



ORIGINAL ARTICLE

# Elevated cerebrospinal fluid nitrite level in human immunodeficiency virus-infected patients with neurosyphilis



Yu-Jung Cheng<sup>a,e</sup>, Hung-Chin Tsai<sup>a,b,e</sup>, Shin-Yu Ye<sup>c</sup>,  
Cheng-Len Sy<sup>a,b</sup>, Kuan-Sheng Wu<sup>a,b</sup>, Jui-Kuang Chen<sup>a,b</sup>,  
Susan Shin-Jung Lee<sup>a,b</sup>, Yao-Shen Chen<sup>a,b,d,\*</sup>

<sup>a</sup> Section of Infectious Diseases, Department of Medicine, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan

<sup>b</sup> Faculty of Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan

<sup>c</sup> Department of Medicine, National Taiwan University Hospital, Taipei, Taiwan

<sup>d</sup> Graduate Institute of Science Education and Environmental Education, National Kaohsiung Normal University, Kaohsiung, Taiwan

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## KEYWORDS

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Nitrite

**Background/Purpose:** Human immunodeficiency virus (HIV) and syphilis coinfection is a common phenomenon. A percentage of neurosyphilis cases is asymptomatic in HIV-infected patients. The diagnosis of neurosyphilis is more difficult because of the alteration of cerebrospinal fluid (CSF) presentation by the HIV itself. The CSF levels of the degradation products of nitric oxide (NO; e.g., nitrate and nitrite) are reportedly elevated in animals and patients with bacterial meningitis. We hypothesized that an elevated CSF nitrite concentration may be present in patients coinfecting with HIV and neurosyphilis.

**Methods:** This cohort study was conducted from January 2007 to June 2008. Forty patients were enrolled and included seven patients in the control group and 33 HIV-infected patients with or without syphilis. Nitrite levels in the serum and the CSF were measured by using the Griess assay.

**Results:** The CSF nitrite levels were significantly higher in HIV-infected patients with neurosyphilis, compared to the control group or patients with HIV infection only or patients with HIV

\* Corresponding author. Section of Infectious Diseases, Department of Medicine, Kaohsiung Veterans General Hospital, 386 Ta-Chung First Road, Kaohsiung 813, Taiwan.

E-mail address: [hchtsai1011@yahoo.com.tw](mailto:hchtsai1011@yahoo.com.tw) (Y.-S. Chen).

<sup>e</sup> Y.-J. Cheng and H.-C. Tsai contributed equally to this study.

and syphilis coinfection ( $p = 0.026$ ). The CSF nitrite levels were correlated with the CSF white blood cell counts (Spearman correlation test,  $r^2 = 0.324$ ;  $p < 0.001$ ). There was no significant difference between different groups in serum nitrite levels.

**Conclusion:** Marked elevation of CSF nitrite level was observed in HIV-infected patients with neurosyphilis.

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## Introduction

Human immunodeficiency virus (HIV) and syphilis coinfection is a common phenomenon among men having sex with men (MSM).<sup>1–3</sup> Each disease alters the clinical presentation of the other. Syphilis may have a negative impact on HIV transmission and on the immune status. It can decrease the CD4 T cell count and increase the viral load in HIV-infected patients.<sup>4–6</sup> Because of the effect of HIV on the immune system, HIV infection may change the presentation, diagnosis, and natural course of syphilis and accelerate its progression to neurosyphilis.<sup>7</sup>

The prevalence of asymptomatic neurosyphilis in HIV-infected patients is high<sup>7,8</sup>; however, diagnosis is difficult and the timing of lumbar puncture remains controversial. The prevalence of neurosyphilis is 0.4% in HIV-infected patients and 23.5% in HIV-infected patients with untreated syphilis.<sup>9</sup> In one study of 117 HIV-infected patients with a diagnosis of neurosyphilis, one-third of the patients were asymptomatic.<sup>7</sup> In another study in Taiwan, which enrolled 121 patients with HIV and syphilis coinfection, 14 of the patients had neurosyphilis and two of the 14 patients were asymptomatic.<sup>8</sup> The diagnosis of neurosyphilis in HIV-infected people may be more challenging because CSF pleocytosis and protein elevation can occur with HIV infection alone.<sup>10,11</sup> Two recent studies report a significant association between serum rapid plasma reagin (RPR) titers of  $\geq 1:32$  and neurosyphilis.<sup>12,13</sup> The U.S. Centers for Disease Control and Prevention recommend that CSF examination should be performed for HIV-infected individuals who are diagnosed with syphilis at the late latent stage or have syphilis with unknown duration, who have neurologic signs or symptoms, or who have suspected treatment failure.<sup>14</sup> For syphilis patients who have HIV coinfection, a CSF leukocyte count greater than 20 cells/ $\mu\text{L}$  is consistent with neurosyphilis.<sup>12</sup>

