NeuroImage: Clinical 20 (2018) 327–335

Contents lists available at ScienceDirect

NeuroImage: Clinical

journal homepage: www.elsevier.com/locate/ynicl

Structural and functional brain imaging in acute HIV

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A B S T R A C T

Background: HIV RNA is identified in cerebrospinal fluid (CSF) within eight days of estimated viral exposure. Neurological findings and impaired neuropsychological testing performance are documented in a subset of individuals with acute HIV infection (AHI). The purpose of this study was to determine whether microstructural white matter and resting-state functional connectivity (rsFC) are disrupted in AHI.

Methods: We examined 49 AHI (100% male; mean age = 30 ± SD 9.9) and 23 HIV-uninfected Thai participants (78% male; age = 30 ± 5.5) with diffusion tensor imaging (DTI) and rsFC acquired at 3 Tesla, and four neuropsychological tests (summarized as NPZ-4). MRI for the AHI group was performed prior to combination antiretroviral treatment (ART) in 26 participants and on average two days (range:1–5) after ART in 23 participants. Fractional anisotropy (FA), mean (MD), axial (AD), and radial diffusivity (RD) were quantified for DTI. Seed-based voxelwise rsFC analyses were completed for the default mode (DMN), fronto-parietal, and salience and 6 subcortical networks. rsFC and DTI analyses were corrected for family-wise error, with voxelwise comparisons completed using *t*-tests. Group-specific voxelwise regressions were conducted to examine relationships between imaging indices, HIV disease variables, and treatment status.

Results: The AHI group had a mean (SD) CD4 count of 421(234) cells/mm^3 plasma HIV RNA of 6.07(1.1) $\log_{10}$ copies/mL and estimated duration of infection of 20(5.5) days. Differences between AHI and CO groups did not meet statistical significance for DTI metrics. Within the AHI group, voxelwise analyses revealed associations between brief exposure to ART and higher FA and lower RD and MD bilaterally in the corpus callosum, corona radiata, and superior longitudinal fasciculus ($p < 0.05$). Diffusion indices were unrelated to clinical variables or NPZ-4. The AHI group had reduced rsFC between left parahippocampal cortex (PHC) of the DMN and left middle frontal gyrus compared to CO ($p < 0.002$). Within AHI, ART status was unrelated to rsFC. However, higher CD4 cell count associated with increased rsFC for the right lateral parietal and PHC seeds in the DMN. Direct associations were noted between NPZ-4 correspond to higher rsFC of the bilateral caudate seed ($p < 0.002$).

Conclusions: Study findings reveal minimal disruption to structural and functional brain integrity in the earliest stages of HIV. Longitudinal studies are needed to determine if treatment with ART initiated in AHI is sufficient to prevent the evolution of brain dysfunction identified in chronically infected individuals.

1. Introduction

HIV is known to impact the central nervous system (CNS) and result in functional and neuropsychological impairment that underlie HIV-associated neurocognitive disorder (HAND) (Heaton et al., 2004). While the introduction of antiretroviral therapy (ART) has decreased the frequency of more severe forms of HAND, the overall prevalence remains unchanged at 30–50% among populations with access to ART (Heaton et al., 2010). Structural and functional brain abnormalities have been reported in later stages of HIV infection; yet, it remains unknown when in the course of HIV infection these structural or functional CNS changes occur (O'Connor et al., 2018; Hakkers et al., 2017). Acute HIV infection (AHI), the first weeks following infection when HIV antibody is often undetectable, is the earliest stage of the

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https://doi.org/10.1016/j.nicl.2018.07.024
Received 21 March 2018; Received in revised form 30 June 2018; Accepted 25 July 2018
Available online 27 July 2018

disease and is distinguished from individuals identified during early or primary HIV (< 1 year of infection) and chronic HIV infection (> 1 year after infection) (Fiebig et al., 2003). In chronic HIV, people with HAND can experience worse performance in executive, attention, working memory, and information processing speed (Woods et al., 2009). Structural brain changes may underlie these cognitive issues, as white and gray matter differences have been seen in older age in fully suppressed chronically infected HIV participants using diffusion tensor imaging (DTI) and volumetric measurements (Underwood et al., 2017; Stebbins et al., 2007; Su et al., 2016; Jernigan et al., 2011; Ances et al., 2012). AHI is also marked by very high plasma viremia, which, when untreated contributes to the morbidity and mortality of HIV and to HIV associated dementia in the chronic phase of disease (Price & Spudich, 2008; Lavreys et al., 2006; Watkins & Treisman, 2015; Sidtis et al., 1993). However, initiation of ART during AHI precedes establishment of viral set-point and lowers viral reservoir levels in peripheral blood mononuclear cells, potentially altering disease trajectory (Ananworanich et al., 2016). Examining early structural and functional alterations in the brain during AHI allows us to understand if early treatment can mitigate changes that result in cognitive impairment in later stages and provides critical knowledge regarding the initial CNS sites of infection that are involved.

