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Altered serum levels of TNF-α, IL-6 and IL-18 in manic, depressive, mixed state of bipolar disorder patients

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ABSTRACT

Bipolar disorder (BD) is associated with alterations of cytokines in the immune system. The aim of this study was to assess the serum levels of TNF-α, IL-6 and IL-18 in manic, depressive, mixed state patients of BD. The correlations between the serum cytokines levels with the demographic characteristics and the psychiatric symptoms were also assessed. We measured serum TNF-α, IL-6 and IL-18 levels using an enzyme-linked immunosorbent assay (ELISA) from 59 BD patients (37 in manic state, 12 in depressive state, 10 in mixed state) and 80 healthy control subjects. The psychotic symptoms of BD were assessed using the Hamilton Depression Scale (HAMD) and the Young Mania Rating Scale (YMRS). The results showed that serum TNF-α and IL-6 levels in manic, depressive and mixed state BD patients were significantly higher than that in controls, while serum IL-18 level was only significantly higher in depressive patients. Serum IL-6 level was significantly positively correlated with YMRS scores in manic episode as well as in mixed episode. When gender and age were added as potentially confounding covariate terms, the differences between controls and each mood state patients were still significant. Our findings provided additional evidence that elevated TNF-α, IL-6 and IL-18 pathway activities may be involved in the psychopathology of BD. Due to the lack of controlling important confounding factors, such as BMI, smoking status and alcohol use, further studies are required to confirm the roles of TNF-α, IL-6 and IL-18.

1. Introduction

Bipolar disorder (BD) is a chronic, severe, and highly disabling psychiatric disorder that affects approximately 2.4% of the population worldwide (Merikangas et al., 2011). BD is typically characterized by recurrent episodes of depressive and manic/hypomanic. The mean age at onset of BD is about 20, and the disease persists at least half of the patient lifetime (Barbosa et al., 2014). In addition, BD is associated with a high rate of medical and psychiatric comorbidities, a progressive deterioration of illness course, and cognitive deficits (Kapczinski et al., 2009). Although there are many promising studies on the psychosocial, biological, and genetic factors contributing to the pathophysiology of BD, its underlying neurobiological mechanisms remain largely unclear.

Converging evidence suggested that immune system abnormity and elevated inflammation response contributed to the psychopathology of BD (Berk, 2009; Goldstein et al., 2009; Berk et al., 2011; Gardner and Boles, 2011; Grande et al., 2012). More specifically, immune system dysregulation has been indicated in both in-vitro studies (Kim et al., 2007; Knijff et al., 2007) and in clinical studies showing alterations of peripheral markers of inflammation (Dickerson et al., 2007; Cunha et al., 2008; Brietzke et al., 2009) and alterations of inflammation related gene characteristics (Padmos et al., 2008; Drexhage et al., 2010). Elevated levels of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), serum soluble IL-6 receptor (sIL-6R) and interleukin-8(IL-8) (O'Brien et al., 2006; Kim et al., 2007; Ortíz-Domingues et al., 2007; Brietzke et al., 2009; Hope et al., 2011; Munkholm et al., 2015), were reported during manic episodes and depressive episodes, suggesting that mania could be a pro-inflammatory state. Previous studies have demonstrated that IL-18, an important modulator of immune response in the CNS, regulated cytotoxic and inflammatory responses pathways under neuropathological conditions and contribute to neuroinflammation and neurodegeneration (Felderhoff-Mueser et al., 2005; Alboni et al., 2010). Elevated levels of interleukin-18 (IL-18), a member of the IL-1family of proinflammatory cytokine, have been found in BD patients (Munkholm et al., 2015). Despite a

Abbreviations: BD, Bipolar disorder; ELISA,, enzyme-linked immunosorbent assay; HAMD, Hamilton Depression Scale; YMRS, Young Mania Rating Scale; CNS, central nervous system; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin 6; sIL-6R, serum soluble IL-6 receptor; IL-18, interleukin-18

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number of evidences for the involvement of cytokines in psychiatric and neurodegenerative disorders, few studies studied different affective states of BD and studies related findings to the psychotic symptoms of BD remained limited. As we known, there has been only one report to study the role of IL-18 in BD (Munkholm et al., 2015). The present study aimed to further investigate serum levels of TNF-α, IL-6 and IL-18 in manic/depressive/mixed states of BD patients in comparison to healthy controls. Then, the correlation between the serum cytokines levels with the subjects' demographic characteristics and psychotic symptoms were analyzed.

2. Methods

2.1. Subjects

The samples were consecutively enrolled in-patients at the Guangzhou Brain Hospital (Guangzhou Huiai Hospital, the Affiliated Brain Hospital of Guangzhou Medical University) from July 2012 to June 2013. Their diagnosis required to be confirmed by at least two senior psychiatrists based on ICD-10 diagnostic criteria within first week of admission. The inclusion criteria were: (a) the primary diagnosis of BD based on ICD-10 diagnostic criteria; (b) between 18 and 65 years of age. Patients with a primary psychiatric diagnosis other than BD, or patients comorbided with current severe physical illness or organic brain disease were excluded. Some patients might experience short term anxiety, some patients might have past history of substance use, those were not excluded due to the high rate of comorbidity.

