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Circulating Toll-Like Receptor 4, Nucleotide-Binding Oligomerization Domain-Like Receptor Protein 3, and Cytokines in Patients with Bipolar Depression: A Case–Control Study

ABSTRACT

Objective: The etiology of bipolar disorder (BD), a complex psychiatric condition, remains uncertain. Previous research has suggested a potential involvement of the host immune system in the development of BD. This study aims to investigate plasma levels of cytokines, circulating toll-like receptor 4 (TLR4), and nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) in patients with bipolar depression.

Methods: This study recruited patients with a depressive episode of BD and healthy controls (HCs). Inflammatory cytokines were quantified using enzyme linked immunosorbent assay (ELISA) analysis.

Results: A total of 26 BD patients with a depressive episode and 14 HCs were enrolled in the study. The findings revealed that individuals with BD with a depressive episode exhibited elevated serum levels of NLRP3 and interleukin-18 compared to HCs. Correlation analyses indicated a favorable association between the frequency of episodes, duration of illness, and TLR4 levels.

Conclusion: The results suggest a connection between cytokines associated with the activation of NLRP3 and their potential impact on the pathogenesis of BD.

Keywords: Bipolar disorder, depression, TLR4, NLRP3, cytokine

Introduction

Bipolar disorder (BD) is a severe, long-term psychiatric disorder marked by recurring periods of (hypo)mania and depression. Bipolar disorder affects 1%-2% of individuals throughout their lifetime, with more than 70% of individuals with BD experiencing its onset before the age of 25 years.[1] Individuals with BD face a suicide risk that is 20-30 times higher than that of the general population. Alarmingly, 30%-50% of individuals with BD attempt suicide, and 15%-20% of them succeed. Statistics indicate that among all psychiatric disorders, BD carries the highest suicide risk.[2] This places a substantial burden on society. However, the underlying mechanisms of BD remain insufficiently understood, and the diagnosis of BD remains a challenge in clinical practice.

To date, emerging research suggests that neuroinflammation plays a significant role in the neuropathogenesis of BD. Both preclinical and clinical studies support the association between neuroinflammation and BD. Patients with BD exhibit higher peripheral plasma levels of pro-inflammatory substances, such as interleukin-6 (IL-6) and tumor necrosis factor (TNF)-α. Post-mortem investigations have found increased levels of IL-1β and nuclear factor kappa-B (NF-κB) levels in frontal cortex of BD patients.[3-5] Additionally, correlations have been observed between serum levels of TNF-α, IL-8, interferon-γ, IL-10, and the functional connectivity of the cerebral cortex in individuals with BD.[6]

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Furthermore, genetic investigations have identified alterations in the expression of numerous genes linked to neuroinflammation in individuals with BD.[7,8] Prior studies have also indicated that both peripheral and central inflammation, driven by the nucleotide-binding oligomerization domain (NOD)-like receptor protein 3 (NLRP3) inflammasome, are associated with the pathophysiology of BD.[9,10] The activation of the NLRP3 inflammasome requires 2 signals. In microglial cells, chronic stress can stimulate the NLRP3 inflammasome and trigger the transcription of pro-IL-1β and pro-IL-18 in circulating monocytes by activating toll-like receptor 4 (TLR4). The activation of NLRP3 leads to the release of mature IL-1β and IL-18 into the peripheral circulation. In neural pathways, released cytokines stimulate primary afferent nerves, such as the vagal nerve, which transmit signals to the central nervous system related to mood regulation.[11] However, limited evidence exists regarding the potential function of the NLRP3 activation pathway in individuals with BD and its connection to changes in plasma cytokine levels.

This pilot study aimed to examine the plasma cytokines, circulating TLR4, and NLRP3 levels in patients with BD during a depressive episode. Our goal was to investigate the potential involvement of the NLRP3 activation pathway in the pathophysiology of BD.

Material and Methods

Participants

Between January 2020 and December 2021, we recruited patients with a depressive episode of BD from both inpatient and outpatient sources within our hospital. Diagnoses were confirmed based on DSM-5 criteria for BD through structured clinical interviews. Eligible participants fell within the age range of 16-45 years. We assessed the severity of depression using the 17-item Hamilton Depression Rating Scale (HDRS-17),[12] with all patients scoring above 14 points. To evaluate mania, we employed the Young Mania Rating Scale (YMRS).[13] Notably, each patient had been free from medication or had not received treatment for a minimum of 3 months.

