Inflammatory Cytokines Changed in Patients With Depression Before and After Repetitive Transcranial Magnetic Stimulation Treatment

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INTRODUCTION

Over the past two decades, there has been increasing evidence that depression is associated with a dysregulated immune system, including abnormalities in inflammatory markers (1). Compared with the healthy control (HC) group, the levels of pro-inflammatory cytokines and proteins in patients with acute phase depression were increased, and the results of the increase in interleukin (IL)-6, tumor necrosis factor (TNF)-α, and C-reactive protein (CRP) levels in the blood of patients...
with depression were relatively consistent (2). A recent meta-analysis of 82 studies, including 3,212 patients with depression and 2,798 HCs, showed that patients with depression had increased peripheral levels of IL-6, TNF-α, IL-10, soluble IL-2 receptor, chemokine ligand 2, IL-13, IL-18, IL-12, IL-1 receptor antagonist, and soluble TNF receptor 2, whereas interferon-gamma (IFN-γ) levels were decreased (3). In general, there is great heterogeneity in the data, which depends on the cytokine composition. This is at least in part due to the failure to consider the clinical course and course of disease, as well as the influence of potential confounding factors, such as comorbidity, drugs, fasting, smoking, and test methods (4–6).

Transcranial Magnetic Stimulation (TMS) is a non-invasive physical regulation method with antidepressant effects (7–10). It has been proven to have clinical application value for patients with depression (9, 11–13), including adolescents (14), the elderly (15, 16), patients with suicidal ideation (17) and patients with anxiety symptoms (18). These pulses can be transmitted at a high frequency (10–20 Hz) or low frequency (less than or equal to 1 Hz). Most clinical TMS treatments used to treat depression are usually performed at frequencies of 10–18 Hz (19, 20). In the early stages, there have been three large, multicenter, randomized pseudo-stimulus-controlled trials, including a total sample of 703 adult patients with major depression. One to four antidepressant treatments were ineffective in the enrolled patients. Two of these studies were industry-sponsored registered trials that led to Food and Drug Administration (FDA) approval of TMS treatment for depression in 2008 (10, 21). The evidence from all three trials was consistent, and TMS had statistically significant and clinically relevant benefits compared with pseudo-stimulation.

Few clinical studies have investigated the effect of rTMS on inflammatory cytokine levels in patients with depression. Langguth et al. (22) reported that peripheral levels of CRP and IL-6 increased after receiving 20-Hz rTMS in an elderly female patient with depression and rheumatoid arthritis, indicating that rTMS enhanced the patient’s peripheral inflammatory response. Zhao et al. (23) recruited 58 elderly patients with depression and 30 HCs. The peripheral levels of IL-1β and TNF were higher in the patient group than in the HC group at baseline. Compared with the non-rTMS treatment group, patients treated with 10-Hz rTMS for 4 weeks had IL-1β, and TNF in the peripheral blood levels decreased significantly.

However, the dynamic changes of inflammatory cytokine in depression before and after the TMS treatment are still largely unknown. The goal of this study was to explore the changes in inflammatory cytokine levels in drug-free patients with depression at recruitment and treated with antidepressants and 4 weeks of active/sham-rTMS treatment and to profile the inflammatory cytokine levels before (baseline) and after (week 2 and week 12) active/sham-rTMS treatment in patients with depression.

MATERIALS AND METHODS

Sample and Study Design

The study participants were drawn from a consecutive clinical sample of adult patients, aged 18–80 years, who presented at the Shanghai Pudong New Area Mental Health Center, Tongji University School of Medicine. The hospital’s Ethical Committee approved the study and it was performed according to the Declaration of Helsinki. All participants provided written informed consent during the recruitment stage of the study. Patients with depression were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5). The inclusion criteria were as follows: (1) age 18–80 years, (2) experienced an acute exacerbation of the symptoms of depression or had a baseline score of at least 14 points on the 24-item Hamilton Rating Scale for Depression (HAMD), (3) satisfied the DSM-5 criteria for depression based on the Structured Clinical Interview for DSM Disorders/Patient Edition, (4) having only one type of antidepressant during rTMS treatment and willing and capable of completing at least 20 sessions. None of the patients had a history of neurosurgery, seizures, head trauma, substance abuse or dependence, psychiatric or neurological disorders other than depression. Patients were excluded from the study if they have current inflammatory disorders, had received past rTMS treatment or other electromagnetic stimulations such as electroconvulsive therapy, ferromagnetic metallic implants, pacemakers.

