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Original Article

Role of IL-1β, IL-6, IL-8 and IFN-γ in pathogenesis of central nervous system neuropsychiatric systemic lupus erythematous

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Abstract: We discussed the role of IL-1β, IL-6, IL-8 and IFN-γ in the pathogenesis of central nervous system neuropsychiatric systemic lupus erythematous (CNS-NPSLE). Serum and cerebrospinal fluid samples were collected from CNS-NPSLE patients, non-CNS SLE patients, patients with intracranial infection and normal subjects. Levels of IL-1β, IL-6, IL-8 and IFN-γ in serum and cerebrospinal fluid were detected by ELISA, and the results were compared across the groups. All subjects received cerebral MRI. The risk threshold for each cytokine in CNS-NPSLE group was set as 2.5%. The positive rates of cytokines for different lesions in cerebral MRI findings in CNS-NPSLE group were compared. The correlations between cytokine levels and cerebral MRI findings were analyzed. All groups did not show significant differences in age and gender (F=1.34, P>0.05; x^2=2.05, P>0.05); The IL-1β, IL-6, IL-8 and IFN-γ levels of serum and cerebrospinal fluid in CNS-NPSLE group were obviously higher than those of the normal control (serum Z_{14}=6.22, 6.04, 6.22, 5.70; cerebrospinal fluid Z_{14}=6.38, 7.10, 6.97, 6.34, P<0.0083); IL-1β, IL-6 and IL-8 levels of cerebrospinal fluid of CNS-NPSLE group were higher than those of the non-CNS SLE group (Z_{12}=2.73, Z_{12}=3.18, Z_{12}=3.86; P<0.0083); IL-1β, IL-6, IL-8 and IFN-γ levels of cerebrospinal fluid of CNS-NPSLE group were higher than those of the serum (Z=3.19, 6.30, 5.44, 3.19, P<0.05); IL-6>20.0679 pg/ml and IL-8>87.1811 pg/ml in the cerebrospinal fluid predicted a higher risk of CNS-NPSLE (x^2=11.98, P<0.05; x^2=4.65, P<0.05); The positive rates of IL-1β and IL-6 in the cerebrospinal fluid of CNS-NPSLE patients with demyelinating diseases were considerably higher than those of CNS-NPSLE patients with normal MRI findings (x^2=10.89, P<0.005; x^2=18.47, P<0.005). The positive rates of IL-6 and IFN-γ in the cerebrospinal fluid of CNS-NPSLE patients presenting with multiple ischemic foci were significantly higher than those with normal MRI findings (x^2=5.56, P<0.005; x^2=14.59, P<0.005). Some cytokines are involved in the pathogenesis of CNS-NPSLE and correlated with cerebral MRI findings in CNS-NPSLE.

Keywords: Neuropsychiatric systemic lupus erythematous (NPSLE), central nervous system (CNS), cytokines, logistic regression analysis

Introduction

Neuropsychiatric systemic lupus erythematous (NPSLE) is the neuropsychiatric involvement in SLE, which affects the central nervous system and/or peripheral nervous system. It is reported that 15-75% of SLE patients suffer from NPSLE [1]. The incidence of NPSLE with the involvement of central nervous system (CNS) is 4% [2]. CNS-NPSLE is the third leading cause of SLE-related death after kidney involvement and secondary infections [3, 4]. Little is known with respect to the pathogenesis of CNS-NPSLE, and autoantibodies and cytokines are believed to play a part. We determined the IL-1β, IL-6, IL-8 and IFN-γ levels in the serum and cerebrospinal fluid (CSF) of CNS-NPSLE patients, non-CNS SLE patients, patients with intracranial infection and normal subjects. The differences in cytokine levels were compared. Logistic regression analysis was performed to analyze the correlations between the levels of these cytokines and MRI findings in CNS-NPSLE. We attempted to clarify the role of these cytokines in the pathogenesis of CNS-NPSLE.