For a better diagnosis of neurosyphilis in HIV-infected patients, some studies suggest using the CSF *Treponema pallidum* hemagglutination assay (TPHA),<sup>15</sup> the CSF fluorescent treponemal antibody (FTA),<sup>16</sup> the percentage of CSF B cells,<sup>16</sup> CSF C-X-C motif chemokine 13 (CXCL13),<sup>17</sup> and CSF matrix metalloproteinases-9 (MMP-9).<sup>18</sup> *T. pallidum* DNA polymerase chain reaction (PCR) analysis of the CSF did not provide more information than conventional CSF analysis.<sup>19</sup> Nitric oxide (NO), which is generated by phagocytes, plays a role in inflammation and immune responses, and may consequently result in neuronal toxicity and dysfunction. Studies have shown that increased NO metabolites are present in patients with meningitis.<sup>20–22</sup> The hypothesis of our study is that neurosyphilis may be correlated with the elevation of NO metabolites in the CSF.

For this purpose, the serum and CSF levels of nitrites were studied in HIV-infected patients with syphilis or neurosyphilis and in control individuals.

## Methods

### Participants

Patients were selected from a cohort of patients and followed from January 2007 to June 2008. Syphilis was defined as a reactive serum test [i.e., venereal disease research laboratory (VDRL) test or RPR test] that was confirmed by a treponemal serological test (i.e., TPHA). Lumbar puncture was performed if the patient had neurological or ophthalmological symptoms or signs, and a lumbar puncture was performed in HIV-infected patients with late latent syphilis or syphilis of unknown duration.<sup>14</sup> Neurosyphilis was defined as a CSF white blood cell (WBC) count of at least 20 cells/ $\mu\text{L}$  or by a reactive CSF VDRL test. Patients were defined as having HIV infection if the enzyme-linked immunosorbent assay (ELISA) and Western blot procedure were both positive.

Among these patients, seven patients were negative for both HIV and syphilis infection and were defined as the control group. To exclude tumor, subarachnoid hemorrhage, inflammatory disease, or meningitis, the patients received a lumbar puncture because of the clinical presentation of fever with headache ( $n = 3$ ) or altered consciousness ( $n = 4$ ). Six patients were HIV-positive without syphilis. They received lumbar puncture because of headache ( $n = 2$ ), altered consciousness ( $n = 1$ ), fever ( $n = 1$ ), limb weakness ( $n = 1$ ), and gait disturbance ( $n = 1$ ). Twenty seven patients had HIV and syphilis coinfection, and eight of these were diagnosed as having neurosyphilis. Plasma HIV-RNA copy number, peripheral blood CD4 + T lymphocyte count, and clinical manifestations were recorded. Serum samples were also tested in another 11 patients, who received anonymous screening for sexually transmitted diseases (STDs) and tested as HIV-1–negative but syphilis positive. The study protocol, including an informed written consent form, was approved by the Commission on Medical Ethics of the Kaohsiung Veterans General Hospital (Kaohsiung, Taiwan).

### Nitrite concentration measurement in the CSF and in serum

The CSF and serum samples were centrifuged and the supernatants were frozen at  $-80^\circ\text{C}$  until assayed. The nitrite levels in CSF and serum were determined by a colorimetric

method,<sup>23</sup> after enzymatic reduction of nitrate to nitrite by using nitrate reductase (1 U/mL) from *Aspergillus* (Boehringer, Mannheim, Germany). The lower limit of detection was 0.2  $\mu\text{mol/L}$ .

### Statistical analysis

All continuous variables were expressed as the median and the interquartile range (IQR). Concentrations of nitrite in the CSF and in the serum of the patients and the controls were compared by the Mann-Whitney *U* test. The concentration of nitrite in the CSF and serum in HIV-infected patients with neurosyphilis, HIV-infected patients with syphilis, HIV-infected patients, and the controls were compared by the Kruskal-Wallis *H* test and the Dunn's multiple comparison test. Correlations between CSF nitrite level and the other variables were calculated by using the Spearman correlation test. A *p* value < 0.05 was considered statistically significant.