Neuropsychological testing abnormalities and neurological exam findings are present in some individuals during AHI, and may be caused by structural brain changes. HIV RNA is detected in the cerebrospinal fluid (CSF) as early as eight days after estimated exposure to HIV, although gross widespread structural CNS differences have not been identified in AHI (Kore et al., 2015; Hellmuth et al., 2016; Valcour et al., 2012; Ragin et al., 2015; Filippi et al., 2001). Standard volumetric techniques explored to date are less sensitive and may not detect changes in this early phase, as Magnetic Resonance Spectroscopy (MRS) suggests cellular inflammation is present in AHI (Sailasuta et al., 2012). Identifying vulnerable neuroanatomy through more sensitive neuroimaging techniques will inform whether microstructural damage exists and could support the identification of initial CNS sites involved.

Few studies have examined neuroanatomical changes occurring during AHI using DTI and resting-state functional magnetic resonance imaging (rs-fMRI). Cao et al. reported reduced regional brain volumes and white matter microstructural neuroimaging abnormalities in a small sample of individuals with HIV disease duration of 0–4 months (Cao et al., 2015). However, the group reported high rates of illicit substance use and mixed ART status. Separate analyses of the same cohort revealed brainstem and third ventricular enlargement in conjunction with parenchymal reduction and reduced microstructural integrity of the white matter quantified by DTI (Ragin et al., 2015). Brain volumes also correlated with cytokine levels (Ragin et al., 2015). This study also postulated engagement of the corpus callosum as the primary site of viral brain infiltration by documenting comparatively lower white matter integrity quantified using DTI compared to controls. Another study detected diminished resting-state functional connectivity (rsFC) of the inferior parietal cortex within the lateral occipital network in primary HIV infection, within 1 year of infection (7 out of 15 were treatment naïve), as compared with controls (Wang et al., 2011). In this study, rsFC within the lateral occipital network was correlated with neuropsychological performance among those with HIV (Wang et al., 2011).

Together, these data suggest that DTI and rs-fMRI may be useful in detecting early alterations in brain structure and function in AHI. Here, we utilize DTI and rs-fMRI to compare 49 AHI participants to 23 demographically similar, uninfected controls to determine whether microstructural white matter and rsFC of cortical and subcortical networks are disrupted during AHI. We examined relationships between both white matter integrity and rsFC and immune system function (CD4:CD8 ratios), viral load (HIV RNA), and cognitive performance (NPZ-4).

2. Methods

2.1. Participant selection

We enrolled participants seeking HIV testing who were determined to be in AHI at the Anonymous clinic of the Thai Red Cross AIDS Research center in Bangkok, Thailand. Most were classified in the primary Fiebig stages of AHI: Fiebig stage I (HIV RNA+, p24 antigen-, HIV IgM-, n = 3), stage II (HIV RNA+, p24 antigen+, HIV IgM-, n = 10), stage III (HIV IgM+, Western Blot -, n = 30), stage IV (HIV IgM+, Western Blot indeterminate, (n = 4)), stage V (Western Blot+ without p31 band, n = 2) and agreed to imaging, laboratory assessments, and clinical follow-up as outlined in a broader protocol set to investigate the immunology and virology of AHI (SEARCH 010/RV 254, ClinicalTrials.gov identifier NCT00796146) (Fiebig et al., 2003; De Souza et al., 2015). Infection duration was calculated by participant's self-reported exposure date, or by taking the median date of multiple potential exposures.

In this study, we compare AHI participants (n = 49) with demographically similar, age matched HIV uninfected controls (n = 23) enrolled from concurrent HIV protocols (RV304/SEARCH 013: NCT01397669). Any AHI participants showing positive serology for syphilis (serum VDRL) at diagnosis were excluded (n = 10). We examined all SEARCH 010/RV254 participants who consented to and underwent neuroimaging on a 3.0 Tesla MRI (dates: 7/6/2015–3/14/2017) were chosen from the greater population of those enrolling in the protocol. All control participants were scanned on the same MRI with identical scanning sequences and parameters. Of the 69 AHI eligible participants, full data were available for 49, after excluding participants due to positive serum syphilis serology (n = 10), and excessive motion during resting-state (n = 7) and DTI (n = 3) scans.

Among a broader set of clinical and laboratory variables captured at baseline, this analysis utilized a four test neuropsychological battery (NPZ-4), CD4 lymphocyte counts, CD8 lymphocyte counts, and plasma HIV RNA (copies/mL). MRI for the AHI group was performed *prior* to combination ART in 26 participants, and a mean (SD) of 2(1.1) days *after* combination ART initiation in 23 participants. The study was approved by institutional review boards from all participating sites all participants also provided written and informed consent.

2.2. Neuropsychological assessment

Our four-category neuropsychological testing battery included of the Grooved Pegboard test non-dominant hand (fine motor function), Color trails 1 (psychomotor speed), Color Trails 2 (executive functioning/set-shifting) and Trail Making A (psychomotor speed). These tests were combined into an overall cognitive performance score (NPZ-4) conducted and tabulated as previously described (Hellmuth et al., 2016).

2.3. HIV disease variables

Quantification of HIV RNA followed standard measures derived from previous work (De Souza et al., 2015). Corresponding plasma HIV RNA, CD4, and CD8 cell counts were measured the day of enrollment into the study and within a mean (SD) 2.6 (Underwood et al., 2017) days of MRI acquisition. Estimated infection duration was calculated using a median of participants' self-reported exposure date(s), as previously described (Valcour et al., 2012).

2.4. Imaging data acquisition

All structural and functional magnetic resonance imaging (MRI) data were acquired using the same Philips Ingenia 3T MRI scanner equipped with