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Expression of CXCL2 in the Serum and Cerebrospinal Fluid of Patients with HIV and Syphilis or Neurosyphilis

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Abstract—The potential mechanisms for blood–brain barrier damage and the diagnosis of neurosyphilis in HIV patients co-infected with syphilis (HIV-S) are unclear. The aim of the study was to determine the expression of CXCL2 in the serum and cerebrospinal fluid (CSF) of HIV-S patients. A total of 34 HIV patients and 7 controls were enrolled in a HIV clinical cohort for diagnosis of neurosyphilis in Taiwan. Serum and CSF concentrations of CXCL2 were determined by ELISA. Neurosyphilis was defined as a CSF white blood cell count of ≧20 cells/μl or a reactive CSF Venereal Disease Research Laboratory (VDRL). Demographics and medical histories were collected. All the patients with HIV-S were males. Most (80 %) had sex with men (MSM) and serum rapid plasma reagin (RPR) titers of ≧1:32. The medium age was 37 (range 21–68)years. The medium CD4 T cell counts at the time of the diagnosis of syphilis were 299 (range 92–434)cells/μl. Eight patients (24 %) had neurosyphilis based on a reactive CSF VDRL test (n=5) or increased CSF white blood cell counts of ≧20 cells/μl (n=3). The concentrations of CSF CXCL2 were significantly higher in patients with HIV and neurosyphilis as compared to HIV with syphilis, HIV, and controls (p=0.012). There were no significant differences in serum concentrations between the four groups. There was a correlation between CSF CXCL2 concentrations with neurosyphilis (p=0.017), CSF white blood cell count (p=0.001), and CSF protein levels (p=0.005). The CSF level of CXCL2 can be used to distinguish those with or without neurosyphilis in HIV infected patients.

KEY WORDS: acquire immunodeficiency syndrome; chemokine; CXCL2; neurosyphilis.

INTRODUCTION

Syphilis can significantly decrease the CD4 cells and increase HIV viral load in HIV-infected individuals [1–3]. HIV may alter the presentation, diagnosis, and natural course of syphilis and accelerate progression to neurosyphilis [4]. The diagnosis of neurosyphilis is based on reactive cerebrospinal fluid (CSF) Venereal Disease Research Laboratory (VDRL). However, the claimed sensitivity is around 50 % only [5–7]. More accurate diagnosis of neurosyphilis can avoid inadequate treatment from under diagnosis. Furthermore, the decision to perform a lumbar puncture to diagnose neurosyphilis in patients with HIV co-infected with syphilis (HIV-S) remains controversial. Several reports have described HIV-infected patients treated for early syphilis who progressed to neurosyphilis [5, 6]. The Centers for Disease Control and Prevention recommends that CSF examinations should be performed in cases of HIV-infected individuals who are diagnosed with late latent syphilis, syphilis of unknown duration, neurologic signs or symptoms, or suspected treatment failure [7]. Two recent studies reported a significant association between serum RPR titers of ≥1:32 and neurosyphilis [8, 9]. It is not known whether all patients should undergo CSF evaluation.

CXCL2 expression *in vitro* is inducible by a variety of inflammatory mediators, including LPS, TNF-α in macrophage, neutrophils, and they have neutrophil-activating

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properties similar to those of IL-8 [10, 11]. We hypothesize that the blood–brain barrier damage mediated by neurosyphilis could cause the inflammatory cell influx into CSF and increase the levels of CXCL2.

This study was designed to obtain clues concerning the pathogenesis and potential markers of neurosyphilis in patients with HIV. To accomplish this goal, we assessed the regulatory response of CXCL2 in the CSF and serum of patients with HIV and syphilis compared to controls. In this report, we provide evidence that the CSF CXCL2 is dysregulated in patients with HIV and neurosyphilis compared to the other groups.