## Results

### Participants

Forty patients were enrolled and were followed from January 2007 to June 2008. Table 1 summarizes the clinical characteristics of the 33 patients with HIV infection and

the seven controls. The CSF white blood cell counts of the control group ranged from 0 cells/ $\mu\text{L}$  to 3 cells/ $\mu\text{L}$ . Of the eight patients with neurosyphilis, one patient was asymptomatic and received lumbar puncture because of a high serum RPR level (1:64). There was no difference in the CD4 cell count or HIV viral load between HIV-infected patients with and without syphilis ( $p = 0.097$  and  $p = 0.792$ , respectively) or between HIV-infected patients with and without neurosyphilis ( $p = 0.571$  and  $0.796$ , respectively). In patients with HIV and syphilis coinfection, the titer of serum RPR was significantly higher in the patients with neurosyphilis ( $p = 0.015$ ).

### CSF white blood cell count and nitrite levels

There was no significant difference in the CSF white blood cell counts between patients with and without HIV infection ( $p = 0.250$ ; Table 2). This may have resulted from the small patient group. The CSF nitrite level was significantly higher in HIV and syphilis coinfecting patients with neurosyphilis than in patients without neurosyphilis ( $p = 0.047$ ). The HIV-infected patients with neurosyphilis had a higher CSF nitrite level than other patient groups ( $p = 0.015$ , compared to all patients without neurosyphilis; and  $p = 0.03$ , compared to HIV-infected patients without neurosyphilis; Fig. 1). The CSF nitrite level was also higher in patients with HIV or syphilis than in patients without these diseases ( $p = 0.043$  and  $0.048$ , respectively).

**Table 1** Clinical characteristics of the HIV-infected patients and the control group

Clinical characteristics	Control group ( $n = 7$ )	HIV-infected patients ( $n = 33$ )
Age (y), median age (IQR)	46 (34–86)	33 (29–43)
Sex		
Male	6 (86)	33 (100)
Risk factors		
MSM	NA	27 (82)
Heterosexual	NA	6 (18)
Indications for lumbar puncture		
Fever	3 (43)	1 (3)
Headache	0	5 (15)
Neurological symptoms <sup>a</sup>	0	5 (15)
Consciousness change	4 (57)	3 (9)
Visual acuity change	0	2 (6)
Serum RPR $\geq 1:32$	0	16 (49)
Late latent syphilis or syphilis of unknown duration	0	1 (3)
CD4 count (cells/ $\mu\text{L}$ ), median count (IQR)	NA	311 (117.5–456)
HIV RNA (copies/mL in log), median (IQR)	NA	3.83 (0–4.33)
Serum RPR titer		
$\geq 1:32$	NA	25 (76)
$< 1:32$	NA	8 (24)
Neurosyphilis		
Reactive CSF VDRL	NA	5 (15)
CSF WBC $\geq 20$ cells/ $\mu\text{L}$ <sup>b</sup>	NA	3 (9)

<sup>a</sup> Neurological symptoms include dizziness, ataxia, limb weakness, and twitching.

<sup>b</sup> One patient with CSF pleocytosis was diagnosed as having syphilitic panuveitis.

Data are presented as *n* (%).

CSF = cerebrospinal fluid; HIV = human immunodeficiency virus; IQR = interquartile range; MSM = men who have sex with men; NA = not available; RPR = rapid plasma reagin; VDRL = Venereal Disease Research Laboratory; WBC = white blood cell count.

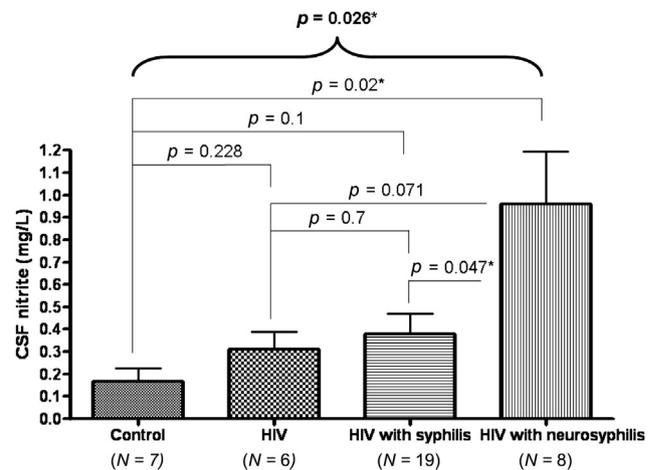
**Table 2** CSF white blood cell count, CSF levels of protein and nitrites, and serum nitrite level in the patient groups

