Research Article

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Interleukin-6 signaling pathway involved in major depressive disorder: selective serotonin reuptake inhibitor regulates IL-6 pathway

Majör Depresif Bozuklukta Yer Alan İnterlökin-6 Sinyal Yolağı: Selektif Serotonin Geri Alım İnhibitörü, IL-6 Yolağını Düzenler

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Abstract

Background: Evidence indicates that pro-inflammatory Th17 and Th1 cells are involved in major depressive disorder (MDD) pathogenesis. Development of Th17 and Th1 are regulated by IL-6 and IFN-γ, respectively. In this study, the levels of IL-6 and IFN-γ, and mRNA expression of related signaling components and, Th17/Th1 transcription factors were investigated in MDD patients with/without selective serotonin reuptake inhibitor (SSRI) medication.

Materials and methods: Forty-six patients and 38 healthy controls (HCs) were recruited. Twenty patients were received the SSRI (sertraline 50–200 mg/day) for at least 1 year, and 26 patients were not received medication. Expression of IL-6R, IFN-γR, JAK1, JAK2, TYK2, STAT1, STAT3, T-bet and RORγt were assessed with Real-Time-PCR. Serum and supernatant levels of IL-6 and IFN-γ were determined using ELISA.

Results and discussion: The serum and supernatant levels of IL-6 were increased in patients without (SSRI−) compared with HCs, while its levels were reduced in SSRI+. Elevated expressions of IL-6R, STAT3 and RORγt were observed in SSRI− compared with HCs. Expressions of IL-6R, STAT3, RORγt and IFN-γR, were decreased in SSRI+ compared to SSRI− patients.

Conclusion: Increased IL-6 involved in MDD, and SSRI regulates IL-6 pathway and IL-6 production. MDD patients may benefit from IL-6/IL-6R targeted therapeutic intervention.

Keywords: Cytokine; Interleukin-6; STAT; T-Lymphocyte; Major depressive disorder.

Öz

Amaç: Bulgular, pro-inflamatuar Th17 ve Th1 hücrelerinin majör depresif bozuk (MDB) patogenezinde önerildiğini göstermektedir. Th17 ve Th1 hücre gelişimleri sırasıyla, IL-6 ve IFN-γ tarafından düzenlenmektedir. Bu çalışmada, SSRI alan veya almayan MDB’lu hastalarda IL-6 ve IFN-γ düzeyleri, ilişkilidir sinyal bileşenlerinin mRNA ifadeleri veTh17 ve Th1 transkripsiyon faktörleri araştırıldı.

Gereç ve Yöntem: Kırk-altı hasta ve 38 sağlıklı kontrol (SKler) değerlendirildi. Yirmi hasta sertralin 50–200 mg/gün) alıyordu ve 26 hasta ilaç kullanmıyordu. IL-6R, IFN-γR, JAK1, JAK2, TYK2, STAT1, STAT3, T-bet ve RORγt ifadeleri, SSRI alan ve almayan MDB’lu hastalarda IL-6 ve IFN-γ düzeyleri, mRNA ifadeleri veTh17 ve Th1 transkripsiyon faktörleri, ELISA kullanılarak belirlendi.

Bulgular ve Tartışma: IL-6 serum ve üst sıvı düzeyleri, SSRI+ grupta azalıyorken, SSRI almayan MDB’lu hastalarda (SSRI−) artışlıktır kontrolle (SKler) kıyaslarda artışlıktır. SSRI+ hastalarda SKler kıyaslarda artışlıktır. IL-6R, STAT3 ve RORγt ifadeleri azaldı. IL-6R, STAT3 ve RORγt ifadeleri SSRI− hastalarda SSRI+ hastalara göre azaldı.
**Introduction**

Major depressive disorder (MDD) is a chronic psychiatric disease that is associated with alteration in immune cell subsets including Th helper (Th) cells [1]. Th cells can differentiate into inflammatory functional subgroups including Th1 and Th17 during an active immune response. Development and differentiation of Th1 and Th17 cells are regulated by IFN-γ and IL-6 cytokines, respectively [2, 3]. The intracellular Janus kinases (JAK) and signal transducer and activator of transcription (STAT) signaling pathway regulates the differentiation and function of Th cells [4]. STAT1 is an important member of STAT family that involved in development of Th1. IFN-γ binds to the surface IFN-γR and activates mainly intracellular Jak1 and Jak2 which leads to activation of STAT1 [4, 5]. STAT1 up-regulates the expression of T-box transcription factor (T-bet), the master transcription factor for Th1 cells [4]. STAT3 is a key signaling molecule involved in Th17 differentiation. IL-6 binds to the IL-6R and activates mainly Jak1, Jak2 and Tyk2 which can activate STAT3 [6]. Activated STAT3 up-regulates the RAR related orphan receptor gamma (ROGY), the master transcription factor for Th17 cells [6].

