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Effects of IL-6 and Cortisol Fluctuations in Post-stroke Depression

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Summary: Depression is an important post-stroke sequela with negative impact on mortality, functional outcome and quality of life. Changes in cytokines have been hypothesized to be associated with the etiology of post-stroke depression (PSD). The altere dhypothalamic-pituitary-adrenal (HPA) functioning is associated with the onset of depression. The activity of HPA could induce the fluctuations of cortisol levels. In this study, we prospectively checked interleukin 6 (IL-6) and cortisol levels in patients with early ischemic stroke. It was hypothesized that early serum IL-6 and cortisol fluctuations in stroke patients were the predictions of PSD. Totally, 100 participants were selected from stroke inpatients consecutively admitted to the Department of Neurology, Tongji Hospital from July 2014 to December 2015. Fifty health people served as the controls. The serum of all the patients was collected at 8:00 am and 4:00 pm respectively one week after stroke. The serum of controls was collected only at 8:00 am. The levels of IL-6 were analyzed by enzyme-linked immunosorbent assay kit, and those of cortisol were detected by chemiluminescence immunoassay. On the 3rd week after stroke, the patients were enrolled to the PSD group and non-PSD group based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-Ⅳ) and The Hamilton Depression Rating Scale (HAMD-21, score>7). The IL-6 level (13.24±2.89 ng/L) was elevated significantly in PSD groups as compared with that in non-PSD group and control group respectively (P<0.05 for both), but there was no significant difference in the IL-6 level between non-PSD group and control group. The patients in both PSD group and non-PSD group had significantly elevated morning cortisol levels in comparison with those in the control group (P<0.05; for PSD, non-PSD and control: 508.86±119.51, 420.83±70.04 and 340.40±76.30 nmol/L respectively). Moreover, afternoon cortisol levels in PSD group were significantly higher than those in non-PSD group, and the morning baseline cortisol levels in these two groups were similar (P>0.05). It was suggested that PSD generally runs a chronic course and is related to a variety of adverse health outcomes including increased disability, morbidity and mortality. This study will help the screening of potential PSD in the early stage.
Key words: post-stroke depression; interleukin 6; cortisol

Depression is an important post-stroke sequela with negative impact on mortality, functional outcome and quality of life[1]. At least one third of stroke patients display mood symptoms at some time after the insult[2, 3]. Despite the high proportion of stroke patients developing mood symptoms, the biological underpinnings of PSD are still not clear. A bulk of interests have recently been addressed to the interaction between the immune system and major depression[4]. Changes in cytokines have been hypothesized to be associated with the etiology of PSD[5]. The altered hypothalamic-pituitary-adrenal (HPA) functioning is associated with the onset of depression[6, 7]. Persistent HPA axis dysregulation occurs in up to 40% stroke patients[8]. The activity of HPA could induce the fluctuations of cortisol levels. In this study, we prospectively checked the IL-6 and cortisol levels in patients with early ischemic stroke. The purpose of this study was to evaluate a cohort of patients with ischemia stroke and compare the IL-6 and cortisol fluctuations between patients with and without PSD during the study period. Our hypotheses were that early serum IL-6 and cortisol fluctuations in stroke patients were the predictions of the PSD.

1 MATERIALS AND METHODS

1.1 Participants

Participants were selected from 130 stroke inpatients consecutively admitted to the Department of Neurology, Tongji Hospital from July 2014 to December 2015. The inclusions were a first-ever stroke confirmed by MRI or CT scan and age between 18–80 years old. Exclusion criteria were: (1) transient ischemic attack; (2) impairment of communication or cognitive function [Mini-mental Status Examination (MMSE) score <15]; (3) history of depression, psychosis, severe substance abuse; (4) complicating with severe medical disorders; (5) taking antidepressants or immunosuppressant at least 6 months prior to the stroke. Fifty health people served as the controls. The serum of all the patients was collected at 8:00 am and 4:00 pm respectively one week after stroke. The serum of controls was collected only at 8:00 am. The HAMD-21 was assessed on patients 3 weeks after stroke. Thirty patients dropped out. Therefore, 100 patients finished the whole investigation. This study pro-

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tocol was approved by the Ethics Committee of Tongji Hospital.

1.2 Determination of IL-6 and Cortisol

Psychiatric interview and HAMD-21 were used to assess the depression at the 3rd week after stroke. Depression diagnosis was determined based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-Ⅳ). Patients were enrolled to the PSD group and non-PSD group according to DSM-Ⅳ and HAMD score (>7). The raters were well trained and had good interrater reliability.