We recruited 70 patients who were randomly and separately assigned to active \( n = 36 \) or sham rTMS \( n = 34 \) conditions. However, 13 patients (seven in the active-rTMS group and six in the sham-rTMS group) could not complete the study and withdrew their consent; thus, 57 patients (29 patients in the active-rTMS group and 28 patients in the sham-rTMS group) were finally included in the subsequent analysis. Independent, third-party assorted participants were enrolled into either the active or sham rTMS groups through computergenerated randomization numbers compiled through simple randomization. The clinical staff and patients were blinded to the assignment, except for one clinical technician who provided active or sham rTMS treatment according to the randomization numbers.

Clinical Assessments

The primary outcome measure for the study was the score on the 24-item version of the HAMD (24). Seven factor scores including anxiety/somatization (items 10–13, 15, and 17), weight loss (item 16), cognitive disturbance (items 2, 3, 9, and 19–21), diurnal variation (item 18), retardation (items 1, 7, 8, and 14), sleep disturbance (items 4–6), and hopelessness (items 22–24) were calculated (25). Clinical assessment were administered at baseline (before rTMS treatment), mid-treatment (2 weeks), and 8 weeks after the last TMS session (12 weeks).

Repetitive Transcranial Magnetic Stimulation

Patients were instructed to sit on a comfortable chair in a quiet room, and rTMS was delivered using a MagPro \( \times 100 \) magnetic stimulator (Medtronic Co., Denmark) equipped with a butterfly coil. In the rTMS group, the stimulation was targeted to the position of the left unilateral dorsolateral prefrontal cortex, stimulated at a frequency of 10 Hz, power (intensity)
level of 120% of the motor threshold, 80 trains, 30 pulses per train, 12-s intertrain-interval, 2,400 pulses per session, and five sessions per week. The same protocol was followed for the sham rTMS group using a fake coil that was unable to produce a magnetic field. The patients were blinded to the treatment received.

Measurement of Inflammatory Cytokines
Venous blood samples were collected in anticoagulant-free tubes between 7 a.m. and 9 a.m. after an overnight fasting. The samples were kept at room temperature for 1 h, followed by centrifugation (3,000 g for 20 min at 4°C) for serum separation. Subsequently, the serum was separated and stored at −80°C until assayed. Serum levels of TNF-α, IFN-γ, IL-4, IL-6, IL-8, IL-2, and CRP-hc for each sample were measured in duplicate using an enzyme-linked immunosorbent assay (ELISA) with a commercial human ELISA kit (Multisciences [Lianke] Biotech, Co., Ltd.), according to the manufacturer’s instructions. The concentrations of TNF-α, IFN-γ, IL-4, IL-6, IL-8, IL-2, and CRP-hc were expressed as picograms of protein per mL of serum. The intra- and inter-assay coefficients of variation were less than 9%.

Data Analysis
The sociodemographic and clinical characteristics of our participants are presented as descriptive statistics, such as percentages and mean scores (standard deviation, SD) (Table 1). Analyses of variance (ANOVA) and χ² tests were used to investigate differences in demographics, clinical variables, and antidepressant medications among the HC, active-rTMS, and sham-rTMS groups. The total and subscale HAMD scores before and after rTMS treatment were displayed and compared using an independent t-test (Table 2). Spearman correlation analysis was used to test the correlations between decreased HAMD scores and decreased cytokine levels. A box-plot diagram was created using GraphPad Prism software to analyze the differences in serum levels of TNF-α, IFN-γ, IL-4, IL-6, IL-8, IL-2, and CRP-hc at baseline in the active/sham-rTMS groups (Figure 1). The paired t-test was used for the self-controlled analysis of serum levels of

### Table 1 | Demographic and clinical variables, comparison among healthy controls (HC), active-rTMS, and sham-rTMS groups.