Previous studies have been indicated that psychiatric disorders including MDD are associated with systemic inflammation [7, 8]. It has been demonstrated that the serum levels of pro-inflammatory cytokines (such as IL-1, IL-6 and TNF-α) are changed in MDD patients [9]. On the other hand, an abnormality of T cells, especially Th1 and Th17 subsets may contribute to the development of MDD [10]. The percentage of CD4+ T cells was increased in patients with MDD [11]. In addition, evidence suggests that the ratio of CD4+/CD8+ was increased in the MDD patients [12]. Studies have been found that the frequency of Th17 (by phenotype: CD4+IL17+) was increased in untreated MDD patients compared to healthy controls subjects (HCs).

Selective serotonin reuptake inhibitors (SSRI) such as sertraline, fluoxetine and citalopram may have beneficial/anti-inflammatory effects on immune system. It has also been reported that the circulating population of CD4+ Th cells and pro-inflammatory cytokines was decreased in MDD patient after treatment with SSRIs [13–16]. Based on the background, studies have been suggested that lymphocytes, especially inflammatory subgroups of Th cells including Th17 and Th1 are more activated in the MDD patients, and their cytokines including IL-6 and IFN-γ are involved in the pathogenesis of MDD. In this study, the mRNA expression levels of IFN-γ, IL-6R, JAK1, JAK2, TYK2, STAT1, STAT3, T-bet and RORγt in isolated peripheral blood mononuclear cells (PBMCs) form MDD patients with or without SSRI treatment were assessed. Moreover, the serum and PBMC supernatant levels of IL-6 and IFN-γ cytokines were investigated. The effects of SSRI medications on the genes expression and cytokines production were also evaluated.

**Materials and methods**

**Participants**

Forty-six patients with MDD were recruited in this study. Twenty patients with MDD were received the SSRI medication including standard dose of sertraline (50–200 mg/day) and 26 patients were not received SSRIs or any other antidepressant medications (SSRI−). The MDD patients were received medications for at least 1 year. Thirty-eight HCs were recruited in the study. The clinical interview confirmed that the HCs did not suffer from any psychiatric disorders/autoimmune disorders and free of any medication for at least 6 months. Further details of the patients and HCs are shown in Table 1. Informed consent was received from all participants and the study was approved by the local Ethics Committee. For this

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**Table 1**: Demographic and clinical data of the patients and HC subjects.

<table>
<thead>
<tr>
<th></th>
<th>MDD</th>
<th>HC</th>
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<tbody>
<tr>
<td>Gender (female/male)</td>
<td>28/18</td>
<td>20/18</td>
</tr>
<tr>
<td>Age (years)</td>
<td>39.95±1.24</td>
<td>39.23±1.34</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.24±4.61</td>
<td>24.10±3.81</td>
</tr>
<tr>
<td>Material status (single/married)</td>
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<td>15/23</td>
</tr>
<tr>
<td>Education (years)</td>
<td>11.96±4.38</td>
<td>14.9±3.27</td>
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<tr>
<td>Family history (negative/positive)</td>
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</tr>
<tr>
<td>Age of onset (years)</td>
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</tr>
<tr>
<td>Duration of disease (years)</td>
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<td>NA</td>
</tr>
<tr>
<td>HAMD</td>
<td>23.02±3.53</td>
<td>NA</td>
</tr>
</tbody>
</table>

The results are presented as mean± standard deviation. *No significant difference was seen in HC compared to MDD. MDD, Major depressive disorder; HC, healthy control; BMI, body mass index; HAMD, Hamilton rating scale for depression; NA, not applicable.
study, ethical approval dated 19.11.2016 with the number IR.UMSHA.REC.1395.373 has been received from the Ethics Committee of our university.

Inclusion criteria were as follows: (1) Diagnosis by the psychiatrist according to the Diagnostic and Statistical Manual Disorder Fourth Edition (DSM-IV) [11]. (2) HAMD score for all patients was more than 17 (HAMD >17) [11]. (3) Age between 18 and 60 years. Exclusion criteria were as follows: (1) Patients with other psychiatric diseases including schizophrenia. (2) Patients with inflammation/autoimmune diseases and allergy diseases. (3) Smoking and alcohol consumption. (4) Taking any other medications. (5) Pregnant women.