The blood of all the participants was collected at 8:00 am. For the analysis of the cortisol fluctuations, blood was also collected at 4:00 pm on the same day. The IL-6 levels were determined by quantitative assays with standard capture enzyme-linked immunosorbent assay kits from Jingmei Biological Engineering Co., Ltd. (China). The cortisol level was detected using standard chemiluminescence immunoassay by SIEMENS Laboratory Diagnostics Co., Ltd. (China). Blood obtained from the antecubital vein was collected in anticoagulant–free tubes after an overnight fasting in the early morning. Assays were performed according to the manufacturer's directions. The results were expressed as concentrations of IL-6 (ng/L) and cortisol (nmol/L) in serum.

1.3 Statistical Analysis

The statistical package for the Social Science (SPSS22.0) for Windows was used to analyse data. Standard statistics included means, medians, standard deviations and percentages for frequency counts. Differences between groups were analyzed by Analysis of variance (ANOVA). *P* values under 0.05 were accepted to indicate significance.

2 RESULTS

100 patients with stroke met the inclusion criteria and completed the follow-up period. The demographic data are shown in table 1. At the end of the study, 34 patients suffered from PSD based on the diagnostic criteria of DSM-Ⅳ, including19 males, and 15 females. Cerebral infarction occurred in 18 cases, and cerebral haemorrhage in 14 cases. The mean ages of the patients in PSD group were 62.4±6.2 years old. There were 66 patients in the non-PSD group with 35 males and 31 females. Cerebral infarction occurred in 39 cases, and cerebral haemorrhage in 27 cases. The mean ages were 64.1±5.1. In the control group, there were 30 males and 20 females with mean age of 63.3±5.2 years old. No significant differences were observed in age and gender across the three groups. The types of stroke showed no significant difference between PSD group and non-PSD group.

Table 1 Baseline data of the subjects

Indexes	PSD (*n*=34)	Non-PSD (*n*=66)	Control (*n*=50)
Gender (male/female)	19/15	35/31	30/20
Age (year)	62.4±6.2	64.1±5.1	63.3±5.2
Stroke type (cerebral infarction /cerebral haemorrhage)	18/14	39/27	–

The level of IL-6 (13.24±2.89 ng/L) was elevated significantly in PSD group as compared with non-PSD group and control group ($P<0.05$ for both). There was no significant difference in the level of IL-6 between non-PSD group and control group (table 2). Furthermore, the cortisol levels in all the patients at 8:00 in the morning were also analyzed. Patients in both the PSD group and non-PSD group had significantly elevated morning cortisol levels in comparison with those in the control group ($P<0.05$; PSD: 508.86±119.51 nmol/L; non-PSD: 420.83±70.04 nmol/L; control: 340.40±76.30 nmol/L). Moreover, patients in the PSD group had higher afternoon cortisol levels than in the non-PSD group, while the morning baseline cortisol levels in those two groups were similar ($P>0.05$), which suggested there existed the alteration of cortisol circadian rhythm in the PSD group (table 3).

Table 2 Comparison of IL-6 levels between groups

Groups	IL-6 (ng/L)
PSD (*n*=34)	13.24±2.89
Non-PSD (*n*=66)	4.56±0.91*
Control (*n*=50)	4.13±0.84*

*$P<0.05$ *vs*. PSD group

Table 3 Cortisol level and circadian rhythm

Groups	Cortisol (nmol/L)	
	8:00	16:00
PSD (*n*=34)	508.89±119.51$^{\Delta}$	460.66±131.11
Non-PSD (*n*=66)	420.83±70.04$^{\Delta}$	247.17±79.20*
Control (*n*=50)	340.40±76.30	–

*$P<0.05$ *vs*. PSD group; $^{\Delta}P<0.05$ *vs*. control group

3 DISCUSSION

PSD generally runs a chronic course and is related to a variety of adverse health outcomes including increased disability, morbidity and mortality[9, 10]. Common mood symptoms after stroke include anxiety and feelings of despair as well as anhedonia[10, 11]. The overall occurrence of PSD was around 20%–50%. In our study, 34 of 100 acute stroke patients met the diagnosis criteria of depression, which was consistent with previous report[12]. This study focused on immune and steroid changes in stroke patients with depression. In the present study, we investigated the changes in IL-6 and cortisol levels one week after stroke. The results indicated that there were significant elevations of IL-6 in patients with PSD and proved the hypothesis the PSD was associated with increased inflammation. On the other hand, patients in PSD group had significantly higher morning cortisol levels than in control subjects and higher afternoon corti-

sol levels than in non-PSD group. Cortisol circadian rhythm disappeared in the PSD group. Although the exact pathophysiology underlying elevated cortisol in PSD remains unclear, it is generally thought that this abnormality is linked to reduced feedback inhibition of HPA axis activity[13].

