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Amyloid beta-42 (Aβ-42), neprilysin and cytokine levels. A pilot study in patients with HIV related cognitive impairments

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ABSTRACT

HIV-associated dementia (HAD) is associated with amyloid-beta (Aβ) deposition. This study measured CSF and plasma amyloid beta-42 (Aβ-42), neprilysin (NEP) and cytokine levels in HIV-related cognitive impairments (HCI), HIV normal cognitive functioning (NF) and non-HIV controls. Our data showed a trend towards detectable plasma Aβ-42 levels more frequently in HCI (67%), when compared to NF (29%) and controls (10%). We showed elevated IL-8 levels in CSF of HCI compared to NF, although not significant values. The data from this pilot study indicates that CSF IL-8 and plasma Aβ-42 may be interesting biomarkers for the presence of HCI.

1. Introduction

HIV-associated dementia (HAD) is rarely seen in patients treated with combination antiretroviral therapy (cART) (Mirza and Rathore, 2012), but more mildly neurocognitive disorders have been observed in 15–50% of patients that are successfully treated with cART (Cysique et al., 2004; Schouten et al., 2011; Simioni et al., 2010). Specific neuropsychometric performance tests can be used to identify and monitor patients with more subtle HIV-associated neurocognitive disorders (HAND) (Clifford and Ances, 2013; Overton et al., 2011); however, these tests acquire specific trained personnel and are labor intensive. Many scientists have therefore been searching for easy accessible biomarkers for HAND, but no reliable markers have yet been found (Price et al., 2013).

Amyloid-β (Aβ) protein isoforms are candidates as biomarkers for HAND. Aβ deposition has been found in brain tissues of HIV infected patients (Achim et al., 2009; Andras and Toborek, 2013; Esiri et al., 1998; Mirza and Rathore, 2012; Rempel and Pulliam, 2005; Soontornniyomkij et al., 2012) and in those with Alzheimer's disease (AD) (van Duinen et al., 1987; Yamada et al., 1988). There are two major soluble Aβ protein isoforms, Aβ-42 and Aβ-40 (Iversen et al., 1995; Iwatsubo et al., 1994), with Aβ-42 being the most neurotoxic compared to Aβ-40 (Klein et al., 1999). Reduced cerebrospinal fluid (CSF) Aβ-42 levels have been found in patients with HAND/AIDS dementia and AD (Brew et al., 2005; Clifford et al., 2009; Hampel et al., 2004), which may be explained by an increased deposition of Aβ-42 (Fagan et al., 2006) or increased ingestion by activated microglial cells (Spies et al., 2012). Plasma Aβ-42 levels were found to be elevated in AD patients with mild cognitive impairment (Cammarata et al., 2009); however, plasma Aβ-42 did not differentiate between AD patients and their age matched controls in other studies (Koyama et al., 2012; Sedaghat et al., 2009).

In the normal brain, Aβ is rapidly degraded by a zinc metalloendopeptidase, referred to as neprilysin (NEP) (Farris et al., 2003; Wang et al., 2006). In AD transgenic Mice it was shown that recombinant soluble neprilysin reduces Aβ accumulation and improves memory impairment in AD transgenic mice (Marr et al., 2003). NEP is inhibited by HIV tat protein, a HIV replicative factor which may explain the accumulation of Aβ in the brains of certain HIV infected patients (Rempel and Pulliam, 2005). Recently it has been demonstrated that HIV tat protein is driving T cell activation and inflammation, also in the central nervous system (Johnson et al., 2013). In AD animal models, pro-

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inflammatory cytokines, especially IL-6 and TNF-alpha were shown to play a key role in neuroinflammation that has a relevance to neurodegeneration (Lin et al., 2014; Tweedie et al., 2012), while different mice studies showed that blocking inflammation attenuates Aβ deposition in the brain (DaSilva et al., 2006; Kiyota et al., 2010). The general consensus is that elevated CSF pro-inflammatory cytokines (Kamat et al., 2012; Yuan et al., 2013) promote cognitive deficits and anti-inflammatory cytokines do not show such distinction (Airoldi et al., 2012; Nolting et al., 2009).

Different mechanisms may therefore contribute to the development of HAND and/or HAD whereby either HIV tat can down-regulate NEP, influence cerebral cytokine levels and predispose to brain amyloid deposition. Therefore we hypothesize that decreased NEP activity will result in increased inflammation and Aβ deposition and decreased CSF Aβ-42 levels in HIV-related cognitive impairments (HCI) patients.

The present study aimed to investigate the role of Aβ-42, NEP, pro- and anti-inflammatory cytokines as biomarkers for HCI in CSF and plasma.

2. Materials and methods

2.1. Study population

This study included 32 patients, among which 22 HIV seropositive patients (19 were on cART) and 10 HIV seronegative controls. HIV positive patients were included if a neuropsychological assessment was available and paired CSF and plasma sample could be retrieved. The 22 HIV positive patients underwent an extensive neuropsychological assessment and then were grouped into 15 patients with significant neurocognitive disorders according to the Frascati criteria (Antinori et al., 2007) and 7 patients with normal neurocognitive functioning. Paired CSF and plasma samples were retrieved from all 32 individuals. The 10 HIV negative controls consisted of samples from patients suspected for neuroborreliosis, all of them tested negative. No intrathecal inflammation as defined by the absence of pleiocytosis or abnormal Q albumin or both was observed in 9 control patients, while 1 control patient had a leukocyte count of 12 cells/mm^3. All patients were recruited from the HIV outpatient clinic at the Radboud University Medical Center (Radboudumc, Nijmegen, The Netherlands). The study protocol was reviewed and approved by the Medical Ethical Committee of the Radboud University Nijmegen Medical Center.