Additionally, we enlisted healthy controls (HCs) from local communities who had no family history of psychiatric conditions. Exclusion criteria for both patients and HCs encompassed: (a) the presence of severe physical diseases (e.g., hypertension, diabetes, acute, and chronic infections); (b) autoimmune diseases; (c) pregnancy or breastfeeding; and (d) a history of substance abuse or the use of immune-regulating drugs. Before enrolling in the study, each participant, along with their legal guardians, provided informed consent. Ethical approval was obtained from the local ethics committee at Hangzhou First People's Hospital (IRB: 2020-K008-01), and all procedures were conducted in accordance with the principles of the Declaration of Helsinki.

MAIN POINTS

- *Patients with bipolar depression exhibited elevated serum levels of nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) and interleukin-18 compared to healthy controls.*
- *Correlation studies indicated a favorable association between the frequency of episodes, duration of illness, and toll-like receptor protein 4 levels.*
- *Cytokines associated with the activation of NLRP3 may have a potential impact on the pathogenesis of BD.*

ELISA Analysis

Venous blood samples (1 mL) were collected from all subjects and promptly stored at −80℃ within 15 minutes of collection. The levels of serum inflammatory markers, including NLRP3, TLR4, IL-18, and IL-1β, were determined using the Human (NLRP3/C1orf7/CIAS1/NALP3/PYPAF1) ELISA Kit (Cusabio, Wuhan, China), Human Toll-Like Receptor 4 ELISA Kit (Cusabio, Wuhan, China), Human IL-18 ELISA Kit (Biotech, Beijing, China), and Human IL-1β ELISA Kit (Biotech). The quantitative sandwich enzyme immunoassay technique was employed in this assay. A microplate was pre-coated with antibodies specific to NLRP3/TLR4/IL-18/IL-1β. Standards and samples were pipetted into the wells, and any NLRP3/ TLR4/IL-18/IL-1β present was bound by the immobilized antibodies. After removing unbound substances, a biotin-conjugated antibody specific to NLRP3/TLR4/IL-18/IL-1β was added to the wells. Subsequently, horseradish peroxidase (HRP) coupled to avidin was introduced after a washing step. A substrate solution was then added to the wells once any unbound avidin-enzyme reagent had been washed away. Color development occurred in proportion to the amount of NLRP3/TLR4/IL-18/IL-1β bound in the initial phase. Color development was halted, and the color intensity was measured. The concentration of these factors was quantified using standard curves as per the manufacturers' instructions.

Statistical Analysis

Demographic, clinical, and inflammatory profiles were subjected to statistical analysis using Statistical Package for Social Science Statistics software, version 20.0 (IBM SPSS Corp.; Armonk, NY, USA). A normality test was conducted to assess the distribution of the data. The HDRS-17 scores conformed to the normal distribution and were presented as mean ± SD. Those that deviated from normality were presented as median with interquartile range (IQR). The Mann–Whitney *U*-test was used to compare the age, TLR4, NLRP3, and cytokine levels between the 2 groups. Categorical data were reported as n (%), and the Fisher's exact test was used for sex, marriage, and education year (>12 years). The relationship between immunological indices and clinical characteristics in patients with BD was analyzed using the Spearman correlation coefficient. A significance level of $P < .05$ was set for statistical significance.

Results

Demographic and Clinical Data

The study included a total of 26 patients with bipolar depression and 14 HCs. No significant differences were observed in terms of gender, age, marital status, education years, or handedness, as demonstrated in Table 1 ($P > .05$). Detailed characteristics pertaining to the severity of depression, age of onset, and duration of illness are also presented in Table 1.

Circulating Toll-Like Receptor 4, Nucleotide-Binding Oligomerization Domain-Like Receptor Protein 3, and Cytokines Changes in Patients with Bipolar Disorder

We conducted ELISA analysis to determine the levels of circulating TLR4, NLRP3, and the cytokines IL-18 and IL-1β. Compared to HCs, patients with bipolar depression exhibited significantly elevated serum levels of NLRP3 and IL-18. ($P < .05$) (see Table 2 and Figure 1).