Materials and methods

Subjects

CNS-NPSLE patients, non-CNS SLE patients, patients with intracranial infection and normal

Table 1. Comparison of serum levels of cytokines among the groups ($\bar{x}\pm s$)

	Cases	IL-1β (pg/ml)	IL-6 (pg/ml)	IL-8 (pg/ml)	IFN-γ (pg/ml)
CNS-NPSLE	30	16.26±5.96	21.68±14.34	143.43±57.89	23.04±18.35
non-CNS SLE	20	14.32±1.11	16.78±34.00	71.52±19.58	24.54±17.09
Intracranial infection	19	19.12±4.69	19.42±5.65	227.82±100.18	19.12±3.83
Normal control	19	11.53±0.75	4.28±0.92	34.55±11.03	4.88±0.91

Note: Corrected P=0.0083.

subjects with complete clinical data from March 2010 to November 2012 were recruited. All 30 cases of CNS-NPSLE were hospitalized at Department of Rheumatology of our hospital, including 3 males and 27 females aged 18-58 years old (26.63±15.38). Diagnosis was made according to 1999 American College of Rheumatology Criteria for SLE Diagnosis [5]. Cases with neuropsychiatric symptoms caused by intracranial infection, hypoxemia, hypertension, uremia, hepatic encephalopathy, electrolyte disturbance and medication were excluded. There were 20 non-CNS SLE cases including 2 males and 18 females aged 14-48 years old (29.11±8.81), who confirmed to 1982 Revised American Rheumatism Association Criteria for the Diagnosis of SLE [6]. These cases presented with no CNS symptoms or infections and tumors. For intracranial infection group, there were 19 cases including 2 males and 17 females aged 16-35 years old (23.47±5.32); 10 cases had bacterial meningitis, and 9 had viral meningitis, all of them were hospitalized at Department of Neurology. Among 19 normal control cases, 6 cases were males and 13 cases were females aged 15-50 years old (25±6.28). They were hospitalized at Department of Neurosurgery.

Method

From each subject 3 ml of CSF and 3 ml of fasting venous serum was collected and centrifuged at 2000 r/min for 5 min. Supernatant was collected and subpackaged in EP tube. The samples were preserved at -80°C. The levels of cytokines in serum and CSF were measured using ELISA kit (eBioscience) in accordance with manufacturer's instruction.

Cerebral MRI

The lesions found by MRI included demyelinating diseases, multiple ischemic foci, cerebral hemorrhage, simple cerebral atrophy and normal.

Statistical analysis

Statistical analysis was performed using the SPSS18.0 software. Nonparametric test was used for measurement data, and x^2 test for count data. The levels of cytokines were expressed as $\bar{x}\pm s$. Comparison of the groups was done using Kraskal-Wallis test. The risk threshold for each cytokine in CNS-NPSLE group was set as 2.5%. Correlations between cytokine levels and pathogenesis of CNS-NPSLE were identified by using logistic regression analysis; P<0.05 was considered as statistically significant difference.

Results

Intergroup differences in age and gender

All groups showed no statistically significant differences in gender and age (F=1.34, P>0.05; x^2=2.05, P>0.05).

Comparison of cytokine levels in serum (**Table 1**) and CSF (**Table 2**) across the groups

Comparison of IL-1β level

The serum IL-1β levels (**Table 1**) of CNS-NPSLE group, non-CNS SLE group and intracranial infection group were significantly higher than that of the control (Z_{14}=6.22, Z_{24}=4.45, Z_{34}=4.99, P<0.0083); but the difference was not significant between CNS-NPSLE group, non-CNS SLE group and intracranial infection group (Z_{12}=1.37, Z_{13}=0.69, Z_{23}=0.61, P>0.0083); CSF IL-1β levels of intracranial infection group, CNS-NPSLE group and non-CNS SLE group were considerably higher than that of the control (Z_{34}=6.14, Z_{14}=6.38, Z_{24}=2.68, P<0.0083); CSF IL-1β levels (**Table 2**) of intracranial infection group and CNS-NPSLE group were significantly higher than those of non-CNS SLE group (Z_{12}=2.73, Z_{23}=2.85, P<0.0083); CSF IL-1β level of intracranial infection group was only insignificantly higher than that of CNS-NPSLE

Table 2. Comparison of CSF levels of cytokines among the groups ($\bar{x}\pm s$)

	Cases	IL-1β (pg/ml)	IL-6 (pg/ml)	IL-8 (pg/ml)	IFN-γ (pg/ml)
CNS-NPSLE	30	24.76±15.65	175.57±124.49	478.89±452.12	32.24±52.19
non-CNS SLE	13	12.13±3.50	35.02±65.00	76.74±18.62	11.06±5.89
Intracranial infection	19	28.16±21.92	73.65±96.67	396.92±312.61	29.08±41.81
Normal control	19	4.20±1.64	3.64± 2.06	25.24±15.31	5.71±6.75

Note: Corrected P=0.0083.

