Cerebrospinal Fluid Cytokines and HIV-Associated Neurocognitive Disorders in Uganda

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Abstract

Background: To better understand the neurocognitive effects of HIV infection in Uganda we compared the expression of various cytokines and neurodegenerative CSF biomarkers among antiretroviral therapy (ART)-naïve adults in Rakai, Uganda.

Methods: CSF from Rakai Community Cohort Study participants, who were HIV-infected, age >20 years, and ART-naïve, was assayed via Luminex ELISA for 17 cytokines and 20 neurodegenerative biomarkers. These Ugandan participants underwent neurological assessment to determine their degree of HIV-Associated Neurocognitive Disorder (HAND).

Results: 78 participants, 38 with CD4⁺ counts of <200 cells/μL and 40 with CD4⁺ counts of 350 -500 cells/μL, were evaluated. Persons with CD4 <200 cells/μL had significantly higher CSF levels of IL-1β, IL-2, IL-4, IL-5, IL-12, GM-CSF, MMP-1, MMP-7 and S100B. Lower CSF levels of Amyloid β42 were found in participants with CD4⁺ <200 cells/μL. S100B was found in higher concentrations in milder forms of neurocognitive impairment; and Amyloid β42 was found in lower concentrations in more severe forms of neurocognitive impairment when stratifying based on HAND classification and adjusting for CD4 count.

Conclusion: Persons with CD4⁺ <200cells/μL have higher levels of inflammatory cytokines (IL-1β, MMP-1, MMP-7, GM-CSF), T-cell growth factor IL-2, Th1 cytokine IL-12, Th2 cytokines (IL-4, IL-5), S100B and lower levels of amyloid β42 in the CSF as compared to those with CD4⁺ >350 cells/μL. The differences in CSF cytokine and neurodegenerative biomarker expression suggest that inflammation plays an important role in the progression of neurocognitive impairment in HIV-infected adults.

Introduction

HIV associated neurocognitive disorders (HAND) are characterized by cognitive, behavioral and motor dysfunction. HAND is a clinical spectrum that includes asymptomatic neurocognitive impairment (ANI), HIV associated mild neurocognitive impairment (MND), and HIV associated dementia (HAD). Despite access to antiretroviral therapy (ART) the overall prevalence of HAND has increased. Whereas more severe forms of neurocognitive impairment, HAD, have decreased, milder forms of neurocognitive impairment, ANI and MND, have increased.

The proposed pathogenesis of HIV associated neurocognitive impairment involves the invasion of HIV infected monocytes-macrophages into the CSF. Infected macrophages release chemokines and cytokines that encourage the disruption of the blood brain barrier, leading to further CSF invasion by inflammatory cells. HIV infected macrophages have been shown to release certain proinflammatory cytokines including; TNF-α, IL-1β, IL-6, IL-8 and IFN-α. Increased levels of IFN-α have been associated with severity in HIV dementia.

The prevalence and severity of neurocognitive impairment has been found to differ between HIV-1 subtypes and regions. Lower rates (0-4%) of severe forms of HAD have been found in India where HIV-1 subtype C predominates, whereas higher rates (15-3%) of HAD have been found in regions where HIV-1 subtype B predominates. The lower rates of HAD in HIV-1 subtype C is thought to occur because of defective monocyte chemotaxis, decreased migration of monocytes into the CSF, and less chemokine and cytokine release resulting in reduced inflammation and less neural damage. Conversely, in HIV-1 subtype B, higher levels of proinflammatory CSF cytokines such as TNF-α and IL-6 have been found.

In Uganda, where HIV-1 subtypes A and D predominate, subtype D has been associated with faster disease progression and risk of developing HIV-associated dementia. In order to better characterize the cellular pathogenesis involved in the development of HAND in Uganda, we analyzed the CSF of 78 HIV+ participants with either moderate or advanced immunosuppression and with no previous history of an opportunistic infection.

Materials & Methods

Study Participants: A prospective cohort of 351 HIV seropositive (HIV+), ART-naïve Ugandans, >20 years of age were recruited from the Rakai Health Sciences Program (RHSP) HIV clinics in the Rakai district of Uganda. 151 participants had advanced immunosuppression (CD4⁺ <200 cells/μL) and 200 participants had moderate immunosuppression (CD4⁺ 350 - 500 cells/μL). Of the 351 HIV+ participants, 78 participants, 38 of which had CD4⁺ < 200 cells/μL, and 40 of which had CD4⁺ of 351-500 cells/μL were randomly selected to have their CSF analyzed.

Neurocognitive Analysis: All participants underwent a comprehensive neurologic assessment to determine HAND classification. The degree of HAND was determined using the American Academy of Neurology and Memorial Sloan Kettering staging system. Neurological assessment included a symptom questionnaire, full exam, and the Ugandan Neuropsychological Test Battery.