| Variables                      | HC                | Active-rTMS     | Sham-rTMS     | Comparison |
|-------------------------------|-------------------|-----------------|---------------|------------|
| Cases (n)                     | 28                | 29              | 28            |            |
| Age [mean (SD)] years         | 56.5 (14.2)       | 58.2 (9.4)      | 55.6 (11.8)   | F = 0.345 0.709 |
| Age range (years)             | 29–76             | 38–72           | 29–77         |            |
| Female [n (%)]                | 23 (82.1)         | 24 (82.8)       | 22 (78.6)     | χ² = 0.189 0.910 |
| Education [mean (SD)] years   | 7.3 (3.6)         | 8.0 (3.2)       | 7.4 (2.8)     | F = 0.395 0.675 |
| Single [n (%)]                | 1 (3.6)           | 3 (10.3)        | 3 (10.7)      | χ² = 1.204 0.548 |
| Married [n (%)]               | 24 (85.7)         | 21 (72.4)       | 21 (75.0)     | χ² = 2.572 0.276 |
| Divorced [n (%)]              | 3 (10.7)          | 3 (10.3)        | 2 (7.1)       | χ² = 0.254 0.881 |
| Widowed [n (%)]               | 0 (0)             | 2 (6.9)         | 2 (7.1)       | χ² = 2.064 0.356 |
| Family history [n (%)]        | 0 (0)             | 3 (10.3)        | 3 (10.7)      | χ² = 3.174 0.205 |
| Age of MD onset [mean (SD)] years | –               | 49.7 (13.7)     | 47.1 (14.8)   | t = 0.639 0.491 |
| Episode numbers [mean (SD)]   | –                 | 2.2 (1.3)       | 3.0 (2.1)     | t = 1.705 0.094 |
| Number of hospitalizations [mean (SD)] | –          | 1.6 (1.0)       | 2.1 (1.8)     | t = 1.457 0.151 |
| Course of MD [mean (SD)] months | –              | 9.1 (10.3)      | 8.0 (6.5)     | t = 0.492 0.625 |
| Baseline HAMD [mean (SD)]     | –                 | 28.5 (3.8)      | 33.1 (4.6)    | t = 4.135 <0.001 |
| HAMD-total score              | –                 | 8.0 (2.9)       | 9.0 (3.0)     | t = 1.281 0.205 |
| HAMD-anxiety/somatization     | –                 | 0.8 (0.8)       | 1.4 (0.8)     | t = 2.744 0.008 |
| HAMD-weight                   | –                 | 3.1 (2.6)       | 4.0 (2.2)     | t = 1.469 0.148 |
| HAMD-cognitive disturbance    | –                 | 2.0 (1.0)       | 2.2 (0.8)     | t = 0.859 0.394 |
| HAMD-retardation              | –                 | 6.3 (1.5)       | 6.5 (1.6)     | t = 0.457 0.650 |
| HAMD-diurnal variation        | –                 | 4.9 (1.7)       | 4.9 (1.3)     | t = 0.012 0.990 |
| HAMD-sleep disorder           | –                 | 4.3 (2.0)       | 4.0 (1.8)     | t = 0.610 0.545 |
| Antidepressants (mg/day) [mean (SD)] | –            | 40.0 (0.0)      | 40.0 (0.0)    | –          |
| Duloxetine                    | –                 | 23.3 (3.9)      | 24.3 (4.3)    | –          |
| Escitalopram                  | –                 | 50.0 (15.8)     | 44.0 (8.9)    | –          |
| Paroxetine                    | –                 | 206.3 (37.5)    | 178.1 (38.8)  | –          |
| Venlafaxine                   | –                 | 54.9 (13.1)     | 51.7 (10.0)   | t = 0.993 0.326 |

HAMD, Hamilton Rating Scale for Depression; HC, healthy control. F/t/χ², F, one-way ANOVA comparison among the three groups; t, independent sample t-test was used to compare between active-rTMS and sham-rTMS groups in patients with depression; χ², κappa test.
TABLE 2 | Hamilton Rating Scale for depression (HAMD) scores at 2 and 12 weeks in the active-rTMS and sham-rTMS groups in patients with depression.