	Patients with/without HIV (n = 33/7)	Patients with/without syphilis (n = 27/13)	Patients with/without neurosyphilis (n = 8/32)	HIV patients with/without neurosyphilis (n = 8/25)	HIV-S patients with/without neurosyphilis (n = 8/19)	p
CSF WBC (cells/ $\mu$ L)	2 (1-7)/2 (1-3)	3 (1-8)/2 (1-3)	12 (3.25-17.5)/2 (1-3)	12 (3.25-17.5)/2 (1-4)	12 (3.25-17.5)/2 (1-4)	0.006*
CSF protein (g/dL)	46 (34-66)/24 (18-48)	43.5 (33.5-63.75)/47 (23.25-55.25)	70.5 (40-116)/43 (26.25-51)	70.5 (40-116)/44 (34-54)	70.5 (40-116)/42 (31-48)	0.026*
CSF nitrite (mg/L)	0.43 (0.24-0.56)/0.17 (0.068-0.345)	0.043* 0.44 (0.25-0.675)/0.28 (0.07-0.413)	0.865 (0.31-1.62)/0.31 (0.12-0.46)	0.865 (0.31-1.62)/0.34 (0.15-0.48)	0.865 (0.31-1.62)/0.34 (0.19-0.49)	0.030* 0.047*
Serum nitrite (mg/L)	1.2 (0.605-1.445)/0.81 (0.35-1.16)	1.23 (0.68-1.48)/0.805 (0.573-1.18)	1.24 (0.68-1.73)/0.97 (0.573-1.315)	1.24 (0.68-1.73)/0.99 (0.575-1.405)	1.24 (0.68-1.73)/1.11 (0.6-1.44)	0.499 0.641

Data are expressed as the median (IQR).

\*Indicates a significant difference ( $p < 0.05$ ).

CSF = cerebrospinal fluid; HIV = human immunodeficiency virus; HIV-S = HIV and syphilis coinfection; IQR = interquartile range; WBC = white blood cell count.



**Figure 1.** The CSF nitrite level is significantly higher in patients with HIV and neurosyphilis than in the other patient groups. CSF = cerebrospinal fluid; HIV = human immunodeficiency virus. \*Indicates a significant difference,  $p < 0.05$ .

### Serum nitrite levels

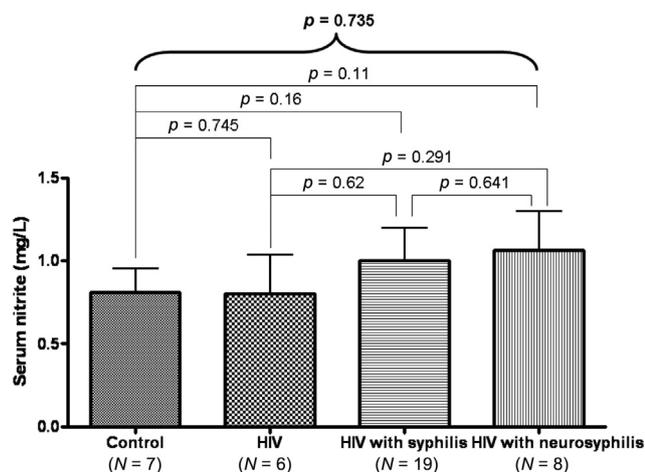
There was no significant difference in the serum nitrite levels between the control group, patients with HIV infection only, patients with HIV infection and syphilis, and patients with HIV infection and neurosyphilis ( $p = 0.735$ ; Fig. 2). In patients with syphilis only (i.e., patients who received an anonymous screen for STDs), the serum nitrites level was significantly lower than in the other patient groups ( $p = 0.004$ ).

### Correlation between CSF nitrites and laboratory parameters

There was an association between the CSF nitrite level and the CSF white blood cell count ( $r^2 = 0.324$ ,  $p < 0.001$ ). However, elevated CSF nitrite levels were observed in HIV-infected patients who had neurosyphilis without CSF pleocytosis. There were six neurosyphilis patients without CSF pleocytosis (i.e., six white blood cell count less than 20 cells/ $\mu$ L), but the CSF nitrite level was remained higher in this patient group in comparison to HIV and syphilis coinfecting patients ( $p = 0.03$ ). There was also a weak but insignificant correlation between the CSF nitrite level and the HIV plasma viral load ( $r^2 = 0.069$ ,  $p = 0.166$ ).