Cytokine Analysis: Participants were given a separate informed consent for collection of CSF by lumbar puncture. Multiplex cytokine profiling of 17 cytokines on 78 specimens and 20 biomarkers on 68 specimens were measured using the Luminex ELISA platform (Human 27-Plex Panel, Bio-Rad, Hercules, CA).

Statistical Analysis: CSF biomarkers are expressed as medians and interquartile ranges (IQR). Differences in CSF biomarker concentrations across CD4 group and HAND classification were evaluated via Wilcoxon rank-sum tests, against an alpha level of 0.05. P-values were adjusted for multiple comparisons using the Holm-Bonferroni method. Logistic regression was also used to evaluate the relationship between CSF biomarkers and HAND classification, adjusted for high or low CD4 count.

Results

	<200 CD4 cells/μL				350-500 CD4 cells/μL				
	Normal & Asymptomatic†	Mild Neurocognitive Disorder	HIV-Associated Dementia	Overall	Normal & Asymptomatic†	Mild Neurocognitive Disorder	HIV-Associated Dementia	Overall	p-value§
N	19	11	8	38	22	11	7	40	
Age	33 (30-37)	38 (26-42)	37 (30-42)	34 (30-41)	37 (27-40)	32 (27-38)	39 (29-45)	37 (27-40)	0.87
Female	8 (42%)	1 (9%)	4 (50%)	13 (34%)	9 (41%)	6 (55%)	6 (86%)	21 (53%)	0.10
Karnofsky Score ≤90	13 (68%)	8 (73%)	6 (75%)	27 (71%)	7 (32%)	3 (27%)	4 (57%)	14 (35%)	<.01
CD4 cells/mL	105 (58-160)	150 (86-184)	151 (135-171)	136 (80-171)	460 (413-476)	426 (380-486)	402 (375-450)	443 (397-476)	N/A

All data presented as median (IQR) or n (%); †Asymptomatic Neurocognitive Impairment;

§P-values originating from Kruskal-Wallis and X2 comparisons of patients across CD4 groups; not stratified by HAND classification.

38 participants had CD4⁺ counts <200 cells/μL, with a median CD4⁺ T-cell count of 136 cells/μL (IQR 80-171).

40 participants had CD4⁺ counts >350 cells/μL, with a median CD4⁺ T-cell count of 443 cells/μL (IQR 397-476).

Participants neurocognitive function was classified as:
- Normal: 38% (30/78)
- Asymptomatic Neurocognitive Impairment: 14% (11/78)
- Mild Neurocognitive Impairment: 28% (22/78)
- HIV-associated dementia: 19% (15/78)

There was no significant differences in age (p=0.87) and sex (p=0.1) between the two groups. Participants with CD4⁺ <200 cells/μL were more likely to have a Karnofsky score below 90 (<0.01).

Table 2: Median CSF Biomarkers in HIV+ Adults in Uganda by CD4 Count

CSF Biomarker	CD4 <200 cells/μL N=38	CD4 350-500 cells/μL N=40	P-value§	Adjusted P-value§§
IL-1β	0.93 (0.93-1.06)	1.10 (1.01-1.26)	<0.001	0.024
IL-2	3.7 (2.3, 5.1)	≤0.55 (≤0.55-1.5)	<0.001	0.003
IL-4	0.47 (0.47-0.68)	≤0.25 (≤0.25-0.46)	<0.001	0.003
IL-5	29 (76%)	13 (32.5%)	<0.001	0.004
IL-6	13.1 (9.7-19.78)	9.645 (5.87-16.95)	0.011	0.283
IL-7	2.3 (1.8-3.5)	1.4 (<0.25-3.5)	0.02	0.469
IL-12	4.7 (3.9-5.6)	≤0.25 (≤0.25-2.4)	<0.001	0.003
MCP-1	1,490 (1,036-2,101)	854 (440-2,305)	0.029	0.645
G-CSF	5.7 (2.8-7.2)	8.1 (4.8-17.2)	0.047	0.934
GM-CSF	176 (145-235)	40 (31-50)	<0.001	0.003
MMP-1	5.98 (4.9-7.1)	≤1.5 (≤1.5-2.89)*	<0.001	0.003
MMP-7	116 (97-129)	48.5 (48.5-48.5)*	<0.001	0.003
MMP-10	11.1 (8.3-15)	7.0 (2.5-13.2)*	0.04	0.834
sVCAM-1	21,652 (14,066-44,122)	13,574 (7,873-26,403)*	0.016	0.384
Amyloid β42	843 (378-1938)	4262 (1648-8118)*	<0.001	0.003
S100B	1,026 (770-1,338)	590 (325-957)*	0.001	0.029

Median values in pg/mL with interquartile range (IQR). *N=30, §Wilcoxon rank-sum test; Chi^2 test for categorical variables, e.g. IL-5; §§Adjusted P values using the Holm-Bonferroni method to adjust for multiple comparisons.

