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Changes in serum TNF-α, IL-18, and IL-6 concentrations in patients with chronic schizophrenia at admission and at discharge

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ABSTRACT

Objective: Schizophrenia is correlated with aberrant cytokine concentrations. The goal of our study was to detect the serum concentrations of tumor necrosis factor (TNF)-α, interleukin (IL)-18, and IL-6 concentrations in patients with chronic schizophrenia in the acute relapse state at admission and at discharge and to analyze the correlations between the three cytokine concentrations with psychosis symptoms.

Methods: Enzyme-linked immunosorbent assay (ELISA) was used to analyze serum concentrations of TNF-α, IL-18, and IL-6 in 68 patients with chronic schizophrenia at admission and at discharge and in 80 controls. The Positive and Negative Syndrome Scale (PANSS) was used to analyze psychosis symptoms of the patients.

Results: Serum concentrations of TNF-α, IL-18, and IL-6 in patients at admission were significantly elevated compared to those in controls. After treatment, IL-6 concentrations in patients at discharge were significantly reduced compared to those in patients at admission, and IL-6 concentrations showed no significant difference between patients at discharge and controls. In contrast, TNF-α and IL-18 concentrations showed no significant difference between patients at discharge and patients at admission, and TNF-α and IL-18 concentrations in patients at discharge were still significantly elevated compared to those in controls. IL-6 concentrations in patients at admission showed a positive correlation with negative scores, and IL-6 concentrations in patients at discharge showed positive correlations with positive, negative, and total scores. Reduction in IL-6 concentrations showed positive correlations with reduction in positive, negative, and total scores in patients at discharge.

Conclusion: Serum concentrations of TNF-α, IL-18, and IL-6 were significantly elevated in patients with chronic schizophrenia in the acute relapse state. After treatment, IL-6 concentrations in patients at discharge were significantly reduced compared to these in patients at admission.

1. Introduction

Schizophrenia (SZ) is a serious psychotic disease that occurs in nearly 1% of the population worldwide [1]. Schizophrenia is manifested as disturbed thinking, perception, belief, language, and social activities that occur during late adolescence or early adulthood and persist throughout the life of the affected individuals [2]. Immune system dysfunction has been implicated in the pathogenesis of schizophrenia [3]. The inflammatory immune response regulated by cytokines may contribute to the psychopathology of schizophrenia through multidimensional mechanisms affecting neurodevelopment, synaptic plasticity, and neurotransmission [3,4]. In particular, increasing evidence has concentrated on the actions of tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1β, and IL-18 as primary changed proinflammatory cytokines in patients with schizophrenia [5–9].

TNF-α, IL-18, and IL-6 are multifunctional proinflammatory cytokines that are secreted primarily through monocytes and macrophages. These three cytokines play key roles in moderating the complicated events implicated in immunity and inflammation. The key roles of TNF-α, IL-18, and IL-6 in regulating the excitability transmission of neuron cell and neurotransmitter metabolisms make them primary candidates for schizophrenia pathogenesis [10]. Patients with chronic schizophrenia exhibited elevated serum concentrations of IL-6, TNF-α, sIL-2R, IL-1β, and IL-18 [6,11–17]. Previous studies have shown that IL-6 was likely to be a state-associated marker, which was elevated during the acute exacerbation state and normalized after antipsychotic treatment. These findings suggested that IL-6 concentrations could be influenced by treatment or disease state [6,9,18–20]. Moreover, IL-6 concentrations were positively correlated with positive symptoms and negative symptoms in schizophrenia [21,22]. The reduction in IL-6 levels in patients with schizophrenia was associated with the alleviation of negative symptoms [23]. Furthermore, high IL-6 concentrations at baseline were associated with therapy resistance and long duration of

Abbreviations: ELISA, enzyme-linked immunosorbent assay; PANSS, Positive and Negative Syndrome Scale; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin 6; IL-18, interleukin-18; sIL-2R, soluble IL-2 receptor.