2.2. Neuropsychological evaluation

A trained neuropsychologist, using validated tasks, performed the neuropsychological evaluation. The assessment covered the following domains; abstract reasoning, working memory, episodic memory, psychomotor speed, visuoconstruction, executive functioning, attention and information processing speed (Janssen et al., 2013). A continuous score was obtained for all 8 domains in HCI and Normal cognitive functioning (NF) groups. HIV patients were defined into two categories 1) HCI and 2) NF, by Frascati criteria (Antinori et al., 2007).

2.3. Laboratory methods

2.3.1. HIV viral load measurement

HIV viral loads in both CSF and plasma compartments were measured by COBAS AmpliPrep/COBAS TaqMan HIV-1 Test, version 2.0, following the manufacturer's instructions (Roche Molecular Systems Inc., USA), with the lower detection limit of 20 copies/ml.

2.3.2. Amyloid beta-42 and neprilysin and cytokines quantification

Aβ-42 concentration in CSF and plasma were quantified by High Sensitivity Human Amyloid beta-42 ELISA (Millipore, Billerica, MA, USA) according to the manufacturer's instructions. NEP activity in CSF and plasma samples was measured using the fluorogenic peptide substrate, Mca-RPPGFSAFK(Dnp)-OH (20 μM) (R&D systems, Minneapolis, MN, USA) in the presence of thiorphan (2 μM) (Sigma Aldrich, Dorset, UK) adapted from the immunocapture-based assay published by Miners et al. (Miners et al., 2008). Concentrations of IL-6, IL-8, IL-10, IL-18, IFN-γ, IL-17 and IL-22 were quantified by Multiplex Fluorescent Bead Immunoassays (Milliplex MAP kit, Millipore, Billerica, MA, USA) according to the manufacturer's instructions. Bio-plex microbead analyzer (Luminex, TX, USA) was used to read the plate according to the manufacturer's protocol. All CSF and plasma samples were analyzed in one batch.

2.3.3. Statistical analysis

Data analysis was performed using the SPSS statistical package (SPSS 20, Inc. Chicago, Illinois, USA). Spearman's correlation was used to determine correlation coefficient between Aβ-42 levels, NEP activity and cytokine levels. Aβ-42 levels, NEP activity and cytokine levels were compared between HCI and NF, HCI and control, NF and control groups pairwise by Mann–Whitney *U*-test. Proportions were compared using Chi-square statistics. All data are expressed as medians.

3. Results

3.1. Baseline characteristics (Table 1)

Almost all HIV patients (16/18) and all controls showed normal Q-albumin (~brain–barrier-function) ratio of no higher than 8.9 indicating normal blood–brain barrier function. Two patients in the HCI group showed high Q-albumin ratios and for four patients no Q-albumin data were available. HIV viral loads were measured in 22 HIV positive patients in both CSF and plasma. Nine patients had detectable viral loads in CSF, 6 of the 9 had viral loads >200 copies/ml. Five of the 9 patients had higher viral load in CSF than in plasma. Nineteen of 22 HIV positive patients were on cART, 2 of 3 patients not on cART were in the HCI group. The median age did not differ between the groups, 44, 49 and 45 years in the HCI, NF and control groups respectively.

3.2. Amyloid beta-42 levels and NEP activity in patients with different cognitive functioning

The CSF levels of Aβ-42 and NEP did not show significant difference between HCI, NF and control groups. Plasma Aβ-42 levels were detectable in 67% (10/15) of HCI patients, but in only 29% (2/7) and 10% (1/10) of NF and control groups respectively (Fig. 1). Plasma Aβ-42 concentrations were significantly different between HCI and controls (25 pg/ml and 16 pg/ml respectively, $p = 0{,}01$). There was no difference in plasma Aβ-42 concentrations between HCI and NF ($p = 0.28$). Positivity rates of plasma Aβ-42 were equal between HCI and NF groups (67% versus 29%, $p = 0.17$), NF and control groups (29% versus 10%, $p = 0.53$), but showed a significant difference between HCI and controls (67% versus 10%, $p = 0.005$). Median plasma NEP activity was elevated in HIV positive patients as compared to HIV negative controls (120 ng/ml versus 88 ng/ml; $p = 0.036$).

3.3. Cytokine production in patients with different cognitive functioning

Most cytokine concentrations in plasma did not differ significantly between HCI and NF groups (Fig. 2a to g). However, plasma IL-10 and IL-22 levels were slightly elevated in HCI patients. Several cytokines (IL-10, IL-17, IL-18, IL-22, IFN-γ) were not detectable or rarely measured (IL-6) in CSF. Remarkably IL-8 showed higher concentrations in CSF when compared to the plasma concentrations (Fig. 2b). Patients with HCI appeared to have higher IL-8 levels in CSF compared to patients with NF (37 pg/ml and 22 pg/ml respectively, $p = 0.057$) and controls (37 pg/ml and 24 pg/ml respectively, $p = 0.11$) (Fig. 2b).