| Variables               | Active-rTMS | Sham-rTMS | Comparison |
|-------------------------|-------------|-----------|------------|
|                         | Mean (SD)   | Mean (SD) | t          |
| Week 2                  |             |           | p          |
| HAMD total score        | 14.41 (2.970) | 17.14 (2.772) | -3.583     | 0.001      |
| HAMD-anxiety/somatization | 4.59 (2.428) | 6.04 (3.727) | -1.733     | 0.090      |
| HAMD-weight             | 0.24 (0.577) | 0.54 (0.683) | -1.746     | 0.086      |
| HAMD-cognitive disturbance | 1.90 (1.800) | 2.14 (1.508) | -0.559     | 0.578      |
| HAMD-retardation        | 0.72 (0.702) | 0.86 (0.803) | -0.666     | 0.508      |
| HAMD-diurnal variation  | 3.14 (1.356) | 3.21 (1.371) | -0.211     | 0.833      |
| HAMD-sleep disorder     | 2.03 (1.569) | 2.46 (1.575) | -1.032     | 0.307      |
| HAMD-desperation        | 1.79 (1.521) | 1.89 (1.133) | -0.280     | 0.781      |
| Week 12                 |             |           |            |
| HAMD total score        | 7.90 (2.257) | 6.54 (2.317) | 2.246      | 0.029      |
| HAMD-anxiety/somatization | 3.62 (2.243) | 2.75 (1.602) | 1.681      | 0.098      |
| HAMD-weight             | 0.07 (0.258) | 0.04 (0.189) | 0.554      | 0.582      |
| HAMD-cognitive disturbance | 0.83 (0.848) | 0.79 (0.957) | 0.175      | 0.862      |
| HAMD-retardation        | 0.45 (0.572) | 0.43 (0.573) | 0.130      | 0.897      |
| HAMD-diurnal variation  | 1.66 (0.897) | 1.46 (1.036) | 0.744      | 0.460      |
| HAMD-sleep disorder     | 0.97 (1.017) | 0.75 (0.799) | 0.887      | 0.379      |
| HAMD-desperation        | 0.31 (0.541) | 0.32 (0.670) | -0.069     | 0.945      |

HAMD, Hamilton Rating Scale for Depression. t, independent sample t-test was used to compare active-rTMS and sham-rTMS groups in patients with depression.

RESULTS

Demographic and Clinical Characteristics
As shown in Table 1, there were no significant differences in baseline age, years of education, marital status, or family history among the active-rTMS, sham-rTMS, and HC groups. Compared with the sham rTMS group, the HAMD total score in the active-rTMS group was significantly lower than that in the pseudo-stimulation group. The main difference in each HAMD factor score is the body weight factor. There was no significant difference in the antidepressant dose between the active-rTMS and sham-rTMS groups. Table 1 lists the demographic and clinical characteristics and antidepressant exposure of the three groups.

Clinical Characteristics Before and After Treatment
As shown in Table 2, the HAMD total score at 2 weeks between the active-rTMS and sham-rTMS groups was statistically different. The total score on the 2-week HAMD in the active-rTMS group was significantly lower than that in the sham-rTMS group. At 12 weeks, the total HAMD score in the active rTMS group was significantly higher than that in the sham rTMS group.

Baseline Inflammatory Cytokines
As shown in Figure 1, the serum levels of TNF-α, IFN-γ, IL-4, and IL-2 at baseline in the HC, active rTMS, and sham rTMS groups were significantly different. The difference mainly lies in the fact that the baseline TNF-α level was significantly lower in the HC group than in the patients with depression in either the active-rTMS or sham-rTMS groups. The level of IFN-γ, IL-4, and IL-2 were significantly higher in HC group than that of the depression group.

Changes of Inflammatory Cytokines Before and After Treatment
The levels of several inflammatory cytokines in the active-rTMS and sham-rTMS groups changed significantly after 2 and 12 weeks of treatment (Table 3). In the self-control analysis of active-rTMS group, the levels of IL-2 and CRP-hc changed significantly after 2 weeks of treatment, the level of CRP-hc changed significantly after 12 weeks of treatment, and the level of TNF-α and IFN-γ changed significantly between week 2 and week 12. In the self-control analysis of sham-rTMS group, IFN-γ changed significantly after 12 weeks of treatment. The level of IFN-γ and IL-4 changed significantly, and the level of TNF-α and CRP-hc changed significantly between week 2 and week 12 (Table 4).

