12**		
		PHIV (n=50) n (%)	**PHEU (n=100)** n (%)	***p***	**PHIV (n=50)** n (%)	**PHEU (n=97)** n (%)	***p***
Very low	≤ 70	9 (18%)	9 (9%)		11 (22%)	15 (16%)	
Below average	71-85	21 (42%)	33 (33%)		19 (38%)	29 (30%)	
Average	86-115	18 (36%)	54 (54%)	0.09	20 (40%)	39 (40%)	0.33
Above average	116-130	2 (4%)	3 (3%)		0 (0)	4 (4%)	
Very high	>130	0	1 (1%)		0	0	

4.3.1.2 Prevalence of global developmental impairment and trajectory status

Prevalence of global developmental impairment (GDI) was shown in Table 20. GDI is defined by having ELC score ≤ 70 and the overall rate of GDI included children who had GDI at enrollment and/or follow-up visit. This study reported the prevalence of any GDI was 18 % (95% CI 11 - 27) in PHEU and 32% (95% CI 20 - 47) with OR 2.14 (95% CI 0.97 – 4.70). At enrolment, the prevalence of GDI was 9% (95% CI 4 - 16) in PHEU and 18% (95%CI 9 - 31%) in PHIV with OR 2.20 (95% CI 0.82 - 6.00). At 12-month visit, the prevalence of GDI was increase in both group [16% (95% CI 9 - 24) in PHEU and 22% (95%CI 12 - 36%) in PHIV with OR 1.50 (95% CI 0.65 - 3.70)]. For trajectory pattern, 82% of PHEU and 68% of PHIV had normal developmental outcome (Figure 5). Seven (14%) PHIV and 9 (9%) PHEU children was emerging GDI at 12-month visit.

Table 20. Prevalence of global developmental impairment of PHIV and PHEU children

Group	Overall (n=150)			Month 0 (n=150)			Month 12 (n=147)		
	% **(95%CI)**	**OR** **(95%CI)**	***p***	**%** **(95%CI)**	**OR** **(95%CI)**	***p***	**%** **(95%CI)**	**OR** **(95%CI)**	***p***
PHEU	18 (11-27)	Ref	-	9 (4-16)	Ref	-	16 (9-24)	Ref	-
PHIV	32 (20-47)	2.14 (0.97-4.70)	0.06	18 (9-31)	2.20 (0.82-6.00)	0.12	22 (12-36)	1.50 (0.65-3.70)	0.37

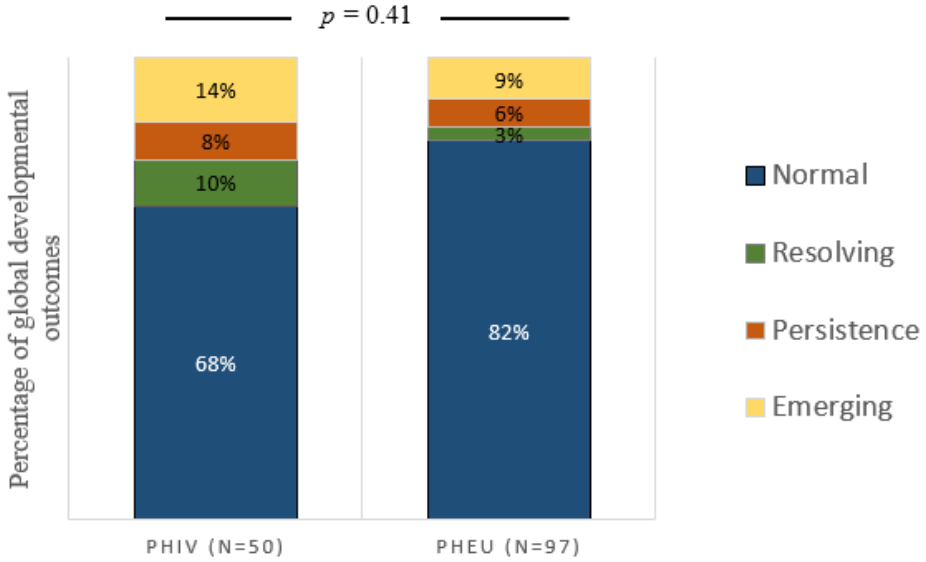

Figure 5. Trajectory pattern of global development outcomes by ELC score of PHIV and PHEU

(ELC, Early learning composite score; Normal = ELC > 70 at month 0 and month 12; Resolving = ELC ≤ 70 at month 0 but ELC > 70 at month 12, Persistence = ELC ≤ 70 at month 0 and month 12; Emerging = ELC > 70 at month 0 but ELC ≤ 70 at month 12)

4.3.1.3 Factors associated with global developmental impairment

Factors associated with global developmental impairment (GDI) are shown in Table 21. Only male gender was associated with GDI [adjusted odd ratio (aOR) 4.65, 95%CI 1.09 to 19.85; p = 0.04]. Other possible risk factors including groups, age, prematurity, nutritional status, smoking, alcohol, maternal ART prophylaxis, parents age, parent education level, primary caregiver age, primary caregiver education, primary caregiver depression score, income, parenting style were not associated as well as HIV parameter in PHIV group including ART regimen, ART duration, CD4+ T cell and HIV-RNA.

Table 21. Factors associated with global developmental impairment by logistic regression

Variable	Univariate		Multivariate	
	OR (95%CI)	*p*	aOR (95%CI)	*p*
Group				
PHEU	Ref	-		
PHIV	1.80 (0.94-3.45)	0.08		
Children				
Male	5.33 (1.36-20.96)	0.02	4.65 (1.09-19.85)	0.04
Age ≤ 36 month	0.83 (0.31-2.18)	0.7		
Preterm (GA 34-37 week)	0.88 (0.26-2.94)	0.83		
Z score weight for age ≤ -2	1.45 (0.09-22.69)	0.79		
Z score height for age ≤-2	2.72 (0.47-15.78)	0.27		
Z score weight for eight ≤ -2	0.48 (0.02-12.91)	0.67		
Z-score head circumference for age ≤ -2	1.09 (0.71-1.69)	0.69		
Anemia	1.92 (0.52-7.07)	0.33		
PHIV children				
Age initiated ART > 3 months old	3.46 (0.77-15.6)	0.11		
ART regimen : PI vs NNRTI	6.03 (0.42-86.1)	0.19		
Duration of ART > 24 months	0.46 (0.09-2.39)	0.36		
CD4+ T cell < 2000 cell/mm^3	0.8 (0.2-3.25)	0.75		
HIV-RNA ≥ 200 copies/mL	0.9 (0.26-3.16)	0.87		
History during pregnancy				
Maternal history of smoking	1.06 (0.79-1.44)	0.69		
Maternal history of alcohol drinking	1.13 (0.82-1.55)	0.46		
Maternal history of no receiving ART prophylaxis	3.47 (0.85-14.13)	0.08		
Parents				
Mother age, year	5.32 (1.23-22.96)	0.03		
Duration of mother's education < 12 years	2.64 (0.77-8.99)	0.12		

Variable	Univariate OR (95%CI)	*p*	Multivariate aOR (95%CI)	*p*
Father age, year	1 (0.92-1.08)	0.93		
Duration of father's education < 12 years	3.19 (0.74-13.7)	0.12		
Divorced/separated/widowed marital status	1.05 (0.26-4.17)	0.95		
Primary caregiver				
Not their biological parents	0.84 (0.25-2.89)	0.79		
Age, year	0.97 (0.92-1.02)	0.24		
Duration of education < 12 years	2.09 (0.62-7.05)	0.23		
Depression score ≥ 9	2.43 (0.70-8.48)	0.16		
Income per family < 10,000 baht/month	3.68 (1.13-11.99)	0.03		
Child rearing				
No Attending daycare/nursery	1.6 (0.55-4.65)	0.39		
No book in their home	1.11 (0.38-3.28)	0.85		
Parenting style				
Authoritative	0.65 (0.31-1.38)	0.27		
Authoritarian	0.91 (0.36-2.34)	0.85		
Permissive	0.71 (0.31-1.64)	0.43		

GA; Gestational age, ART;antiretroviral therapy, NNRT; non-nucleoside reverse transcriptase therapy, PI; protease inhibitor

4.3.1.4 Predictors of changing early learning composite score

Predictors of decreasing ELC scores included income less than 10,000 Baht/month (adjusted coefficient -3.16, 95%CI -5.89 to -0.44, $p = 0.02$) and no nursery school attendance (adjusted coefficient -2.83, 95% CI -5.05 to -0.60, $p = 0.01$) (Table 22).

Table 22. Predictor of changing early learning composite score by linear regression

Variable	Univariate		Multivariate	
	Coef (95%CI)	*p*	Coef (95%CI)	*p*
Group				
PHEU	Ref			
PHIV	-0.51 (-2.82 to 0.84)	0.67		
Children				
Male	-1.18 (-3.35 to 0.98)	0.28		
Age	0.05 (-0.02 to 0.13)	0.18		
Prematurity (GA 34-37 week)	-0.14 (-0.85 to 0.58)	0.71		
Z score weight for age ≤ -2	1.04 (-4.39 to 6.47)	0.71		
Z score height for age ≤ -2	-1.01 (-4.56 to 2.54)	0.58		
Z score weight for eight ≤ -2	1.04 (-5.56 to 7.65)	0.76		
Z-score head circumference for age ≤ -2	0.61 (-2.69 to 3.90)	0.72		
Anemia	1.59 (-1.41to 4.58)	0.30		
PHIV children				
Age initiated ART > 3 months old	0.67 (-0.17 to 1.52)	0.12		
ART regimen : PI vs NNRTI	-0.62 (-5.54 to 4.29)	0.80		
Duration of ART > 24 months	0.05 (-0.14 to 0.24)	0.61		
CD4+ T cell < 2000 cell/mm^3	-0.12 (-0.38 to 0.14)	0.36		
HIV-RNA ≥ 200 copies/mL	-3.34 (-7.42 to 0.73)	0.11		
History during pregnancy				
Maternal history of smoking	-0.02 (-0.62 to 0.58)	0.95		
Maternal history of alcohol drinking	-0.24 (-0.89 to 0.41)	0.48		
Maternal history of no receiving ART prophylaxis	-0.07 (-2.9 to 2.76)	0.96		
Parents				
Mother age, year	0.11 (-0.03 to 0.25)	0.13		
Duration of mother's education < 12 years	0.29 (0.02 to 0.58)	0.04		
Father age, year	0.10 (-0.02 to 0.22)	0.11		

Variable	Univariate Coef (95%CI)	*p*	Multivariate Coef (95%CI)	*p*
Duration of father's education < 12 years	0.24 (-0.07 to 0.55)	0.13		
Divorced/separated/widowed marital status	-0.29 (-2.85 to 2.26)	0.82		
Primary caregiver				
Not their biological parent	0.86 (-1.44 to 3.15)	0.46		
Age, year	0.10 (-0.16 to 0.35)	0.46		
Duration of education < 12 years				
Depression score ≥ 9	-0.17 (-0.47 to 0.12)	0.26		
Income per family < 10,000 baht/month	-2.88 (-5.38 to -0.37)	0.02	-3.16 (-5.89 to -0.44)	0.02
Child rearing				
No Attending daycare/nursery	-3.57 (-5.74 to -1.4)	0.001	-2.83 (-5.05 to -0.60)	0.01
No book in their home	-1.16 (-3.71to 1.38)	0.37		
Parenting style				
Authoritative	0.52 (-1.13 to 2.17)	0.54		
Authoritarian	-0.84 (-2.80 to 1.13)	0.41		
Permissive	0.52 (-1.09 to 2.13)	0.53		

GA; Gestational age, ART;antiretroviral therapy, NNRT; non-nucleoside reverse transcriptase therapy, PI; protease inhibitor

4.3.2 Gross motor

4.3.2.1 Gross motor developmental quotient

Due to limitation of MSEL, gross motor domain was assessed only developmental age less than 34 month old. Gross motor score will be presented as gross motor developmental quotient (GMDQ). Mean (SD) of GMDQ was 86.5 (16.3) in PHIV and 78.4 (14.5) in PHEU, $p = 0.009$ at the first assessment and decline at 12-month visit, 77.1 (15.8) in PHIV *vs* 80.0 (11.5) in PHEU, $p = 0.38$ (Table 23). PHEU children had significant greater decline of GMDQ than PHIV children as shown in mean difference (95% CI) between at enrolment and 12-month visit was -11.4 (-15.0 to -7.8) in PHEU and -1.9 (-7.1 to 3.4) in PHIV, $p = 0.004$. The frequency of GMDQ according to descriptive category is shown in Table 24. Most PHIV and PHEU children had below average to average gross motor development outcome.

Table 23. Gross motor developmental quotient of PHIV and PHEU

Gross motor developmental quotient	**N**	**PHIV**	**N**	**PHEU**	***p***
Mean (SD)					
• month 0	40	78.4 (14.5)	79	86.5 (16.3)	0.009
• month 12	26	77.0 (15.8)	53	80.0 (11.5)	0.38
Mean difference (95% CI)		-1.9 (-7.1 to 3.4)		-11.4 (-15 to -7.8)	0.004

Table 24. Frequency of gross motor developmental quotient (GMDQ) according to descriptive category in PHIV and PHEU

Descriptive category	**GMDQ**	**Month 0**			**Month 12**		
		PHIV (n=40) n (%)	**PHEU (n=79)** n (%)	***p***	**PHIV (n=26)** n (%)	**PHEU (n=53)** n (%)	***p***
Very low	≤ 70	10 (25%)	15 (19%)		5 (20%)	10 (19%)	
Below average	71-85	15 (37.5%)	22 (28%)		15 (60%)	21 (40%)	
Average	86-115	15 (37.5%)	40 (51%)	0.55	5 (20%)	22 (41%)	0.15
Above average	116-130	0 (0)	1 (1%)		0	0	
Very high	>130	0 (0)	1 (1%)		0	0	

4.3.2.2 Prevalence of gross motor impairment

Due to limitation of MSEL, gross motor domain was assessed only developmental age less than 34 month old. Gross motor impairment was defined as gross motor developmental quotient ≤ 70 (Table 25). Only 79 PHEU and 40 PHIV children were analyzed at enrolment as well as 53 PHEU and 26 PHIV were analyzed at 12-month visit. The overall rate of gross motor impairment included children who had gross motor impairment at enrollment and/or follow-up visit. The prevalence of any gross motor impairment was 27% (95% CI 17 - 38) in PHEU and 35% (95%CI 21 - 52) with OR 1.5 (95% CI 0.7 - 3.4). At enrolment, the prevalence of gross motor impairment was 19% (95% CI 11 - 29) in PHEU and 25% (95% CI 13 - 41%) in PHIV with OR 1.4 (95%CI 0.6 - 3.5). At 12-month visit, the prevalence of gross motor impairment was stable in PHEU as 19% (95% CI 9 - 32) and decline in PHIV as 20% (95%CI 7 - 41%)] with OR 1.1 (95% CI 0.3 - 3.6)].

Table 25. Prevalence of gross motor impairment of PHIV and PHEU children

Group	Overall			Month 0			Month 12		
	N	% (95%CI)	OR (95%CI)	N	% (95%CI)	OR (95%CI)	N	% (95%CI)	OR (95%CI)
PHEU	79	27 (17-38)	Ref	79	19 (11-29)	Ref	53	19 (9-32)	Ref
PHIV	40	35 (21-52)	1.5 (0.7-3.4)	40	25 (13-41)	1.4 (0.6-3.5)	26	20 (7-41)	1.1 (0.3-3.6)

4.3.3 Fine motor

4.3.3.1 Fine motor T-score of PHIV and PHEU children

Mean (SD) of fine motor T-score was 43.1 (11.3) in PHIV and 46.7 (11.7) in PHEU, p = 0.07 at enrollment. PHIV children had increased fine motor T-score at 12-month visit [mean (SD) 48.3 (14.8)] while PHEU children had declined T-score [mean (SD) 46.1 (13.6) in PHEU, p = 0.38] (Table 26). Thus, there was significant different in mean difference between PHIV and PHEU, p = 0.03. Mean difference (95% CI) between at enrolment and 12-month visit was 5.2 (0.9 to 9.4) in PHIV and -0.4 (-3.4 to 2.6) in PHEU. The frequency of fine motor T-score according to descriptive category is shown in Table 27. Most PHIV and PHEU children had average fine motor development outcome.

Table 26. Fine motor T-score of PHIV and PHEU

Fine motor T-score	PHIV	PHEU	*p*
Mean (SD)			
• month 0	43.1 (11.3)	46.7 (11.7)	0.07
• month 12	48.3 (14.8)	46.1 (13.6)	0.38
Mean difference (95% CI)	5.2 (0.9-9.4)	-0.4 (-3.4 to 2.6)	0.03

Table 27. Frequency of fine motor T-score according to descriptive category in PHIV and PHEU

Descriptive category	T-score	Month 0			Month 12		
		PHIV (n=50)	PHEU (n=100)	*p*	PHIV (n=50)	PHEU (n=97)	*p*
		n (%)	n (%)		n (%)	n (%)	
Very low	≤ 30	6 (12%)	8 (8%)		10 (20%)	18 (19%)	
Below average	31-40	15 (30%)	26 (26%)		6 (12%)	16 (16%)	
Average	41-60	25 (50%)	59 (59%)	0.47	15 (30%)	38 (39%)	0.42
Above average	61-70	4 (8%)	4 (4%)		19 (38%)	25 (26%)	
Very high	>70	0 (0)	3 (3%)		0	0	

4.3.3.2 Prevalence of fine motor impairment

The prevalence of any fine motor impairment was 23% (95% CI 15 - 32) in PHEU and 24% (95%CI 13 - 38) with OR 1.1 (95% CI 0.5 - 2.4) (Table 28). At enrollment, the prevalence of fine motor impairment was 8% (95% CI 4 - 15) in PHEU and 12% (95% CI 5 - 24%) in PHIV with OR 1.6 (95%CI 0.5 - 4.8). At 12-month visit, the prevalence of fine motor impairment was increase in both group as 19% (95% CI 11 - 28) in PHEU and 20% (95% CI 10 - 34%) in PHIV with OR 1.1 (95% CI 0.5 - 2.6).

Table 28. Prevalence of fine motor impairment of PHIV and PHEU children

Group	Overall, n=150		Month 0, n=150		Month 12, n=147	
	% (95%CI)	OR (95%CI)	% (95%CI)	OR (95%CI)	% (95%CI)	OR (95%CI)
PHEU	23 (15-32)	Ref	8 (4-15)	Ref	19 (11-28)	Ref
PHIV	24 (13-38)	1.1 (0.5-2.4)	12 (5-24)	1.6 (0.5-4.8)	20 (10-34)	1.1 (0.5-2.6)

4.3.4 Visual reception

4.3.4.1 Visual reception T-score of PHIV and PHEU children

Mean (SD) of visual reception T-score was 40.2 (10.8) in PHIV and 47.3 (12.7) in PHEU, $p = 0.001$ at the first assessment. PHIV children had increased visual reception T-score at 12-month visit [mean (SD) 47.6 (14.7)] while PHEU children had stable T-score [mean (SD) 47.8 (14.2) in PHEU, $p = 0.95$] (Table 29). Thus, there was significant different in mean difference between PHIV and PHEU, $p = 0.02$. Mean difference (95% CI) between at

enrollment and 12-month visit was 7.3 (2.7 to 12.1) in PHIV and 0.8 (-2.3 to 3.9) in PHEU. The frequency of visual reception T-score according to descriptive category is shown in Table 30. Most PHIV and PHEU children had below average to average visual reception development outcome at enrolment and average to above average outcome at 12-month visit.

Table 29. Visual reception T-score of PHIV and PHEU

Visual reception T-score	**PHIV**	**PHEU**	***p***
Mean (SD)			
• month 0	40.2 (10.8)	47.3 (12.7)	0.001
• month 12	47.6 (14.7)	47.8 (14.2)	0.95
Mean difference (95% CI)	7.3 (2.7 to 12.1)	0.8 (-2.3 to 3.9)	0.02

Table 30. Frequency of visual reception T-score according to descriptive category in PHIV and PHEU

Descriptive category	**T-score**	**Month 0**			**Month 12**		
		PHIV (n=50) n (%)	**PHEU (n=100)** n (%)	***p***	**PHIV (n=50)** n (%)	**PHEU (n=97)** n (%)	***p***
Very low	≤ 30	7 (14%)	8 (8%)		9 (18%)	18 (19%)	
Below average	31-40	24 (48%)	22 (22%)		10 (20%)	15 (15%)	
Average	41-60	17 (34%)	57 (57%)	0.004	13 (26%)	33 (34%)	0.72
Above average	61-70	2 (4%)	11 (11%)		18 (36%)	29 (30%)	
Very high	>70	0 (0)	2 (2%)		0 (0)	2 (2%)	

4.3.4.2 Prevalence of visual reception impairment

The prevalence of any visual reception impairment was 23% (95% CI 15 - 33) in PHEU and 24% (95%CI 13 - 38) with OR 1.1 (95% CI 0.5 - 2.4) (Table 31). At enrolment, the prevalence of visual reception impairment was 8% (95% CI 4 - 15) in PHEU and 14% (95% CI 6 - 27%) in PHIV with OR 1.9 (95% CI 0.6 - 5.5). At 12-month visit, the prevalence of fine motor impairment was stable in both group as 19% (95% CI 11 - 28) in PHEU and 18% (95% CI 9 - 31%) in PHIV with OR 1.0 (95% CI 0.4 - 2.3).

Table 31. Prevalence of visual reception impairment of PHIV and PHEU children

Group	Overall, n=150		Month 0, n=150		Month 12, n=147	
	% (95%CI)	OR (95%CI)	% (95%CI)	OR (95%CI)	% (95%CI)	OR (95%CI)
PHEU	23 (15-33)	Ref	8 (4-15)	Ref	19 (11-28)	Ref
PHIV	24 (13-38)	1.1 (0.5-2.4)	14 (6-27)	1.9 (0.6-5.5)	18 (9-31)	1.0 (0.4-2.3)

4.3.5 Receptive language

4.3.5.1 Receptive language T-score of PHIV and PHEU children

Mean (SD) of receptive language T-score was 41.5 (9.2) in PHIV and 44.2 (11.4) in PHEU, p = 0.15 at the first assessment. Both PHIV and PHEU children had declined in receptive language T-score at 12-month visit [mean (SD) 39.6 (6.9) in PHIV and mean (SD) 41.0 (9.4) in PHEU, p = 0.33] (Table 32). Mean difference (95% CI) between at enrolment and 12-month visit was -2.0 (-4.9 to 1.0) in PHIV and -3.2 (-5.3 to -1.0) in PHEU, p =0.51. The frequency of receptive language T-score according to descriptive category is shown in Table 33. Most PHIV and PHEU children had average developmental outcome at enrolment and below to average development outcome at 12-month visit.