Cell isolation

Blood samples (10 mL) from the patients and HCs were collected in EDTA tubes (BD, San Jose, CA, USA). PBMCs were isolated using Ficoll histopaque 1077 (Sigma, St. Louis, MO, USA) density gradient centrifugation, as described previously [17]. The PBMCs were washed twice with phosphate-buffered saline (PBS) at 1500 rpm for 10 min and stored in the complete cell media for further culture or molecular experiments. Viability of cells was determined using trypan blue. Also 5 mL of blood samples were collected and stored at −80°C for serum separation. The serum samples were collected in EDTA tubes (BD, San Jose, CA, USA). PBMCs were isolated using Ficoll histopaque 1077 (Sigma, St. Louis, MO, USA) for 4 days at 37°C in a 5% CO2 incubator. The dose of mitogen was determined based on previous studies [17, 18]. After 4 days, supernatant of the cells were collected and stored at −80°C for the cytokine assay. Supplemented RPMI 1640 (Biosera, Kansas, MO, USA) with 2 mM L-glutamine, 10,000 U/mL penicillin, 100 mg/mL streptomycin (Biosera, Kansas City, MO, USA), and 10% heat-inactivated fetal bovine serum (Biosera, Kansas, MO, USA) was used as complete cell culture medium [19].

Gene expression

Total RNAs were extracted from fresh PBMCs using RNA mini kit (Qiagen, USA) according to the manufacturer’s instructions. Concentration of RNA was assessed using NanoDrop spectrophotometer (A & E Lab, UK). The samples with purity of ≥1.8 were used for cDNA synthesis. cDNA synthesis was performed using reverse transcriptase kit ( Takara, Nohihihagi, Kusatsu, Shiga, Japan) according to the manufacturer’s instructions. mRNA gene expression of the receptors (IFN-γR and IL-6R), JAKs-STATs (JAK1, JAK2, TYK2, STAT1, STAT3) and transcription factors (T-bet and RORγt) were determined with the SYBR Green (Ampliqon, Odense, Denmark) real time polymerase chain reaction (Real Time PCR) method. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for normalize PCR products and for compare mRNA expressions between the samples. To evaluate the mRNA expression of the genes, three-step Real Time PCR was conducted in a Light Cycler (Roche 96 System, Germany). PCR amplification included an initial incubation step at 95°C followed by 40 cycles of denaturation for 15 s at 95°C, annealing for 30 s at 58°C and extension for 30 s at 72°C. Forty cycles were performed and final extension at 72°C for 10 min. The relative mRNA expression level for each sample were determined using the 2−ΔΔCT method [20].

The specific oligonucleotide primers were used in the Real Time PCR as follows: (A) human IFN-γR (PubMed no. NM_000416.2), sense primer is 5′-GATTCAGTGCCAGTTAGCATTCC-3′, and the antisense primer is 5′-ACCAGGCTAAGCACTAGAAAAAGTAG-3′, (B) human IL-6R (PubMed no. NM_00565.3), sense primer is 5′-CATCTTTCTACAGACTACGGTTGAG-3′, and the antisense primer is 5′-TCGCTCCACTCGCCTG-3′, (C) human JAK1 (PubMed no. NM_00321857.1), sense primer is 5′-GGTCTCCCACAACACCATC-3′, and the antisense primer is 5′-GGTCTTTTATCCTCCTACAG-3′, (D) human JAK2 (PubMed no. NM_00322199.1), sense primer is 5′-ATATTGGTGGAGACAAGAGACAG-3′, and the antisense primer is 5′-CTATCGCATAAATCCGCTGGTG-3′, (E) human TYK2 (PubMed no. NM_003331.4.), sense primer is 5′-CATAATGCTTTCCCTGAGGTTATC-3′, and the antisense primer is 5′-AGCACAGCTGAACACTGAAG-3′, (F) human STAT1 (PubMed no. NM_007315.3), sense primer is 5′-AACATGCTGGTGGCGGAAC-3′, and the antisense primer is 5′-GGACATCAGCGGTAAG-3′, (G) human STAT3 (PubMed no. NM_213662.1), sense primer is 5′-TTGAGAGGACGATCATC-3′, and the antisense primer is 5′-GATTCAGTGC-3′, (H) human STAT5 (PubMed no. NM_001322199.1), sense primer is 5′-AGCAGAGCATCAGCGTGA-3′, and the antisense primer is 5′-GAGCTTGCTGG-3′, (I) human RORγt (PubMed no. NM_001001523.1), sense primer is 5′-CCTTACAGCCAGTTTCGTCAT-3′, and the antisense primer is 5′-CAGTTAGCGATT-3′, (J) human GAPDH.
Serum and supernatant cytokine analysis

Serum and supernatant levels of IL-6 and IFN-γ were measured using enzyme-linked immunosorbent assay kits (ELISA MAX™ Deluxe set, Biolegend, San Diego, USA) according to the manufacturer’s instructions. The ELISA kits used in this study were suitable for serum and cell culture supernatant experiments.