Correlation Between Decreased Depressive Symptoms and Inflammatory Cytokines After Transcranial Magnetic Stimulation Treatment
After the active or sham TMS treatment, the decrease in depressive symptoms of the patients (n = 57) were negatively correlated with the decreased levels of TNF-α after 2 weeks of treatment. While the decrease in depressive symptoms were negatively correlated with the decreased levels of IL-2 at 12 weeks. The changes of all the other inflammatory cytosines were not...
FIGURE 1 | Baseline inflammatory cytokine levels in healthy controls (HC), active-rTMS (TMS), and sham-rTMS (no-TMS) groups in patients with depression.
TABLE 3 | The levels of inflammatory cytokines at baseline, 2 and 12 weeks in the active-rTMS and sham-rTMS groups in patients with depression.

| Variables | Baseline vs. week 2 | Baseline vs. week 12 | Week 2 vs. week 12 |
|-----------|---------------------|----------------------|------------------|
|           | Mean | SD | Mean | SD | Mean | SD |
| Active-rTMS | TNF-α | 8.106 | 3.202 | 9.905 | 7.457 | 6.980 | 2.883 |
|            | IFN-γ | 7.014 | 5.164 | 6.752 | 2.151 | 5.733 | 2.013 |
|            | IL-4  | 10.479 | 3.871 | 15.648 | 39.190 | 8.318 | 4.441 |
|            | IL-6  | 3.486 | 2.928 | 3.960 | 2.360 | 3.813 | 1.657 |
|            | IL-8  | 202.341 | 54.149 | 338.238 | 276.709 | 260.658 | 274.033 |
|            | IL-2  | 410.345 | 290.926 | 470.586 | 150.389 | 432.069 | 173.358 |
| Sham-rTMS | CRP-hc | 0.139 | 0.192 | 0.299 | 0.312 | 0.366 | 0.253 |
| TNF-α | 7.333 | 1.860 | 8.291 | 2.856 | 6.580 | 2.507 |
| IFN-γ | 9.172 | 5.562 | 6.248 | 1.998 | 5.500 | 2.140 |
| IL-4 | 17.169 | 17.126 | 12.060 | 17.279 | 7.171 | 3.596 |
| IL-6 | 3.375 | 1.591 | 4.339 | 4.332 | 4.792 | 3.423 |
| IL-8 | 175.739 | 179.681 | 514.150 | 1097.508 | 275.171 | 233.763 |
| IL-2 | 448.536 | 93.633 | 475.464 | 117.294 | 508.429 | 151.898 |
| CRP-hc | 0.271 | 0.701 | 0.193 | 0.161 | 0.428 | 0.400 |

TABLE 4 | Self-controlled comparisons for serum levels of inflammatory cytokines before and after treatment in patients with depression.

| Variables | Baseline vs. week 2 | Baseline vs. week 12 | Week 2 vs. week 12 |
|-----------|---------------------|----------------------|------------------|
|           | t | p | t | p | t | p |
| Active-rTMS | TNF-α | -1.148 | 0.261 | 1.745 | 0.092 | 2.109 | 0.044 |
|            | IFN-γ | 0.300 | 0.767 | 1.266 | 0.216 | 2.171 | 0.039 |
|            | IL-4  | -0.705 | 0.487 | 1.790 | 0.084 | 1.018 | 0.317 |
|            | IL-6  | -0.897 | 0.377 | -0.521 | 0.606 | 0.274 | 0.786 |
|            | IL-8  | -1.093 | 0.284 | -0.492 | 0.627 | 1.262 | 0.217 |
|            | IL-2  | -2.278 | 0.031 | -0.557 | 0.582 | 1.068 | 0.294 |
|            | CRP-hc | 2.448 | 0.021 | -3.681 | 0.001 | -0.874 | 0.390 |
| Sham-rTMS | TNF-α | -1.562 | 0.130 | 1.201 | 0.24 | 2.456 | 0.021 |
|            | IFN-γ | 2.602 | 0.015 | 3.402 | 0.002 | 1.474 | 0.152 |
|            | IL-4  | 1.070 | 0.294 | 3.084 | 0.005 | 1.409 | 0.170 |
|            | IL-6  | -1.087 | 0.287 | -1.872 | 0.072 | -0.406 | 0.688 |
|            | IL-8  | -1.604 | 0.120 | -1.725 | 0.096 | 1.138 | 0.265 |
|            | IL-2  | -0.941 | 0.355 | -1.774 | 0.087 | -1.043 | 0.306 |
|            | CRP-hc | 0.567 | 0.575 | -1.151 | 0.26 | -3.346 | 0.002 |