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hospitalization [13,24]. Together, these results suggested that IL-6 may be implicated in the response of the patient to antipsychotic therapy and could be regarded as a potential marker of cure response in schizophrenia. In contrast, TNF-α was likely to be considered as a trait marker, which shows no significant alterations after antipsychotic treatment [9,19,20]. Compared with the more frequently analyzed cytokines, the reports on the effect of antipsychotics on IL-18 are limited. A previous study indicated that IL-18 exhibited a positive correlation with general psychopathology of patients with chronic schizophrenia, but antipsychotics have no effects on IL-18 concentrations [17].

In the present study, the serum cytokine concentrations of TNF-α, IL-18, and IL-6 and psychosis symptoms of patients with schizophrenia were assessed during the first week of admission as baseline and in the week before the discharge. The aims of our study were to assess the following in patients with chronic schizophrenia in the acute relapse state: (1) the serum levels of inflammatory cytokines TNF-α, IL-18, and IL-6 at admission and at discharge; (2) the correlation of inflammatory cytokine concentrations at admission with the duration of admission; (3) the correlation of the cytokine levels at admission and at discharge with the severity of the psychotic symptoms; and (4) the association of the changes in these cytokine concentrations with the alleviations of psychotic symptoms.

2. Methods

2.1. Subjects

Sixty-eight inpatients with chronic schizophrenia in the acute relapse state were recruited from the Affiliated Brain Hospital of Guangzhou Medical University as described in previous studies [6,9,13]. The inclusion criteria for all patients were as follows: (1) diagnosed as schizophrenia on the basis of International Classification of Diseases-tenth edition (ICD-10) diagnostic criteria; (2) free of currently occurring allergies, autoimmune disorders, and infections; (3) free of immunosuppressive or anti-inflammatory agent use; and (4) free of past history of substance use. The diagnosis of these patients was made by two senior psychiatrists on the basis of ICD-10 diagnostic criteria during the first week of admission and the week before discharge. Eighty healthy subjects were recruited by advertisements. The inclusion criteria for healthy controls were as follows: (1) free of personal or family history (including first-or second-level family members) of psychiatric diseases; (2) free of any current or past neurological or medical diseases or traumatic brain injury; (3) free of currently occurring allergies, autoimmune disorders, and infections; and (4) free of immune suppressive or anti-inflammatory drug use. The study was approved by the Ethics Committee of the Affiliated Brain Hospital of Guangzhou Medical University. The investigation was carried out according to the latest version of the Declaration of Helsinki. Each subject signed informed consent to join the study.

The psychosis symptoms of patients with chronic schizophrenia were evaluated using the Positive and Negative Syndrome Scale (PANSS) [25] by psychiatrists. The inter-class correlation coefficient (ICC) between raters on the PANSS ranged from 0.90 to 0.96. All patients had been receiving atypical antipsychotic drugs, including risperidone (n = 8), clozapine (n = 14), quetiapine (n = 3), aripiprazole (n = 6), olanzapine (n = 15), ziprasidone (n = 3), clozapine and olanzapine (n = 4), clozapine and risperidone (n = 6), olanzapine and risperidone (n = 5), olanzapine and quetiapine (n = 2), and clozapine and quetiapine (n = 2). The antipsychotic treatment was clinician-led, and the treating physicians were blind to the cytokine levels during the treatment. No anti-inflammatory or immunosuppressive agents were used.

2.2. Measurement of cytokine concentrations

The blood samples were obtained from the patients and healthy subjects between 7:00 a.m. and 9:00 a.m. in the fasting state and stored at room temperature within 2 h. Serum was obtained by centrifuging the blood samples at 4000 rpm for 15 min; the serum was then separated and stored at −80 °C before use.

Serum TNF-α, IL-18, and IL-6 concentrations were tested using eBioscience ELISA kits (catalog numbers: BMS223, BMS213, and BMS267, respectively, eBioscience, San Diego, USA). The sensitivities were 0.13 pg/mL for TNF-α, 0.03 pg/mL for IL-6, and 9 pg/mL for IL-18. The range of the standard curves varied from 0.31 to 20 pg/mL for TNF-α, from 0.09 to 5 pg/mL for IL-6, and from 19.5 to 1250 pg/mL for IL-18. Duplicate tests were performed for standards and samples. Absorbance at 450 nm was measured using a microplate reader.