MATERIAL AND METHODS

Patients and Controls

Subjects were selected from a cohort of HIV-infected patients followed from August 2011 to December 2012. They were eligible for enrollment if they had syphilis, defined as a reactive serum (VDRL or rapid plasma reagin (RPR)) confirmed by a treponemal serological test (fluorescent treponemal antibody-absorbed test or *T. pallidum* particle agglutination). Lumbar punctures were performed if the patients had neurological or ophthalmological symptoms or signs or any stage of syphilis. Neurosyphilis was defined as a CSF white blood cell count ≧20 cells/μl or reactive CSF VDRL [7–9]. The plasma HIV RNA load and CD4 cell counts were quantified using a Cobas Amplicor HIV-1 Monitor Test, version 1.5 (Roche Diagnostics Corporation, Indianapolis, IN) and FACS Flow (Becton Dickinson), respectively. Plasma HIV-RNA viral load, peripheral blood CD4+T lymphocyte count, and clinical manifestations were recorded. Six HIV-1 infected patients without syphilis all had normal CSF appearance and they underwent lumbar puncture because of mental decline (n=3), headache (n=2), and change of consciousness (n=2). The control group consisted of seven patients without HIV and syphilis who underwent a lumbar puncture for a presumptive diagnosis of meningitis (n=3) and altered consciousness (n=4). Their CSF examinations were normal except in one patient who had slight elevations of CSF protein. CSF samples were centrifuged and the supernatants were frozen at –80 °C until assayed. The study protocol, including informed consent, was approved by the Commission on Medical Ethics of the Kaohsiung Veterans General Hospital.

Table 1. Demographic Data and VDRL Titers among 34 Patients with HIV Infection and Syphilis/Neurosyphilis (Including Six Patients with HIV Only)

Clinical parameters	Patients (n=34)
Sex	
Male (%)	34 (100 %)
Median age (range)	37 (21–68)
HIV risk factors	
MSM	27
Bisexual	3
Heterosexual	4
Median CD4 [IQR] (cells/ml)	299 (92–433)
Median HIV RNA level [IQR] (copies/ml)	79,192 (40–53,400)
HIV only	6 (18 %)
HIV with syphilis/neurosyphilis	28 (82 %)
Serum RPR ≥1:32	25
Serum RPR <1:32	3
HIV with neurosyphilis (%)	8 (24 %)
Reactive CSF VDRL	5
CSF WBC ≧20/μl	3

Enzyme-linked Immunosorbent Assays for CXCL2

Concentrations of CXCL2 in serum and CSF were determined using ELISA Kits (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. ELISA plate wells were coated with antibodies that captured CXCL2 from the added serum or CSF. Biotinylated detection antibodies and streptavidin-labeled horseradish peroxidase (HRP) were then added. Substrate (3, 3′, 5, 5′-tetramethylbenzidine) solution was used for visualization. The reactions were stopped with 2 N sulfuric acid. Optical density (OD) values were read at 450 nm.

Table 2. Clinical Manifestations among 28 Patients with HIV Infections and Syphilis/Neurosyphilis

Clinical manifestations	Patients (numbers)
Ophthalmic symptoms	2
Neurological signs	7
Primary syphilis	0
Secondary syphilis	7
Tertiary syphilis	0
Early latent syphilis	0
Late latent syphilis and syphilis with unknown duration	14
Image study show brain parenchyma damage	2
Maculosquamous rash	7
Headache	7
Lymphoadenopathy	2
Condyloma lata	3

Table 3. CD4, CSF White Blood Cell Counts, and Protein Levels in Patients with HIV Infection, HIV and Syphilis, HIV and Neurosyphilis, and Controls

	CSF (median, IQR)				
	HIV (n=6)	HIV with syphilis (n=20)	HIV with neurosyphilis (n=8)	Control (n=7)	p
CD4	79 (15, 587)	365 (170, 585)	179 (84, 342)		
CSF WBC (cumm)	2 (0.75, 5)	2 (1, 4)	12 (3, 38)	2 (1, 3)	*0.024
CSF protein (mg/dl)	50 (47, 95.5)	43 (34, 53)	70.5 (40, 116)	28 (21.75, 0.25)	*0.012