Table 32. Receptive language T-score of PHIV and PHEU

Receptive language T-score	PHIV	PHEU	*p*
Mean (SD)			
• month 0	41.5 (9.2)	44.2 (11.4)	0.15
• month 12	39.6 (6.9)	41.0 (9.4)	0.33
Mean difference (95% CI)	-2.0 (-4.9 to 1.0)	-3.2 (-5.3 to -1.0)	0.51

Table 33. Frequency of receptive language T-score according to descriptive category in PHIV and PHEU

Descriptive category	T-score	Month 0			Month 12		
		PHIV (n=50)	PHEU (n=100)	*p*	PHIV (n=50)	PHEU (n=97)	*p*
		n (%)	n (%)		n (%)	n (%)	
Very low	≤ 30	7 (14%)	15 (15%)		4 (8%)	8 (9%)	
Below average	31-40	14 (28%)	24 (24%)		27 (54%)	46 (47%)	
Average	41-60	27 (54%)	54 (54%)	0.96	19 (38%)	38 (39%)	0.47
Above average	61-70	2 (4%)	6 (6%)		0 (0)	5 (5%)	
Very high	>70	0 (0)	1 (1%)		4 (8%)	0 (0)	

4.3.5.2 Prevalence of receptive language impairment

The prevalence of any receptive language impairment was 18% (95% CI 11 - 27) in PHEU and 18% (95% CI 9 - 31) in PHIV with OR 1.0 (95% CI 0.4 - 2.4) (Table 34). At enrolment, the prevalence of receptive language impairment was 15% (95% CI 9 - 24) in PHEU and 14% (95% CI 6 - 27%) in PHIV with OR 0.9 (95% CI 0.3 - 2.4). At 12-month visit, the prevalence of receptive language impairment was decline in both group as 8% (95% CI 4 - 16) in PHEU and 8% (95% CI 2 - 19%) in PHIV with OR 1.0 (95% CI 0.3 - 3.4).

Table 34. Prevalence of receptive language impairment of PHIV and PHEU children

	Overall, n=150		**Month 0, n=150**		**Month 12, n=147**	
Group	**%**	**OR**	**%**	**OR**	**%**	**OR**
	(95%CI)	**(95%CI)**	**(95%CI)**	**(95%CI)**	**(95%CI)**	**(95%CI)**
PHEU	18	Ref	15	Ref	9	Ref
	(11-27)		(9 -24)		(4-16)	
PHIV	18	1.0	14	0.9	8	1.0
	(9-31)	(0.4-2.4)	(6-27)	(0.3-2.4)	(2-19)	(0.3-3.4)

4.3.6 Expressive language

4.3.6.1 Expressive language T-score of PHIV and PHEU children

Mean (SD) of expressive language T-score was 36.9 (7.9) in PHIV and 39.7 (10.3) in PHEU, p = 0.10 at the first assessment. PHIV children had increased mean expressive language T-score at 12-month visit [mean (SD) 37.6 (10.2)] while PHEU children had declined T-score [mean (SD) 37.8 (10.6) in PHEU, p = 0.77] (Table 35). Mean difference (95% CI) between at enrolment and 12-month visit was 0.3 (-2.3 to 3.0) in PHIV and -2.0 (-4.2 to 0.2) in PHEU, p = 0.19. The frequency of expressive language T-score according to descriptive category is shown in Table 36. Most PHIV and PHEU children had below average to average development outcome.

Table 35. Expressive language T-score of PHIV and PHEU

Expressive language T-score	**PHIV**	**PHEU**	***p***
Mean (SD)			
• month 0	36.9 (7.9)	39.7 (10.3)	0.10
• month 12	37.2 (10.2)	37.8 (10.6)	0.77
Mean difference (95% CI)	0.3 (-2.3 to 3.0)	-2.0 (-4.2 to 0.2)	0.19

Table 36. Frequency of expressive language T-score according to descriptive category in PHIV and PHEU

Descriptive category	T-score	Month 0			Month 12		
		PHIV (n=50)	PHEU (n=100)	*p*	PHIV (n=50)	PHEU (n=97)	*p*
		n (%)	n (%)		n (%)	n (%)	
Very low	≤ 30	8 (16%)	19 (19%)		12 (24%)	28 (29%)	
Below average	31-40	25 (50%)	33 (33%)		14 (28%)	33 (34%)	
Average	41-60	17 (34%)	46 (46%)	0.21	24 (48%)	35 (36%)	0.55
Above average	61-70	0 (0)	2 (2%)		0 (0)	1 (1%)	
Very high	>70	0 (0)	0 (0)		0 (0)	0 (0)	

4.3.6.2 Prevalence of expressive language impairment

The prevalence of any expressive language impairment was 34% (95% CI 25 - 44) in PHEU and 28% (95%CI 16 - 43) in PHIV with OR 0.8 (95% CI 0.4 - 1.6) (Table36). At enrolment, the prevalence of receptive language impairment was 19% (95% CI 12 - 28) in PHEU and 16% (95%CI 7 - 29%) in PHIV with OR 0.8 (95%CI 0.3-2.0). At 12-month visit, the prevalence of expressive language impairment was increase in both group as 29% (95% CI 20 - 39) in PHEU and 24% (13 - 38%) in PHIV with OR 0.8 (95% CI 0.4 - 1.7).

Table 36. Prevalence of expressive language impairment of PHIV and PHEU children

Group	Overall, n=150		Month 0, n=150		Month 12, n=147	
	% (95%CI)	OR (95%CI)	% (95%CI)	OR (95%CI)	% (95%CI)	OR (95%CI)
PHEU	34 (25-44)	Ref	19 (12-28)	Ref	29 (20-39)	Ref
PHIV	28 (16-43)	0.8 (0.4-1.6)	16 (7-29)	0.8 (0.3-2.0)	24 (13-38)	0.8 (0.4-1.7)

Summary of developmental score and prevalence of developmental impairment are shown in Figure 6 and 7.

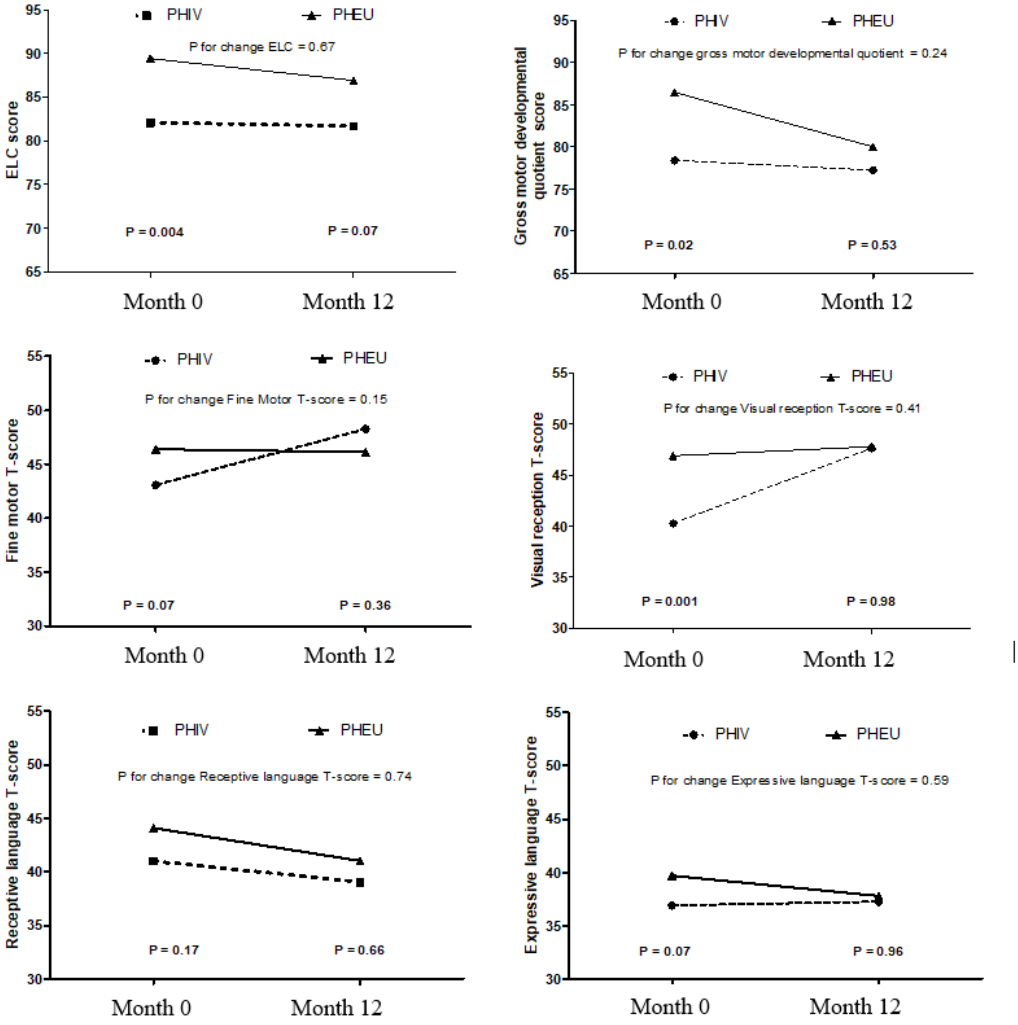

Figure 6. Comparison of Mullen Scales of Early Learning outcomes overtime in PHIV and PHEU children

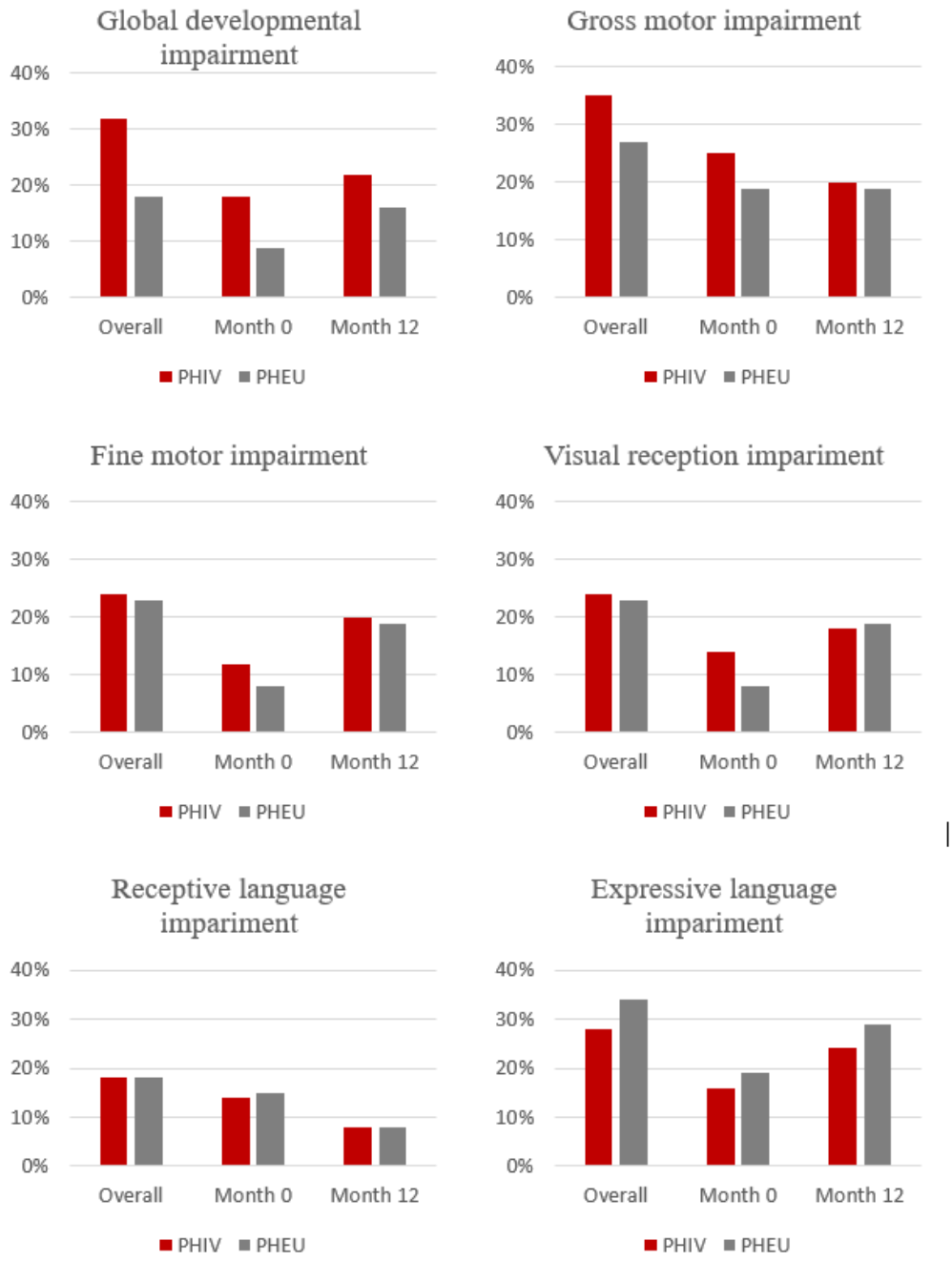

Figure 7. Comparison of prevalence of developmental impairment between PHIV and PHEU

4.3.7 Subgroup analysis in early ART PHIV, standard ART PHIV and PHEU

PHIV children were categorized by time at ART initiation. Early ART PHIV group initiated ART at age ≤ 3 months. Standard ART PHIV group initiated ART at ages > 3 to ≤12 months. Baseline characteristic of early ART and standard ART PHIV are shown in Table 37. Twenty seven PHIV children was defined as early ART PHIV and 23 PHIV children as

standard ART PHIV. Early ART PHIV Median (IQR) age initiated ART was 2.1 (1.5-2.8) months in early ART PHIV and 5.3 (4.2-6.7) months in standard ART PHIV, *p*-value < 0.001. Current ART regimen and HIV parameter were not different between early ART and standard ART PHIV.

Table 37. Baseline characteristics of early ART PHIV and standard ART PHIV

	Month 0			**Month 12**		
Variable	**Early ART PHIV (n=27)**	**Standard ART PHIV (n=23)**	***p***	**Early ART PHIV (n=27)**	**Standard ART PHIV (n=23)**	***p***
Age initiated ART, months, median (IQR)	2.1 (1.5-2.8)	5.3 (4.2-6.7)	<0.001	NA	NA	NA
Mode of infection						
• In utero	7 (26%)	1 (4%)	0.10	NA	NA	NA
• Peripartum	4 (15%)	3 (13%)		NA	NA	NA
• Unknown	16 (59%)	19 (83%)		NA	NA	NA
Current ART regimen, n (%)			0.84			0.83
• PI-based	23 (85%)	19 (83%)		23 (85%)	19 (83%)	
• NPV-based	4 (15%)	4 (17%)		3 (11%)	4 (17%)	
• Integrase inhibitor-based	0	0		1 (4%)	0	
CD4+ T cell count (cells/µL), median (IQR)	1943 (1370-2885)	1725 (1340-2363)	0.37	1570 (1239-1818)	1409 (1121-1829)	0.42
HIV RNA <200 copies/mL, n (%)	19 (70%)	18 (78%)	0.53	19 (70%)	16 (70%)	0.95

ART; antiretroviral therapy, PIs; protease inhibitors, NVP; nevirapine

Comparison of baseline characteristics was shown in Table 38. Median (IQR) age at the first assessment was 27 (19 - 42) months in PHEU, 25 (18 - 30) months in early ART PHIV and 35 (28 - 41) months in standard ART PHIV, $p = 0.01$. There was no significant difference for gender, birth weight, prematurity and anemic status. However, there were statistically significant difference for WAZ, HAZ, HCAZ and MUACZ.

Table 38. Baseline characteristics of PHEU, early ART PHIV and standard ART PHIV

Median (IQR) or n (%)	**PHEU**	**Early ART PHIV**	**Standard ART PHIV**	***p***
At enrollment	n=100	n=27	n=23	
Age, months	27 (19-42)	25 (18-30)	35 (28-41)	0.01
Sex: male	45 (45%)	16 (59%)	12 (53%)	0.43
Low birth weight (birth weight < 2500 g)	18 (18%)	7 (30%)	8 (30%)	0.26
Preterm (GA 34 - < 37 weeks)	32 (32%)	9 (33%)	10 (44%)	0.59
Weight for age Z-score	-0.3 (-0.9 to 0.5)	-0.3 (-1.2 to 0.4)	-0.7 (-1.5 to -0.4)	0.01
Height for age Z-score	-0.6 (-1.3 to 0.1)	-0.8 (-1.6 to -0.2)	-1.3 (-2 to -0.9)	0.002
Head circumference for age Z-score	-0.8 (-1.5 to -0.1)	-0.8 (-2.3 to 0.1)	-1.6 (-2.4 to -0.2)	0.06
Mid upper arm circumference for age Z-score	-0.3 (-1.0 to 0.9)	-0.8 (-1.7 to 0)	-0.3 (-1.1 to 0.4)	0.03
Anemia (Hb < 11 g/dl)	14 (14%)	7 (26%)	5 (22%)	0.27
No nursery school attendance	67 (67%)	22 (81%)	12 (52%)	0.09
At 12-month visit	n=97	n=27	n=23	
Weight for age Z-score	-0.2 (-0.9 to 0.4)	-0.6 (-1.6 to 0)	-0.8 (-1.3 to -0.3)	0.003
Height for age Z-score	-0.8 (-1.3 to -0.2)	-0.6 (-1.5 to 0)	-1.4 (-2 to -0.7)	0.03
Head circumference for age Z-score	-0.3 (-1 to 0.5)	-0.8 (-1.4 to -0.4)	-0.8 (-1.6 to -0.5)	0.002
Mid upper arm circumference for age Z-score	0.2 (-0.6 to 0.9)	-0.4 (-1.4 to 0.4)	-0.2 (-0.7 to 0.3)	0.04
Anemia (Hb < 11 g/dl)	12 (12%)	5 (19%)	2 (9%)	0.57
No nursery school attendance	52 (54%)	18 (67%)	9 (39%)	0.15

PHEU; Perinatally HIV exposed uninfected children, PHIV; Perinatally HIV infected children, Early ART PHIV; children with early initiated antiretroviral therapy within 3 months of age, Standard ART PHIV; children with initiated antiretroviral therapy within 3-12 months of age, GA; gestational age, *p-value*; *p*-value among PHEU, early PHIV, and standard PHIV

4.3.7.1 Neurodevelopmental scores

Comparison of developmental scores among groups of study participants is shown in Table 39 and Figure 8. Mean (SD) ELC scores at enrollment were 90 (16), 83 (11), 81 (19) in PHEU, early ART PHIV, and standard ART PHIV children, respectively, with significant differences among 3 groups (p = 0.02) and between PHEU and standard ART PHIV (p = 0.01). However, no group differences were observed at 12-month visit among 3 groups and when compared to PHEU [Mean (SD) 87 (15), 81 (15), and 82 (16) in PHEU, early ART PHIV, and standard ART PHIV children, respectively, p > 0.05]. Mean ELC score declined overtime in PHEU and early ART PHIV children [mean difference -2.7 (95% CI -5.4 to 0.05 in PHEU and -2.1 (95% CI -8.5 to 4.2) in early ART PHIV group]. Mean scores increased in standard ART PHIV children [mean difference 1.6 (95% CI -4.2 to 7.4)].

Standard ART PHIV children had significantly lower gross motor developmental quotient when compared to PHEU children at enrollment (p < 0.001): however, no difference was observed at the 12-month visit. Early ART PHIV children had comparable performances in all domains when compared to PHEU children, except for lower visual reception T-score at enrollment [mean (SD) 41 (9) vs. 47 (13), p = 0.01]. On the contrary, standard ART PHIV children had lower scores in all domains with significant differences in gross motor and visual reception domain when compared to PHEU children at the enrolment visit. However, no differences were observed at the 12-month visit. Standard ART PHIV children showed a significant increase in fine motor T-score (mean difference 7.6 (95% CI 1.4 to 13.7) and visual reception T score (mean difference 10.5 (95% CI 3.4 to 17.6) from month 0 to month 12.

Table 39. Comparison of neurodevelopmental scores among PHEU, early ART PHIV and standard ART PHIV

	Month 0			Month 12			Mean differences	
	Mean (SD)	***P1***	***P2***	**Mean (SD)**	***P1***	***P2***	**Mean differences (95%CI)**	***P1***
Early learning composite								
PHEU	90 (16)	Ref		87 (15)	Ref		-2.7 (-5.4 to 0.1)	Ref
Early ART PHIV	83 (11)	0.05	0.02	81 (15)	0.09	0.15	-2.1 (-8.5 to 4.2)	0.86
Standard ART PHIV	81 (19)	0.01		82 (16)	0.20		1.6 (-4.2 to 7.4)	0.19
Gross motor developmental quotient								
PHEU	87 (16)	Ref		80 (12)	Ref		-11.4 (-15 to -7.8)	Ref
Early ART PHIV	84 (11)	0.41	0.002	80 (11)	0.99	0.21	-2.1 (-9.1 to 4.8)	0.01
Standard ART PHIV	71 (16)	<0.001		71 (23)	0.08		-1.3 (-11 to 8.4)	0.045
Fine motor T-score								
PHEU	47 (12)	Ref		46 (14)	Ref		-0.4 (-3.4 to 2.6)	Ref
Early ART PHIV	44 (10)	0.21	0.25	47 (14)	0.84	0.48	3.2 (-2.9 to 9.3)	0.27
Standard ART PHIV	43 (13)	0.12		50 (16)	0.23		7.6 (1.4 to 13.7)	0.02
Visual reception T score								
PHEU	47 (13)	Ref		48 (14)	Ref		0.8 (-2.3 to 3.9)	Ref
Early ART PHIV	41 (9)	0.01	0.008	45 (14)	0.41	0.43	4.7 (-1.8 to 11.2)	0.26
Standard ART PHIV	40 (13)	0.01		51 (16)	0.42		10.5 (3.4 to 17.6)	0.01
Receptive Language T-score								
PHEU	44 (11)	Ref		41 (9)	Ref		-3.2 (-5.3 to -1)	Ref
Early ART PHIV	43 (8)	0.54	0.26	40 (8)	0.59	0.58	-2.8 (-6.9 to 1.3)	0.88
Standard ART PHIV	40 (11)	0.09		39 (5)	0.32		-1 (-5.5 to 3.6)	0.37
Expressive Language T-score								
PHEU	40 (10)	Ref		38 (11)	Ref		-2 (-4.2 to 0.2)	Ref
Early ART PHIV	38 (7)	0.44	0.17	38 (10)	0.87	0.77	0.1 (-3.5 to 3.7)	0.36
Standard ART PHIV	36 (9)	0.06		36 (11)	0.51		0.7 (-3.7 to 5)	0.27

P1: *P*-value when compared to PHEU, P2: *P*-value among 3 groups

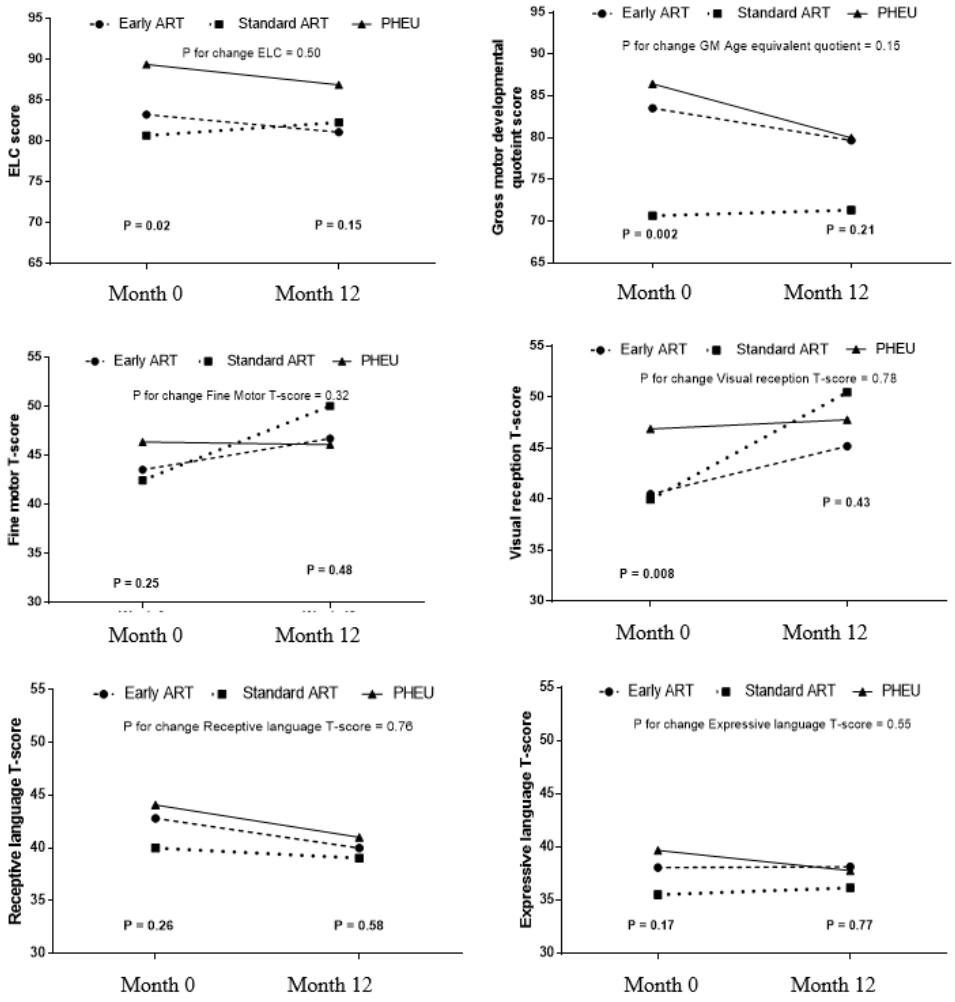

Figure 8. Comparison of Mullen Scales of Early Learning outcomes overtime in PHEU, early ART PHIV, and standard ART PHIV children
p-value; compare among 3 groups

4.3.7.2 Prevalence of global and individual developmental impairment

The prevalence of overall GDI was 18% (95% CI 11-27) and 32% (95% CI 20-47) in PHEU and PHIV children, respectively (p = 0.06). For the subgroup analysis, 22% (95% CI 9-42) of early ART PHIV and 44% (95% CI 23-66) of standard ART PHIV children had overall GDI (Table 40). There were no significant differences in the rate of overall GDI in early ART PHIV compared to the PHEU group, at enrollment and at 12-month visit (p = 0.62, p = 0.79 and p = 0.70 respectively). PHIV children with standard ART had a higher prevalence of overall GDI compared to PHEU children (p = 0.01), specifically only at study enrollment (p = 0.009). The rate of GDI in the standard ART group declined at 12-month visit and was comparable to PHEU children (p = 0.23).