Table 3. Parameters of logistic regression analysis and tests

Variable	$\hat{\beta}$	$SE(\hat{\beta})$	Wald x^2	P	$\hat{OR}=exp(\hat{\beta})$
Constant term	-1.492	0.462	10.42	0.001	
X_1	-1.917	3.301	0.34	0.561	0.147
X_2	1.498	1.563	0.92	0.338	4.473
X_3	0.589	1.351	0.19	0.663	1.802
X_4	-1.046	3.158	0.11	0.741	0.351
N_1	1.674	1.415	1.4	0.237	5.332
N_2	4.083	1.179	11.98	0.001*	59.308
N_3	4.846	2.246	4.65	0.031*	127.202
N_4	-2.889	2.300	1.58	0.209	0.056

Note: *P<0.05 indicated statistical significance. X_1, X_2, X_3 and X_4 stands for serum IL-1β, IL-6, IL-8 and IFN-γ; N_1, N_2, N_3 and N_4 stands for GSF IL-1β, IL-6, IL-8 and IFN-γ.

group (Z_{13}=0.41, P>0.0083); CSF IL-1β level of CNS-NPSLE group was higher than serum IL-1β level (Z=3.19, P<0.05).

Comparison of IL-6 level

Serum IL-6 levels (**Table 1**) of CNS-NPSLE group, non-CNS SLE group and intracranial infection group were significantly higher than that of the control (Z_{14}=6.04, Z_{24}=5.20, Z_{34}=5.75, P<0.0083); however, CNS-NPSLE group, non-CNS SLE group and intracranial infection group did not show obvious differences in serum IL-6 level (Z_{12}=0.63, Z_{13}=0.41, Z_{23}=0.94, P>0.0083); CSF IL-6 levels (**Table 2**) of CNS-NPSLE group and intracranial infection group were obviously higher than that of the control (Z_{14}=7.11, Z_{34}=3.67, P<0.0083); CSF IL-6 level of non-CNS SLE group was insignificantly higher than that of the control (Z_{24}=2.85, P>0.0083); CSF IL-6 level of intracranial infection group was much higher than that of the non-CNS SLE group (Z_{12}=3.19, P<0.0013<-0.0083); CSF IL-6 level was not considerably different between intracranial infection group and non-CNS SLE group (Z_{23}=0.45, P>0.0083); (3) CSF IL-6 level of NPSLE group was higher than serum L-6 level (Z=6.30, P<0.05).

Comparison of IL-8 level

Serum IL-8 levels (**Table 1**) of CNS-NPSLE group, non-CNS SLE group and intracranial infection group were significantly higher than that of the control (Z_{34}=6.14, Z_{14}=6.21, Z_{24}=2.64, P<0.0083); serum IL-8 levels of intracranial infection group and CNS-NPSLE group were considerably higher than that of non-CNS SLE group (Z_{23}=5.08, Z_{12}=3.38, P<0.0083); serum IL-8 level of intracranial infection group was not significantly higher than that of CNS-NPSLE group (Z_{13}=2.22, P>0.0083); CSF IL-8 levels (**Table 2**) of CNS-NPSLE group and intracranial infection group were significantly higher than that of the control (Z_{14}=6.97, Z_{34}=5.76, P<0.0083); no obvious difference was observed in CSF IL-8 level between non-CNS SLE group and the control (Z_{24}=2.12, P>0.0083); CSF IL-8 level of CNS-NPSLE group was considerably higher than serum IL-8 level (Z=5.44, P<0.05).