2.3. Data analysis

All data were analyzed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA). The difference in gender between patients and healthy subjects was determined by the chi-square test. The difference in age between patients and healthy subjects was determined using independent *t*-test. The difference of cytokine concentrations between patients at admission and patients at discharge was evaluated by the paired *t*-test. The difference of cytokine concentrations between healthy subjects and patients at admission or at discharge was evaluated by a covariance analysis with age and gender as covariates. Pearson's correlation analysis was performed to determine the correlation between cytokine concentrations with demographic and clinical characteristics in patients with schizophrenia. Further multiple linear regression analysis was applied to detect the correlation between cytokine concentrations with demographic and clinical characteristics in patients with schizophrenia. All of the presented variables from Table 1 were included in regression models. Categorical variables were converted into dummy variables in regression models. Multiple comparisons were corrected by Bonferroni's corrections [26]. $p < 0.05$ was considered significant.

3. Results

3.1. Clinical characteristics

Clinical characteristics of patients with schizophrenia and healthy subjects are shown in Table 1. Sixty-eight inpatients with chronic schizophrenia in the acute relapse state and 80 healthy subjects were recruited in the study. All subjects were of Chinese ethnicity. The duration of illness and mean age at onset (SD) in the patient group were 10.56 (9.73) and 23.81 (8.21) years, respectively. The gender and age showed significant differences between patients and controls (Table 1, $p = 0.001$). PANSS scores (positive, negative, and general subscores, total score) in patients at discharge were significantly reduced compared to those in patients at admission (Table 1, $p = 0.001$).

3.2. Cytokine concentrations

As shown in Table 2, serum IL-6 concentrations in patients at admission were significantly elevated compared to those in healthy subjects. After treatment, IL-6 concentrations were significantly reduced in patients at discharge compared to these in patients at admission, and IL-6 concentrations showed no significant difference between patients at discharge and controls. TNF-α and IL-18 concentrations in patients at admission were significantly elevated compared to those in healthy subjects. After treatment, TNF-α and IL-18 concentrations showed no significant difference between patients at discharge and patients at admission, and TNF-α and IL-18 concentrations in patients at discharge were still significantly elevated compared to those in healthy subjects ($p < 0.05$) (Fig. 1, Table 2).

Then, the patients were split into two groups according to a cutoff value of PANSS [25]. One group of patients with moderate symptoms (58 ≤ PANSS total score ≤ 75, n = 10), another group of patients with severe symptoms (PANSS total score>75, n = 58). The age and gender showed significant differences between the control group and the

Table 1
Demographic and clinical characteristics of patients with schizophrenia and healthy controls.

	Patients with schizophrenia (N = 68)		Healthy controls (N = 80)	t/x^2	p
Age** (years ± SD)	34.29 ± 11.17		26.77 ± 5.37	$t = -5.073$	0.001
Gender** (male/female)	29/39		65/15	$x^2 = 23.636$	0.001
Alcohol use N(%)[a]	10 (14.71%)				
Smoking N(%)[b]	15 (22.06%)				
Age at onset (years ± SD)	23.81 ± 8.21				
Duration of illness (years ± SD)	10.56 ± 9.73				
Duration of admission (days ± SD)	68.85 ± 45.66				
Number of hospitalization (±SD)	1.99 ± 1.67				
Antipsychotic dose (chlorpromazine equivalents, mg/day ± SD)	723.89 ± 465.67				
PANSS score[c]	Admission	Discharge			
P subscore** (±SD)[d]	24.78 ± 5.19	13.29 ± 3.83		$t = 17.578$	0.001
N subscore** (±SD)[e]	23.53 ± 7.16	18.04 ± 5.98		$t = 7.556$	0.001
G subscore** (±SD)[f]	47.03 ± 7.78	32.54 ± 7.22		$t = 14.871$	0.001
Total score** (±SD)	95.33 ± 14.62	64.00 ± 13.97		$t = 16.586$	0.001

[a] The number of episodes consuming alcohol not less than once during one month before admission.
[b] The number of smoking not less than once during one month before admission.
[c] Positive and Negative Symptom Scale score.
[d] PANSS positive symptom subscale score.
[e] PANSS negative symptom subscale score.
[f] PANSS general psychopathology subscale score.
** Denotes $p < 0.01$.