* $p<0.05$

Statistical Analysis

All of the continuous variables were expressed as median and interquartile range (IQR). The concentrations of CXCL2 in CSF and serum in HIV infected patients with neurosyphilis, HIV with syphilis, HIV only, and controls were compared by the Kruskal–Wallis H test. The association between CXCL2, clinical parameters, and CSF laboratory abnormalities was analyzed with Pearson correlation test. A p value of <0.05 was considered to be statistically significant.

RESULTS

Characteristics of the Study Subjects and Clinical Manifestations

The study was conducted from August 2011 to December 2012. During this period 34 patients were identified as having HIV with or without syphilis. Among them, 28 patients had HIV and syphilis or neurosyphilis. Thirty-four had a lumbar puncture. None of the patients had any opportunistic infections. The clinical characteristics of the 34 patients are summarized in Tables 1 and 2. The indications for lumbar puncture were neurological symptoms or signs (n=7), late latent syphilis or syphilis of unknown durations (n=14), and a higher serum RPR titer (≧1:32) (n=25). Eight (24 %) were diagnosed as neurosyphilis based on the reactive CSF VDRL test (n=5) and elevated CSF white blood cell count of more than 20 cells/μl (n=3). The treatment for HIV and syphilis consisted of benzathine penicillin 2.4 million unit intramuscularly weekly for three consecutive weeks (n=20). The treatment for HIV and neurosyphilis consisted of intravenous penicillin G 3 million unit every 4 h for 14 days (n=5). Three patients in the HIV and neurosyphilis group received only benzathine penicillin 2.4 million units intramuscularly weekly for 3 weeks and all had favorable outcomes. Twelve patients received antiretroviral therapy. Six HIV-1 infected patients without syphilis all had normal CSF appearance and they underwent lumbar puncture because of mental decline (n=3), headache (n=2), and change of consciousness (n=2). The control group consisted of seven patients without HIV and syphilis who underwent a lumbar puncture for a presumptive diagnosis of meningitis (n=3) and altered consciousness (n=4). The CSF examinations were normal except in one patient who had slight elevations of CSF protein. The CD4 count, CSF white blood cell count and protein levels are summarized in Table 3. The CSF white blood cell count and protein levels were higher in the HIV and neurosyphilis group (p=0.024 and p=0.012,

Table 4. Serum and CSF CXCL2 Concentrations in Patients with HIV, HIV with Syphilis, HIV with Neurosyphilis, and Controls

	Median, IQR				
	HIV (n=6)	HIV with syphilis (n=20)	HIV with neurosyphilis (n=8)	Control (n=7)	p
Serum CXCL2 (ng/ml)	15.91 (9.12, 25.31)	16.72 (11.50, 21.57)	14.60 (5.10, 21.26)	14.38 (13.41, 23.36)	0.625
CSF CXCL2 (ng/ml)	0.04 (0.05, 0.096)	0.02 (0.01, 0.03)	0.14 (0, 1.38)	0.015 (0.01, 0.105)	*0.012

* $p<0.05$. The data was expressed as median (interquartile range)

respectively, Kruskal–Wallis H test) compared to HIV with syphilis, HIV only, and HIV negative groups (Table 3). There was no correlation between the serum RPR titer with neurosyphilis.

Concentrations of CXCL2 in Serum and CSF

The CSF concentrations of CXCL2 were significantly higher in patients with HIV and neurosyphilis as compared to the other three groups. There was a 9.7-fold increase in the levels of CSF CXCL2 in HIV infected patients with neurosyphilis compared to the levels in patients with syphilis. There were no significant differences in the serum concentrations of CXCL2 between HIV and neurosyphilis, HIV with syphilis, HIV only, and HIV negative groups (Table 4 and Figs. 1 and 2). Three of the eight HIV infected patients with neurosyphilis had a follow-up lumbar puncture 6 months after presentation. All of the three patients had normal CSF appearances and the levels of CXCL2 in the CSF were all decreased to undetectable level compared to the levels of pretreatment.