Previous studies had found there was a significant positive correlation between depression and the level of IL-6[14, 15]. Increasing evidence has suggested that cytokines may play a role in the pathophysiology of depression[16]. The "cytokine theory of depression" proposes that proinflammatory cytokines acting as neuromodulators may mediate the behavioural and neurochemical features of depression[4, 17, 18]. Moreover, in the depression by these cytokines, the symptoms disappear after the treatment is finished or after antidepressant therapy[19]. Cytokines reduce 5-HT levels by lowering the availability of its precursor tryptophan (TRP) through activation of the TRP-metabolising enzyme indoleamine-2,-3-dioxygenase (IDO)[4]. The central action of cytokines may also account for the HPA axis hyperactivity that is frequently observed in depressive disorders, as proinflammatory cytokines may cause HPA axis hyperactivity by disturbing the negative feedback inhibition of circulating corticosteroids (CSs) on the HPA axis[4]. Proinflammatory cytokines are important in normal synaptic and neural plasticity; but, high levels may have an adverse effect on cellular processes leading to neuronal loss and neuronal atrophy, affecting behavioural systems[20].

The common opinion in psychoneuroimmunological studies is that just as there is an increase in proinflammatory cytokines levels as a response to encounter a pathogen or to tissue damage, psychosocial stressors also cause an increase in the cytokine levels and mediate the communication between the immune system and the brain. In the current study, biologically based changes in brain function or psychological factors secondary to losses caused by stroke have been the main mechanisms proposed to explain the development of PSD. In the monoamine theory of depression[21], stroke could induce anatomical or biological changes, which may disrupt the serotonergic input arising from the dorsal and caudal raphe nuclei to the hypothalamus, amygdala, hippocampus, striatum, brain-stem and neocortex. All these areas are believed to mediate depression[22]. Inflammatory response has been hypothesized to link the brain ischemic injury and depressed mood disorders[5]. Overexpression of proinflammatroy cytokines has been observed following acute cerebral ischemia[23, 24]. Proinflammatory cytokines also stimulate the secretion of corticotrophin-releasing hormone, adrenocorticotropic hormone and cortisol in HPA[25, 26]. The dysregulation of the HPA has an important role in the depressive disorders[26, 27]. The increase in IL-6 might be the contributor to this phenomenon in patients with ischemic stroke.

Abnormalities of cortisol secretion had been reported in patients with PSD[28, 29]. Consistent with these, increased cortisol levels were also found in PSD patients. IL-6 stimulates the HPA axis, which leads to increased levels of cortisol in the peripheral blood[30]. The elevated level of cortisol might contribute to the impairments in neuroplasticity and cellular resilience, and down-regulation of the glucocorticoid receptor's sensitivity[31, 32]. Increased glucocorticoid levels may worsen ischaemic brain damage[33]. Furthermore, hyper-cortisolism has been reported to predict the later development of PSD[34]. Elevated levels of both IL-6 and cortisol have been correlated to functional outcome and/ or survival in less homogenous patient populations[35, 36].

There is the potential limitation to our study. Although the patients were not in an infectious state when the sera samples were collected, we cannot consider other factors which confound the measurement of the IL-6, such as environment, circadian rhythm or coeducations. The further study will be performed to help us understand this situation.

In conclusion, the levels of both IL-6 and cortisol at 8:00 am were increased in the sera of PSD patients as compared with those in the non-PSD patients. The alteration of cortisol circadian rhythm was also found in the patients with PSD. All these will help the screening of potential PSD in the early stage. Neuroendocrine disturbances are common and often profound early after stroke. By interfering with the cytokine release, brain damage might be lessened and neuropsychiatric disturbances reduced.

Conflict of Interest Statement

The authors declare no conflict of interest.

REFERENCES

1 Lu W, Bian Q, Song YY, *et al*. Prevalence and related risk factors of anxiety and depression among Chinese college freshmen. J Huazhong Univ Sci Technol (Med Sci), 2015,35(06):815-822

2 Hachinski V. Post-stroke depression, not to be underestimated. Lancet, 1999,353(9166):1728

3 Carson AJ, MacHale S, Allen K, *et al*. Depression after stroke and lesion location: a systematic review. Lancet, 2000,356(9224):122-126

4 Schiepers OJ, Wichers MC, Maes M. Cytokines and major depression. NEURO-PSYCHOPH, 2005,29(2): 201-217

5 Spalletta G, Bossu P, Ciaramella A, *et al*. The etiology of poststroke depression: a review of the literature and a new hypothesis involving inflammatory cytokines. Mol Psychiatry, 2006,11(11):984-991

6 Carroll BJ, Feinberg M, Greden JF, *et al.* A specific laboratory test for the diagnosis of melancholia: standardization, validation, and clinical utility. JAMA Psychiatry, 1981,38(1):15-22

7 Holsboer F, Von Bardeleben U, Gerken A, *et al.* Blunted corticotropin and normal cortisol response to human corticotropin-releasing factor in depression. N Engl J Med, 1984,311(17):1127

8 Katon WJ. Epidemiology and treatment of depression in patients with chronic medical illness. Dialogues Clin Neurosc, 2011,13(1):7-23