Correlation Analyses

Further exploration was conducted to examine the relationships between immunological indices and clinical characteristics such as

Table 1. Demographic and Clinical Details of Recruited Subjects

Demographic and Clinical Indexes		BD n = 26	%	HCs n = 14	%	*P*
Sex	Female	22	84.62	12	85.71	1.000
	Male	4	15.38	2	14.29	
Age (years, median [IQR])		19.00 (6.25)		21.00 (5.50)		.864
Married, %		6 (23.08)		2 (14.29)		.689
Education year (>12 years) %		7 (26.92)		5 (35.71)		.720
Right hand, %		26 (100)		14 (100)		-
HDRS-17 score (mean ± SD)		20.13 ± 5.18		-		-
YMRS score (median [IQR])		0.50 (1.25)		-		-
Onset age (years, median [IQR])		13.50 (5.00)		-		-
Duration of illness (years, median [IQR])		4.00 (2.25)		-		-

BD, bipolar disorder; HCs, healthy controls; HDRS-17, 17-item Hamilton Depression Rating Scale; IQR, interquartile range; YMRS, Young Mania Rating Scale.

age of onset, duration of illness, number of episodes, HDRS-17, and YMRS scores. Among patients with BD, a positive correlation was observed between the blood concentration of TLR4 and both the duration of illness ($P < .001$ and $r = .697$) and the number of episodes ($P = .016$ and $r = 0.469$).

Discussion

Our study revealed increased levels of serum NLRP3 and IL-18 in patients with bipolar depression. Correlation analyses indicated a positive association between TLR4 levels and both the frequency of episodes and the duration of illness. Nucleotide-binding oligomerization domain-like receptor protein 3 is recognized as vital for host inflammatory responses, and BD has been linked to NLRP3 gene variations.[14] Neuroinflammation, exacerbated by the NLRP3 inflammasome, exacerbates depressive-like behaviors. A review recently examined the connection between inflammation and mitochondrial dysfunction in BD.[15] In a rat model of depression, ketamine was found to suppress NLRP3 inflammasome activation.[16] Additionally, the NLRP3 inflammasome has been shown to be inhibited by and bound to fluoxetine.[17] Physical therapy involving acupuncture has been shown to have antidepressant effects by reducing NLRP3 inflammasome and inflammatory factor levels in the prefrontal cortex of depression-prone rats.[18]

Our finding indicated increased levels of circulating IL-18 in patients with bipolar depression. Activation of the NLRP3 inflammasome can stimulate the release of IL-1β and IL-18.[19] Prolonged stress in the hippocampus results in NLRP3 inflammasome activation and overexpression of inflammatory mediators such as IL-1β, IL-6, and IL-18.[20] Elevated IL-1β and IL-18 levels are often observed in the central nervous system (CNS) during CNS infection, brain trauma, and neurodegenerative diseases.[21,22] Patients with major depressive disorder (MDD) and chronic stress models yield similar outcomes.[11] A study also noted that IL-18 levels were substantially higher in BD compared to MDD, while IL-1β levels were significantly higher in MDD than in

Table 2. The Serum Nucleotide-Binding Oligomerization Domain-Like Receptor Protein 3, Toll-Like Receptor 4, Interleukin-18, and Interleukin-1β Levels in Patients with Bipolar Depression and Healthy Controls.

Inflammatory Indexes	BD	HCs	*P*
NLRP3 (ng/mL, median [IQR])	0.82 (2.44)	0.02 (0.08)	<.001
TLR4 (ng/mL, median [IQR])	5.36 (5.57)	4.09 (3.95)	.192
IL-18 (pg/mL, median [IQR])	351.05 (304.57)	241.67 (164.45)	.016
IL-1β (pg/mL, median [IQR])	0.00 (0.00)	0.00 (2.51)	.278

BD, bipolar disorder; HCs, healthy controls; IL, interleukin; IQR, interquartile range; NLRP3, nucleotide-binding oligomerization domain-like receptor protein 3; TLR4, toll-like receptor 4.