Statistical analyses

Statistical analyses were calculated with SPSS (version 21 software) and the graphs were drawn with Graph Pad Prism (version 6.07 software). Data were checked for normality by the Shapiro-Wilk test. ANOVA was used to assess differences among groups. Posthoc Bonferroni test was applied for multiple comparisons. The results are shown as the mean ± standard error of the mean (SEM). The p-value < 0.05 was considered significant.

Results and discussion

The level of cytokines in serum and PBMC culture supernatants of MDD patients compared to healthy individuals

We first assessed the serum and culture supernatants levels of IL-6 and IFN-γ in the patients and HCs. PBMCs were stimulated with PHA for 4 days and the level of cytokines were determined. The result showed that the serum level of IL-6 was significantly enhanced in total MDD patients compared to HCs (p = 0.02, Figure 1A). Its supernatant levels was slightly increased in total MDD patients compared to HCs, but did not reach statistical

Figure 1: Levels of IL-6 and IFN-γ in the serum and supernatants of MDD patients and HCs.

For assessed the level of cytokines in culture supernatant, PBMCs were isolated and stimulated by PHA (5 mg/mL) for 4 days. The serum level of IL-6 (A), supernatant level of IL-6 (B), serum level of IFN-γ (C) and supernatant level of IFN-γ (D) were measured in MDD patients (20 SSRI− and 16 SSRI+) and HCs (n = 19). MDD, Major depressive disorder; SSRI, selective serotonin reuptake inhibitor; HC, healthy control. Data are shown as mean ± SEM. Data was tested using the ANOVA and p < 0.05 was considered significant.
mRNA expression levels of IFN-γ receptor (IFN-γR) and IL-6R in MDD patients compared to healthy individuals

According to the alterations in cytokine levels, we next assessed the mRNA expression levels of IFN-γR and IL-6R and related Th1 and Th17 JAK-STAT intracellular pathway components, in total MDD, SSRI− and SSRI+ subgroups and HCs. Total RNA was extracted from isolated PBMCs and the mRNA gene expression levels was assessed using Real Time PCR.

The results showed that the mRNA expression level of IL-6R was higher in SSRI− subgroup compared to HCs (p = 0.04, Figure 2A). The mRNA level of IL-6R expression showed a significant decrease in the SSRI+ compared to SSRI− group (p = 0.003, Figure 2A). No significant difference was seen in its expression between the SSRI− and HC group.

The mRNA expression level of IFN-γR was significantly reduced in SSRI+ compared with SSRI− group (p = 0.046, Figure 1B). No significant differences were determined in its expression levels between the total MDD patients or SSRI− subgroup and HC subjects (Figure 2B).

mRNA expression levels of JAKs and STATs in MDD patients compared to healthy individuals

According to alterations in cytokines and their receptors, we next analyzed the mRNA expression of JAK1, JAK2, TYK2, STAT1 and STAT3 in the patient and HC subjects. No significant differences were observed in the expression of JAK1, JAK2 and TYK2 between the patient subgroups and HC subjects (Figure 3A–C). Also, the mRNA expression levels of JAK1, JAK2 and TYK2 did not changed between SSRI− and SSRI+ subgroups. There was no significant difference in expression of STAT1 in the MDD patients, SSRI− and SSRI+ subgroups compared to HCs. The STAT1 mRNA expression did not differ significantly between the patient groups (Figure 3D). In contrast, the expression of STAT3 was significantly increased in SSRI− MDD patients compared to HCs (p = 0.02, Figure 1E). The gene level of STAT3 was significantly down-regulated in SSRI+ subgroup compared to SSRI− subgroup (p = 0.005, Figure 3E). The STAT3 mRNA expression in SSRI+ patients did not significantly differ with HC subjects.

mRNA expression levels of T-bet and RORγt transcription factors in MDD patients compared to healthy individuals

After evaluation of IL-6 and IFN-γ JAK-STAT pathway components expression, we next detected the mRNA expression of Th7 and Th1 transcription factors. The mRNA expression of RORγt and T-bet was assessed in the patients and HC subjects.