9 Morris PL, Robinson RG, Andrzejewski P, *et al*. Association of depression with 10-year poststroke mortality. Am J Psychiatry, 1993,150:124

10 Loubinoux I, Kronenberg G, Endres M, *et al.* Post-stroke depression: mechanisms, translation and therapy. J Cell Mol Med, 2012,16(9):1961-1969

11 Li W, Ling S, Yang Y, *et al.* Systematic hypothesis for post-stroke depression caused inflammation and neurotransmission and resultant on possible treatments. Neuro Endocrinol Lett, 2014,35(2):104-109

12 Dafer RM, Rao M, Shareef A, *et al.* Poststroke depression. Top Stroke Rehabil, 2008,15(1):13-21
13 Belanoff JK, Kalehzan M, Sund B, *et al.* Cortisol activity and cognitive changes in psychotic major depression. Am J Psychiatry, 2001,158(10):1612-1616
14 Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol, 2006,27(1):24-31
15 Demir S, Atli A, Bulut M, *et al.* Neutrophil-lymphocyte ratio in patients with major depressive disorder undergoing no pharmacological therapy. Neuropsychiatr Dis Treat, 2015,11:2253
16 Marques AH, Cizza G, Sternberg E. Brain-immune interactions and implications in psychiatric disorders. Rev Bras Psiquiatr, 2007,29:s27-s32
17 Capuron L, Gumnick JF, Musselman DL, *et al.* Neurobehavioral effects of interferon-α in cancer patients: phenomenology and paroxetine responsiveness of symptom dimensions. Neuropsychopharmacology, 2002, 26(5):643-652
18 Knuth B, Radtke V, Rocha P, *et al.* Prevalence of depression symptoms and serum levels of interleukin-6 in hemodialysis patients. Psychiatry Clin Neurosci, 2014,68(4):275-282
19 Dantzer R, O'Connor JC, Freund GG, *et al.* From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci, 2008,9(1):46-56
20 Khairova RA, Machado-Vieira R, Du J, *et al.* A potential role for proinflammatory cytokines in regulating synaptic plasticity in major depressive disorder. Int J Neuropsychopharmacol, 2009,12(4), 561-578
21 Meyer JH, Ginovart N, Boovariwala A, *et al.* Elevated monoamine oxidase a levels in the brain: an explanation for the monoamine imbalance of major depression. JAMA Psychiatry, 2006,63(11):1209-1216
22 Ressler KJ, Nemeroff CB. Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. Depress Anxiety, 2000,12(Supp 1):2-19
23 Wang Q, Tang XN, Yenari MA. The inflammatory response in stroke. J Neuroimmunol, 2007,184(1):53-68
24 Mehta SL, Manhas N, Raghubir R. Molecular targets in cerebral ischemia for developing novel therapeutics. Brain Res Rev, 2007,54(1):34-66
25 Webster JI, Tonelli L, Sternberg EM. Neuroendocrine regulation of immunity. Annu Rev Immunol, 2002,20(1):125-163
26 Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol Psychiatry, 2009,65(9):732-741
27 Nolen-Hoeksema S. Gender differences in depression. CDPS, 2001,10(5):173-176
28 Gunnar MR, Vazquez DM. Low cortisol and a flattening of expected daytime rhythm: Potential indices of risk in human development. Dev Psychopathol, 2001,13(03): 515-538
29 Robinson RG, Spalletta G. Post-stroke depression: a review. Can J Psychiatry, 2010,55(6):341-349
30 Flier JS, Underhill LH, Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. N Engl J Med, 1995,332(20):1351-1363
31 Sale MV, Ridding MC, Nordstrom MA. Cortisol inhibits neuroplasticity induction in human motor cortex. J Neurosci, 2008,28(33):8285-8293
32 Hunsberger JG, Austin DR, Chen G, *et al.* Cellular mechanisms underlying affective resiliency: the role of glucocorticoid receptor- and mitochondrially-mediated plasticity. Brain Res, 2009,1293:76-84
33 Meisel C, Schwab JM, Prass K, *et al.* Central nervous system injury-induced immune deficiency syndrome. Nat Rev Neurosci, 2005,6(10):775-786
34 Juster R-P, Bizik G, Picard M, *et al.* A transdisciplinary perspective of chronic stress in relation to psychopathology throughout life span development. Dev Psychopathol, 2011,23(03):725-776
35 Hoffmann SC, Stanley EM, Cox ED, *et al.* Ethnicity greatly influences cytokine gene polymorphism distribution. Am J Transplant, 2002,2(6):560-567
36 Lutgendorf SK, Weinrib AZ, Penedo F, *et al.* Interleukin-6, cortisol, and depressive symptoms in ovarian cancer patients. J Clin Oncol, 2008,26(29):4820-4827

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