Table 2
Comparison of cytokine concentrations between patients with schizophrenia and healthy controls.

	Patients with schizophrenia (N = 68)		Healthy controls (N = 80)
	Admission	Discharge	
Serum TNF-α (pg/ml)	12.15 ± 4.01*(p = 0.013)	11.30 ± 3.66*(p = 0.021)	2.24 ± 1.44
Serum IL-6 (pg/ml)	5.61 ± 1.97* (p = 0.012)	1.62 ± 0.19 ** (p = 0.001)	0.45 ± 0.28
Serum IL-18 (pg/ml)	73.60 ± 13.92*(p = 0.025)	68.47 ± 13.31*(p = 0.011)	33.79 ± 6.35

* Denotes $p < 0.05$ vs healthy controls.
** Denotes $p < 0.01$ vs patients at admission.

moderate symptom group or the severe symptom group. The difference in IL-6 concentrations between healthy controls and the severe symptom patients at admission or the moderate symptom patients at admission was evaluated by a covariance analysis with age and gender as covariates. Also, the difference in IL-6 concentrations between healthy controls and the severe symptom patients at discharge or the moderate symptom patients at discharge was evaluated by a covariance analysis with age and gender as covariates. We found that IL-6 concentrations were significantly elevated in the severe/moderate symptom patients at admission than those in healthy controls ($p = 0.006$ and 0.012, respectively). IL-6 concentrations showed no significant difference between the severe/moderate symptom patients at discharge and healthy controls ($p = 0.148$ and 0.205, respectively).

3.3. Correlations of cytokine concentrations with clinical characteristics

IL-6 concentrations showed a positive correlation with PANSS negative scores ($r = 0.275$, $p < 0.01$) in patients at admission (Table 3). Further, IL-6 concentrations in patients at discharge showed positive correlations with positive ($r = 0.473$, $p < 0.01$), negative ($r = 0.435$, $p < 0.01$), and total scores ($r = 0.331$, $p < 0.01$) (Table 3). There were significantly positive correlations between reduction in IL-6 concentrations and reduction in PANSS positive ($r = 0.260$, $p < 0.01$), negative ($r = 0.366$, $p < 0.01$), and total scores ($r = 0.326$, $p < 0.01$) in patients at discharge (Table 4). IL-6 concentrations in patients at admission and at discharge were not associated with the duration of admission. Further, there were no significant associations between IL18 and TNF-α

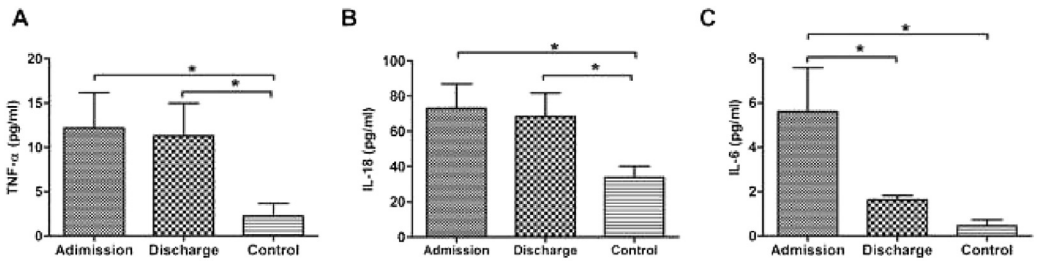

Fig. 1. Serum TNF-α (A), IL-18 (B), and IL-6 (C) levels in patients with schizophrenia (admission patients, n = 68; discharge patients, n = 68) and healthy controls (n = 80). The error bars represent standard deviations. * denotes $p < 0.05$.

Table 3
Correlation analysis between cytokine concentrations with demographic and clinical characteristics of patients with schizophrenia at admission and at discharge.