The paired t-test was used for self-controlled analysis of serum levels of inflammatory cytokines pre- and post-treatment in patients in the active and sham rTMS groups. The level of statistical significance was set at a two-tailed p-value of 0.05.

significantly correlated with the decreased depressive symptoms in the patients (Table 5).

DISCUSSION

Main Findings
In this study, we hypothesized that, in association with depressive symptom improvements, serum inflammatory cytokine levels in patients with depression would be changed after active rTMS treatment compared with the sham rTMS group. The main findings supported the assumption that the change patterns of inflammatory cytokine levels highly varied after rTMS treatment. In the active rTMS group, the serum levels of CRP-hc and IL-2 significantly changed at week 2, and the change last through the 12 weeks period, which were not seen in the sham-rTMS group, suggesting that rTMS can change serum inflammatory cytokine levels in patients with depression. Furthermore, the improvement of depressive symptoms in the patients was negatively correlated with the decreased levels of TNF-α during the TMS treatment at 2 weeks and negatively correlated with decreased IL-2 levels at 12 weeks after the treatment. Besides, the baseline TNF-α level was significantly higher and IFN-γ, IL-4, and IL-2 were lower in patients with depression compared with the HC group. After the to the best of our knowledge, this is the first study to examine dynamic changes in the levels of inflammatory cytokines following active rTMS and sham-rTMS in patients with depression.

TNF-α and Depression
This study found that the concentration of TNF-α in the depression group was significantly higher than that in the HC
resulting in reduced availability of serotonin in patients with and serotonin-degrading enzyme indoleamine-2,3-dioxygenase, α serotonin uptake (27). Second, TNF-α plays a pathogenic role in depression, and the TNF-α levels of antidepressant treatment and last through the whole 12 weeks of follow-up in the active-rTMS groups. CRP is a non-specific acute-phase protein, and its level increases in response to an inflammatory response. A meta-analysis of cross-sectional studies confirmed that the average concentrations of CRP in patients with depressive episodes were higher than those in the control group (36). Cheng et al. reported that in patients treated with antidepressants, baseline CRP levels were significantly correlated with treatment response at 2 weeks. Higher CRP levels were associated with poorer treatment outcomes. After 6 weeks, CRP levels in both treatment groups increased significantly (37). It is speculated that CRP may serve as a useful marker of inflammation in the antidepressant treatment of depression (38).

**IFN-γ and Depression**

In this study, IFN-γ decreased after 2 and 12 weeks of antidepressant treatment with sham-rTMS and decreased from 2 to 12 weeks in the active-rTMS group. Chen et al. (39) found that the baseline IFN-γ levels in patients with depression were significantly higher than those in the control group. In the antidepressant treatment group, a decrease in IFN-γ levels was observed after 8 weeks of treatment. The decrease in IFN-γ level was not statistically significant in the remission group, while the decrease in IFN-γ levels was statistically significant in the non-remission group. Dahl et al. (40) found that, compared with HC, the IFN-γ level of untreated patients with depression was significantly higher. In the group of patients who achieved remission, IFN-γ levels decreased significantly compared to baseline levels. These results suggest that IFN-γ may be involved in the pathogenesis of depression and in the therapeutic mechanism of antidepressant treatment.