The trajectory pattern of global developmental outcome is shown in Figure 9. Typical development was reported among 82%, 77% and 57% in PHEU, early ART PHIV and standard ART PHIV children, respectively. Four PHIV children with standard ART (17%) had resolving GDI at the 12-month visit as did 1(4%) early PHIV child and 3 (3%) PHEU children. PHIV children had a higher rate of emerging GDI when compared to PHEU children (13-15% vs. 9%). Three standard PHIV children (13%) had persistent GDI while 1 early ART (4%) and 6 PHEU (6%) demonstrated persistent GDI pattern.

Table 40. Prevalence of global developmental impairment by Mullen Scales of Early Learning among PHEU, early ART PHIV, and standard ART PHIV children

	Overall (N=150)			**Month 0 (N=150)**			**Month 12 (N=147)**		
Group	**%** **(95%CI)**	**OR** **(95%CI)**	***P***	**%** **(95%CI)**	**OR** **(95%CI)**	***P***	**%** **(95%CI)**	**OR (95%CI)**	***P***
PHEU	18 (11-27)	Ref	-	9 (4-16)	Ref	-	16 (9-24)	Ref	-
Early ART PHIV	22 (9-42)	1.3 (0.5-3.7)	0.62	7 (1-24)	0.8 (0.2-4.0)	0.79	19 (6-38)	1.2 (0.4-3.8)	0.70
Standard ART PHIV	44 (23-66)	3.5 (1.3-8.2)	0.01	30 (13-53)	4.4 (1.4-13.6)	0.009	26 (10-48)	1.9 (0.7-5.7)	0.23

PHEU; Perinatally HIV exposed uninfected children, PHIV; Perinatally HIV infected children, Early ART PHIV; children with early initiated antiretroviral therapy within 3 months of age; Standard ART PHIV; children with initiated antiretroviral therapy within 3-12 months of age, OR; Odds ratio

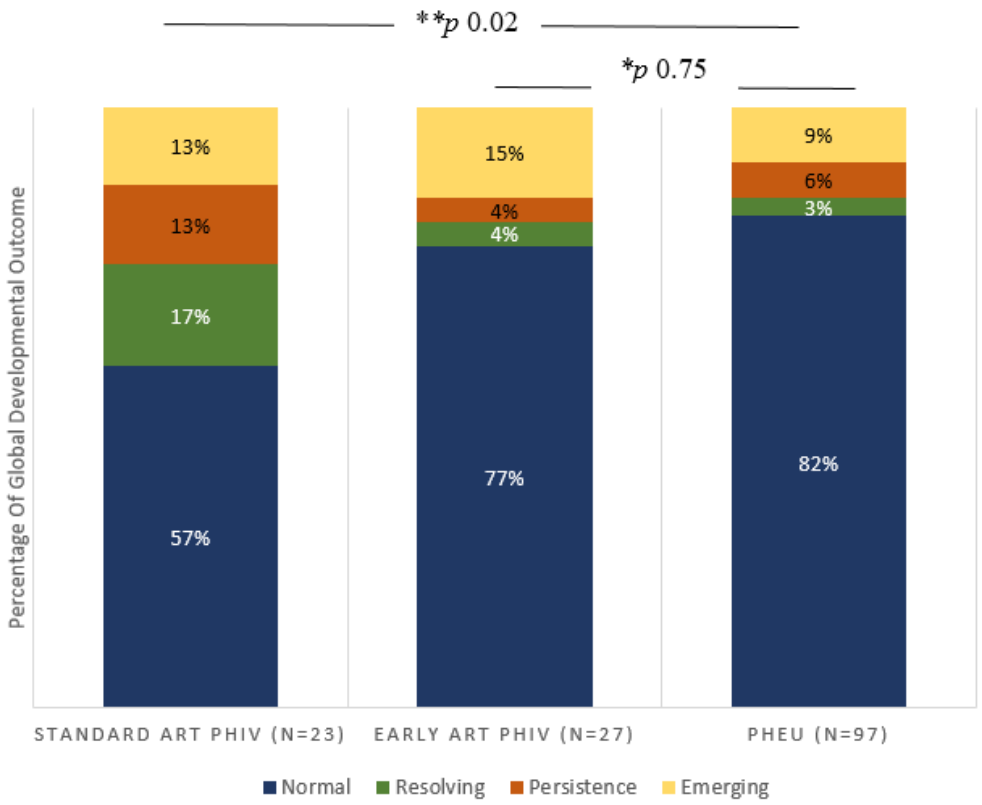

Figure 9. Trajectory pattern of global developmental outcome by ELC score of standard ART PHIV, early ART PHIV and PHEU children

(ELC, Early learning composite; Normal = ELC > 70 at month 0 and month 12; Persistence = ELC ≤ 70 at month 0 and month 12; Resolving = ELC ≤ 70 at month 0, but ELC > 70 at month 12, Emerging = ELC > 70 at month 0, but ELC ≤ 70 at month 12), *p-value between PHEU and early ART PHIV, **p-value between PHEU and standard ART PHIV)

Prevalence of individual domain impairment is shown in Table 41 and Figure 10. Prevalence of impairment in all individual domains among early ART PHIV was comparable to PHEU children. In contrast, prevalence of gross motor impairment was higher in the standard ART PHIV children compared to the PHEU group at enrollment (44% vs. 19%, p = 0.04) but was comparable at the 48-week visit (25% vs. 19%, p = 0.69). Prevalence rates of fine motor, visual reception, receptive and expressive language impairment were not significantly different in standard ART PHIV children when compared to PHEU children. There were no children with standard ART PHIV who had receptive language impairment at week 48.

Table 41. Prevalence of individual domain impairment among PHEU, early ART PHIV and standard ART PHIV

	N	PHEU % (95%CI)	N	Early ART PHIV % (95%CI)	*P*	N	Standard ART PHIV % (95%CI)	*P*
Gross motor impairment (GM developmental quotient ≤ 70)								
Overall	79	27 (17-38)	24	25 (10-47)	0.88	16	50 (25-75)	0.07
Enrollment	79	19 (11-29)	24	13 (3-32)	0.47	16	44 (20-70)	0.04
12-month visit	53	19 (9-32)	18	18 (4-43)	0.91	8	25 (3-65)	0.69
Fine motor impairment (T score ≤ 30)								
Overall	100	23 (15-32)	27	19 (6-38)	0.95	23	30 (13-53)	0.95
Enrollment	100	8 (4-15)	27	7 (1-24)	0.92	23	17 (5-39)	0.65
12-month visit	97	19 (11-28)	27	15 (4-34)	0.18	23	26 (10-48)	0.42
Visual reception impairment (T score ≤ 30)								
Overall	100	23 (15-33)	27	16 (6-38)	0.62	23	30 (13-53)	0.46
Enrollment	100	8 (4-15)	27	7 (1-24)	0.92	23	22 (8-44)	0.06
12-month visit	97	19 (11-28)	27	15 (4-34)	0.65	23	22 (8-44)	0.73
Receptive language impairment (T score ≤ 30)								
Overall	100	18 (11-27)	27	19 (6-38)	0.95	23	17 (5-39)	0.95
Enrollment	100	15 (9-24)	27	11 (2-29)	0.61	23	17 (5-39)	0.78
12-month visit	97	8 (4-16)	27	17 (5-39)	0.78	23	0 (0-15)	NA
Expressive language impairment (T score ≤ 30)								
Overall	100	34 (25-44)	27	22 (9-42)	0.25	23	35 (16-57)	0.94
Enrollment	100	19 (12-28)	27	7 (1-24)	0.17	23	26 (10-48)	0.45
12-month visit	97	29 (20-39)	27	22 (9-42)	0.50	23	26 (10-48)	0.79

p-value when compared to PHEU children

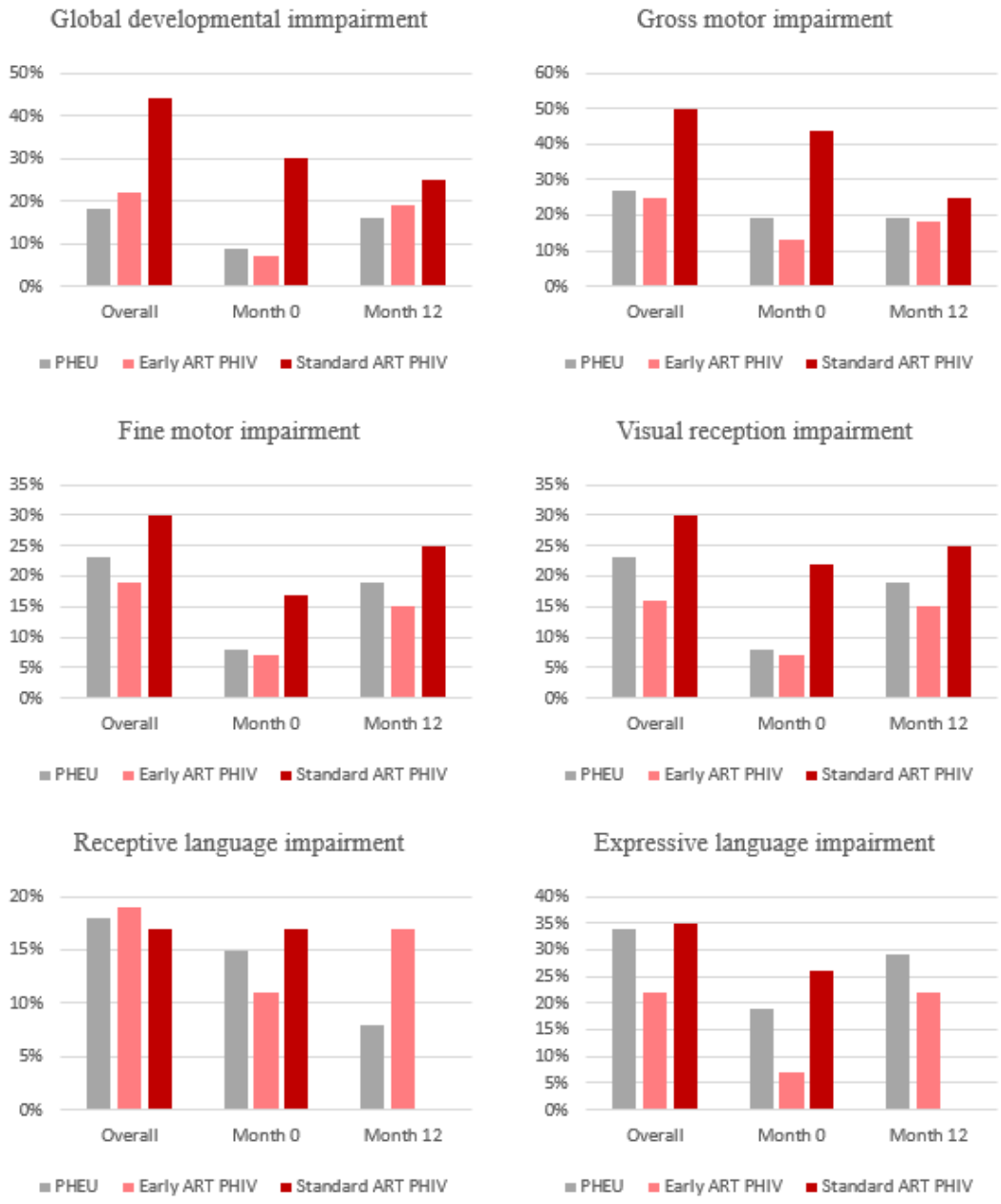

Figure 10. Comparison prevalence of global developmental impairment and each individual impairment among PHEU, early ART PHIV and standard ART PHIV

4.4. Neurobehavioral outcomes

Neurobehavioral outcomes by Child Behavior Checklist (CBCL) were analyzed in children who age ≥ 18 months old. Neurobehavioral outcomes are presented in each problems by DSM-IV oriented and syndrome scales, follow by group problems as internalizing, externalizing and total problems.

4.4.1 DSM-oriented and syndrome scale

Raw score of each behavioral problem is shown in Table 42. There was no difference between PHIV and PHEU, except somatic complaints at enrollment. Mean (SD) somatic complaints raw score was 5.1 (2.5) in PHIV and 3.8 (2.5) in PHEU at enrollment, respectively. However, this difference was resolved at 12-month visit.

Table 42. Comparison of each behavioral problem raw score by DSM-oriented and syndrome scales

	At enrolment			**12-month-visit**			***p*[b]**
Mean (SD)	**PHIV** n=80	**PHEU** n=41	***p*[a]**	**PHIV** n=80	**PHEU** n=41	***p*[a]**	
DSM-oriented (range)							
Affective problem (0-20)	4.2 (2.4)	4.4 (2.4)	0.68	4.1 (2.8)	4.7 (3.1)	0.26	0.37
Anxiety problem (0-20)	5.1 (2.6)	5.4 (2.9)	0.63	4.9 (2.2)	5.2 (3.2)	0.62	0.87
Pervasive development problem (0-26)	3.9 (2.5)	4.4 (2.8)	0.32	3.8 (2.5)	4.6 (3.3)	0.14	0.29
Attention-deficit/ hyperactivity problem (0-12)	6.1 (2.5)	6.6 (2.7)	0.33	6.4 (2.7)	6.4 (2.8)	0.99	0.51
Oppositional defiant problem (0-12)	4.9 (1.6)	4.6 (2.4)	0.56	4.5 (1.8)	4.6 (2.6)	0.93	0.76
Syndromes scales							
Emotionally-reactive (0-18)	4.4 (2.5)	4.5 (2.9)	0.98	4.1 (2.1)	4.5 (3.4)	0.52	0.54

Mean (SD)	At enrolment			12-month-visit			p^b
	PHIV	PHEU	p^a	PHIV	PHEU	p^a	
	n=80	n=41		n=80	n=41		
Anxious/depressed (0-16)	4.1 (2.6)	4.0 (2.2)	0.73	3.4 (2.1)	3.7 (2.6)	0.41	0.36
Somatic complaints (0-22)	5.1 (2.5)	3.8 (2.5)	0.006	4.3 (2.5)	4.1 (2.8)	0.57	0.50
Withdrawn (0-16)	2.7 (2.0)	3.2 (2.0)	0.23	2.9 (2.3)	3.4 (2.1)	0.27	0.45
Sleep problems (0-14)	3.8 (2.0)	4.2 (2.5)	0.29	3.9 (2.0)	3.9 (2.9)	0.87	0.66
Attention problems (0-10)	3.5 (1.8)	4.3 (2.2)	0.04	3.9 (1.9)	4.1 (2.4)	0.64	0.49
Aggressive behavior (0-38)	13.3 (5.0)	14.0 (6.7)	0.59	13.2 (4.9)	13.1 (7.0)	0.92	0.57

p^a for comparison mean raw score between 2 group at each week using two sample independent t-test, p^b for comparison mean raw score change overtime using random effect linear regression to.

Prevalence of each behavioral problem by DSM-oriented and syndrome scales is shown in Table 43. Most common problems were somatic complaints (14-27%), affective problem which included dysthymia and depression (12-22%), withdrawn (10-15%), anxiety problem (5-17%) and attention problems (2-18%). There was no difference among groups and each visits, except sleep problems at 12-month visit. Nine PHEU children had sleep problems while no one in PHIV group had.

Table 43. Comparison of prevalence of each behavioral problem by DSM-oriented and syndrome scales

	At enrolment			12-month-visit			p^b
n (%)	**PHIV** n=80	**PHEU** n=41	p^a	**PHIV** n=80	**PHEU** n=41	p^a	
DSM-oriented (T-score ≥ 70)							
Affective problem	7 (17%)	15 (19%)	0.82	5 (12%)	17 (22%)	0.21	0.36
Anxiety problem	5 (12%)	12 (15%)	0.67	2 (5%)	13 (17%)	0.09	0.17
Pervasive development problem	4 (10%)	12 (15%)	0.57	3 (7%)	15 (19%)	0.11	0.09
Attention-deficit/ hyperactivity	2 (5%)	6 (8%)	0.72	1 (2%)	8 (10%)	0.16	0.18
Oppositional defiant problem	0	5 (6%)	0.17	1 (2%)	8 (10%)	0.16	0.08
Syndromes scales (T-score ≥ 70)							
Emotionally-reactive	1 (2%)	4 (5%)	0.66	2 (5%)	9 (11%)	0.33	0.23
Anxious/depressed	2 (5%)	3 (4%)	0.77	0	5 (6%)	0.16	0.49
Somatic complaints	11 (27%)	11 (14%)	0.08	9 (22%)	12 (15%)	0.36	0.08
Withdrawn	4 (10%)	11 (14%)	0.77	6 (15%)	12 (15%)	0.94	0.63
Sleep problems	1 (2%)	5 (6%)	0.66	0 (0%)	9 (11%)	0.03	0.06
Attention problems	1 (2%)	14 (18%)	0.09	5 (12%)	12 (15%)	0.66	0.18
Aggressive behavior	1 (2%)	7 (9%)	0.26	2 (5%)	6 (8%)	0.71	0.29

4.4.2 Internalizing, externalizing and total problems

T-score of internalizing, externalizing and total problems between PHIV and PHEU children is shown in Table 44. PHIV and PHEU children had similar mean (SD) T score on the CBCL, with internalizing of 61.3 (7.3) vs 59.3 (9.6) at enrolment and 59.3 (7.6) vs 69.6 (9.6) at 12-month visit, externalizing of 55.6 (7.5) vs 57.6 (9.8) at enrollment and 55.4 (7.2) vs 55.5 (10.1) at 12-month visits and total problems of 59.5 (7.5) vs 59.7 (9.8) at enrollment and 58.0 (7.9) vs 58.7 (10.6) at 12-month visit, $p > 0.05$. Overall, PHIV and PHEU children had mean CBCL score that were within normal range (<64).

Table 44. Comparison of T-score of internalizing, externalizing and total problems between PHIV and PHEU children

Mean (SD)	**At enrolment**			**12-month-visit**			p^b
	PHIV n=80	**PHEU** n=41	p^a	**PHIV** n=80	**PHEU** n=41	p^a	
Internalizing (29-100)	61.3 (7.3)	59.3 (9.6)	0.25	59.3 (7.6)	59.6 (9.6)	0.88	0.47
Externalizing (28-100)	55.6 (7.5)	57.6 (9.8)	0.26	55.4 (7.2)	55.5 (10.1)	0.96	0.44
Total problems (28-100)	59.5 (7.5)	59.7 (9.8)	0.94	58.0 (7.9)	58.7 (10.6)	0.71	0.85

The internalizing, externalizing and total problems T score ≥ 64 typically suggest behavioral problems in clinical range and ≥ 60 suggest in border-line range. Prevalence of internalizing, externalizing and total problems in PHIV and PHEU children are shown in Table 45. PHIV and PHEU children were not different rate of internalizing problems (32 - 34% vs 38 - 39%). PHEU children seem to have more prevalence of externalizing than PHIV children (18 - 19% vs 10 - 12%). Prevalence of total problems was 20 - 29% in PHIV and 28 - 30% in PHEU.

Table 45. Comparison of prevalence of clinical range internalizing, externalizing and total problems between PHIV and PHEU children

	At enrolment			12-month-visit			p^b
n (%)	**PHIV** n=80	**PHEU** n=41	p^a	**PHIV** n=80	**PHEU** n=41	p^a	
Clinical range (T-score ≥ 64)							
Internalizing	13 (32%)	31 (39%)	0.45	14 (34%)	30 (38%)	0.68	0.29
Externalizing	5 (12%)	15 (19%)	0.36	4 (10%)	14 (18%)	0.25	0.28
Total problems	8 (20%)	24 (30%)	0.22	12 (29%)	22 (28%)	0.87	0.74
Borderline range (T-score ≥ 60)							
Internalizing	25 (61%)	36 (45%)	0.09	22 (54%)	38 (48%)	0.56	0.11
Externalizing	15 (37%)	13 (16%)	0.01	10 (24%)	24 (30%)	0.49	0.23
Total problems	24 (59%)	36 (45%)	0.16	18 (44%)	32 (41%)	0.72	0.21

Trajectory pattern of clinical-range behavior problems between PHIV and PHEU children is shown in Figure 11. From study entry to the follow-up visit, 51% PHIV and 54% PHEU met the CBCL behavioral cutoff clinical range criteria at internalizing, 19% PHIV and 25% PHEU at externalizing as well as 41% PHIV and 38% PHEU at total problems.