Figure 2: Gene expression level of IL-6R and IFN-γR in MDD patients and HCs.
PBMCs were isolated from MDD patients (26 SSRI− and 20 SSRI+) and HCs (n = 38). Total RNA was extracted from PBMCs and the expression of IL-6R (A) and IFN-γR (B) determined using Real Time PCR. MDD, Major depressive disorder; SSRI, selective serotonin reuptake inhibitor; HC, healthy control. Data are shown as mean ± SEM. Data was tested using the ANOVA and p < 0.05 was considered significant.
The mRNA expression level of RORγt was significantly up-regulated in SSRI− MDD patients compared to HCs (p ≤ 0.001, Figure 4A). The mRNA expression of RORγt was markedly lower in SSRI+ group compared with its expression in SSRI− MDD patients (p ≤ 0.001, Figure 4A). No significant changes were observed in RORγt expression between the other groups. No significant differences were observed in mRNA expression of T-bet in the MDD patients, SSRI− and SSRI+ subgroups compared to HC subjects (Figure 4B). The expression of T-bet did not differ between the patients groups (Figure 4B). No significant differences were observed between the genders in the parameters examined in this study (data not shown).

Several studies have been indicated that the serum levels of pro-inflammatory cytokines including IL-6, TNFα and IL-1 and the frequency of Th1 and Th17 cells are changed in MDD patients [9, 11]. To our knowledge there was no report that evaluate mRNA expression levels of intracellular IL-6 and IFN-γ signaling pathway components related to Th1 and Th17 cell subsets in the patients.
On the other hand, studies have been revealed that SSRI antidepressant medications have an anti-inflammatory effects on immune system but molecular mechanisms underlie that are unclear. In current study, IL-6 and IFN-γ cytokines and gene expression of related signaling pathway components were investigated. The effects of SSRI medication on genes expression and IL-6 and IFN-γ cytokine secretion were also evaluated.

The main finding of the present study were as follows: an elevated serum and supernatants level of IL-6 cytokine was observed in SSRI− MDD patients, while its levels was reduced in SSRI+ subgroup. The mRNA level of IL-6R, STAT3 and RORγt was up-regulated in SSRI− MDD patients compared to HCs. The mRNA expression levels of IL-6R, STAT3 and RORγt transcription factor were down-regulated in MDD patients who treated with SSRI medications.

We observed that the serum level of IL-6 was elevated in SSRI+ subgroup, compared to HC subjects, in agreement with previous study [15]. Moreover, the production of IL-6 cytokine from isolated and PHA-activated PBMCs was enhanced in SSRI− subgroup. The boosting of cytokine responses in PBMCs by PHA in many ways mimics the initial T cell adaptive immune responses. We found that the serum and supernatant level of IL-6 was diminished in the SSRI+ group compared to SSRI− group. The levels of serum IL-6 was markedly enhanced in total MDD patients compared to HCs, which could be as a result of elevated IL-6 in SSRI− subgroup. Also, IL-6 supernatant levels was slightly increased in total MDD patients compared with HCs, but did not reach statistical significance, which could be as a result of sample distribution or of sample size or high level production of IL-6 from HCs. This result indicated that consuming of SSRI medication may be reduce the production level of IL-6 in SSRI+ MDD patients, which can be one of the anti-inflammatory mechanisms of SSRI medications. The anti-inflammatory role of SSRIs has been reported before in vitro; the level of TNF-α was decreased due to the effect of SSRI [21].

Biological effect of IL-6 is mediated through its interaction with the surface receptor. We observed that gene expression of IL-6R was slightly increased in SSRI− MDD patients compared to HC subjects. Previous studies showed that the expression of IL-1R1, TNFRI and TNFR2 were elevated in schizophrenia patients [22], and the level of sIL-2R was increased in MDD patients without treatments [23]. We also found that the mRNA expression level of IL-6R was significantly decreased in SSRI+ compared with SSRI− group. It’s possible that the elevated IL-6R gene expression in SSRI− MDD patients is associated with increased IL-6 cytokine levels. This feedback loop is kept in check by the cells. It is only suggestive of one possibility to illustrate how a positive feedback could cause a cytokine effects on expression of related receptor. On the other hand, these results indicated that SSRI medication may also suppress inflammation by reducing IL-6R gene expression in MDD patients.