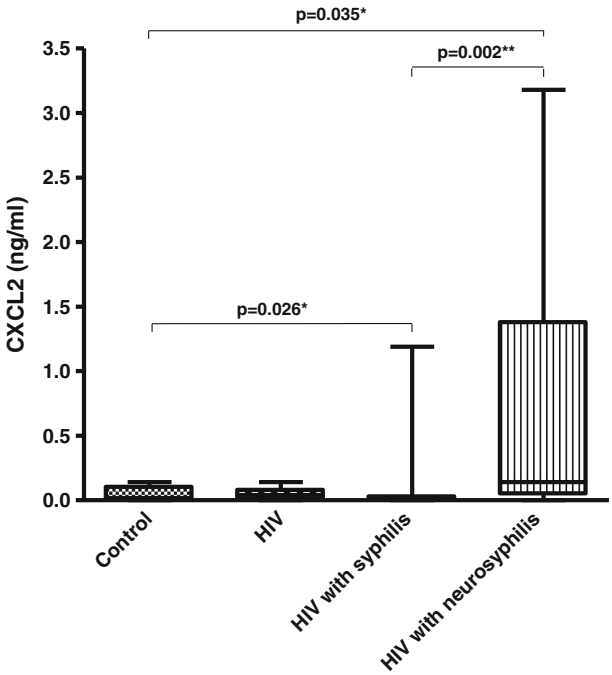

Fig. 1. The CSF concentrations of CXCL2 in controls, patients with HIV infection, HIV with syphilis, and HIV with neurosyphilis were shown here. There were significantly higher CSF CXCL2 levels in patients with HIV and neurosyphilis as compared to HIV with syphilis and HIV negative control. (The CXCL2 levels were expressed as median.*Indicates a significant difference, *$p<0.05$; **$p<0.01$).

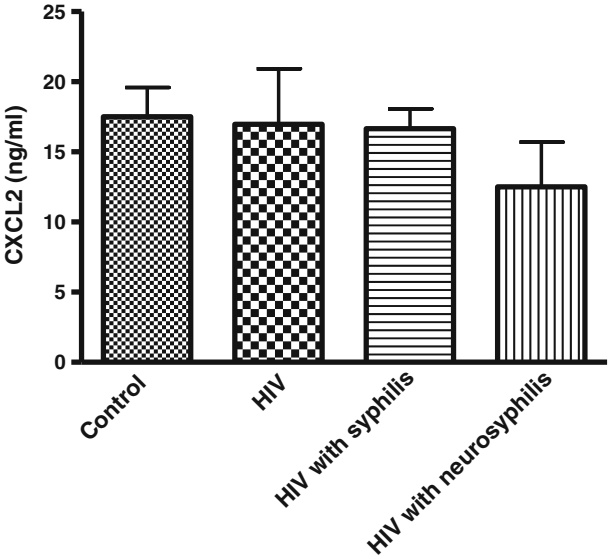

Fig. 2. The serum concentrations of CXCL2 in controls, patients with HIV infection, HIV with syphilis, and HIV with neurosyphilis were shown here (the CXCL2 levels were expressed as median). There were no significant differences in the serum concentrations of CXCL2 between HIV and neurosyphilis, HIV with syphilis, HIV only, and HIV negative groups.

Correlation of CXCL2 Concentrations with Clinical and CSF Parameters

There was a correlation between CSF CXCL2 concentrations with neurosyphilis ($p=0.017$), CSF white blood cell count ($p=0.001$), and CSF protein levels ($p=0.005$). There was no correlation between serum CXCL2 concentrations with any parameters tested (Table 5).