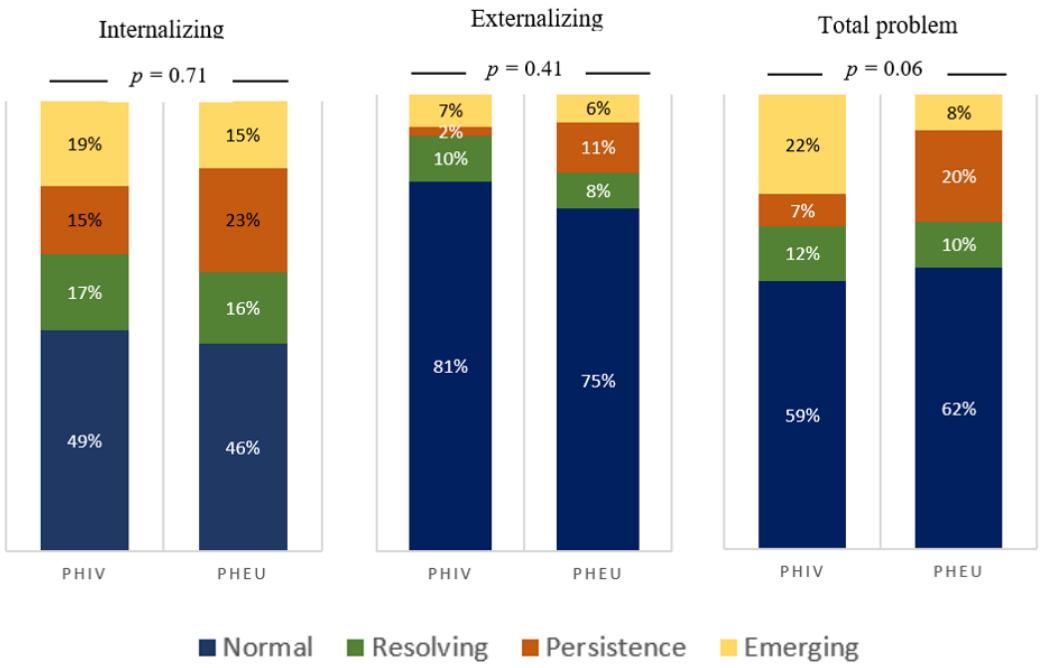

Figure 11. Trajectory pattern of clinical range behavior problems between PHIV and PHEU children

(Normal = T-score ≥ 64 at month 0 and month 12; Persistence = T-score ≥ 64 at month 0 and month 12; Clearance = T-score < 63 at month 0 but T-score ≥ 64 at month 12, Emerging = T-score ≥ 64 at month 0 but T-score < 64 at month 12)

4.4.3. Risk factors of behavioral problems

Risk factors associated with internalizing, externalizing and total problems are shown in Table 46-48. Risk factors of internalizing problems were primary caregiver's depression (aOR 3.09, 95% CI 1.11 - 8.63, p = 003) and authoritarian parenting style (aOR 3.01, 95% CI 1.38 - 6.59, p =0.01). Risk factors of externalizing problems were primary caregiver's duration of education (aOR 5.64, 95% CI 1.07 – 29.61, p = 0.04), primary caregiver's depression (aOR 5.71, 95% CI 1.46 - 22.28, p = 01) and authoritarian parenting style (aOR 7.58, 95% CI 1.90 - 30.28, p =0.004). Risk factor of total problems were primary caregiver's depression (aOR 7.38, 95% CI 2.02 – 26.97, p = 0.003) authoritarian parenting style (aOR 4.01, 95% CI 1.48 - 10.83, p = 0.006).

Table 46. Risk factors associated with internalizing problems

Variable	Univariate		Multivariate	
	OR (95%CI)	*P*	aOR (95%CI)	*P*
Male	1.19 (0.52-2.72)	0.68		
Age ≤ 36 month	0.98 (0.47-2.04)	0.95		
Preterm (GA 34-37 week)	0.48 (0.19-1.19)	0.11		
ELC score ≤ 70	1.91 (0.69-5.27)	0.21		
Group				
PHEU	Ref			
PHIV	0.73 (0.30-.75)	0.48		
PHIV children				
Age started ART > 3 months old	1.96 (0.58-6.69)	0.28		
ART regimen : PI vs NNRTI	2.22 (0.4-12.2)	0.36		
Duration of ART > 24 months	1.08 (0.26-4.43)	0.92		
CD4+ T cell < 2000 cell/mm^3	0.99 (0.29-3.37)	0.99		
HIV-RNA ≥ 200 copies/mL	0.38 (0.06-2.3)	0.29		
Primary caregiver				
Not biological parents	0.68 (0.28-1.67)	0.4		
Age, year	0.97 (0.94-1.01)	0.15		
Duration of education < 12 years	1.88 (0.79-4.48)	0.16		
Depress depression score ≥ 9	3.79 (1.39-10.33)	0.01	3.09 (1.11-8.63)	0.03
Income per family < 10,000 baht/month	0.75 (0.28-2.02)	0.57		
Child rearing				
No Attending daycare/nursery	0.94 (0.44-2.01)	0.87		
No book in their home	1.26 (0.54-2.94)	0.6		
Parenting style				
Authoritative	1.68 (0.92-3.05)	0.10		
Authoritarian ≥ 2	3.50 (1.57-7.77)	0.002	3.01(1.38-6.59)	0.01
Permissive ≥ 3	3.33 (1.5-7.39)	0.003		

GA; gestational age, ELC; Early learning composite score, ART; antiretroviral therapy, PI; protease inhibitor, NNRTI; non-nucleotide reverse transcriptase inhibitor

Table 47. Risk factors associated with externalizing problems

Variable	Univariate		Multivariate	
	OR (95%CI)	*P*	aOR (95%CI)	*P*
Male	2.13 (0.51-8.98)	0.3		
Age ≤ 36 month	1.12 (0.34-3.66)	0.85		
Preterm (GA 34-37 week)	0.73 (0.16-3.33)	0.68		
ELC score ≤ 70	0.93 (0.17-4.97)	0.93		
Group				
PHEU	Ref			
PHIV	0.42 (0.09-1.99)	0.27		
PHIV children				
Age started ART > 3 months old	1.15 (0.21-6.38)	0.87		
ART regimen : PIs vs NNRTI	NA			
Duration of ART > 24 months	5.66 (0.42-76.57)	0.19		
CD4+ T cell < 2000 cell/mm^3	1.15 (0.17-7.72)	0.89		
HIV-RNA ≥ 200 copies/mL	0.43 (0.03-5.24)	0.51		
Primary caregiver				
Not their biological parents	2.76 (0.61-12.4)	0.19		
Age, year	1.07 (1.00-1.14)	0.04		
Duration of education < 12 years	6.84 (1.14-41.19)	0.04	5.64 (1.07-29.61)	0.04
Depress depression score ≥ 9	8.01(1.96-32.78)	0.004	5.71 (1.46-22.28)	0.01
Income per family < 10,000 baht/month	0.65 (0.12-3.61)	0.62		
Child rearing				
No Attending daycare/nursery	3.68 (0.84-16.09)	0.08		
No book in their home	2.13 (0.54-8.32)	0.28		
Parenting style				
Authoritative	1.7 (0.63-4.61)	0.3		
Authoritarian ≥ 2	9.36 (2.29-38.27)	0.002	7.58 (1.90-30.28)	0.004
Permissive ≥ 3	4.05 (1.25-13.08)	0.02		

GA; gestational age, ELC; Early learning composite score, ART; antiretroviral therapy, PIs; protease inhibitor, NNRTI; non-nucleotide reverse transcriptase inhibitor

Table 48. Risk factors associated with total problems

Variable	Univariate		Multivariate	
	OR (95%CI)	*P*	aOR (95%CI)	*P*
Male	2.18 (0.68-6.94)	0.19		
Age ≤ 36 month	0.93 (0.36-2.38)	0.88		
Preterm (GA 34-37 week)	0.52 (0.15-1.76)	0.29		
ELC score ≤ 70	3.78 (1.05-13.61)	0.04		
Group				
PHEU	Ref			
PHIV	0.74 (0.22-2.48)	0.63		
PHIV children				
Age started ART > 3 months old	0.81 (0.26-2.51)	0.72		
ART regimen : PIs vs NNRTI	5.29 (0.59-47.57)	0.14		
Duration of ART > 24 months	1.3 (0.33-5.14)	0.71		
CD4+ T cell < 2000 cell/mm^3	0.62 (0.21-1.89)	0.41		
HIV-RNA ≥ 200 copies/mL	0.28 (0.05-1.54)	0.14		
Primary caregiver				
Not their biological parent	1.11 (0.33-3.77)	0.87		
Age, year	1.01 (0.96-1.06)	0.7		
Duration of education < 12 years	3.89 (1.1-13.75)	0.04		
Depression score ≥ 9	9.62 (2.53-36.5)	0.001	7.38 (2.02-26.97)	0.003
Income per family < 10,000 baht/month	1.00 (0.27-3.71)	0.99		
Child rearing				
No Attending daycare/nursery	1.62 (0.57-4.56)	0.36		
No book in their home	1.06 (0.36-3.15)	0.92		
Parenting style				
Authoritative	6.17 (1.14-33.43)	0.04		
Authoritarian ≥ 2	4.75 (1.77-12.75)	0.002	4.01(1.48-10.83)	0.006
Permissive ≥ 3	3.2 (1.27-8.07)	0.01		

GA; gestational age, ELC; Early learning composite score, ART; antiretroviral therapy, PIs; protease inhibitor, NNRTI; non-nucleotide reverse transcriptase inhibitor

4.4.4. Predictors of changing in behavioral scores

Predictors of changing in internalizing, externalizing and total problems scores are shown in Table 49-51. Internalizing scores were increasing with primary caregiver's depression score (coef 0.29, 95% CI 0.09 to 0.49, p = 0.01) and authoritarian parenting style (coef 1.73, 95% CI 0.41 to 3.05, p = 0.01), yet these internalizing scores were decreasing with ELC score (coef -0.06, 95% CI -0.11 to -0.02, p = 0.01). Externalizing scores were increasing with primary caregiver's depression score (coef 0.28, 95% CI 0.07 to 0.49, p = 0.01), authoritative parenting style (coef 1.17, 95% CI 0.08 to 2.27, p = 0.04) and authoritarian parenting style (coef 2.61, 95% CI 1.24 to 3.99, p <0.001), yet these externalizing scores were decreasing with age (coef -0.08, 95% CI -0.14 to -0.02, p = 0.01). Total behavior scores were increasing with primary caregiver's depression score (coef 0.37, 95% CI 0.15 to 0.58, p = 0.001), authoritative parenting style (coef 1.24, 95% CI 0.14 to 2.34, p < 0.001) and authoritarian parenting style (coef 2.34, 95% CI 0.99 to 3.70, p = 0.001), yet these total problems scores were decreasing with age (coef -0.06, 95% CI -0.12 to -0.01, p = 0.01) and ELC score (coef -0.05, 95% CI -0.1 to -0.004, p = 0.03)

Table 49. Predictors of changing in internalizing scores

Variable	Univariate		Multivariate	
	Coef (95%CI)	***P***	**Coef (95%CI)**	***P***
Male	0.48 (-0.96 to 1.93)	0.51		
Age	-0.03 (-0.09 to 0.02)	0.24		
GA	-0.12 (-0.6 to 0.36)	0.62		
ELC score	-0.07 (-0.12 to -0.03)	0.002	-0.06 (-0.11 to -0.02)	0.01
Group				
PHEU	Ref			
PHIV	-0.56 (-2.09 to 0.97)	0.47		
PHIV children				
Age started ART	0.3 (-0.2 to 0.81)	0.24		
ART regimen : PIs vs NNRTI	5.35 (-0.35 to 8.35)	0.11		
Duration of ART	0.02 (-0.11 to 0.16)	0.71		
CD4+ T cell count per 100	0.07 (-0.1 to 0.23)	0.42		
HIV-RNA >=200 copies/mL	-1.24 (-4.12 to 1.64)	0.40		
Primary caregiver				
Not their biological, parent	0.38 (-1.16 to 1.92)	0.63		
Age, year	0.02 (-0.05 to 0.08)	0.64		
Duration of education, years	-0.12 (-0.29 to 0.04)	0.15		
Depression score	0.37 (0.17 to 0.58)	<0.001	0.29 (0.08 to 0.49)	0.01
Income per family < 10,000 baht/month	1.34 (-0.38 to 3.07)	0.13		
Child rearing				
No Attending daycare/nursery	0.62 (-0.83 to 2.07)	0.40		
No book in their home	0.68 (-1.04 to 2.41)	0.44		
Parenting style				
Authoritative	1 (-0.09 to 2.09)	0.07		
Authoritarian	2.33 (1.02 to 3.65)	<0.001	1.73 (0.41 to 3.05)	0.01
Permissive	1.58 (0.52 to 2.64)	0.004		

GA; gestational age, ELC; Early learning composite score, ART; antiretroviral therapy, PIs; protease inhibitor, NNRTI; non-nucleotide reverse transcriptase inhibitor

Table 50. Predictors of changing in externalizing scores

Variable	Univariate		Multivariate	
	Coef (95%CI)	*P*	Coef (95%CI)	*P*
Male	0.81 (-0.72 to 2.34)	0.3		
Age	-0.07 (-0.13 to 0)	0.04	-0.08 (-0.14 to -0.02)	0.01
GA	-0.25 (-0.78 to 0.27)	0.34		
ELC score	-0.03 (-0.08 to 0.01)	0.17		
Group				
PHEU	Ref			
PHIV	0.64 (-0.98 to 2.26)	0.44		
PHIV children				
Age started ART	0.3 (-0.27 to 0.87)	0.31		
ART regimen : PIs vs NNRTI	3 (-0.65 to 6.66)	0.11		
Duration of ART	-0.04 (-0.18 to 0.11)	0.63		
CD4+ T cell count per 100	0.06 (-0.12 to 0.24)	0.52		
HIV-RNA >=200 copies/mL	-1.89 (-5 to 1.22)	0.23		
Primary caregiver				
Not their biological parent	0.82 (-0.8 to 2.44)	0.32		
Age, year	0.01 (-0.06 to 0.08)	0.77		
Duration of education, years	-0.11 (-0.29 to 0.07)	0.22		
Depression score	0.36 (0.15 to 0.58)	0.001	0.28 (0.07 to 0.49)	0.01
Income per family < 10,000 baht/month	-0.07 (-1.89 to 1.75)	0.94		
Child rearing				
No Attending daycare/nursery	1.03 (-0.5 to 2.56)	0.19		
No book in their home	1 (-0.82 to 2.83)	0.28		
Parenting style				
Authoritative	1.74 (0.6 to 2.87)	0.003	1.17 (0.08 to 2.27)	0.04
Authoritarian	3.15 (1.79 to 4.52)	<0.001	2.61 (1.24 to 3.99)	<0.001
Permissive	2.29 (1.19 to 3.39)	<0.001		

GA; gestational age, ELC; Early learning composite score, ART; antiretroviral therapy, PIs; protease inhibitor, NNRTI; non-nucleotide reverse transcriptase inhibitor

Table 51. Predictors of changing in total problems scores

Variable	Univariate		Multivariate	
	Coef (95%CI)	*P*	Coef (95%CI)	*P*
Male	0.95 (-0.58 to 2.47)	0.22		
Age	-0.06 (-0.12 to 0.01)	0.05	-0.06 (-0.12 to -0.01)	0.04
GA	-0.05 (-0.57 to 0.46)	0.84		
ELC score	-0.06 (-0.11 to -0.01)	0.01	-0.05 (-0.1 to -0.004)	0.03
Group				
PHEU	Ref			
PHIV	-0.16 (-1.77 to 1.45)	0.85		
PHIV children				
Age started ART	0.37 (-0.2 to 0.95)	0.2		
ART regimen : PIs vs NNRTI	5.27 (1.74 to 8.81)	0.11		
Duration of ART	-0.02 (-0.17 to 0.13)	0.76		
CD4+ T cell count per 100	0.09 (-0.09 to 0.28)	0.33		
HIV-RNA >=200 copies/mL	-2.47 (-5.71 to 0.77)	0.14		
Primary caregiver				
Not their biological parent	0.6 (-1.02 to 2.22)	0.47		
Age, year	0 (-0.07 to 0.07)	0.96		
Duration of education, years	-0.12 (-0.3 to 0.06)	0.2		
Depression score	0.47 (0.25 to 0.68)	0.001	0.37 (0.15 to 0.58)	0.001
Income per family < 10,000 baht/month	0.88 (-0.95 to 2.7)	0.35		
Child rearing				
No Attending daycare/nursery	0.99 (-0.53 to 2.52)	0.2		
No book in their home	1.43 (-0.38 to 3.24)	0.12		
Parenting style				
Authoritative	1.64 (0.5 to 2.78)	0.005	1.24 (0.14 to 2.34)	<0.001
Authoritarian	3.17 (1.81 to 4.54)	<0.001	2.34 (0.99 to 3.7)	0.001
Permissive	2.07 (0.97 to 3.18)	<0.001		

GA; gestational age, ELC; Early learning composite score, ART; antiretroviral therapy, PIs; protease inhibitor, NNRTI; non-nucleotide reverse transcriptase inhibitor

4.4.5 Subgroup analysis among early ART PHIV, standard ART PHIV and PHEU

4.4.5.1 Behavior scores

Raw score of individual behavioral problem and T score of overall problems are shown in Table 52. There was no difference among early ART PHIV, standard ART PHIV and PHEU, except somatic complaints at enrollment. Mean (SD) somatic complaints was 3.8 (2.5), 4.9 (3.0) and 5.3 (2.1) in PHEU, early ART PHIV and standard ART at enrollment, respectively, $p = 0.02$. However, no difference reported at 12-month visit.

4.4.5.2 Prevalence behavior problems

Prevalence of individual behavioral problems and overall problems are shown in Table 53. There was no difference among early ART PHIV, standard ART PHIV and PHEU. Most common problems were somatic complaints, affective problem, withdrawn, anxiety problem and attention problems.

Table 52. Raw score of individual behavior problems and T score of overall problems among PHEU, early ART PHIV and standard ART PHIV

Mean (SD)	At enrolment				12-month-visit				p^b
	PHEU n=80	Early ART PHIV n=19	Standard ART PHIV n=22	p^a	PHEU n=80	Early ART PHIV n=19	Standard ART PHIV n=22	p^a	
DSM-oriented (range)									
Affective problems (0-20)	4.4 (2.4)	4.5 (2.5)	3.9 (2.4)	0.66	4.7 (3.1)	3.7 (2.9)	4.4 (2.6)	0.44	0.22
Anxiety problems (0-20)	5.4 (2.9)	5.3 (2.2)	5.0 (2.9)	0.84	5.2 (3.2)	4.6 (2.2)	5.1 (2.2)	0.76	0.65
Pervasive development problems (0-26)	4.4 (2.8)	3.6 (1.8)	4.1 (3)	0.53	4.6 (3.3)	3.9 (1.9)	3.7 (3.1)	0.33	0.41
Attention-deficit/ hyperactivity problems (0-12)	6.6 (2.7)	6.6 (2.4)	5.8 (2.7)	0.39	6.4 (2.8)	6.4 (2.7)	6.5 (2.7)	0.99	0.70
Oppositional defiant problems (0-12)	4.6 (2.4)	4.8 (1.4)	4.9 (1.8)	0.85	4.6 (2.6)	4.6 (1.3)	4.5 (2.2)	0.98	0.84
Syndromes scales									
Emotionally-reactive (0-18)	4.5 (2.9)	4.5 (2.0)	4.4 (2.8)	0.99	4.5 (3.4)	3.6 (1.9)	4.5 (2.3)	0.52	0.31
Anxious/depressed (0-16)	4.0 (2.2)	4.1 (2.5)	4.1 (2.7)	0.94	3.7 (2.6)	3.2 (2.2)	3.5 (2.0)	0.67	0.32
Somatic complaints (0-22)	3.8 (2.5)	4.9 (3.0)	5.3 (2.1)	0.02	4.1 (2.8)	4.4 (3.0)	4.2 (2.1)	0.86	0.61
Withdrawn (0-16)	3.2 (2.0)	2.5 (1.6)	2.9 (2.3)	0.39	3.4 (2.1)	2.8 (1.5)	3.0 (2.8)	0.53	0.45

Mean (SD)		At enrolment				12-month-visit				p^b
	PHEU n=80	Early ART PHIV n=19	Standard ART PHIV n=22	p^a	PHEU n=80	Early ART PHIV n=19	Standard ART PHIV n=22	p^a	
Sleep problems (0-14)	4.2 (2.5)	4.4 (2.1)	3.2 (1.9)	0.17	3.9 (2.9)	3.5 (1.8)	4.1 (2.1)	0.75	0.82
Attention problems (0-10)	4.3 (2.2)	3.7 (1.2)	3.3 (2.1)	0.09	4.1 (2.4)	3.9 (1.8)	3.9 (2.1)	0.90	0.63
Aggressive behavior (0-38)	14.0 (6.7)	13.9 (4.5)	12.9 (5.4)	0.75	13.1 (7.0)	12.7 (4)	13.6 (5.7)	0.91	0.84
Grouping									
Internalizing (29-100)	59.3 (9.6)	60.9 (6.6)	61.5 (8.0)	0.51	59.6 (9.6)	58.4 (8.3)	60.2 (7.0)	0.80	0.65
Externalizing (28-100)	57.6 (9.8)	56.1 (6.0)	55.1 (8.8)	0.50	55.5 (10.1)	55.1 (5.8)	55.6 (8.4)	0.98	0.39
Total problems (28-100)	59.7 (9.8)	60.2 (6.9)	59.0 (8.1)	0.91	58.7 (10.6)	56.9 (8.0)	59.0 (7.9)	0.74	0.83

PHEU; Perinatally HIV exposed uninfected children, PHIV; Perinatally HIV infected children, Early ART PHIV; children with early initiated antiretroviral therapy within 3 months of age; Standard ART PHIV; children with initiated antiretroviral therapy within 3-12 months of age

Table 53. Prevalence of behavioral problems among PHEU, early ART PHIV and standard ART PHIV

n (%)	At enrolment				12-month-visit				
	PHEU n=80	**Early ART PHIV** n=19	**Standard ART PHIV** n=22	***p*[a]**	**PHEU** n=80	**Early ART PHIV** n=19	**Standard ART PHIV** n=22	***p*[a]**	***p*[b]**
DSM-oriented (T-score ≥ 70)									
Affective problems	15 (19%)	3 (16%)	4 (18%)	0.96	17 (22%)	3 (16%)	2 (9%)	0.44	0.64
Anxiety problems	12 (15%)	2 (11%)	3 (14%)	0.89	13 (17%)	1 (5%)	1 (5%)	0.27	0.39
Pervasive development problems	12 (15%)	1 (5%)	3 (14%)	0.67	15 (19%)	0	3 (14%)	0.1	0.14
Attention-deficit/ hyperactivity	6 (8%)	1 (5%)	1 (5%)	0.86	8 (10%)	0	1 (5%)	0.46	0.39
Oppositional defiant problems	5 (6%)	0	0	0.50	8 (10%)	0	1 (5%)	0.46	0.27
Syndromes scales (T-score ≥ 70)									
Emotionally-reactive	4 (5%)	0	1 (5%)	0.61	9 (11%)	0	2 (9%)	0.40	0.72
Anxious/depressed	3 (4%)	0 (0)	2 (9%)	0.38	5 (6%)	0	0	0.50	0.98
Somatic complaints	11 (14%)	4 (21%)	7 (32%)	0.13	12 (15%)	5 (26%)	4 (18%)	0.46	0.22
Withdrawn	11 (14%)	1 (5%)	3 (14%)	0.71	12 (15%)	2 (11%)	4 (18%)	0.87	0.52
Sleep problems	5 (6%)	1 (5%)	0	0.69	9 (11%)	0	0	0.10	0.24

n (%)	At enrolment				12-month-visit				
	PHEU n=80	**Early ART PHIV** n=19	**Standard ART PHIV** n=22	***p*[a]**	**PHEU** n=80	**Early ART PHIV** n=19	**Standard ART PHIV** n=22	***p*[a]**	***p*[b]**
Attention problems	14 (18%)	0	2 (9%)	0.12	12 (15%)	2 (11%)	3 (14%)	0.87	0.31
Aggressive behavior	7 (9%)	0	1 (5%)	0.64	6 (8%)	0	2 (9%)	064	0.89
Grouping (T-score ≥ 64)									
Internalizing	31 (39%)	4 (21%)	9 (41%)	0.31	30 (38%)	6 (32%)	8 (36%)	0.87	0.78
Externalizing	15 (19%)	3 (16%)	2 (9%)	0.56	14 (18%)	1 (5%)	3 (14%)	0.39	0.35
Total problems	24 (30%)	5 (26%)	3 (14%)	0.31	22 (28%)	5 (26%)	7 (32%)	0.91	0.58

PHEU; Perinatally HIV exposed uninfected children, PHIV; Perinatally HIV infected children, Early ART PHIV; children with early initiated antiretroviral therapy within 3 months of age; Standard ART PHIV; children with initiated antiretroviral therapy within 3-12 months of age

4.5 Neuroanatomical outcomes

Twenty PHIV children were performed MRI scan. Median (IQR) age of these PHIV children was 29.5 (25.8-33.0) months old and median (IQR) age of initiated ART was 3.2 (1.8-4.6) months old. Four children had detectable HIV-RNA (>200 copies/ml) at 1st assessment (PID 12, 91, 54 and 59) and 5 children at 12-month visit (PID 12, 28, 54, 59 and 94). Median (IQR) ELC score by MSEL was 81 (65-85) at 1st assessment and 79 (72-93) at 12-month visit. Six and five PHIV children were GDI at 1st and 12-month visit, respectively. Median (IQR) total problem score by CBCL was 61 (55-64) at 1st assessment and 58 (51-62) at 12-month visit. Five PHIV children had total behavior problems T score ≥ 64 at both visit.