**Inflammatory Cytokines and Repetitive Transcranial Magnetic Stimulation**

This study found that rTMS had an effect on various inflammatory factors during antidepressant treatment, which was significantly different from that in the sham-rTMS group. An interesting study by Zhao et al. (23) reported the effects of rTMS on IL-1β and TNF-α levels in elderly patients with refractory depression. In their study, serum levels of IL-1β and TNF-α were measured before the study, 48 h, and 1, 2, 3, and 4 weeks after the first TMS treatment. The levels of IL-1β and TNF-α gradually decreased and were significantly lower than those in the control group. Serum factors of healthy individuals were not affected by rTMS. The levels of IL-1β and TNF-α were positively correlated with the HAMD score. These results suggested that rTMS may reduce serum IL-1β and TNF-α levels during antidepressant treatment. Interestingly, our results showed that IL-2 levels in the depression patients was significantly decreased at the baseline and increased after 2 weeks of active TMS treatment. Furthermore, the long-term improvement of depressive symptoms in the patients was significantly correlated with the change of IL-2 levels, in supporting of the pathological role of IL-2 in the development and TMS treatment of depression.

**Limitations**

The present study had a few limitations. First, the sample size of the diagnostic subgroup was small. The number of patients in each group was smaller after stratification according to active rTMS and sham rTMS. Another limitation is the uncertainty over how accurately serum inflammatory cytokine levels reflect levels in the brain. Third, due to ethical reasons, all the depressive patients in this study were administered by antidepressants besides the TMS or sham-TMS treatments, so the effects of

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**Table 5 | Correlations between decreased HAMD score and decreased serum levels of inflammatory cytokines before and after TMS treatment in patients with depression.**

| Inflammatory cytokines | Baseline-week 2 Δ HAMD | Baseline - week 12 Δ HAMD |
|------------------------|-------------------------|--------------------------|
|                        | r          | p        | r          | p        |
| ΔTNF-α                 | −0.272     | 0.040    | −0.089     | 0.511    |
| ΔIFN-γ                 | −0.117     | 0.387    | −0.143     | 0.289    |
| ΔIL-4                  | 0.134      | 0.920    | 0.280      | 0.035    |
| ΔIL-6                  | −0.022     | 0.869    | 0.081      | 0.551    |
| ΔIL-8                  | −0.029     | 0.831    | −0.008     | 0.955    |
| ΔIL-2                  | −0.103     | 0.446    | −0.340     | 0.010    |
| ΔCRP-hc                | 0.019      | 0.886    | −0.163     | 0.226    |

High-Sensitivity C-Reactive Protein and Depression

We found significant increases in CRP-hc levels after 2 weeks of TMS treatment and last through the whole 12 weeks of follow-up in the active-rTMS groups. CRP is a non-specific acute-phase protein, and its level increases in response to an inflammatory response. A meta-analysis of cross-sectional studies confirmed that the average concentrations of CRP in patients with depressive episodes were higher than those in the control group (36). Cheng et al. reported that in patients treated with antidepressants, baseline CRP levels were significantly correlated with treatment response at 2 weeks. Higher CRP levels
antidepressants on serum cytokines could not be excluded. The current study relied only on the assessment of depressive symptoms, which is inadequate.

**CONCLUSION**

Despite these limitations, we performed a careful evaluation of depressive symptoms, as well as dynamic measuring of the serum inflammatory cytokines throughout the active/sham-rTMS treatment period, thus obtaining reliable results. This study preliminarily explored the characteristics of inflammatory cytokines in patients with depression and assessed the dynamic changes of these cytokines 2 and 12 weeks after active/sham-rTMS treatment. Our results revealed that there were significant changes in several inflammatory cytokines in patients with depression compared with HC, and the changes in inflammatory cytokines in the rTMS treatment group followed different patterns compared with the sham control group, suggesting that the antidepressant effect of rTMS is related to the changes in inflammatory cytokines.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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**ETHICS STATEMENT**

The studies involving human participants were reviewed and approved by the Ethical Committee of Shanghai Pudong New Area Mental Health Center. The patients/participants provided their written informed consent to participate in this study.

**AUTHOR CONTRIBUTIONS**

QW designed the study and drafted the manuscript. LZ conceived the analysis. WH, ML, NZ, and XH performed the analysis and interpretation of the results. MS, JQ, YS, and XT collected the data. HM and WL supervised the experiment and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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