Comparison of IFN-γ level

Serum IFN-γ levels (**Table 1**) of non-CNS SLE group, CNS-NPSLE group and intracranial infection group were remarkably higher than that of the control (Z_{24}=6.19, Z_{14}=5.70, Z_{34}=4.71, P< 0.0083); pairwise differences among non-CNS SLE group, CNS-NPSLE group and intracranial infection group were of no statistical significance (Z_{12}=1.09, Z_{13}=0.48, Z_{23}=1.42, P> 0.0083); CSF IFN-γ levels of CNS-NPSLE group, intracranial infection group and non-CNS SLE group were higher than that of the control, showing statistical significance (Z_{14}=6.34, Z_{34}=5.14, Z_{24}=3.56, P<0.0083); pairwise differences among the three groups were not signifi-

Table 4. Correlation between MRI findings and cytokine levels in CNS-NPSLE

MRI findings	Cases	Positive IL-1β		Positive IL-6		Positive IL-8		Positive IFN-γ	
		Cases	%	Cases	%	Cases	%	Cases	%
Demyelinating diseases	16	16	100[b]	14	87.5	11	68.75	13	81.25[b]
Multiple ischemic foci	10	8	80[a]	6	60	9	90	9	90[a,b]
Bleeding foci	1	0	0[a]	0	0[a]	1	100	0	0[a]
Simple cerebral atrophy	2	1	50[a]	1	50[a]	0	50	1	50[a]
Normal	1	0	0	0	0[a]	0	0	0	0

a: Compared with demyelinating diseases, P<0.005; *b*: Compared with the control, P<0.005.

cant (Z_{12}=1.73, Z_{13}=0.64, Z_{23}=1.08, P>0.0083); CSF IFN-γ level (**Table 2**) of CNS-NPSLE group was significantly higher than serum IFN-γ level (Z=3.19, P<0.05).

Analysis of risk thresholds of each cytokine in CNS-NPSLE

The serum levels of IL-1β, IL-6, IL-8 and IFN-γ in CNS-NPSLE group corresponding to 2.5% risk threshold were 11.4983, 13.3371, 59.4457 and 14.3462, denoted by X_1, X_2, X_3 and X_4, respectively; CSF levels of IL-1β, IL-6, IL-8 and IFN-γ corresponding to 2.5% risk threshold were 7.9335 pg/ml, 20.0679 pg/ml, 87.1811 pg/ml and 6.3245 pg/ml, denoted by N_1, N_2, N_3 and N_4, respectively. It can be seen from **Table 3** that when CSF IL-6>20.0679 pg/ml or CSF IL-8>87.1811 pg/ml, the risk of CNS-NPSLE increased (x^2=11.98, P<0.05; x^2=4.65, P< 0.05).

Correlation between cytokine levels and cerebral MRI findings (**Table 4**).

The rate of MRI abnormalities was 96.7% among 30 CNS-NPSLE cases, including 16 cases of demyelinating diseases, 10 cases of multiple ischemic foci, 1 case of bleeding foci, 2 cases of simple cerebral atrophy and 1 normal case. The positive rate of CSF IL-1β in CNS-NPSLE cases with demyelinating diseases was significantly higher than that of CNS-NPSLE cases with normal MRI findings (x^2=10.889, P<0.005); the positive rates of CSF IL-6 in cases with demyelinating diseases and multiple ischemic foci were considerably higher than those of the control (x^2=18.471, P<0.005; x^2=5.556, P<0.005). CNS-NPSLE cases with normal or abnormal MRI findings did not differ significantly in positive rate of CSF IL-8 (x^2=10.00, P>0.005). The positive rate of CSF IFN-γ in CNS-NPSLE cases with multiple ischemic foci was considerably higher than that of CNS-NPSLE cases with normal MRI findings (x^2=14.59, P<0.005).

Discussion

We have very limited knowledge about the pathogenesis, diagnosis and treatment of CNS-NPSLE among all forms of multiple system involvement in SLE [7]. CSF test and cerebral MRI can facilitate the differentiating diagnosis of CNS-NPSLE to a large extent, but biomarkers of CNS-NPSLE have not established yet. The role of autoantibodies and cytokines in the pathogenesis of CNS-NPSLE is being highlighted in recent years [8]. According to some reports, the CSF levels of IL-1, IL-6, TGF-α and IFN-γ are increased in CNS-NPSLE patients [9]. We measured serum and CSF levels of relevant cytokines in CNS-NPSLE patients with the purpose of clarifying the correlation between cytokine levels, CNS-NPSLE and cerebral MRI findings.