Multiple high signal intensity lesion on T2/FLAIR were documented in 13 PHIV children (65%), predominantly in frontal and parietal area (Table 54 and Figure 12). One PHIV (PID 51) had periventricular leukomalacia who was initiated ART at 3.4 months old and virological suppression at assessments (Figure 13). This PHIV child was a term infant (gestational age 38 weeks) with normal Apgar score (8 at 1 minute and 9 at 5 minutes after birth), had severe developmental impairment (ELC 49-52) and severe gross motor impairment (gross motor developmental quotient 23-29). The other 6 children had normal MRI brain results.

Table 54. Baseline characteristics, neurodevelopmental, neurobehavioral and neuroanatomical outcomes of 20 PHIV children (MRI group)

PID	Age at 1st visit (mo)	Age initiated ART (mo)	ELC		Total behavior T score		MRI result	
			At 1st visit	At 12-month visit	At 1st visit	At 12-month visit	At 1st visit	At 12-month visit
1	30	3.7	76	70	60	41	A tiny focal hypersignal intensity (SI) on T2WI/T2WI FLAIR at subcortical white matter of posterior aspect of right superior frontal gyrus, probably nonspecific white matter change	Unchanged A tiny focal hypersignal intensity (SI) on T2WI/T2WI FLAIR at subcortical white in right frontal lobe and newly seen another one lesion at subcortical white matter in left frontal lobe probably nonspecific white matter change

PID	Age at 1st visit (mo)	Age initiated ART (mo)	ELC		Total behavior T score		MRI result	
			At 1st visit	At 12-month visit	At 1st visit	At 12-month visit	At 1st visit	At 12-month visit
9	37	6.4	66	82	48	46	Normal MRI brain	A tiny focal hypersignal intensity (SI) on T2WI/T2WI FLAIR at subcortical white matter of insular lobe, probably nonspecific white matter change
10	45	2.5	88	101	70	45	Normal MRI brain	Few T2/FLAIR hyperintense foci in right external capsule and right frontal white matter, non-specific white matter change
11	30	2.9	58	73	62	45	Normal MRI brain	Newly seen a few tiny abnormal signal intensity lesions at subcortical white matter of bilateral frontal lobe,

PID	Age at 1st visit (mo)	Age initiated ART (mo)	ELC		Total behavior T score		MRI result	
			At 1st visit	At 12-month visit	At 1st visit	At 12-month visit	At 1st visit	At 12-month visit
								probably nonspecific white matter change
12	26	1.2	75	74	51	48	No demonstrable abnormality of the brain Mega cisterna magna, anatomic variation	Unchanged T2/FLAIR hyperintense areas and foci indeep bilateral parietal white matter and centrum semiovale
28	28	5.0	82	61	63	52	Normal MRI brain	Normal MRI brain
34	30	1.6	94	85	68	53	Normal MRI brain	Normal MRI brain
36	55	4.8	83	78	62	58	Normal MRI brain	Normal MRI brain
37	24	2.9	88	104	74	59	A few foci of hyper SI on T2WI/T2WI FLAIR at subcortical white matter of anterior aspect of right superior frontal gyrus, probably nonspecific white	No significant change of abnormal SI at subcortical white matter in right frontal lobe, probably nonspecific white matter change

PID	Age at 1st visit (mo)	Age initiated ART (mo)	ELC		Total behavior T score		MRI result	
			At 1st visit	At 12-month visit	At 1st visit	At 12-month visit	At 1st visit	At 12-month visit
							matter change	
38	29	4.2	63	72	62	56	Normal MRI brain	No significant change of a few abnormal SI foci at subcortical white matter of bilateral frontal lobes, probably nonspecific white matter change
40	26	2.2	94	92	57	53	Normal MRI brain	Normal MRI brain
44	33	1.8	84	102	52	58	A few foci of hyper SI on T2WI/T2WI FLAIR at subcortical white matter of anterior aspect of right	No significant change of a few abnormal SI at subcortical white matter in right frontal lobe, probably nonspecific

PID	Age at 1st visit (mo)	Age initiated ART (mo)	ELC		Total behavior T score		MRI result	
			At 1st visit	At 12-month visit	At 1st visit	At 12-month visit	At 1st visit	At 12-month visit
							superior frontal gyrus, probably nonspecific white matter change	white matter change
48	30	1.7	82	80	46	66	A few foci and patchy areas of low SI on T1QI and high SI on T2WI/FLAIR at deep white matter of bilateral parietal regions, possible to be demyelination Linear T2WI-hyperintense area at periventricular white matter of bilateral peritrigonal regions, probably terminal zone of myelination	No significant change of several foci and patchy areas of low SI on T1SI and high SI on T2WI/FLAIR at subcortical and deep white matter of bilateral frontal and parietal regions, possible to be demylination. Unchanged linear T2WI-hyperintense area at periventricular white matter of bilateral peritrigonal regions, probably terminal zone of myelination

PID	Age at 1st visit (mo)	Age initiated ART (mo)	ELC		Total behavior T score		MRI result	
			At 1st visit	At 12-month visit	At 1st visit	At 12-month visit	At 1st visit	At 12-month visit
51	20	3.4	49	52	56	69	Giotic-encephalomalacic change at bilateral centrum semiovale and periventricular white matter along bilateral lateral ventricles, possible to be periventricular leukomalacia with some cystic change.	No signficiant change of gliotic-encephalomalacic change at bilateral centrum semiovale and periventricular white matter along bilateral lateral ventricles, possible to be periventricular leukomalacia with some cystic change
54	34	4.6	56	63	61	58	Normal MRI brain	Normal MRI brain
59	33	11.3	81	100	46	61	Normal MRI brain	Normal MRI brain
69	24	4.2	91	87	61	64	Normal MRI brain	A few T2/FLAIR hyperintense foci in deep and periventricular white matter of bilateral frontal lobe, parietal lobe and peritrigonal region, probably non-specific white matter

PID	Age at 1st visit (mo)	Age initiated ART (mo)	ELC		Total behavior T score		MRI result	
			At 1st visit	At 12-month visit	At 1st visit	At 12-month visit	At 1st visit	At 12-month visit
								change
75	24	1.5	79	95	65	59	Normal MRI brain	A few T2 hyperintense foci at subcortical and deep white matter in left frontal and right parietal lobe, probably nonspecific white matter change.

PID	Age at 1st visit (mo)	Age initiated ART (mo)	ELC		Total behavior T score		MRI result	
			At 1st visit	At 12-month visit	At 1st visit	At 12-month visit	At 1st visit	At 12-month visit
91	25	1.9	80	77	56	70	Multiple foci of high SI on T2WI/T2WI FLAIR at subcortical and deep white matter of bilateral frontal and parietal lobes, probably nonspecific white matter change Patchy T2WI-hyperintense areas at bilateral peritrigonal white matter, probably terminal zone of myelination	Multiple foci and small patchy areas of T2 hyperintensity at subcortical, deep and periventricular white matter of bilateral frontal, parietal and temporal lobes, possible to be demyelination, ventricular dilation

PID	Age at 1st visit (mo)	Age initiated ART (mo)	ELC		Total behavior T score		MRI result	
			At 1st visit	At 12-month visit	At 1st visit	At 12-month visit	At 1st visit	At 12-month visit
94	28	8.1	55	61	71	68	A few focal hypersignal intensity (SI) on T2WI/T2WI FLAIR at subcortical white matter of bilateral frontal lobes, probably nonspecific white matter change	Several T2 hyperintense foci at subcortical white matter of bilateral frontal and left parietal lobe, probably non-specific white matter change

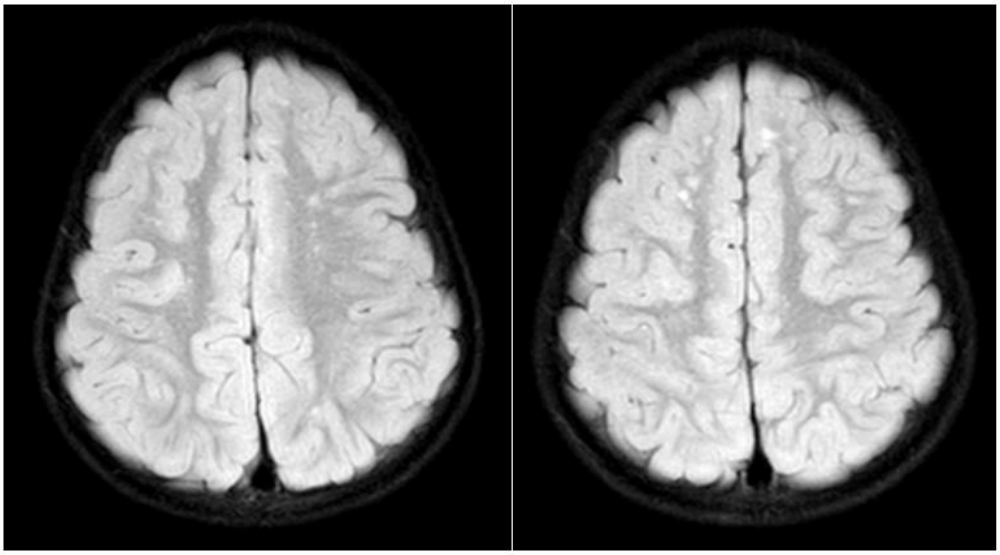

Figure 12. MRI brain reported several T2/FLAIR hyperintense foci at subcortical white matter probably non-specific white matter change

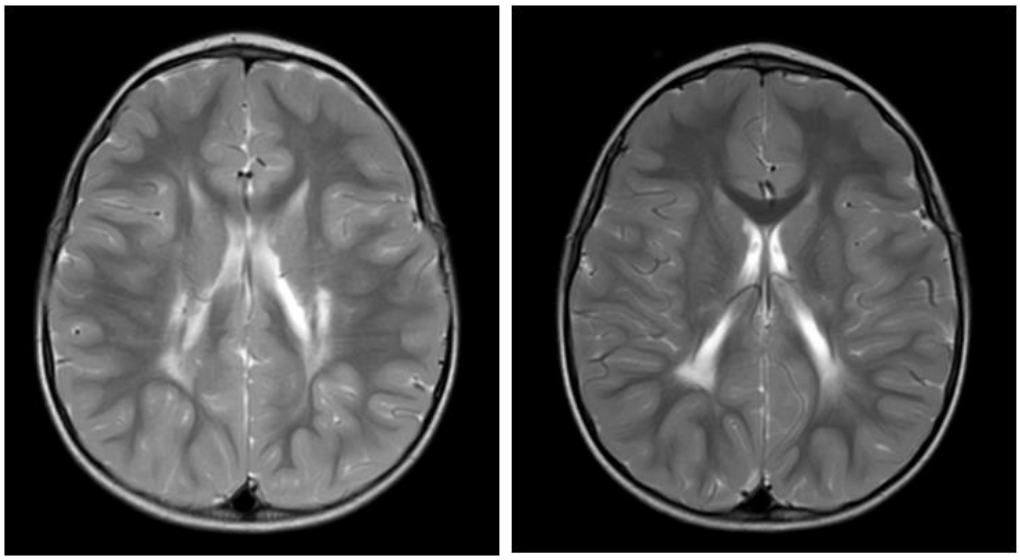

Figure 13. MRI brain of PHIV child (PID 51) showed periventricular leukomalacia

CHAPTER 5
Discussion

This chapter will discuss the results including baseline characteristic, neurodevelopmental outcomes and neurobehavioral outcomes which compare between PHIV and PHEU children as well as and neuroanatomical outcomes in PHIV children. It will also discuss the strength, limitation, implication, clinical recommendation and recommendation for future research.

5.1 Baseline characteristics

5.1.1 Baseline characteristics of children

There was no significant difference between PHIV and PHEU children for infant demographics (gender, age, gestational age, birth weight). PHIV children was slightly less than birth weight than PHEU children (median 2723 vs 2845 g, *p*-value = 0.06). Maternal and child ART regimen for PMTCT were different between groups with unsurprisingly. The difference of maternal ART impact to child HIV infected status e.g. mother who have no ART during pregnancy are tend to have HIV infected child. The difference of child ART prophylaxis regimen was caused by 1) according to PMTCT program e.g. PHIV children usually previously defined as high risk group so they usually had got combination regimen while PHEU children who previously usually defined as low risk group so they had got AZT monotherapy and 2) child with no ART prophylaxis tends to have HIV infection.

Median (IQR) age at ART initiation in PHIV children was 2.9 (1.9-5.1) months old in this study and only 8 PHIV children was initiated ART after 6 months old. In Thailand, early infant diagnosis scale-up program was rolled out by the National Health and Security Office via the National AIDS Program since 2006. Median (IQR) age at ART initiation was 14.2 (10.2-25.6) months in 2006-2007 and decreased to 6.1 (4.2-9.2) months in 2013 [97]. Children, participating in this study, were born during 2012-2016. The trend of age at ART initiation was decreased. The reduction of age ART initiation reflects the improvement of PMTCT program and early infant diagnosis in Thailand. However, only 70-74% of PHIV children had HIV viral suppression. In 2014, the UNAIDS and partners launched the 90-90-90 targets; the aim was to diagnosed 90% of all HIV-positive persons, provide ART for 90% of those diagnosed, and achieve viral suppression for 90% of those treated by 2020. In 2017, UNAIDS fact sheet reported 81% among people accessing treatment were virally suppressed [23]. To get and maintain virological suppression are still challenge in young children.

5.1.2 Anthropometric data and nutritional status

Even though our PHIV children had initiated ART within 3 months old, they had significantly lower growth parameter than PHEU children. However, all WAZ, HAZ, HCAZ, MUACZ were between -2 to 0 SD Z-score. The rate of underweight, stunting and microcephaly in this study was less than several Sub-Saharan African studies [98-100]. Previous studies reported more rapid growth recovery in PHIV with early initiation ART and longer duration of ART improves growth outcome [100, 101]. However, several factors affect the malnutrition. PHIV children in this study had increased underweight and stunting rate at 12-month visit while PHEU had better outcome.

This study reported higher rate of anemia in PHIV than PHEU at enrolment and improved at 12-month visit. We investigated causes of anemia such as thalassemia and adverse effect from AZT. One child had changed AZT to d4T due to AZT associated anemia. One child had homozygous hemoglobin E, six children have hemoglobin E trait and six children were suspected alpha thalassemia trait. Other anemic children were prescribed the iron supplement and 50% of them had a good response. Anemia is a common complication of HIV infection and a systemic review reported PHIV compared to PHEU children were at significantly higher rate of anemia [6, 102].

5.1.3 Baseline characteristics of parents and primary caregiver data

Even PHEU group was chosen as comparison group to diminish baseline socioeconomic confounder, this study still reported the difference of parent age, maternal education level, marital status and income per family. However, there was no difference in primary caregiver's age, education and depression status as well as rate of attending to nursery or pre-school. These different background of PHIV and PHEU children previously reported in other studies including studies in resource-rich and resource-limiting countries [72]. However, the secondary analysis included all these variable.

5.1.4 Child rearing history and parenting style

The rate of attending nursery or preschool was increase overtime without difference between groups. This might explained by increasing age of children to meet the standard criteria of attending preschool. The increasing of having children books at home is due to providing the children books from the study to all participants. In term of parenting style, authoritative parenting style scores were more in PHIV than PHEU children.

5.2 Neurodevelopmental outcomes

This study demonstrated that the rate of GDI and the rate of individual domains impairment in PHIV children who initiated ART within 12 month-old were comparable to PHEU children at overall, enrollment visit and 12-month visit. However, the sub-study analysis demonstrated that the rate of GDI in PHIV children who initiated ART within 3 month-old were comparable with PHEU, while PHIV children who initiated ART during 3-12 months old were higher rate than PHEU children. This study suggests that early initiated ART preserved neurodevelopmental outcomes and emphasizes the effect of ART initiation.

ELC score, gross motor developmental quotient and visual reception in all PHIV children were significantly lower than PHEU children at enrollment. These different scores were resolved at 12-month visit. Predictors of changing developmental scores was socioeconomic status and nursery school attendance. This result implies that neurodevelopmental outcome is dynamic and can stimulate.

5.2.1 Global developmental outcomes

Previous studies reported PHIV young children displayed poorer mean developmental score than PHEU [6, 18, 41]. This study demonstrated that PHIV children who initiated ART within 12 months had poor mean ELC score than PHEU at both visit but only significant difference at enrollment because PHIV children had stable mean ELC score and PHEU children had lower ELC score at 12-month visit. The result in sub-study analysis reported that only standard ART PHIV group, who was initiated ART during 3-12 months old, had significantly poorer mean ELC score than PHEU at enrollment. These results are similar to CHER trial in South Africa that PHIV children with mean age of initiated ART was 2.1 months had no difference general neurodevelopmental score when compare with PHEU but defer ART PHIV group with mean age of initiated ART was 7.8 months had lower general neurodevelopmental score in early life and catch-up later. All PHIV children in this studies, which median age of initiated ART was 2.9 months, still had difference neurodevelopmental score, however, subgroup of PHIV group which median initiated ART was 2.1 months old had no difference developmental score [8, 65]. These results are consisted with the studies in Kenya and South Africa which reported PHIV infants who initiated ART with median age 4 months old had delay developmental milestone than healthy unexposed children in the first few years of life [43, 64]. This provide evidence for a narrow window of time to initiated ART during infancy to reserve neurodevelopmental outcome [18]. It is hypothesized to be related that HIV was neurotropic virus that attack brain very early [12].

The rate of GDI among PHIV children has varied in earlier studies depend on several factor such as the characteristics of children, neurodevelopmental tool and cut-off of the result. A recent meta-analysis of neurodevelopment in young children born to HIV-infected mothers which focused on the assessment by the Bayley Scales of Infant Development (BSID), reported that severe developmental impairment, defined as -2SD below the mean on BSID, was 21-35%; most studies did not indicate timing of onset of ART[41, 43, 53, 62, 103, 104]. This study showed the rate of GDI in PHIV children was 18-22% and was comparable to PHEU children. However, a trend of increasing rate of GDI overtime was observed in both PHIV and PHEU group. The result in the subgroup analysis showed lower rate of GDI among those children with early initiation of ART vs standard initiation of ART PHIV children. In term of trajectory pattern, PHIV group demonstrated lower rate of normal developmental pattern than PHEU group (64% vs 82%, $p = 0.02$). In term of the descriptive category of ELC score, 60% of PHIV and 42-46% of PHEU had ELC score below average. The high rate of this impairment give the concern about early child development program in these vulnerable children.

On the secondary analysis, we examined whether HIV treatment, viral load status, socioeconomic variables, growth parameters and child rearing style were associated with neurodevelopmental outcomes. On multivariate GEE logistic analysis, male children was significantly associated with increased risk of GDI. Male sex is previously reported as one of prognostic factor of GDI in children younger than 5 years [105]. Even, the study design did not sex-matched for the comparison PHEU group, there were no different rate of gender between groups. HIV characteristics were not reported as the factor of GDI in this study. PHIV children with viral suppression had been reported better neurodevelopmental outcome than those without suppression [18, 49, 51, 56, 64, 106]. Even growth parameters were significant lower in PHIV children, these parameters were not associated with developmental outcomes. This may explained by the low rate of stunting, underweight and microcephaly [11, 107, 108].

Poor socioeconomic and no attending nursery or preschool have been shown to decline neurodevelopmental score. Poverty is the known factor of poor child development which children with the poverty context increase risk to expose biological and psychosocial risks that affect development through changes in brain structure and function [13]. Another factor, early learning opportunity by caregivers or school personnel facilitates early cognitive development. Attending nursery or school has been shown to improve neurodevelopmental score, given increased opportunities for developmental stimulation. Previous studies showed

child stimulation in home-based or school-based intervention effectively improved early childhood developmental outcome [11, 21, 22, 109, 110].

This study and CHER study found that PHIV children demonstrate catch up overtime. We hypothesized about this improvement that the first is that longer duration of ART may be associated. However, the secondary analysis did not reported this association. Second, the co-intervention may play the role as PHIV children have frequent regular schedule to get the ART every 3 months, this is the opportunity to ask and advice about neurodevelopmental outcomes while PHEU children may have longer duration to visit every 6-12 months when age > 2 years old as Thai schedule well child clinic. Third, even this study is the prospective observational study, if the participants were detected any abnormality, they will have the procedure to solve that problem. The developmental pediatrician, who assessed the outcomes, always suggest all caregivers how to stimulate their children and refer them to therapeutic service. (5 PHIV and 10 PHEU children refer to developmental stimulation services). Forth, primary caregivers were assessed depression status and refer to psychiatrist to evaluate. Even some primary caregivers did not reach the cut off of depression, they also have mental support by nurse staffs. This data supported that the simple interventions may affect the neurodevelopmental outcomes and overcome the socioeconomic limitation in PHIV children.