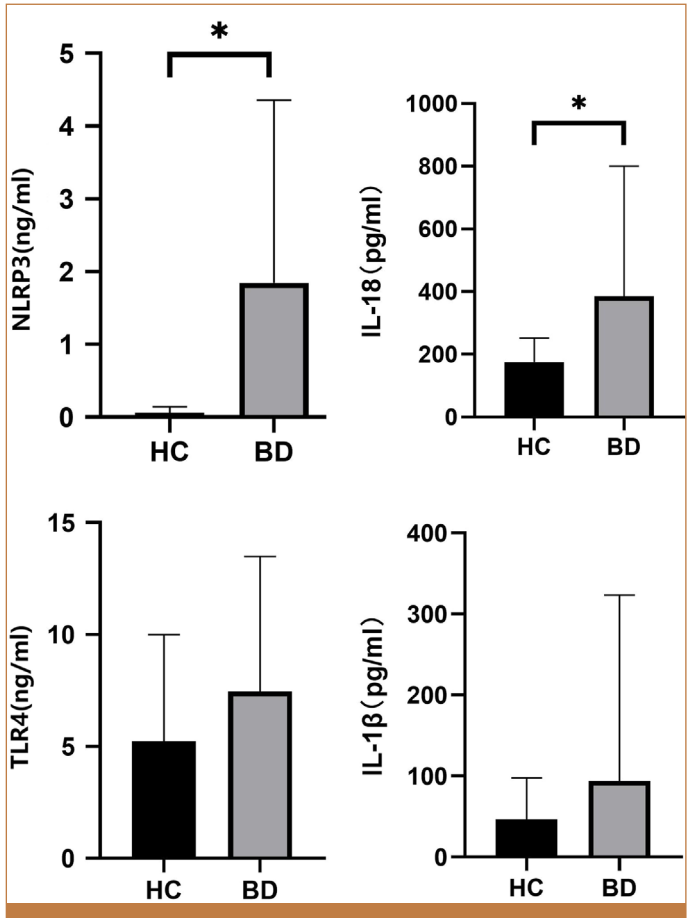

Figure 1. Serum levels of NLRP3, TLR4, IL-18, and IL-1β in patients with bipolar depression. Compared to HCs, circulating levels of NLRP3 and IL-18 were significantly higher in patients with bipolar depression than in HCs ($P < .05$). BD, bipolar disorder; IL, interleukin; HCs, healthy controls; NLRP, nucleotide-binding oligomerization domain-like receptor protein 3; TLR4, toll-like receptor 4.

BD.[23] Furthermore, IL-6 and IL-18 levels were significantly higher in individuals with BD experiencing manic or hypomanic states compared to depressive and euthymic states, suggesting these 2 substances may serve as indicators of manic episodes.[24] In the midbrain of individuals with BD, cytokine mRNAs and proteins showed higher levels of IL-1β, IL-6, and IL-18 in high-inflammatory subgroups than in low-inflammatory subgroups.[25]

Previous studies have indicated that chronic stress has the capacity to activate brain pattern recognition receptors, leading to an increase in TLR4 levels. Furthermore, experiments involving TLR4 deletion in mice have shown that this can mitigate the rise in cytokines and chemokines induced by stress in the hippocampus.[26] Additionally, multiple investigations have demonstrated that peripheral blood mononuclear cells from depressed patients exhibit higher TLR4 levels.[27] Moreover, research examining the polymorphism of the TLR4 gene in BD has revealed a genetic link between BD and TLR4, with identified polymorphisms of the TLR4 gene in patients with BD.[28] In our study, we found no significant difference in TLR4 levels between BD patients and HCs. This result may be attributed to the constraints imposed by the limited number of participants. Furthermore, we observed a positive correlation between the TLR4 level and both the frequency of episodes and the duration of the illness. Prior research has indicated that the TLR4 mRNA expression level is associated with the severity of depression.[29] These findings lend support to the notion that TLR4-mediated inflammation is interconnected with emotional regulation.

It is worth acknowledging several limitations in our study. The statistical power was inevitably compromised by the small sample size, which represents a notable drawback. Our study's cross-sectional design means that we cannot ascertain whether changes in inflammatory variables are causative or consequential. To gain a more comprehensive understanding of the relationship between inflammatory variables and BD, longitudinal studies involving individuals in manic or remission states are warranted.

Based on the findings from our investigation, it was observed that patients with bipolar depression exhibited elevated serum levels of NLRP3 and IL-18. These results imply that the activation of NLRP3 and its associated cytokines may contribute to the etiology of BD. It is advisable that future studies pay closer attention to the role of immunological dysfunction in patients with BD.

Availability of Data and Materials: *The data supporting the conclusions of this article are available from the corresponding author upon request.*

Ethics Committee Approval: *This study was approved by Ethics Committee of Hangzhou First People's Hospital (Approval No: 2020-K008-01, Date: January 20, 2020).*

Informed Consent: *Written informed consent was obtained from the participants and legal guardians who agreed to take part in the study.*

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