DISCUSSION

In this study, we found that the CSF concentrations of CXCL2 were significantly higher in patients with HIV and

Table 5. Correlation of CSF/Serum CXCL2 and CSF Parameters, CD4 and, Viral Load

Variable	CSF CXCL2		Serum CXCL2	
	r	*p*	*r*	*p*
HIV and syphilis	0.2	0.228	−0.132	0.458
HIV and neurosyphilis	0.384	*0.017	−0.207	0.239
CD4	−0.273	0.137	−0.057	0.782
Plasma viral load	−0.056	0.764	−0.04	0.844
CSF WBC	0.645	*0.001	0.011	0.952
CSF protein	0.480	*0.005	−0.255	0.182

* $p<0.05$

neurosyphilis when compared to HIV with syphilis, HIV only, and HIV negative groups. There was an association between CSF CXCL2 concentrations and neurosyphilis, CSF protein levels and white blood cell counts.

GROβ (CXCL2, MIP-2α) expression *in vitro* is inducible by a variety of inflammatory mediators, including LPS, TNF-α in macrophage, neutrophils, and they have neutrophil-activating properties similar to those of IL-8 [10, 11]. In the animal model of experimental autoimmune encephalomyelitis (EAE), the elevated mRNA levels of the chemokine MIP-2 was detected in the CNS of diseased mice, whereas no chemokine expression could be measured in asymptomatic mice. Activated astrocytes were shown to be the main source of MIP-2 before and during cellular CNS infiltration [11]. MIP-2 was shown to induce massive neutrophil migration into the CNS [11].

The cells and chemokines involved in patients with neurosyphilis are still unclear. Previous reports found elevations of CXCL13 in CSF of patients with neuroborreliosis (NB), and also in patients with neurosyphilis [12, 13]. CXCL13 is a chemokine that directs B-cell traffic [13]. Marra et al. showed that B cells were increased in the CSF of patients with neurosyphilis, as compared to patients without neurosyphilis [14]. Another study showed that changes in CD3+CD8+ lymphocyte number and NK cell numbers were consistent with clinical outcomes in the course of neurosyphilis and the T lymphocytes and NK cells are involved in inflammatory injury of neurosyphilis [15]. Our study showed that the CSF CXCL2 expression was increased in patients with HIV and neurosyphilis and was associated with CSF inflammatory cell influx. Taken together, B and T cells, NK cells, chemokines (such as CXCL2, CXCL13), and neutrophil were involved in the pathogenesis of neurosyphilis. It is likely that *T. pallidum* produces a lipoprotein that acts to stimulate CXCL2 production by activated astrocytes then mediated the inflammatory cell influx into CSF and increase the levels of CXCL2. This hypothesis was consistent with our findings which showed that the increase in CSF CXCL2 concentrations was found in patients with HIV and neurosyphilis when compared to the other three groups.

There were a number of limitations to our study that should be noted. Our sample size and control group were small. We did not observe any correlation between the serum RPR titer with neurosyphilis. There was also no difference between the symptom severity and levels of CXCL2. We did not include HIV seronegative patients with syphilis. The etiology of this dysregulation would not easily clarified if not inclusion of syphilis, but no HIV group. However, it is not feasible and ethical to perform a lumbar puncture in those patients without CNS symptoms. Furthermore, we did not undergo follow-up lumbar puncture in most of the patients and, therefore, it was difficult to see the dynamic changes after treatment and the clinical impact was hard to evaluate. However, elevated CSF concentrations of CXCL2 can be used as the additional tool to differentiate HIV infected patients with syphilis and neurosyphilis.

In conclusion, we found that CXCL2 was dysregulated in patients with HIV and syphilis regardless of the standard clinical and laboratory criteria for neurosyphilis. It is possible that CSF CXCL2 concentrations may help differentiate patients with HIV and neurosyphilis, but the sensitivity and specificity of this type of testing would need to be determined before it can be considered as a useful marker.

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Conflicts of interest. The authors report no financial conflicts of interest.

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