5.2.2 Individual outcomes

Rate of individual domains impairment was not significantly different in both group. However, rate of gross motor impairment seemed to higher in PHIV children than PHEU children while rate of expressive language impairment seemed to higher in PHEU children than PHIV children. Gross motor and expressive language are the most common domains impairment in PHIV and PHEU which previously reported the critical domain. However, they can catch-up overtime. This suggest that neurodevelopment was dynamic and PHIV had potential to improve development.

Gross motor

Gross motor is usually reported as the critical domain impairment in PHIV. After the ART era, the mean motor scores improve from > 2SD to 1- 2 SD below the population mean which consisted with this study reported. However, according to CHER study, gross motor score was significant lower in PHIV when compare with PHEU even though initiated ARV within 3 months [8]. The result in the sub-study reinforced that timing ART contributed to gross motor impairment. This study reported significant lower score at enrolment, particularly in standard ART PHIV and then improved at 4-5 years old. However, the rate of impairment between PHIV and PHEU children was not different.

Gross motor skills depend on relatively larger muscle groups, incorporate an element of strength and do not depend as much on precise movement coordination as fine motor skills. Children who are HIV+ might be slightly deficient in areas associated with generalized strength and conditioning, which could explain the deficit in their gross motor skills [111]. Besides, our team reported the correlation between corpus callosum abnormalities and gross motor deficit in PHIV children [112]. The structure of corpus callosum may be related to motor function in preschool healthy children [113, 114]. This structure has also been routinely implicated as a brain pathway disrupted with HIV infection [115].

Fine motor

This study reported mean score of fine motor in PHIV and PHEU was from 0 to 1 SD below population mean. It is interesting to note that PHIV children significantly improved mean score at 12-month visit.

Fine motor functions are the collective skill and activities that involve using the hands and fingers to work together to perform precise and refined movement. Fine motor carry out after a period of gross motor activities. The possible reason why PHIV might have performed well with fine motor is that timing of developing fine motor is during the suppressive stage of HIV infection and PHIV children have already received ART for a while. The other possible reason is the trunk is supported during testing for the upper extremity. The stability provided by this support might have allowed for a higher level of performance by PHIV children [111]. Finally, the possible is the child rearing culture in Thai usually stimulates fine motor skill as the low prevalence of fine motor delay in previous Thai healthy study [116].

Visual reception

Mean visual reception score in PHIV children was significantly lower when compare with PHEU children at enrollment. The significantly improvement was reported, thus mean visual reception score at 12-month visit was comparable with PHEU and normal population mean. In contrast with CHER study, PHIV children were comparable visual reception score at enrollment then detected visual reception impairment at age 60 months old in PHIV when compare with PHEU [65]. The difference of developmental assessment method may play the role and the long term follow up is needed. In term of timing of ART initiation and visual reception, no difference between early and standard ART was reported as CHER study [65].

Visual reception refers to the information that is perceived through the eye and was not similar to visual acuity. This skill is a complex process includes the ability to distinguish difference color and shape perception, spatial relation, visual analysis, visual synthesis,

conceptualizing and memory. Good visual perception is an important skill especially for school success. It is crucial to close monitor and proper stimulation in this skill.

Receptive language and Expressive language

Language skill were widely reported deficit in PHIV children older than 3 years in both resource-rich and resource-limiting setting. This study result was consisted with previous studies reported mean score from 1 to 2 SD below the population mean in both receptive language and expressive language [18]. However, this study did not report higher rate of language impairment in PHIV when compare to PHEU [20, 43, 59, 63]. PHEU children seems to have higher rate of expressive language impairment. Beside, as the other domain, the mean score declined overtime. The expressive score in both group revealed quite low in this study.

5.3 Neurobehavioral outcomes

PHIV and PHEU children had similar prevalence of behavior problems included DSM-oriented scale, syndromes scale, internalizing, externalizing and total problems. One-third of PHIV and PHEU have been reported any behavior problem. The most common problems were somatic complaints and affective problem. Risk factors of internalizing, externalizing and total problems were primary caregiver's depression and authoritarian parenting style. Besides, primary caregiver's education was additional risk factor of externalizing behavior. The negative predictors of behavioral score were primary caregiver's depression, authoritarian parenting style and authoritative parenting style.

5.3.1 DSM-oriented and syndrome scales

In term of DSM-oriented and syndrome scales, there was no different rate of behavioral problem between PHIV and PHEU pre-school age children. Previous studies demonstrated PHIV children were at risk for anxiety, depression, attention deficit and hyperactivity. This study reported the greater problems in somatic complaints. Even PHIV children initiated ART within 1 year and no obvious health problem, somatic complaint still was the greatest problems in our study but this rate was less than previous reported in PHIV children who were not on therapy [74]. The prevalence of ADHD by DSM-oriented in PHIV children in the present study was comparable with general Thai population (2-8%) [117, 118]. whereas the previous study reported high prevalence of ADHD in PHIV children [52].

5.3.2 Internalizing, externalizing and total problems

Internalizing included emotionally-reactive, anxious/depressed, somatic complaints and withdrawn. Externalizing included attention problems and aggressive behavior. Total

problem included internalizing, externalizing, sleep problems and other problems such as jealous, fears and shy.

This study reported no difference behavioral score and clinical range problem between PHIV and PHEU children which similar to previous studies [69, 73, 75, 79]. As comparison to PREDICT study and another study, which most participants aged more than 5 years old, PHIV had significantly higher mean (SD) T score and borderline-clinical range problem of CBCL internalizing, externalizing and total problems when compare to PHEU children particularly in interruptive and hyperactive behaviors [9, 71]. The difference timing of initiation ART may play the role as PREDICT study also reported PHIV children who initiated ART after 1 year of life perform neurodevelopmental outcome worse than PHEU. The other possible cause are the mental ability in preschool children is still not affected as much as in school children and behavioral problems may be difficult to evaluate in young children [119]. We suggest that there should be a long term follow up until school age.

The proportion of PHIV and PHEU preschool children who were identifies with behavior problem using CBCL borderline/clinical cut-off in this study is higher than previous studies [73, 74]. Mechanisms that increase risk for child behavioral include genetic, biological causes and environmental context including family life and socioeconomic status. This may be accounted for the different social context. Most PHIV and PHEU families were low socio-economic status and caregiver education. The contribution may be difficult to determine. However, as this result, HIV-exposed children, whether HIV-infected or uninfected, are at increased risk for negative behavior outcomes [67, 68].

Risk factor of behaviors problem and predictors of changing behavior scores

The association between caregiver's depression and child behavioral outcomes has been document in numerous healthy and HIV children studies [79, 120-125]. This study demonstrated caregiver's depression was significantly related to higher levels of internalizing, externalizing and total problems. Notably the association between caregiver's depression and externalizing problems was stronger than with internalizing problems [[120]]. The psychopathology of this association is proposed that maternal depression is related to increase parenting stress, parent-child dysfunction, low attention to child emotional expression, and low positive and high negative emotion in the context of parenting [121, 123].

Parenting strategy in rearing children has a significant impact on children's behavior. This study and previous studies demonstrated that authoritarian parenting style was negative predictor of behavior problem [126-129]. Authoritarian parents attempt to control the attitudes and behavior of their children in an absolute standard. Children are supposed to

follow very strict rule defined by their parents. While authoritative parents tend to display both high control and high levels of warmth to their children as well as reasonable and nurturing. Authoritative parenting has been associated with great child competence and self-control. Thus, it is surprising that authoritative parenting style was negative predictor of behavior problem in this study. Besides, lower developmental performance score predicted lower internalizing score. This data is contrast to previous studies that lower cognitive performance predict greater behavior problem [9, 52, 67]. It is possible that behavioral problem as a result of developmental impairment may only manifest in older children, particularly with regard to externalizing [79].

This study did not find associated of factors related to ART (e.g. time to initiation, HIV viral loads, CD4 count) and behavioral outcome [19, 52, 70, 78, 130]. It will likely be through interactions with other biologic and psychosocial variables.

5.4 Neuroanatomical outcomes

Twenty PHIV children were randomly to perform MRI scan. The baseline characteristic of these 20 PHIV children were comparable with all PHIV children in age, gender, CD4 + T cell count, HIV RNA status, ELC score and total behavior problem score. Most children had nonspecific white matter change which predominantly in frontal and parietal lobes. Superficial white matter change in PHIV children were previously reported in CHER study [85]. However, no difference in neurodevelopmental scores nor neurobehavioral scores in children with and without this white matter change. The relationship between developmental impairment and CNS abnormality in HIV remains inconsistently reports. PHIV children with global developmental impairment did not always correlated with brain imaging findings in all studies [131]. The etiology of abnormal brain imaging is likely to be multifactorial including 1) HIV associated factor such as severity, stage of diseases, ART therapy and 2) non-HIV associated factors such as prenatal exposure, previous CNS infections, nutrition and social environment. This study reported only minor abnormality in MRI brain. The early initiated ART in HIV children may produce neuroprotective effect that overall imaging finding in these children did not show obvious abnormality. The microstructure and diffusion tension imaging will be further analyzed.

Periventricular leukomalacia (PVL) is characterized by diffuse injury of deep cerebral white matter and results in cerebral palsy in 60-100% of survivors [132, 133]. The classic neuropathology of PVL related to hypoxia-ischemia and reperfusion. The potential risk factors included prematurity, low Apgar score, apnea and seizure [133]. However, PVL in PHIV children rarely reported. Neuroimaging in children with HIV encephalopathy typically

described as global cerebral atrophy and/or basal ganglia calcifications [36, 84]. It may be difficult to distinguish the causes of these abnormalities such as from prenatal and perinatal injury. Four PHIV children were reported asymmetrical white matter change in the peritrigonal region which is susceptible to global hypoxic insults and is also a terminal zone of maturation, yet all children in this study had no report of birth asphyxia by Apgar score at birth.

5.5 Strength and limitations

The strengths of this study include documentation of timing of ART initiation, excellent retention of both study groups, and consideration of multiple demographic and psychosocial factors that potentially influence child developmental outcomes. Children were assessed the neurodevelopmental outcome with the MSEL which our researcher teams have currently used it at King Chulalongkorn Memorial Hospital and the mean MSEL in healthy was 100-110 (unpublished data) which correlates with the other normal healthy. The CBCL was used for neurobehavioral assessment which widely used in Thailand. Besides, primary caregivers were provided education about nurturing care and children were refer to improve developmental outcomes.

Several limitation need to be kept in mind when interpreting the finding even we attempt to minimized confounding variables. First is the timing of assessment in each children was in different age. Neurodevelopment outcome in each age group are different task. However, the comparison PHEU group was age-matched control and the data analysis used T-score which convert from age. Second, even though we chose PHEU as comparable group with expectations of similar background, there were significant differences among PHIV and PHEU children with regard to family characteristics and socioeconomic status which may lead to underestimation of the effect of HIV and ART exposure on child development. However, the potential confounding effects were controlled for at least in part by the multivariate analysis. Third, the relatively brief time of follow up also requires consideration given the dynamic and multifactorial nature of child development during the early years of life. Forth, there are few participants in sub-study that may affect less power to detect difference between groups.

5.6 Implications

Since 2010, the WHO and Thai National Guideline recommends HIV DNA PCR for early infant diagnosis and immediate ART in those infected regardless of symptoms and CD4+ T cell [3, 134]. However, only 83% of infants are treated within the first year of life due to limited infrastructure and resources [97]. Careful assessment of precise timing of ART during

infancy and neurodevelopmental outcomes could provide tangible results to motivate clinicians and policy makers towards implementing very early ART in infants as recommended by treatment guidelines. Missed opportunities for early ART may lead to not only medical problems but also early developmental problems that have potential to affect later outcomes and quality of life. This study emphasizes that it is essential to establish system to early diagnosis and early treatment in PHIV infant. However, this vulnerable PHIV children should had close monitor in developmental milestone and early stimulation.

5.7 Clinical recommendations

Early diagnosis and early initiated ART as early as possible in PHIV children need to be emphasized as the outcomes in early initiated ART children have comparable to HIV uninfected children.

Early childhood development program should be integrated to HIV clinic to encourage and support primary caregiver to nurturing care the PHIV and PHEU children since HIV-exposed children are vulnerable to developmental and behavioral problems. Early intervention both in home-based and school- based management should be encouraged.

PHEU children should continue evaluate in neurodevelopmental and neurobehavioral outcomes even they are not infected.

Caregiver of PHIV and PHEU should have been screened for depression.

5.8 Recommendation for future research

Conducting longitudinal studies to obtain long term neurodevelopmental and neurobehavioral outcomes in PHIV children with early ART treatment and PHEU children

Neuroanatomical outcome with brain microstructure should be further analysis and find out the correlation with neurodevelopmental and neuroanatomical outcomes.

CHAPTER 6
Conclusions

Although early initiation of antiretroviral therapy (ART) in perinatally HIV infected (PHIV) infants significantly reduces morbidity and mortality, neurodevelopmental and neurobehavioral problems are still issues of concern. This study primarily aims to compare neurodevelopmental outcomes by the Mullen Scales of Early Learning test and neurobehavioral outcomes by the Child Behavioral Checklist between PHIV children who initiated ART within 12 months of life and perinatally HIV-exposed uninfected (PHEU) children. The secondary aims are to assess the outcomes by timing of ART, to delineate factors and predictors affected with neurodevelopmental and neurobehavioral outcomes and to describe neuroanatomical outcome in PHIV children.

Fifty PHIV and 100 PHEU were well-match for gender, age, gestational age, birth weight, primary caregiver's age and education. However, growth was significantly different between PHIV and PHEU as well as baseline socioeconomic included parents age, maternal education and parenting styles. Most PHIV children initiated ART within 3 months old and 70% PHIV had undetectable HIV-RNA at assessments.

This study demonstrated that the prevalence of global developmental impairment and behavioral problem were comparable between PHIV and PHEU children. However, PHIV children initiated ART after 3 month-old have higher rate of GDI when compare to PHEU children. The other important finding is the improvement of neurodevelopmental outcomes overtime in PHIV children with the co-interventions during the study period such as nurturing care and stimulation education for primary caregiver, the regular visit to health care service and mental support for primary caregiver. Psychosocial factors mainly contributed to these outcomes. Predictors of decreasing developmental scores were no nursery school attendance and poor incomes while predictors of increasing behavioral scores were high caregiver depression score and high authoritarian parenting style. Timing of ART initiation, CD4+ T cell level and HIV-RNA level were not reported as factors affected with neurodevelopmental and neurobehavioral outcomes. MRI results in PHIV reported that most children had nonspecific white matter change predominantly in frontal and parietal lobes.

This study emphasized that time of ART initiation is important and should as early as possible to improve the neurodevelopmental and neurobehavioral outcome. All stakeholders should make an effort to establish the early diagnosis and early initiation ART in PHIV infants. Besides, all PHIV and PHEU children should had close monitor about neurodevelopmental and neurodevelopmental outcomes such as integrating well child care

service into the HIV clinic service as well as provide the education about the early stimulation. Screening depression in primary caregiver should administer in the HIV clinic and give the proper management. All these interventions aim to improve children and family's quality of life.

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Appendix A

Case Record Form

	Screening	Subject ID:I__I__I I__I__I__I Initials: I__I__I *first last*

Date of visit: I__I__I I__I__I I__I__I__I__I
dd mm yy

Date of birth: I__I__I I__I__I I__I__I__I__I **Age** I__I__I months
dd mm yy

Written informed assent/consent is obtained	☐ Yes ☐ No	Date: I_I_I I_I_I I_I_I__I__I *dd mm yyyy*

Eligibility Criteria

Inclusion Criteria (all answer must be 'Yes')

		Yes	No
1.	Age 12-56 months old	☐	☐
2.	Born to HIV-infected	☐	☐
3.	Caregivers give written informed consent	☐	☐
	For PHIV group		
1.	Documented HIV infection (Positive HIV DNA PCR)	☐	☐
2.	Have initiated ART < 1 year, and have ≥ 1 year of ART	☐	☐
	For PHEU group		
1.	Documented negative HIV DNA PCR test	☐	☐

Exclusion Criteria (all answer must be 'No')

		Yes	No
1.	Gestational age < 34 weeks	☐	☐
2.	Major congenital anomalies and genetic diseases	☐	☐
3.	Current neurologic diseases (CNS infection, neoplasm)	☐	☐
4.	Head injury with a loss of consciousness of greater than one hour or known long-term cognitive sequelae	☐	☐
5.	Persistent and active AIDS-defining opportunistic infection or autoimmune disease within 30 days prior to enrol (stable treated opportunistic infections on maintenance therapy, minor infections such as oral thrush will be allowed)	☐	☐

Visit Month 0

Subject ID:I__I__I I__I__I__I

Initials: I__I__I
first last

☐ **PHIV group** ☐ **PHIV MRI group** ☐ **PHEU**

Date of visit: I__I__I I__I__I I__I__I__I__I
dd mm yyyy

Demographic Data

Date of birth: I__I__I I__I__I I__I__I__I__I
dd mm yyyy

Gender: ☐ Male ☐ Female

Ethnic group: ☐ Thai ☐ Other specify:_____

Pregnancy History

1. **History antenatal care (ANC)** ☐ No ☐ Yes
2. **Maternal illness history**
   a. Date of HIV diagnosis I__I__I I__I__I I__I__I__I__I
   dd mm yyyy
   b. Timing of known HIV infection ☐ Before pregnancy
   ☐ During pregnancy
   ☐ After pregnancy
   c. HIV-related illness during pregnancy ☐ None

Description	**Start Date** (dd mm yy)	**Stop date** (tick if ongoing) (dd mm yy)	**Hospitalized**
	_____/_____/_____	_____/_____/_____ ☐	☐ No ☐ Yes
	_____/_____/_____	_____/_____/_____ ☐	☐ No ☐ Yes
	_____/_____/_____	_____/_____/_____ ☐	☐ No ☐ Yes
	_____/_____/_____	_____/_____/_____ ☐	☐ No ☐ Yes

d. Medical event history during pregnancy (non HIV-related Illness) ☐ None

Description	**Start Date** (dd mm yy)	**Stop date** (tick if ongoing) (dd mm yy)	**Hospitalized**
	_____/_____/_____	_____/_____/_____ ☐	☐ No ☐ Yes
	_____/_____/_____	_____/_____/_____ ☐	☐ No ☐ Yes
	_____/_____/_____	_____/_____/_____ ☐	☐ No ☐ Yes
	_____/_____/_____	_____/_____/_____ ☐	☐ No ☐ Yes

e. History of CD4 count during pregnancy
☐ None

Start Date (dd mm yy)	**Result** (cell/mm^3)	**Result** (%)
_____/_____/_____		
_____/_____/_____		
_____/_____/_____		
_____/_____/_____		

Visit Month 0	Subject ID:I__I__I I__I__I__I Initials: I__I__I *first last*

f. History of HIV RNA during pregnancy ☐ None

Start Date (dd mm yy)	**Result** (copies/ml)	**Result** (log)
_____/_____/______		
_____/_____/______		
_____/_____/______		
_____/_____/______		

3. **Maternal medication history during pregnancy**

a. Antiretroviral drugs (ARVs) History ☐ None

ARV Name	**Start Date** (dd mm yy)	**Stop date** (tick if ongoing) (dd mm yy)	**If stop ART, Indicate reason** *(can tick more than one)*
	___/_____/_____	___/_____/_____ ☐	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7___________
	___/_____/_____	___/_____/_____ ☐	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7___________
	___/_____/_____	___/_____/_____ ☐	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7___________
	___/_____/_____	___/_____/_____ ☐	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7___________
	___/_____/_____	___/_____/_____ ☐	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7___________
	___/_____/_____	___/_____/_____ ☐	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7___________

Note: 1: Clinical failure, 2: Immunological failure, 3: Virological failure, 4: Socioeconomic problem, 5: Adherence, 6: Toxicity, 7: Other (please specify)

b. Medication history (Concomitant Medication) during pregnancy ☐ None

Medication Name	**Start Date** (dd mm yy)	**Stop date** (tick if ongoing) (dd mm yy)
	_____/_____/______	_____/_____/______ ☐
	_____/_____/______	_____/_____/______ ☐
	_____/_____/______	_____/_____/______ ☐
	_____/_____/______	_____/_____/______ ☐
	_____/_____/______	_____/_____/______ ☐

4. **History of substance use** ☐ None

Substance Name	**Start Date** (mm yy)	**Stop date** (tick if ongoing) (dd mm yy)
	_____/_____/______	_____/_____/______ ☐
	_____/_____/______	_____/_____/______ ☐
	_____/_____/______	_____/_____/______ ☐
	_____/_____/______	_____/_____/______ ☐
	_____/_____/______	_____/_____/______ ☐

	Visit Month 0	Subject ID:I__I__I I__I__I__I Initials: I__I__I *first last*

5. **History of smoking** ☐ No ☐ Yes, specify

Start Date (mm yy)	**Stop date** (tick if ongoing) (dd mm yy)	**Average total Cigarette/day**
_____/_____/_____	_____/_____/_____ ☐	

6. **History of alcohol**

Start Date (mm yy)	**Stop date** (tick if ongoing) (dd mm yy)	**Average amount/day**
_____/_____/_____	_____/_____/_____ ☐	

7. **Parity** I__I__I
8. **Gestation age** I__I__I weeks
9. **Mode of delivery** ☐ Normal labor
   ☐ Vacuum
   ☐ Forceps
   ☐ Cesarean section due to _____
10. **APGAR Score at 1, 5 min** I__I , I__I ☐ No document
11. **Birth Weight** I__I__I__I__I g
12. **Birth Head circumference** I__I__I. I__I cm
13. **Birth Length** I__I__I. I__I cm
14. **Postpartum complication** ☐ None

Description	**Start Date** (dd mm yy)	**Stop date** (tick if ongoing) (dd mm yy)
	_____/_____/_____	_____/_____/_____ ☐
	_____/_____/_____	_____/_____/_____ ☐
	_____/_____/_____	_____/_____/_____ ☐
	_____/_____/_____	_____/_____/_____ ☐

Children History

1. **PMTCT prophylaxis regimen** ☐ None

Medication Name	**Start Date** (dd mm yy)	**Stop date** (tick if ongoing) (dd mm yy)
	___/_____/_____	_____/_____/_____
	___/_____/_____	_____/_____/_____
	___/_____/_____	_____/_____/_____

2. **Laboratory of HIV status**
   a. 1st PCR ☐ Positive ☐ Negative
   Date: I__I__I I__I__I I__I__I__I__I
   dd mm yyyy

   b. 2nd PCR ☐ Positive ☐ Negative
   Date: I__I__I I__I__I I__I__I__I__I
   dd mm yyyy