### Discussion

In our study, the CSF nitrite levels were significantly higher in HIV-infected patients with neurosyphilis than in other patient groups. There was also a correlation between the CSF nitrite levels and the WBC count. The CSF nitrite levels remained significantly elevated, even after we excluded two neurosyphilis patients with CSF pleocytosis. It is clinically difficult to diagnose neurosyphilis in HIV-infected patients with a single marker. Despite the use of traditional CSF markers such as VDRL, WBC count, and protein level, our study showed that an elevated CSF nitrite level is also present in patients with neurosyphilis.



**Figure 2.** No significant difference exists in the serum nitrite levels of the patients in the control group, in patients with HIV, in patients with HIV and syphilis, and in patients with HIV and neurosyphilis. HIV = human immunodeficiency virus.

The cellular site of CSF nitrite production in our patients with HIV and neurosyphilis is unknown. Nitrites can be generated by a variety of cell types such as neutrophils, microglia, endothelial cells, astrocytes, neurons, vascular smooth muscle cells, and bacterial cells.<sup>24</sup> Tumor necrosis factor alpha (TNF- $\alpha$ ) in the CSF was also involved in the induction of arginine-dependent NO production in the CSF compartment of patients with bacterial meningitis.<sup>24</sup> It is possible that the elevated levels of CSF nitrites in our patients were because of the influx of WBCs, cytokines, and other unidentified factors.

The clinical impact of NO in the CSF of patients with meningitis has been discussed in detail in several studies. One study, which enrolled 73 patients (including 26 patients with meningitis), reported negative findings with regards to elevated CSF nitrite levels in patients with meningitis.<sup>25</sup> The clinical information of the patients was limited in that study.

By contrast, more studies have shown marked elevation of the NO level in the CSF during central nervous system (CNS) infection with various kinds of pathogens. Infection by HIV-1 alone may increase NO production, possibly because of monocyte activation associated with lipopolysaccharide or TNF- $\alpha$ , or through interactions with astroglial cells.<sup>26</sup> The dysregulation or overexpression of NO consequently contributes to neurologic damage associated with various pathologies.<sup>27</sup> However, another study shows no significant increase in the CSF levels of NO metabolites in HIV-infected patients, but the increase in the metabolites was significant in patients with bacterial meningitis or viral meningitis.<sup>20</sup> Another study also reports a significantly greater NO concentration in the CSF in patients with bacterial meningitis than in patients with viral meningitis.<sup>22</sup>

In an animal model, the expression of inducible NO synthase (NOS) in the neurons and astrocytes was correlated with the detection of bovine herpes virus 5.<sup>28</sup> In one study, blood and CSF samples were collected from 61 patients with meningitis and 64 healthy individuals; the results showed markedly elevated endothelial NOS mRNA in the patient group.<sup>29</sup> Another study reports a significantly

positive correlation between the nitrite level and granulocyte counts in the CSF of 100 pediatric patients with viral and bacterial meningitis.<sup>21</sup>

In our study, an elevation in the CSF nitrite level was present in patients with HIV and syphilis infection, but the difference was most significant in patients with neurosyphilis, despite underlying HIV infection. There were no significant differences between patients with and without neurosyphilis in the serum nitrite levels. Why CSF nitrite levels were higher in patients with neurosyphilis cannot be explained, although serum NO metabolites may penetrate into the CSF during CNS inflammation. Our study showed a low correlation between the CSF nitrite levels and CSF white blood cell counts. The increase in NO metabolites may just be a byproduct of CSF pleocytosis; however, an increased CSF nitrite level was present in neurosyphilis patients without CSF pleocytosis. The results of this study imply that increased NO metabolites in the CSF indicates a local immune response to CNS infection and that neurosyphilis may contribute to this phenomenon to a greater degree than HIV infection in coinfecting patients.

There were a number of limitations in this study. Our sample size and control group were small. Patients with diseases that cause CSF pleocytosis such as bacterial meningitis and tuberculosis meningitis were not included. We did not collect the CSF data in syphilis patients who did not have HIV infection; however, it is unfeasible and unethical to perform lumbar puncture in such patients without CNS symptoms. Furthermore, we did not collect CSF data in the neurosyphilis patients after they received antibiotic therapy; as a result, CSF nitrite response to the therapy remains unknown.

In conclusion, the CSF nitrite levels were significantly higher in HIV-infected patients with neurosyphilis than in patients without neurosyphilis. More trials are needed to explain further the pathophysiology and clinical applications of this phenomenon.

## Conflicts of interest

The authors declare that there are no conflicts of interest.

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