- IL-1β, IL-2, IL-4, IL-5 IL-12, and GM-CSF was found in significantly higher concentrations in participants with CD4⁺ T-cell count <200 cells/μL.
- MMP-1, MMP-7 and S100B was found in higher concentrations in participants with CD4⁺ T-cell count <200 cells/μL.
- Amyloid β42 was found in lower concentrations in participants with CD4⁺ T-cell count <200 cells/μL.

Table 3: CSF Biomarkers in HIV+ Adults in Uganda Stratified by HAND Classification

CSF Biomarker	Normal & Asymptomatic Neurocognitive Impairment N=41	Mild Neurocognitive Disorder & HIV-associated Dementia N=37	Univariate P-value §	Odds Ratio (95% CI) *	Adjusted P-Value §
INF-γ	≤1.1 (≤1.1-19.4)	4.2 (≤1.1-51.6)	0.07	2.7 (0.8 -9.3)	0.10
G-CSF	5.68 (2.8-11.2)	7.2 (4.8-12.1)	0.38	1.5 (0.6-3.8)	0.45
TNF-α	5.32 (2.8-7.35)	5.35 (2.8-9.1)	0.50	1.2 (0.7-1.9)	0.50
Amyloid β42	1578 (574-5028)**	1352 (601-4110)***	0.29	0.5 (0.3-1.0)	0.05
S100B	1101 (699-1348)**	775 (418-1033)***	0.02	0.5 (0.2-0.9)	0.02

Median values in pg/mL with interquartile range (IQR).
*Independent t-test across HAND classification on log2 transformed data. §Logistic regression predicting Mild Neurocognitive Disorder or HIV-associated Dementia diagnosis, adjusted for high or low CD4⁺ T-cell count. **N=32; ***N=36

Comparison of CSF biomarkers stratified by HAND classifications and adjusting for CD4⁺ T-cell count revealed the following:

- S100B was found in higher concentrations in NML and ANI participants.
- Amyloid β42 was found in lower concentrations in MND and HAD participants.
- We found no differences in the concentrations of INF-γ, G-CSF and TNF-α between the different HAND groups after adjusting for CD4⁺ T-cell count.

Discussion

In our study we have demonstrated that HIV-infected, ART-naïve individuals with CD4⁺ T-cell counts <200 cells/μL have increased levels of CSF inflammatory cytokines: IL-1β, IL-2, IL-4, IL-5, IL-12, and GM-CSF. Previous work on cytokine expression has found an association between the development of HAND and increased CSF levels of Th1 (IL-2, IL-12) and Th2 (IL-4, IL-6) cytokines. Increased levels of IL-1β has been documented in patients with HAD as well as Alzheimer's disease, multiple sclerosis, delirium and dementia. IL-2 has been found in plaques of patients with multiple sclerosis. IL-4 has been found to be strongly expressed in the CSF of patients with brain injury, infection, and neurodegenerative diseases. GM-CSF producing CD4⁺ T-cells contribute to chronic neuro-inflammation leading to neurodegeneration.

Higher concentrations of matrix metalloproteinases (MMPs) 1 and 7 were found in individuals with CD4⁺ T-cell counts <200 cells/μL. MMPs are important in neuro-inflammation, increasing permeability of the blood brain barrier causing edema, hemorrhage, and cell death. MMPs have been implicated in neurodegenerative diseases such as vascular cognitive impairment, Alzheimer's disease and Parkinson's disease, as well as being elevated in HIV+ patients with neurologic deficits.

Increased levels of S100B was found in individuals with CD4 <200 cells/μL. When comparing between HAND classifications, higher levels of S100B were seen in those with NML and ANI. S100B is expressed in astrocytes and oligodendrocytes and increased CSF concentrations may represent astroglial pathology in neurodegenerative diseases. Increased concentrations, however, may also represent a neuroprotective repair mechanism.

Amyloid β42 was found in lower concentrations in individuals with CD4⁺ T cell count <200 cells/μL. When comparing between HAND classification and adjusting for CD4⁺ count, lower levels of Amyloid β42 was found in those with MND and HAD. β-Amyloid is highly neurotoxic and is responsible for neuronal loss and dysfunction in Alzheimer's disease.

A limitation of our study is the sample size as preliminary analysis was conducted on a small subset the Rakai Cohort participants.

Conclusion

In Uganda, HIV-infected, ART-naïve individuals with advanced immunosuppression (CD4⁺ <200 cells/μL) expressed higher levels of pro-inflammatory CSF cytokines (IL-1β, IL-2, IL-4, IL-5, IL-12, GM-CSF), MMP 1 and 7, and S100B. Lower levels of Amyloid β42 was found in individuals with CD4⁺ <200 cells/μL. Higher levels of CSF cytokine expression with advanced immunosuppression suggest that inflammation plays an important role in HIV-associated neurocognitive impairment.

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