	Visit Month 0	Subject ID:I__I__I I__I__I__I Initials: I__I__I *first last*

PHIV group: PCR positive

a. Mode of infection ☐ *in utero* ☐ peripartum ☐ unknown

b. HIV-related illness ☐ None

Description	**Start Date** (dd mm yy)	**Stop date** (tick if ongoing) (dd mm yy)	**Hospitalized**
	_____/_____/_____	_____/_____/_____ ☐	☐ No ☐ Yes
	_____/_____/_____	_____/_____/_____ ☐	☐ No ☐ Yes
	_____/_____/_____	_____/_____/_____ ☐	☐ No ☐ Yes
	_____/_____/_____	_____/_____/_____ ☐	☐ No ☐ Yes

c. Medical event history (non HIV-related Illness) ☐ None

Description	**Start Date** (dd mm yy)	**Stop date** (tick if ongoing) (dd mm yy)	**Hospitalized**
	_____/_____/_____	_____/_____/_____ ☐	☐ No ☐ Yes
	_____/_____/_____	_____/_____/_____ ☐	☐ No ☐ Yes
	_____/_____/_____	_____/_____/_____ ☐	☐ No ☐ Yes
	_____/_____/_____	_____/_____/_____ ☐	☐ No ☐ Yes

d. Antiretroviral drugs (ARVs) History

ARV Name	**Start Date** (dd mm yy)	**Stop date** (tick if ongoing) (dd mm yy)	**If stop ART, Indicate reason** *(can tick more than one)*
	___/_____/____	___/___/_____ ☐	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7_____
	___/___/____	___/___/_____ ☐	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7_____
	___/_____/____	___/_____/_____ ☐	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7_____
	___/_____/____	___/_____/_____ ☐	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7_____

Note: 1: Clinical failure, 2: Immunological failure, 3: Virological failure, 4: Socioeconomic problem, 5: Adherence, 6: Toxicity, 7: Other (please specify)

e. Medication history (Concomitant Medication) ☐ None

Medication Name	**Start Date** (dd mm yy)	**Stop date** (tick if ongoing) (dd mm yy)
	___/_____/_____	_____/_____/_____ ☐
	___/_____/_____	_____/_____/_____ ☐
	___/_____/_____	_____/_____/_____ ☐
	___/_____/_____	_____/_____/_____ ☐

	Visit Month 0	Subject ID:I__I__I I__I__I__I Initials: I__I__I *first last*

f. History of CD4 count ☐ None

Start Date (dd mm yy)	**Result** (cell/mm³)	**Result** (%)
_____/_____/_____		
_____/_____/_____		
_____/_____/_____		
_____/_____/_____		

g. History of HIV RNA ☐ None

Start Date (dd mm yy)	**Result** (copies/ml)	**Result** (log)
_____/_____/_____		
_____/_____/_____		
_____/_____/_____		
_____/_____/_____		

PHEU group: PCR negative

a. Medical event history ☐ None

Description	**Start Date** (dd mm yy)	**Stop date** (tick if ongoing) (dd mm yy)	**Hospitalized**
	_____/_____/_____	___/_____/_____ ☐	☐ No ☐ Yes
	_____/_____/_____	___/_____/_____ ☐	☐ No ☐ Yes
	_____/_____/_____	___/_____/_____ ☐	☐ No ☐ Yes
	___/___/_____	___/_____/_____ ☐	☐ No ☐ Yes

b. Medication history ☐ None

Medication Name	**Start Date** (dd mm yy)	**Stop date** (tick if ongoing) (dd mm yy)
	___/_____/_____	_____/_____/_____ ☐
	___/_____/_____	_____/_____/_____ ☐
	___/_____/_____	_____/_____/_____ ☐
	___/_____/_____	_____/_____/_____ ☐
	___/_____/_____	_____/_____/_____ ☐

	Visit Month 0	Subject ID:I__I__I I__I__I__I Initials: I__I__I *first last*

Family History

1. **Mother History**
   a. Birth Date I__I__I I__I__I I__I__I__I__I
   dd mm yyyy
   b. Vital status
   ☐ Alive ☐ Dead ☐ Unknown
   c. Highest level of education
   ☐ No education ☐ Primary school ประถมศึกษา
   ☐ High school มัธยมศึกษา ☐ Vocational certificate ปวช
   ☐ High vocational certificate ปวส ☐ Bachelor degree ปริญญาตรี
   ☐ Master degree ปริญญาโท ☐ Postgraduated masters สูงกว่าปริญญาโท
   d. Employment ☐ Unemployed
   ☐ Employed, specify_____
2. **Father History**
   a. Birth Date I__I__I I__I__I I__I__I__I__I
   dd mm yyyy
   b. Vital status
   ☐ Alive ☐ Dead ☐ Unknown
   c. Highest level of education
   ☐ No education ☐ Primary school ประถมศึกษา
   ☐ High school มัธยมศึกษา ☐ Vocational certificate ปวช
   ☐ High vocational certificate ปวส ☐ Bachelor degree ปริญญาตรี
   ☐ Master degree ปริญญาโท ☐ Postgraduated masters สูงกว่าปริญญาโท
   d. Employment ☐ Unemployed ☐ Employed, specify_____
3. **Marital status:**
   ☐ Married ☐ Divorced/Separated ☐ Widowed
4. **Primary caregiver**
   ☐ mother ☐ father
   ☐ relative person, specify_____ ☐ Not relative person, specify_____
   If not mother and father,
   1. Birth Date I__I__I I__I__I I__I__I__I__I
   dd mm yyyy
   2. Highest level of education
   ☐ No education ☐ Primary school ประถมศึกษา
   ☐ High school มัธยมศึกษา ☐ Vocational certificate ปวช
   ☐ High vocational certificate ปวส ☐ Bachelor degree ปริญญาตรี
   ☐ Master degree ปริญญาโท ☐ Postgraduated masters สูงกว่าปริญญาโท
   3. Employment ☐ Unemployed ☐ Employed, specify_____
   4. Onset of taking care children: I__I__I I__I__I__I__I
   mm yyyy

	Visit Month 0	Subject ID:I__I__I I__I__I__I Initials: I__I__I *first last*

5. **Number of person in family**

Total _____ persons
Adults_____ persons
Children age < 18 yr_____ persons (including a participant)

6. **Income per family**: (Bath/month)
   - ☐ <10,000
   - ☐ 10,000 – 25,000
   - ☐ 25,001 – 50,000
   - ☐ 50,001 – 75,000
   - ☐ 50,001 – 100,000
   - ☐ >100,000

7. **Family History of developmental and behavior disorder**
   - ☐ No
   - ☐ Yes, specify as following
     - ☐ Father; Diagnosis_____
     - ☐ Mother, Diagnosis_____
     - ☐ Brother, Diagnosis_____
     - ☐ Sister, Diagnosis_____
     - ☐ Other, specify_____Diagnosis_____

   a. Delay speech
   b. Global delay development
   c. Attention deficit
   d. Autism
   e. Learning disorder
   f. Psychological problem (schizophrenia, depression, anxiety)
   g. Other, specify_____

Child rearing history

1. Language use in home
   ☐ Thai ☐ Dialect (ภาษาท้องถิ่น) ☐ English ☐ Other, specify _____
2. Attending daycare/nursery/preschool
   ☐ No ☐ Day care/nursery ☐ Preschool
3. Activity with children: Reading book with children
   ☐ None ☐ < 3 times/month ☐ 1-2 times/week ☐ ≥ 3 times/week ☐ Everyday
4. How many books does your child own?
   ☐ None ☐ 1-2 books ☐ 3-5 books ☐ > 10 books

Measurements

			Not Done
Body Weight	I___I___I.I___I	kg.	☐
Height	I___I___I___I.I___I	cm.	☐
Head circumference	I___I___I___I.I___I	cm.	☐

	Visit Month 0	Subject ID:I__I__I I__I__I__I Initials: I__I__I *first last*

Physical Examination

General appearance	☐ Normal ☐ Abnormal*	Specify:_____
Skin	☐ Normal ☐ Abnormal*	Specify:_____
Ear, nose, throat	☐ Normal ☐ Abnormal*	Specify:_____
Cardiovascular System	☐ Normal ☐ Abnormal*	Specify:_____
Respiratory System	☐ Normal ☐ Abnormal*	Specify:_____
Gastrointestinal System	☐ Normal ☐ Abnormal*	Specify:_____
Neurological System	☐ Normal ☐ Abnormal*	Specify:_____
Musculoskeletal System	☐ Normal ☐ Abnormal*	Specify:_____
Other specify:	☐ Normal ☐ Abnormal*	Specify:_____

Laboratory *(Data within 1 month before visit month 0 are allowed)*

1. Hematology Sample Collection Date I__I__I I__I__I I__I__I__I
dd mm yyyy

Test	Results	Unit	Not Done
Hemoglobin	I___I___I___I___I___I.I___I___I___I	g/dl	☐
Hematocrit	I___I___I___I___I___I.I___I___I___I	%	☐
MCV	I___I___I___I___I___I.I___I___I___I	f/l	☐
WBC	I___I___I___I___I___I.I___I___I___I	10^3/ul	☐
Neutrophil	I___I___I___I___I___I.I___I___I___I	10^3/ul	☐
Lymphocyte	I___I___I___I___I___I.I___I___I___I	10^3/ul	☐
Monocyte	I___I___I___I___I___I.I___I___I___I	10^3/ul	☐
Platelet	I___I___I___I___I___I.I___I___I___I	10^3/ul	☐

	Visit Month 0	Subject ID:I__I__I I__I__I__I Initials: I__I__I *first last*

2. Reticulocyte count Sample Collection Date I__I__I I__I__I I__I__I__I__I
dd mm yyyy

Test	Results	Unit	Not Done
Reticulocyte count	I___I___I.I___I	%	☐
Correct reticulocyte count	I___I___I.I___I	%	☐

Laboratory for PHIV group *(Data within 1 month before visit month 0 are allowed)*

1. Immunology Sample Collection Date I__I__I I__I__I I__I__I__I__I
dd mm yyyy

Test	Results	Unit	Not Done
%CD4	I___I___I___I___I___I.I___I___I___I	%	☐
Absolute CD4	I___I___I___I___I___I.I___I___I___I	cells/ul	☐

2. HIV virology Sample Collection Date I__I__I I__I__I I__I__I__I__I
dd mm yyyy

Test	Results	Unit	Not Done
HIV-RNA	☐> ☐< ☐= I___I___I___I___I___I___I___I	copies/ml	☐

Parenting Style

Parenting Style and Dimensions Questionnaire completed at this visit?

☐ Performed ☐ Not performed

PHQ-9

PHQ-9 completed at this visit?

☐ Performed ☐ Not performed

Neurodevelopmental and Neurobehavioral test (Indicate reason if not done)

Mullen Scale of Early Learning completed at this visit?

☐ Performed ☐ Not performed

Child behavioral checklist completed at this visit? (for subjects age 18-60 months)

☐ Performed ☐ Not performed ☐ Not applicable

MRI brain (Indicate reason if not done) ***only subgroup MRI***

MRI brain Date I__I__I I__I__I I__I__I__I__I
dd mm yyyy

☐ Performed ☐ Not performed, specify _____

	Visit Month 12	Subject ID:I__I__I I__I__I__I Initials: I__I__I *first last*

☐ **PHIV group** ☐ **PHIV MRI group** ☐ **PHEU**

Date of visit: I__I__I I__I__I I__I__I__I__I
dd mm yyyy

Medical History:

a. Has there been any change in the HIV-related Illnesses history since the last visit? ***(only PHIV group)***

☐ Yes ☐ No

If Yes, please complete those events.

Description	**Start Date** (dd mm yy)	**Stop date** (tick if ongoing) (dd mm yy)	**Hospitalized**
	____/____/____	____/____/____ ☐	☐ No ☐ Yes
	____/____/____	____/____/____ ☐	☐ No ☐ Yes
	____/____/____	____/____/____ ☐	☐ No ☐ Yes
	____/____/____	____/____/____ ☐	☐ No ☐ Yes

b. Has there been any change in medical history since the last visit?

☐ Yes ☐ No

If Yes, please complete those events

Description	**Start Date** (dd mm yy)	**Stop date** (tick if ongoing) (dd mm yy)	**Hospitalized**
	____/____/____	____/____/____ ☐	☐ No ☐ Yes
	____/____/____	____/____/____ ☐	☐ No ☐ Yes
	____/____/____	____/____/____ ☐	☐ No ☐ Yes
	____/____/____	____/____/____ ☐	☐ No ☐ Yes

Medication History

a. Has there been any change in Antiretroviral drug(s) (ARV) history since the last visit? ***(only PHIV group)***

☐ Yes ☐ No

If Yes, please complete those events.

ARV Name	**Start Date** (dd mm yy)	**Stop date** (tick if ongoing) (dd mm yy)	**If stop ART, Indicate reason** *(can tick more than one)*
	___/____/____	___/____/____ □	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7_____
	___/____/____	___/____/____ □	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7_____
	___/____/____	___/____/____ □	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7_____
	___/____/____	___/____/____ □	☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7_____

Note: 1: Clinical failure, 2: Immunological failure, 3: Virological failure, 4: Socioeconomic problem, 5: Adherence, 6: Toxicity, 7: Other (please specify)

	Visit Month 12	Subject ID:I__I__I I__I__I__I Initials: I__I__I *first last*

b. Has there been any change in medication history (Concomitant medication) since the last visit?

☐ Yes ☐No

If Yes, please complete those events.

Medication Name	**Start Date** (dd mm yy)	**Stop date** (tick if ongoing) (dd mm yy)
	___/_____/_____	_____/_____/_____ ☐
	___/_____/_____	_____/_____/_____ ☐
	___/_____/_____	_____/_____/_____ ☐
	___/_____/_____	_____/_____/_____ ☐
	___/_____/_____	_____/_____/_____ ☐

History of primary caregiver

Primary caregiver

Has there been any change in a primary caregiver since the last visit?

☐ Yes ☐No

If yes, please specify

☐ mother ☐ father

☐ Relative person, specify_____ ☐ Not relative person, specify_____

,

a. Birth Date I__I__I I__I__I I__I__I__I__I
dd mm yyyy

b. Highest level of education

☐ No education ☐ Primary school ประถมศึกษา

☐ High school มัธยมศึกษา ☐ Vocational certificate ปวช

☐ High vocational certificate ปวส ☐ Bachelor degree ปริญญาตรี

☐ Master degree ปริญญาโท ☐ Postgraduated masters สูงกว่าปริญญาโท

c. Employment ☐ Unemployed ☐ Employed, specify_____

d. Onset of taking care children: I__I__I I__I__I__I
mm yyyy

1.Number of person in family

Total _____ persons

Adults_____ persons

Children age < 18 yr_____ persons (including a participant)

2.Income per family: (Bath/month)

☐ <10,000 ☐ 10,000 – 25,000

☐ 25,001 – 50,000 ☐ 50,001 – 75,000

☐ 50,001 – 100,000 ☐ >100,000

	Visit Month 12	Subject ID:I__I__I I__I__I__I Initials: I__I__I *first last*

Child rearing history

1. Language use in home
☐ Thai ☐ Dialect (ภาษาท้องถิ่น) ☐ English ☐ Other, specify _____

2. Attending daycare/nursery/preschool
☐ No ☐ Day care/nursery ☐ Preschool

3. Activity with children: Reading book with children
☐ None ☐ < 3 times/month ☐ 1-2 times/week ☐ ≥ 3 times/week ☐ everyday

4. How many books does your child own?
☐ None ☐ 1-2 books ☐ 3-5 books ☐ > 10 books

Measurements

			Not Done
Body Weight	I___I___I.I___I	kg.	☐
Height	I___I___I___I.I___I	cm.	☐
Head circumference	I___I___I___I.I___I	cm.	☐

Physical Examination

General appearance	☐ Normal ☐ Abnormal*	Specify:_____
Skin	☐ Normal ☐ Abnormal*	Specify:_____
Ear, nose, throat	☐ Normal ☐ Abnormal*	Specify:_____
Cardiovascular System	☐ Normal ☐ Abnormal*	Specify:_____
Respiratory System	☐ Normal ☐ Abnormal*	Specify:_____
Gastrointestinal System	☐ Normal ☐ Abnormal*	Specify:_____
Neurological System	☐ Normal ☐ Abnormal*	Specify:_____
Musculoskeletal System	☐ Normal ☐ Abnormal*	Specify:_____
Other specify:	☐ Normal ☐ Abnormal*	Specify:_____

**Remark: If "Abnormal", please record the event on the AE OR HIV-related Illnesses form .*

Visit Month 12

Subject ID:I__I__I I__I__I__I

Initials: I__I__I
first last

Laboratory

1. **Hematology** Sample Collection Date I__I__I I__I__I I__I__I__I__I
dd mm yyyy

Test	Results	Unit	Not Done
Hemoglobin	I___I___I___I___I___I.I___I___I	g/dl	☐
Hematocrit	I___I___I___I___I___I.I___I___I	%	☐
MCV	I___I___I___I___I___I.I___I___I	f/l	☐
WBC	I___I___I___I___I___I.I___I___I	10^3/ul	☐
Neutrophil	I___I___I___I___I___I.I___I___I	%	☐
Lymphocyte	I___I___I___I___I___I.I___I___I	%	☐
Monocyte	I___I___I___I___I___I.I___I___I	%	☐
Platelet	I___I___I___I___I___I.I___I___I	10^3/ul	☐

2. Reticulocyte count Sample Collection Date I__I__I I__I__I I__I__I__I__I
dd mm yyyy

Test	Results	Unit	Not Done
Reticulocyte count	I___I___I.I___I	%	☐
Correct reticulocyte count	I___I___I.I___I	%	☐

Laboratory for PHIV group

1.Immunology Sample Collection Date I__I__I I__I__I I__I__I__I__I
dd mm yyyy

Test	Results	Unit	Not Done
%CD4	I___I___I___I___I___I.I___I___I___I	%	☐
Absolute CD4	I___I___I___I___I___I.I___I___I___I	cells/ul	☐

2. HIV virology Sample Collection Date I__I__I I__I__I I__I__I__I__I
dd mm yyyy

Test	Results	Unit	Not Done
HIV-RNA	☐> ☐< ☐= I___I___I___I___I___I___I___I	copies/ml	☐

	Visit Month 12	Subject ID:I__I__I I__I__I__I Initials: I__I__I *first last*

Parenting Style

Parenting Style and Dimensions Questionnaire completed at this visit?

☐ Performed ☐ Not performed

PHQ-9

PHQ-9 completed at this visit?

☐ Performed ☐ Not performed

Neurodevelopmental test (Indicate reason if not done)

Mullen Scale of Early Learning completed at this visit?

☐ Performed ☐ Not performed

Child behavioral checklist completed at this visit? (for subjects age 18-60 months)

☐ Performed ☐ Not performed ☐ Not applicable

MRI brain (Indicate reason if not done) ***(only subgroup PHIV MRI)***

MRI brain Date I__I__I I__I__I I__I__I__I__I

dd mm yyyy

☐ Performed ☐ Not performed, specify _____

Appendix B

Parenting Style and Dimension Questionnaire

Parents' attitude to children, manners and behaviors directly affect the children's personality and temperament shaping as well as mental health development. Parenting Style and Dimensions Questionnaire (PSDQ) was developed by Robinson and Mandleco, which was internationally recognized as one of the scales with parents as the respondents to evaluate the parenting style and is demonstrated to have good reliability and validity.

PSDQ is with 32 self-report items and measuring continuous scales of authoritative (15 items), authoritarian (12 items) and permissive parenting (5 items).

The PSDQ-Thai version was translated by Dr.Weerasak Chonchaiya and used in King Chulalongkorn Memorial Hospital Longitudinal Cohort, Thailand.

Quality assurance

The investigator will be check that primary caregivers complete all 32 items.

Protocol

- The primary caregiver is asked to describe their parenting style using a 5-point scale (ranging from "never" to "always" (code 1 to 5)) in the PSDQ-Thai version.
- The primary caregiver is defined as the caregiver with the most responsibility for caring for the child.
- The primary caregiver reports the questionnaire by themselves. If they can't read, the trained nurse will be read the questions or if they do not understand the questions, the trained nurse will explain the questions.

แบบประเมินลักษณะและมิติการเลี้ยงดูลูก

Parenting Style and Dimension Questionnaire (PSDQ) ฉบับย่อภาษาไทย

แบบสอบถามนี้มีจุดประสงค์เพื่อประเมินว่าคุณในฐานะที่เป็นผู้ดูแลหลักของเด็ก แสดงพฤติกรรมต่างๆต่อลูกบ่อยเพียงใด กรุณาอ่านคำถามแต่ละข้อและพิจารณาว่าคุณแสดงพฤติกรรมเหล่านี้อย่างไร โดยทำเครื่องหมาย x ลงในช่องคำตอบที่ตรงกับพฤติกรรมของคุณมากที่สุด

คำถาม	ไม่เคย	นานๆ ครั้ง	ครึ่งหนึ่งของทั้งหมด	บ่อยมาก	สม่ำเสมอ
1.ฉันตอบสนองต่อความรู้สึกและความต้องการของลูก					
2.ฉันใช้การลงโทษทางการในการฝึกวินัยลูก					
3.ฉันคำนึงถึงความต้องการของลูกก่อนที่จะขอให้เขาทำอะไรบางอย่าง					
4.เมื่อลูกถามฉันว่าทำไมเขาต้องทำตามที่ฉันสั่ง ฉันจะตอบว่าเพราะแม่บอกให้ทำ หรือแม่เป็นแม่ของลูก และแม่อยากให้ลูกทำ					
5.ฉันอธิบายลูกว่าฉันรู้สึกต่อพฤติกรรมที่ดีและไม่ดีของลูกอย่างไรบ้าง					
6.ฉันตีก้นลูกเมื่อเขาไม่เชื่อฟัง					
7.ฉันส่งเสริมให้ลูกพูดเกี่ยวกับปัญหาต่างๆของตนเอง					
8.ฉันพบว่ามันเป็นการยากที่จะฝึกวินัยลูกของฉัน					
9.ฉันส่งเสริมให้ลูกแสดงความเป็นตัวของตัวเองอย่างเป็นอิสระถึงแม้ว่าฉันจะไม่เห็นด้วยก็ตาม					
10.ฉันลงโทษลูกโดยการจำกัดสิทธิพิเศษของเขาโดยแทบไม่อธิบายเหตุผลใดๆ					
11.ฉันเน้นถึงเหตุผลของกฎต่างๆ					
12.ฉันปลอบและเข้าใจเมื่อลูกอารมณ์เสีย					
13.ฉันตะโกนหรือตะคอกใส่ลูกเมื่อเขาแสดงพฤติกรรมที่ไม่เหมาะสม					
14.ฉันชมเมื่อลูกทำดี					
15.ฉันยอมลูกเมื่อเขาสร้างความวุ่นวายในบางสิ่งบางอย่าง					
16.ฉันระเบิดความโกรธใส่ลูก					
17.ฉันขู่ลูกว่าจะลงโทษเขาบ่อยกว่าที่ทำจริง					

คำถาม	ไม่เคย	นานๆ ครั้ง	ครึ่งหนึ่งของทั้งหมด	บ่อยมาก	สม่ำเสมอ
18.ฉันคำนึงถึงความชอบของลูกด้วยในการวางแผนภายในครอบครัว					
19.ฉันคว้าตัวลูกไว้เมื่อเขาไม่เชื่อฟัง					
20.ฉันบอกลูกว่าจะลงโทษแต่ไม่ทำจริง					
21.ฉันคำนึงถึงความเห็นของลูกโดยส่งเสริมให้เขาแสดงมันออกมา					
22ฉันยอมให้ลูกมีส่วนร่วมในกฎต่างๆภายในครอบครัว.					
23.ฉันดุด่าและวิจารณ์ลูกเพื่อให้เขาปรับปรุงตัวเอง					
24.ฉันตามใจลูก					
25.ฉันให้เหตุผลแก่ลูกว่าทำไมเขาควรทำตามกฎ					
26.ฉันขู่ลูกว่าจะลงโทษโดยแทบไม่ให้เหตุผล					
27.ฉันใช้เวลากับลูกอย่างอบอุ่นและใกล้ชิด					
28.ฉันลงโทษลูกโดยการปล่อยให้อยู่เพียงลำพังโดยแทบไม่อธิบายเหตุผลใด					
29.ฉันช่วยลูกให้เข้าใจถึงผลกระทบของพฤติกรรมของเขาโดยส่งเสริมให้ลูกพูดเกี่ยวกับผลที่จะตามมาจากสิ่งที่เขาทำ					
30.ฉันดุด่าหรือวิจารณ์เมื่อพฤติกรรมของลูกไม่เป็นไปอย่างที่ฉันคาดหวัง					
31.ฉันอธิบายถึงผลที่จะตามมาจากพฤติกรรมของลูก					
32.ฉันตบลูกเมื่อเขาแสดงพฤติกรรมไม่เหมาะสม					

ได้รับอนุญาตให้แปลและเรียบเรียบโดย นพ.วีระศักดิ์ ชลไชยะ ภาควิชากุมารเวชศาสตร์ คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กันยายน 2558.