The Hamilton Depression Scale (HAMD) (Hamilton, 1967) and The Young Mania Rating Scale (YMRS) (Young et al., 1978) were used within first week of admission to assess manic and depressive symptoms respectively. All patients had been receiving stable oral doses of one or more atypical antipsychotic medications. A complete medical history, physical examination, and routine laboratory tests were obtained from electronic medical records to rule out possible medical conditions and possible other psychiatric conditions. All subjects were free of ongoing infections or allergies, autoimmune disorders and use of anti-inflammatory or immunosuppressive agents. Healthy controls were recruited through advertisements. Healthy controls were free of traumatic head injury and any past or present major medical or neurological illness. None of the healthy control subjects presented a personal or family history of psychiatric disorder. This study was approved by the Ethics Committee of Guangzhou Brain hospital, and was in accordance with the Helsinki Declaration as revised 1989. Written informed consent was obtained from each participant.

2.2. Serum TNF-α, IL-6 and IL-18 measurement

Venous blood sample was collected in the fasting from each subject using standard venipuncture technique between 7:00 a.m. and 9:00 a.m. Serum was obtained by centrifuged at 4000 rpm for 15 min, then aliquoted and stored at -80 °C until assay.

Serum levels of TNF-α, IL-6 and IL-18 were measured by using commercial enzyme linked immunosorbent (ELISA) kits (eBioscience, San Diego, USA), in accordance with the manufacturer's instructions. The sensitivities of TNF-α, IL-6 and IL-18 were 2.3 pg/mL, 0.92 pg/mL and 9 pg/mL, with inter-assay variation coefficients of 7.4%, 5.2% and 8.1%, and intra-assay variation coefficients of 6.0%, 3.4% and 6.5%, respectively. No cross-reactivity was detected. Standard curve concentrations were calculated in duplicate for each plate. All samples were assayed in duplicate. Absorbencies were measured using a microtiter plate reader (Bio-Rad iMark) set at 450 nm.

2.3. Statistical analysis

All statistical analyses were performed using SPSS15.0 for Windows (SPSS Inc., Chicago, IL, USA). Chi-square test was used to examine the difference of gender variables between BD patients and healthy controls. Age variables in the patient and control groups were analyzed by Non-parametric analyses using the two-sided Mann-Whitney *U* tests. In our study, data of serum levels of cytokines were ranked. ANOVA Bonferroni post-hoc statistical tests were used for pairwise comparisons of serum levels of TNF-α, IL-6 and IL-18 in multiple groups. Then, we further compared the TNF-α, IL-6 and IL-18 levels in multiple groups using a univariate analysis of covariance (ANCOVA) with gender and age as covariates. Spearman's correlation analysis was used to assess the relationships between serum levels of TNF-α, IL-6 and IL-18 and clinical characteristics in BD patient groups. A power analysis was done to estimate the effectiveness of sample size. A Data are presented as mean $\pm$ SD. Differences at $p < 0.05$ level (two tailed) were considered significant.

Table 1
Clinical and demographic characteristics of bipolar patients in different mood states and healthy controls.

	Bipolar patients				Healthy controls (N=80)	*p*-value
	Total patients (N=59)	Manic patients (N=37)	Depressive patients (N=12)	Mixed patients (N=10)		
Age (years ± SD)	32.35 ± 12.47	35.14 ± 13.60	28.58 ± 8.74	26.60 ± 8.68	26.77 ± 5.37	0.146
Age range (years)	18–62	18–62	20–43	18–49	18–48	
Gender (male/female)	38/21*	27/10	10/2	1/9*	65/15	0.002
Education (years ± SD)	11.68 ± 3.29	11.32 ± 3.63	12.00 ± 2.69	12.60 ± 2.59		
Duration of admission (days ± SD)	53.32 ± 32.76	48.97 ± 30.08	56.50 ± 43.81	65.60 ± 26.34		
Duration of illness (years ± SD)	8.07 ± 5.39	9.83 ± 8.21	5.53 ± 3.19	4.59 ± 4.31		
Age at onset (years ± SD)	24.54 ± 9.7	25.37 ± 10.80	24.00 ± 8.01	22.10 ± 7.15		
Antipsychotic dose (chlorpromazine equivalents, mg/day ± SD)	283.33 ± 201.8	336.48 ± 261.80	187.58 ± 154.50	201.60 ± 176.82		
HAMD(± SD)	15.98 ± 9.65	12.40 ± 6.66	27.75 ± 11.52	15.10 ± 4.74		
YMRS (± SD)	28.06 ± 12.33	34.13 ± 8.78	12.00 ± 9.67	24.90 ± 5.82		
SerumTNF-α (pg/mL ± SD)	11.37* ± 9.24	13.33* ± 10.05	3.24* ± 3.76	13.91* ± 14.31	2.24 ± 3.92	0.017
Serum IL-6 (pg/mL ± SD)	1.21* ± 0.84	1.47* ± 1.07	0.71* ± 1.11	0.83* ± 0.87	0.45 ± 0.89	0.024
Serum IL-18 (pg/mL ± SD)	49.47 ± 20.67	43.63 ± 53.86	65.94* ± 70.57	51.33 ± 51.48	33.79 ± 36.84	0.045

* Denotes $p < 0.05$ vs healthy controls.