	TNF-α		IL-6		IL-18	
	Admission	Discharge	Admission	Discharge	Admission	Discharge
Age (years)	0.164	0.225	0.008	−0.208	0.098	0.067
Gender	0.036	0.123	−0.041	−0.173	−0.088	−0.219
Alcohol use[a]	−0.007	−0.023	−0.058	−0.004	0.201	0.010
Smoking[b]	0.046	−0.010	0.117	0.145	0.064	−0.145
Age at onset (years)	−0.082	−0.022	0.119	−0.163	−0.020	0.003
Duration of illness (years)	0.155	0.193	−0.093	−0.180	0.132	0.075
Duration of admission (days)	0.046	−0.099	−0.103	−0.172	0.083	0.157
Number of hospitalizations	−0.085	−0.130	0.201	−0.073	−0.163	−0.190
Antipsychotic dose (chlorpromazine equivalents, mg/day)	−0.078	0.050	−0.025	0.168	−0.005	0.022
Admission P subscore[c]	−0.045		0.071		−0.130	
Admission N subscore[d]	0.184		0.275**(p = 0.007)		−0.018	
Admission G subscore[e]	−0.040		0.023		−0.060	
Admission total score	0.048		0.131		−0.087	
Discharge P subscore[c]		0.054		0.473**(p = 0.001)		0.218
Discharge N subscore[d]		0.157		0.435**(p = 0.001)		0.196
Discharge G subscore[e]		0.074		−0.202		0.159
Discharge total score		0.117		0.331**(p = 0.004)		0.221

[a] The number of episodes consuming alcohol not less than once during one month before admission.
[b] The number of smoking not less than once during one month before admission.
[c] PANSS positive symptom subscale score.
[d] PANSS negative symptom subscale score.
[e] PANSS general psychopathology subscale score.
** Denotes $p < 0.01$.

concentrations in patients at admission and at discharge with clinical characteristics ($p > 0.05$).

The multiple linear regression data revealed that IL-6 concentrations showed a positive correlation with PANSS negative scores in patients at admission ($\beta = 0.351$, $p < 0.01$, $R^2 = 0.212$) (Table 5). There were also significantly positive associations between IL-6 concentrations with PANSS positive, negative, and total scores in patients at discharge ($\beta = 0.254$, 0.231, and 0.412, respectively, $p < 0.01$, $R^2 = 0.235$) (Table 5). Furthermore, there were significantly positive correlations between reduction in IL-6 concentrations with reduction in PANSS positive, negative, and total scores in patients at discharge ($\beta = 0.292$, 0.372, and 0.224, respectively, $p < 0.01$, $R^2 = 0.227$) (Table 5).

4. Discussion

In our study, we indicated that serum TNF-α, IL-18, and IL-6 concentrations were significantly elevated in patients with chronic schizophrenia in the acute relapse state at admission compared to those in controls. After antipsychotic treatment, IL-6 concentrations were significantly reduced in patients at discharge compared to these in patients at admission, and IL-6 concentrations in patients at discharge were not significantly different from those in controls. In contrast, TNF-α and IL-18 concentrations showed no significant difference between patients at discharge and patients at admission, and TNF-α and IL-18 concentrations in patients at discharge were still significantly elevated compared to those in healthy subjects. IL-6 concentrations in patients at admission showed a positive correlation with negative scores. Further, IL-6 concentrations in patients at discharge showed positive correlations with positive, negative, and total scores. Reduction in IL-6 concentrations showed positive associations with reduction in positive, negative, and total scores in patients at discharge.

Table 4
Correlation analysis between altered cytokine concentrations with duration of admission as well as with altered PANSS scores before and after treatment.

	△TNF-α	△IL-6	△IL-18
△P subscore[a]	−0.126	0.260**(p = 0.007)	0.021
△N subscore[b]	0.096	0.366**(p = 0.002)	−0.050
△G subscore[c]	−0.171	0.147	−0.105
△Total score	−0.104	0.326**(p = 0.004)	−0.066
Duration of admission	−0.138	0.097	0.133

△ represents changes in cytokine concentrations or PANSS scores from admission to discharge.
[a] PANSS positive symptom subscale score.
[b] PANSS negative symptom subscale score.
[c] PANSS general psychopathology subscale score.
** Denotes $p < 0.01$.