Appendix C

Patient Health Questionnaire-9

Depression is the most common mental disorder among HIV-infected adults with higher than general population. Therefore, there is a significant opportunity to have a negative impact on the mother-infant relationship and lead to poor infant developmental and behavioral outcomes.

PHQ-9 is with 9 self-report items to screen and diagnose depression. The primary caregiver is asked to complete the questionnaire about feeling of depression

The validated PHQ-9 Thai version was translated by Dr.Manote Lotrakul and widely used in Thailand. The primary caregiver is defined as the caregiver with the most responsibility for caring for the child. It will take time around 5 minutes.

Quality assurance

The investigator will be check that primary caregivers complete all 9 items.

Protocol

- The primary caregiver is asked to describe their mental health using a 4-point scale (ranging from “not at all” to “nearly every day”) in PHQ-Thai version.
- The primary caregiver is defined as the caregiver with the most responsibility for caring for the child.
- The primary caregiver reports the questionnaire by themselves. If they can’t read, the trained nurse will be read the questions or if they do not understand the questions, the trained nurse will explain the questions.

แบบสอบถามสุขภาพจิตใจของผู้เลี้ยงดู PHQ-9

ในช่วง **2 สัปดาห์**ที่ผ่านมา ท่านมีอาการดังต่อไปนี้ บ่อยแค่ไหน

	ไม่เลย	มีบางวัน ไม่บ่อย	มีค่อนข้าง บ่อย	มีเกือบ ทุกวัน
1. เบื่อ ทำอะไรก็ไม่เพลิดเพลิน				
2. ไม่สบายใจ ซึมเศร้า หรือท้อแท้				
3. หลับยาก หรือหลับๆตื่นๆ หรือหลับมากไป				
4. เหนื่อยง่าย หรือไม่ค่อยมีแรง				
5. เบื่ออาหาร หรือกินมากเกินไป				
6. รู้สึกไม่ดีกับตัวเอง คิดว่าตัวเองล้มเหลว หรือเป็นคนทำให้ตัวเองหรือครอบครัวผิดหวัง				
7. สมาธิไม่ดี เวลาทำอะไร เช่น ดูโทรทัศน์ ฟังวิทยุ หรือทำงานต้องใช้ความตั้งใจ				
8. พูดหรือทำอะไรช้าจนคนอื่นมองเห็น หรือกระสับกระส่าย จนท่านอยู่ไม่นิ่งเหมือนเคย				
9. คิดทำร้ายตนเอง หรือคิดว่าถ้าตายๆไปเสียคงจะดี				

ถ้าท่านตอบว่ามีอาการไม่ว่าในข้อใดก็ตาม อาการนั้นๆทำให้ท่านมีปัญหาในการทำงาน การดูแลสิ่งต่างๆในบ้าน หรือการเข้ากับผู้คน หรือไม่

ไม่มีปัญหาเลย	มีปัญหาบ้าง	มีปัญหามาก	มีปัญหามากที่สุด

Appendix D

Mullen Scale of Early Learning test

The Mullen Scales of Early Learning (MSEL) test is a measure of cognitive function for infants and preschool-age children from birth through age 68 months. Information about cognitive function is generated in 5 distinct areas (visual reception, fine motor, receptive language, expressive langue and gross motor skill). The results are reported using T scores to interpret results as standard score for each part. This also provides an early learning composite score that is referred to as an estimate of overall intelligence.

Quality assurance

A training of examiners (physicians and nurses) in the use of the Mullen Scales of Early Learning test is conducted by a behavioral-developmental physician. The test will be followed by a manual guideline and an item administration book of MSEL. If the children are not co-operative, the test will be re-schedule. All examiners will test in approximately the same way and yield comparable results. Dr Chonchaiya will be observed the examiner and recheck scoring system (by direct observe or VDO recording).

The raw score will be converted into T-score by using program and It will be rechecked by another data entry person.

Protocol

- The testing environment is set up to be fun for the child and includes room to play/interact pleasantly with research staff.
- The children will be in stable mood and with their caregiver.
- The examiners are blinded to HIV status of children.
- The test performs around 15-60 minutes depend on age.

Appendix E

Child Behavioral Checklist protocol

Childhood behavioral checklist (CBCL) test is a well-standardized and widely used 100 item rating scale for the identification of behavior problem in children aged 1 year 6 months – 5 years old. Eight cluster behaviors problems are assessed and further categorized into internalizing(including emotionally reactive, anxious/depressed, somatic complaints and withdrawn), externalizing (including attention problems and aggressive behavior), and total problems (including internalizing problems, externalizing problems, sleep problems, and other problems)

The validated CBCL-Thai version was translated by Dr.Orawan Louthrenoo and widely used in Thailand.

Quality assurance

The investigator will be check that the primary caregiver completes all 99 items. The raw score will be converted into T-score by using a program and it will be rechecked by another data entry person.

Protocol

- The primary caregiver is asked to describe how much a particular behavior describes their children within the past 2 months, using a 3-point scale (ranging from “not true” to “very true or often true”) in the CBCL-Thai version.
- The primary caregiver is defined as the caregiver with the most responsibility for caring for the child.
- The primary caregiver reports the questionnaire by themselves. If they can’t read, the trained nurse will be read the questions or if they do not understand the questions, the trained nurse will explain the questions.

แบบสำรวจพฤติกรรมเด็กอายุ 1.5-5 ปี

เพศ อายุ_____ ปี เชื้อชาติ_____ ☐ ชาย ☐ หญิงวันที่_____ วันเดือนปีเกิด_____	ผู้ตอบแบบสำรวจนี้ เพศ____เกี่ยวข้องกับเด็กโดยเป็น ☐ บิดา/มารดาโดยสายเลือด ☐ บิดา/มารดาเลี้ยง ☐ บิดา/มารดาบุญธรรม
กรุณาตอบแบบสำรวจนี้ตามความคิดเห็นของท่านเกี่ยวกับพฤติกรรมของเด็ก โดยเพิ่มเติมแต่ละข้อได้ในที่ว่างหน้าที่ 2	☐ ปู่ ย่า / ตา ยาย ☐ อื่นๆ (ระบุ)_____

ข้างล่างนี้เป็นข้อต่าง ๆ ที่จะใช้อธิบายลักษณะเด็ก **ในขณะนี้หรือในระยะ เดือนที่ผ่านมา** 2 กรุณาวงกลมเลข 2 **ถ้าเป็นจริงหรือบ่อยมาก** วงกลมเลข 1 ถ้า**เป็นจริงบางครั้ง** และถ้า**ไม่เป็นจริงเลย** วงกลมเลข 0 กรุณาตอบทุกข้อให้ได้มากที่สุด แม้บางข้อท่านอาจรู้สึกว่าไม่เกี่ยวกับเด็กของท่านก็ตาม **0 = ไม่จริง (เท่าที่ท่านทราบ) 1 = จริงบางครั้ง 2 = จริงหรือบ่อยมาก**

0	1	2	1.	เจ็บหรือปวด (ไม่มีสาเหตุทางร่างกาย **ไม่รวม** ปวดท้องหรือปวดศีรษะ)	0	1	2	30. อิจฉาบ่อย
0	1	2	2.	แสดงท่าทางเล็กกว่าอายุ	0	1	2	31. กินหรือดื่มสิ่งที่ไม่ใช่อาหาร ไม่รวมลูกอม (อธิบาย)_____
0	1	2	3.	กลัวที่จะลองสิ่งใหม่ ๆ	0	1	2	32. กลัวสัตว์ สถานการณ์ หรือสถานที่เฉพาะ (อธิบาย)_____
0	1	2	4.	หลีกเลี่ยงการสบตามกับผู้อื่น				
	1	2	5.	ไม่มีสมาธิ ไม่สามารถตั้งใจได้นาน	0	1	2	33. ถูกกระทบความรู้สึกได้ง่าย
0	1	2	6.	นั่งนิ่งไม่ได้ ยุกยิก หรือเคลื่อนไหวมาก	0	1	2	34. ได้รับบาดเจ็บหรือเกิดอุบัติเหตุบ่อย
0	1	2	7.	ไม่พอใจเมื่อสิ่งของถูกย้ายจากที่ประจำ	0	1	2	35. เข้ากลุ่มการต่อสู้
0	1	2	8.	ไม่สามารถอดทนรอ อยากได้ทุกอย่างทันที	0	1	2	36. เข้าร่วมทุกเรื่อง
0	1	2	9.	เคี้ยวสิ่งที่กินไม่ได้	0	1	2	37. รู้สึกเดือดร้อนมากเมื่อแยกจากพ่อแม่
0	1	2	10.	ติดผู้ใหญ่หรือต้องพึ่งพาผู้ใหญ่มาก	0	1	2	38. นอนหลับยาก
0	1	2	11.	ต้องการให้ช่วยเหลืออยู่ตลอด	0	1	2	39. ปวดศีรษะ (ไม่มีสาเหตุทางร่างกาย)
0	1	2	12.	ท้องผูก ไม่ถ่ายอุจจาระ(เมื่อไม่เจ็บป่วย)	0	1	2	40. ทุบตีผู้อื่น
0	1	2	13.	ร้องไห้บ่อย	0	1	2	41. กลั้นหายใจ
0	1	2	14.	โหดร้ายรังแกสัตว์	0	1	2	42. ทำร้ายสัตว์หรือผู้อื่นโดยไม่ได้ตั้งใจ
0	1	2	15.	ต่อต้าน	0	1	2	43. ท่าทางไม่มีความสุขโดยไม่มีเหตุผล
0	1	2	16.	เรียกร้องให้ได้ดังต้องการทันที	0	1	2	44. อารมณ์โกรธเกรี้ยว
0	1	2	17.	ทำลายสิ่งของของตนเอง	0	1	2	45. คลื่นไส้ วิงเวียน (ไม่มีสาเหตุทางร่างกาย)
0	1	2	18.	ทำลายของที่เป็นของครอบครัวหรือผู้อื่น	0	1	2	46. แสดงกิริยากังวล(อธิบาย)_____
0	1	2	19.	ท้องเสีย ถ่ายเหลว (เมื่อไม่เจ็บป่วย)	0	1	2	47. กังวล ตึงเครียด
0	1	2	20.	ไม่เชื่อฟัง	0	1	2	48. ฝันร้าย
0	1	2	21.	เดือดร้อนถ้ากิจวัตรประจำวันถูกเปลี่ยนแปลง	0	1	2	49. กินจุ
0	1	2	22.	ไม่ยอมนอนคนเดียว	0	1	2	50. เหนื่อยง่าย
0	1	2	23.	ไม่ตอบเมื่อมีผู้อื่นพูดด้วย	0	1	2	51. แสดงความกลัวรุนแรงไม่มีเหตุผล
0	1	2	24.	กินได้น้อย (อธิบาย)_____	0	1	2	52. เจ็บป่วยเวลาถ่าย (ไม่มีสาเหตุทางกาย)
0	1	2	25.	เข้ากับเด็กอื่นไม่ได้	0	1	2	53. ทำร้ายร่างกายผู้อื่น
0	1	2	26.	ไม่รู้วิธีเล่นสนุก ทำท่าเหมือนผู้ใหญ่	0	1	2	54. แคะจมูก แกะเการ่างกาย (อธิบาย)
0	1	2	27.	ไม่รู้สึกผิดเมื่อกระทำผิด				
0	1	2	28.	ไม่ยอมไปไหนนอกบ้าน				
0	1	2	29.	หงุดหงิดง่าย				**กรุณาตอบทุกข้อ แล้วเปิดหน้าต่อไป**

กรุณาตอบทุกข้อ

0 = ไม่จริง (เท่าที่ท่านทราบ) **1 = จริงบางครั้ง** **2 = จริงหรือบ่อยมาก**

0	1	2	55. เล่นอวัยวะเพศบ่อย	0	1	2	79. อารมณ์เปลี่ยนเร็วระหว่างเศร้าและสนุก
0	1	2	56. เคลื่อนไหวไม่คล่องแคล่ว งุ่มง่าม	0	1	2	80. มีพฤติกรรมแปลก
0	1	2	57. ปัญหาเกี่ยวกับตา (ไม่มีสาเหตุทางร่างกาย) (อธิบาย)_____	0	1	2	81. ดื้อรั้น ขุ่นเคือง หรือหงุดหงิด
				0	1	2	82. อารมณ์หรือความรู้สึกเปลี่ยนแปลงเร็ว
0	1	2	58. การลงโทษไม่ได้เปลี่ยนแปลงพฤติกรรมเด็ก	0	1	2	83. โกรธง่าย
0	1	2	59. เปลี่ยนจากกิจกรรมหนึ่งไปอันอื่นรวดเร็ว				84.
0	1	2	60. ผื่นหรือปัญหาผิวหนัง (ไม่มีสาเหตุทางร่างกาย)	0	1	2	85. ละเมอพูดหรือร้องไห้เวลาหลับ
0	1	2	61. ไม่ยอมกินอาหาร	0	1	2	86. ร้องไห้อาละวาดหรืออารมณ์รุนแรง
0	1	2	62. ไม่ยอมแล่นกีฬาที่ต้องเคลื่อนไหว	0	1	2	87. ห่วงเรื่องความเรียบร้อยหรือสะอาดมาก
0	1	2	63. โขกศีรษะหรือโยกตัวซ้ำ ๆ	0	1	2	88. หวาดกลัวหรือกังวลมาก
0	1	2	64. ไม่ยอมเข้านอนตอนกลางคืน	0	1	2	89. ไม่ให้ความร่วมมือ
0	1	2	65. ขัดขืนการฝึกขับถ่าย (อธิบาย)_____	0	1	2	90. เฉื่อย เคลื่อนไหวช้า หรือไม่มีแรง
0	1	2	66. กรีดร้องบ่อย	0	1	2	91. ไม่มีความสุข ซึมเศร้า
0	1	2	67. ดูเหมือนไม่ตอบสนองต่อการแสดงความรัก	0	1	2	92. ทำเสียงดัง อึกทึก
0	1	2	68. ระวังตัวมากหรือรู้สึกอับอายง่าย	0	1	2	93. อารมณ์เสียเมื่อพบคนหรือสถานการณ์ใหม่ ๆ (อธิบาย)_____
0	1	2	69. เห็นแก่ตัวหรือไม่ยอมแบ่งปัน				
0	1	2	70. ไม่ค่อยแสดงความรักต่อคนอื่น	0	1	2	94. อาเจียน (ไม่มีสาเหตุทางร่างกาย)
0	1	2	71. แสดงความสนใจต่อสิ่งต่าง ๆ รอบตัวน้อย	0	1	2	95. ตื่นบ่อยตอนกลางคืน
0	1	2	72. แสดงความกลัวต่อการบาดเจ็บน้อย	0	1	2	96. เดินเที่ยวไปเรื่อย
0	1	2	73. เงียบ ขี้อาย	0	1	2	97. ต้องการได้รับความสนใจมาก
0	1	2	74. นอนหลับน้อยกว่าเด็กอื่นช่วงกลางวันและหรือ/กลางคืน	0	1	2	98. อ้อน ขี้แย
0	1	2	75. ละเลงหรือเล่นอุจจาระ	0	1	2	99. แยกตัวเอง ไม่ยุ่งเกี่ยวกับผู้อื่น
0	1	2	76. ปัญหาการพูด (อธิบาย)_____	0	1	2	100. วิตกกังวล
0	1	2	77. มองเหม่อหรือเหมือนคิดหมกมุ่น	0	1	2	101. กรุณาเขียนปัญหาอื่น ๆ ที่พบในเด็กแต่ไม่อยู่ในข้อข้างต้น
0	1	2	78. ปวดท้อง (ไม่มีสาเหตุทางร่างกาย)				102.

กรุณาตอบทุกข้อ ขีดเส้นใต้สิ่งที่ท่านเป็นห่วง

เด็กมีความเจ็บป่วยหรือพิการหรือไม่ (ทั้งทางร่ายกายหรือจิตใจ)☐ ไม่มี ☐ มี – กรุณาอธิบาย:

Appendix F

Neuroimaging acquisition protocol

Safety: At enrollment and again just prior to scanning, all subjects will be carefully screened by the study coordinator for standard MRI contraindications, such as the presence of aneurysm clips, non-removable ferrous metal, or implanted metal fragments. Universal MRI safety precautions will be observed. We WILL NOT employ gadolinium enhancement. Ear protection will be provided.

Quality Assurance:

I. Prior to enrolling any children will complete the following scans, have reviewed that they are cleared for enrollment:
    a. scan the spherical GE phantom within the 8 channel head coil.
    b. scan the cylindrical phantom using single channel head coil.
    c. scan the human phantom using the appropriate structural MRI protocol as below (T1 and T2 weighted sequences)

II. Once monthly, scan the cylindrical phantom using single channel head coil. Data will be sent to UCLA to determine if machine calibration is needed. Protocol for this cylindrical phantom (which contain dopes water) is Axial plane, 2D spin echo (fast), FOV-24cm, matrix size = 256x128, phase FOV=0.75, 1 excitation, 5 mm slice thickness, slice spacing = 1mm, TE=min, TR=min, number of slices should cover the entire phantom (prescribe from 3-plane localizer).

Should excessive drift be noted, maintenance of the MRI will be planned prior to further imaging. Using the same adult control at longitudinally will add further control for between in within machine drift, which can be adjusted in the final analyses.

Data security:

MRIs will be acquired WITHOUT subject names on the header to allow sharing with UCLA. Instead, only a study ID, date, and visit number will be used. These will be captured on CDroms with subject ID and visit number noted on the disc for permanent archival in Thailand. The discs will be stored with the secure participant files at the Infectious Diseases office. The secure-copy internet protocol (scp) requires password access to the secure LONI site within an account designated for this project and accessible only to the appropriate investigators at UCLA and the study staff uploading data from Thailand. Essentially, only study investigators with need for access will have access to this account. No identifying information will be used in any correspondence, including emails.

Important considerations for children:

Our prescription is carefully designed for maximal gain of optimally acquired data using two principles: (1) a hierarchical manner of sequencing to acquire the most important data first and (2) duplication of some series. Because irregularities in the T1-weighted sequence, such as motion artifact, can be identified in real-time, technicians will immediately determine quality and repeat the scan if needed. The DTI sequences are obtained in duplicate, since these data can be combined to improve our scalar metrics and to ensure that motion does not deem the data unusable. Our final sequence will be the T2-weighted sequences.

The most important consideration in children is motion artifact. Young children may experience discomfort for having to lie still in a close-space during MRI. We will perform scans when children are sleepy and more likely to fall asleep in the scanner. Parents will be allowed to with participants in the MRI suite with each child, to provide support and reminders not to move. Active and continued correspondence with children will be encouraged, as will breaks between sequences, if needed. If children are uncomfortable or express fear in lying in the MRI scanner (particularly the 3T scanner that is very loud during scans), with parental consent, our pediatric anesthesiologists will be always available to provide light general anesthesia.

The children will be evaluated health conditions by pediatricians and pediatric anesthesiologists. For safety, an intravenous line will be put in place for the MRI in case of emergency and need for administration of intravenous medications. If children are uncomfortable in lying in the MRI scanner, with caregivers' consent, the pediatric anesthesiologists will provide light general anesthesia by laryngeal mask airway. Children who receive light general anesthesia will be monitored at least 6 hours after MRI.

If children cannot tolerate the MRI or the caregivers feel uncomfortable, they may ask for the MRI to be stopped at any time without this affecting his/her medical care in the future. However, they can continue in the developmental and behavioral tests. The investigators will use any results that are available.

MRI acquisition protocol:

For comfort and to minimize motion during imaging, the subject's head and neck will be relaxed and stabilized, with leg and/or back support provided as needed. Correct positioning will be observed in order to ensure consistency across scans. Investigators will notify the technical team of all system upgrades to ensure that they do not impact longitudinal outcomes.

The proposed MRI prescription at KCMH on a 3 Tesla Philips MRI scanner using an 8-channel head coil is as follows and in the following order:

1) **3-plane localizer** for setting up examination and **reference scan** for multi-coil calibration and image reconstruction.
2) **3D T1-weighted sequence:** isometric with SENSE, Sagittal plane, T1-weighted 3D turbo field echo (T1W 3D TFE), repetition time TR/echo time TE= 8.1ms/3.7ms, flip angle 8°, voxel size=1.00x1.00x1.00 mm^3, 160 slices with no gap. Acquisition time ~ 8 minutes.
3) **Diffusion Tensor Imaging (DTI)**: A single-shot EPI sequence is used for DTI: TR/TE=9396ms/ 92ms, flip angle 90°, NSA (number of signals averaged)=2, FOV (AP/RL/FH)=256x256x140 mm^3, SENSE parallel imaging (R=2). EPI factor=67, acquisition voxel size=2.0x2.0x2.0 mm^3, 32 diffusion-encoding directions (high) with b=1000 s/mm^2, 70 2-mm thick axial slices, no gap. An image without diffusion gradients (b=0) is also acquired. Acquisition time ~ 12 minutes.
    a. **DTI b0 Only**: An additional, separate b=0 volume without diffusion gradients is acquired: TR/TE=9396ms/92 ms, FOV (AP/RL/FH) =256x256x140 mm^3, acquisition voxel size=2.0x2.0x2.0 mm^3, 70 axial slices, number of b-factors=1, max-b-factor=0 s/mm^2, nex (number of signals averaged) =1.

4) **FLAIR (T2-weighted) (optional)**: axial plane, TR/TE= 11000ms/125ms, TI=2800ms, voxel size =0.70 x1.06 x 6.0 mm^3, 20 slices, 6mm slice thickness, slice gap = 1 mm. Acquisition time ~ 5 minutes.

The MRI scan will be performed with 3D T1-weight sequence for TBM (time ~ 8 minutes) then diffusion tensor imaging (DTI) (time ~ 12 minutes) as well as optionally for FLAIR (T2-weighted) (time ~ 5 minutes). Therefore the typical total acquisition time is around 20 minutes. If MRI include FLAIR (T2-weighted) and repeated some series if needed, the acquisition time is around 25-45 minutes. The MRI prescriptions may be modified as appropriate as long as the maximum acquisition time remains unchanged.

VITA

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