Whether the pathogenesis of CNS-NPSLE is related to IL-1β is not fully confirmed [10, 11]. Our study indicated that the serum and CSF IL-1β levels in CNS-NPSLE patients were obviously higher than those of the control, so it was inferred that IL-1β is associated with pathogenesis of CNS-NPSLE. Furthermore, the positive rate of CSF IL-1β in cases presenting with demyelinating diseases was significantly higher than that of other cases, which was suggestive of the role of CSF IL-1β in demyelinating diseases. The CSF IL-1β level of CNS-NPSLE group was higher than that of non-CNS SLE group and also higher than serum IL-1β level of CNS-NPSLE group. CSF IL-1β produced by inflammatory cells at local sites may induce demyelinating diseases. However, CNS-NPSLE group and intracranial infection group did not differ significantly in terms of CSF IL-1β level, suggesting

the non-specificity of IL-1β as an indicator of CNS-NPSLE.

IL-6 can cause blood vessel dilation and the increase of vascular permeability, thereby leading to cerebral edema and neuropsychiatric symptoms [2]. The serum IL-6 levels of CNS-NPSLE group, intracranial infection group and non-CNS SLE group were significantly higher than that of the control; CSF IL-6 level of CNS-NPSLE group was significantly higher than those of the intracranial infection group and control. The positive rates of CSF IL-6 in CNS-NPSLE cases with demyelinating diseases and multiple ischemic foci were considerably higher than that those with normal MRI findings. It was implied that a large amount of IL-6 was produced locally to act on the nerve cells or to increase blood-brain barrier permeability, leading to brain injury [12].

SLE patients may present with an elevation in serum IL-8 level, which is related to mobility in SLE [13, 14]. It is reported that IL-8 may be involved in the pathogenesis of CNS-NPSLE by increasing blood-brain barrier permeability [15]. We showed that serum and CSF IL-8 levels of CNS-NPSLE cases were higher than those of the control and non-CNS SLE group, which further demonstrated the correlation between IL-8 and CNS-NPSLE. This agreed with the previous reports [9, 14]. We also found that CSF IL-8 level in CNS-NPSLE group was significantly higher than that of intracranial infection group and control. Serum IL-8 level may be a more specific indicator of CNS-NPSLE than serum and CSF IL-8 levels.

IFN-γ can trigger or aggravate SLE and shows a significant correlation with mobility in SLE [16]. In our study, both serum IFN-γ and CSF IFN-γ levels of non-CNS SLE group and CNS-NPSLE group were much higher than those of the control. However, the differences between non-CNS SLE group and CNS-NPSLE group were not significant. The positive rate of CSF IFN-γ in CNS-NPSLE cases with multiple ischemic foci was considerably higher than that of other cases. This may suggest the role of IFN-γ in inducing multiple ischemic foci.

SLE combined with CNS infection can be hardly differentiated from CNS-NPSLE. Our results indicated that CSF IL-6 and IL-8 levels of CNS-NPSLE cases were significantly higher than those of the intracranial infection group. CSF IL-6 and IL-8 levels may help the differentiating diagnosis, and a large-sample analysis is required to determine the threshold. As shown by logistic regression analysis, CSF IL-6> 20.0679 pg/ml and CSF IL-8>87.1811 pg/ml in SLE cases can predict an increased risk of CNS-NPSLE. That is to say, an elevation in CSF IL-6 and CSF IL-8 levels increases the risk of CNS-NPSLE.

Cytokines are mediators of immuno-inflammatory response. All presentations of SLE including CNS-NPSLE may be mediated by cytokines. CNS-NPSLE is associated with abnormal levels of a variety of cytokines. Over-expression of some pro-inflammatory cytokines is responsible for symptoms and abnormal imaging findings of CNS-NPSLE. Among them, CSF IL-6 and IL-8 levels are more specific indicators of CNS-NPSLE. Differentiating diagnosis of CNS-NPSLE can be made by combining clinical presentations, imaging findings, CSF testing and cytokine detection.

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Disclosure of conflict of interest

None.

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