Elevated serum concentrations of TNF-α, IL-18, and IL-6 have been reported in patients with schizophrenia [6,11–17]. Two meta-analyses indicated that IL-6 and TNF-α concentrations were elevated in inpatients with the acute relapse state [6,9]. In agreement with these studies, we found that patients with chronic schizophrenia in the acute relapse state presented elevated serum concentrations of TNF-α, IL-18, and IL-6 at admission, providing further support that inflammatory immune response may be implicated in the psychopathology of schizophrenia. Previous studies have also shown positive correlations between IL-6 concentrations with negative and positive symptoms in patients with schizophrenia [21,22]. In addition, there were positive correlations between the IL-6 gene polymorphism with reduced hippocampal volume in patients with schizophrenia and genetic vulnerability to schizophrenia [27,28]. We found that IL-6 concentrations in patients at admission showed a positive association with negative scores, and IL-6 concentrations in patients at discharge showed positive correlations with positive, negative, and total scores; this indicated that patients with elevated IL-6 concentrations may be inclined to show serious symptoms and further indicated the important role of IL-6 in the pathological process of schizophrenia. In the present study, there were no significant associations between TNF-α and IL-18 concentrations in patients at admission and at discharge with psychotic symptoms.

Many studies have shown that typical or atypical antipsychotic medications may affect cytokine concentrations [29–32]. Flupentixol, quetiapine, risperidone, aripiprazole, chlorpromazine, clozapine, and olanzapine could reduce serum IL-10, IL-6, IL-1β, IL-2, and TNF-α concentrations [6,9,18,32–38], thus suggesting antipsychotic-related anti-inflammatory effects in patients with schizophrenia. There was also a positive association between the reduction in IL-6 concentrations and the amelioration of negative symptoms [23]. In our study, we indicated

Table 5
Multiple linear regression analysis between cytokine levels with demographic and clinical characteristics in patients with schizophrenia.

	β (IL-6 at admission)	β (IL-6 at discharge)	β (ΔIL-6)
Age (years)	0.096	−0.143	0.092
Gender	−0.045	−0.021	−0.078
Alcohol use[a]	−0.052	−0.041	0.198
Smoking[b]	0.097	0.091	0.056
Age at onset (years)	0.102	−0.102	−0.031
Duration of illness (years)	−0.062	−0.078	0.122
Duration of admission (days)	−0.101	−0.089	0.065
Number of hospitalizations	0.034	−0.123	−0.151
Antipsychotic dose (chlorpromazine equivalents, mg/day)	−0.056	0.076	−0.002
Admission P subscore[c]	0.055		
Admission N subscore[d]	0.351**(p = 0.003)		
Admission G subscore[e]	0.030		
Admission total score	0.112		
Discharge P subscore[c]		0.254**(p = 0.007)	
Discharge N subscore[d]		0.231**(p = 0.008)	
Discharge G subscore[e]		−0.102	
Discharge total score		0.412**(p = 0.001)	
ΔP subscore[c]			0.292**(p = 0.006)
ΔN subscore[d]			0.372**(p = 0.001)
ΔG subscore[e]			0.042
ΔTotal score			0.224**(p = 0.008)
R^2	0.212	0.235	0.227

[a] The number of episodes consuming alcohol not less than once during one month before admission.
[b] The number of smoking not less than once during one month before admission.
[c] PANSS positive symptom subscale score.
[d] PANSS negative symptom subscale score.
[e] PANSS general psychopathology subscale score.
** Denotes p < 0.01.

that IL-6 concentrations were significantly reduced in patients at discharge compared to those in patients at admission and reduced IL-6 concentrations showed positive associations with the reduction in PANSS positive, negative, and total scores in patients at discharge; this suggests that the improvement of acute relapse symptoms by antipsychotic medications may be correlated with a reduction in IL-6 concentrations. IL-6 may be a state marker for the acute relapse stage and could be considered as a potential biomarker of cure response in schizophrenia. Further studies are needed to determine whether amplifying of antipsychotic therapy with anti-inflammatory medications in patients with schizophrenia with elevated IL-6 concentrations at admission would lead to better treatment outcomes. Hence, assessment of changes in the cytokine concentrations before and after treatment may be helpful to identify different subtypes of patients with schizophrenia, predict treatment responses to antipsychotics, and identify the potential application of anti-inflammatory medications as auxiliary agents to antipsychotic drugs.

In our study we indicated that TNF-α and IL-18 concentrations showed no significant difference between patients at discharge and patients at admission, and TNF-α and IL-18 concentrations in patients at discharge were still significantly elevated compared with those in control after antipsychotic treatment, which was consistent with previous reports indicating that TNF-α and IL-18 concentrations show no significant alterations after antipsychotic treatment [9,17,19,20]. TNF-α and IL-18 may be independent biomarkers of psychosis and trait markers of schizophrenia [39]. These findings also suggested that the influence of antipsychotic agents on cytokines was complicated and varied, which may have diverse influence on these cytokines and different immune responses may occur during the different stages of schizophrenia. Compared to IL-6, TNF-α and IL-18 may be less responsive to antipsychotic drugs.

It remained unclear whether the alterations in cytokine concentrations directly resulted from the effects of the antipsychotic drugs or whether they were an outcome of clinical symptom improvement. It has been reported that antipsychotic drugs such as chlorpromazine, haloperidol, risperidone, or clozapine may directly affect the actions of macrophage cells and microglia cells that produce cytokines [40,41]. In addition, various neurotransmitters were produced by immune cells, and neurotransmitters such as dopamine, serotonin, and opioid receptors could change the action of cytokines of these immune cells [42,43]. In our study, we indicated that reduced IL-6 concentrations showed positive associations with the reduction in PANSS positive, negative, and total scores in patients at discharge; IL-6 concentrations were significantly elevated in the severe/moderate symptom patients at admission than those in healthy controls. IL-6 concentrations showed no significant difference between the severe/moderate symptom patients at discharge and healthy controls. These findings suggest that there was a close relationship between the reduction in IL-6 concentrations ant the improvement of acute relapse symptoms. Since the sample size in our study is relatively small, and most patients were with severe symptom, the effect of symptom severity on IL-6 concentrations could not be completely clarified. Further studies with larger sample sizes are essential to clarify the relationships among antipsychotics, symptoms severity and changes in IL-6 concentrations.

Our study has some limitations. First, the present study only assessed three pro-inflammatory cytokines. Future studies should assess more cytokines, including anti-inflammatory cytokines. Second, the relatively small sample size of our study may limit the detection of the actual relationships between clinical characteristics and altered inflammatory cytokines. Future studies with larger sample sizes are essential to confirm findings in our study. Third, we did not control potential confounding elements such as smoking, drinking alcohol, body mass index (BMI) and C-reactive protein (CRP) in controls as well as BML and CRP in patients. A previous study has demonstrated that smoking and BMI could be correlated with elevated or reduced concentrations of cytokines in patients with schizophrenia [31,38,44]. The current findings should be regarded as preliminary in nature. It cannot be excluded, that findings are due to residual confounding issues, since relevant confounders of cytokine levels have not been investigated and included in the statistical analysis. Further studies by controlling related confounding factors are essential to confirm the effect of cytokine changes in patients with schizophrenia and seek better treatment methods. Fourth, there was

no control of the use of antipsychotic agents in patients with schizophrenia. All patients in this study were on antipsychotic treatment before entering the study. Further study in first-episode or drug-naive patients with a uniform treatment is essential to prove the correlation between antipsychotic drugs and serum concentrations of TNF-α, IL-18, and IL-6.

5. Conclusions

In summary, our study revealed that patients with chronic schizophrenia in the acute relapse state showed elevated TNF-α, IL-18, and IL-6 concentrations compared to those in healthy subjects. After antipsychotic drug treatment, IL-6 concentrations were significantly reduced in patients at discharge compared to those in patients at admission, while TNF-α and IL-18 concentrations showed no significant difference between patients at discharge and patients at admission. Reduction IL-16 concentrations in parallel with improvements in positive, negative, and total symptoms suggested that IL-6 could be used as a state-related marker and a possible treatment response predictor in patients with schizophrenia.

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