The signal transduction of IL-6 involves in activation of intracellular JAK1, JAK2, and TYK2, that leads to activation of STAT3. STAT3 activates RORγt, transcription factor for differentiation and function of Th17 [24]. The STAT3 gene expression was slightly up-regulated in untreated MDD patients compared to HCs and consuming of SSRI medication may be down-regulated its levels in the patients. It’s possible that the increased IL-6R gene expression in SSRI− MDD patients responsible for up-regulated STAT3 gene expression through other JAKs molecules in the patients, but further complementary studies in protein levels are required to confirm this possibility.

Previous study showed a potential role of Th17 cells in the autoimmune process in MDD patients. It has been shown that RORγt gene expression was increased in MDD patients without treatment. In addition, by flow cytometric analysis, MDD patients revealed a significant increase in peripheral Th17 cell number, and showing an imbalance of Th17/Treg ratio [1]. We confirmed that the level of RORγt mRNA expression was elevated in SSRI− group compared to the HCs. A reduction in gene expression of RORγt was seen in SSRI+ group compared to SSRI− group, which may be caused by reduced expression of IL-6R and STAT3. This result indicated that consuming the SSRI medications can down-regulate the enhanced expression of IL-6R, STAT3 and RORγt in MDD patients; another possible anti-inflammatory effects of SSRI medications. In this study, no significant differences were observed in the expression of JAK1, JAK2 and TYK2 between the patients and HCs. It is possible that the activation of STAT3 may regulate by other stimulatory molecules (such as JAK3) or inhibitory molecules (such as suppressor of cytokine signaling proteins, SOCS). Further studies are necessary to confirm these possibility.

Studies examining the level of IFN-γ in MDD have reported inconsistent data, including elevation [15], reduction [13] or no abnormalities [25], thereby demonstrating the importance of patient selection (treatments profile or status of the patients). Moreover, the frequency of Th1 (by phenotype: CD4+/IFN-γ+) was decreased in MDD patients without treatments compared to the healthy individuals [26]. The recent meta-analysis study also investigated the peripheral cytokine alterations in MDD. The results suggest that levels of IL-6 significantly elevated in MDD patients, while IFN-γ levels may be slightly reduced in the MDD group compared to HCs [13]. It is possible
that various types of the assay methods across laboratories, medication status, and potential confounders (such as body mass index) may have a different effects on the cytokine levels. We observed that the serum and PBMCs culture supernatants level of IFN-γ did not changed in MDD patients compared to HC subjects. SSRI medications did not change the level of IFN-γ in the serum and supernatants.

IFN-γsignaling is triggered by its binding to the IFN-γR [27]. This leads to the activation of JAK1, JAK2 and STAT1 [28]. Activated-STAT1 leads to expression of T-bet, specific transcription factor for differentiation of Th1 cells. In this study, no significant differences were seen in the mRNA expression levels of IFN-γR, JAK1, JAK2, STAT1, and T-bet in MDD patients compared to HCs. The present study demonstrated that gene expression of IFN-γR was decreased in SSRI+ group compared to SSRI− group. The anti-inflammatory effects of SSRI medications may lead to reduction in mRNA expression of IFN-γR in SSRI− group.

Further studies are warranted to confirm the observations in isolated CD4+ T cell cultures. The effects of antidepressant medications on phosphorylation level of JAKs and STATs, related to Th1 and Th17 cells should be investigated by flow cytometry. Future studies should be evaluated/compared the gene expression of IFN-γ and IL-6 intracellular pathway components, before and after treatment with antidepressant in MDD patients.

Conclusion

Studies indicated that pro-inflammatory IL-6 and Th17 cells are involved in MDD pathogenesis. SSRI medications have an anti-inflammatory effects on the immune system; the molecular mechanisms underlie that are unclear. In this study, we observed that the serum and supernatant level of IL-6 cytokine was elevated in SSRI+ MDD patients, and its level was decreased in patients who have undergone SSRI treatment (SSRI− group). The mRNA level of STAT3 and RORγt, involved in downstream signaling of IL-6/IL-6R interaction was increased in SSRI+ MDD patients, while their expression was reduced in MDD patients with SSRI treatment (SSRI− group). The results indicate that intracellular IL-6 signaling pathway components become more active in MDD patients and this pathway are associated with MDD pathogenesis. Moreover, SSRI treatment can inhibit gene expression of IL-6 signaling pathway components and may reduce the development of Th17, but further studies are needed to investigate the differentiation of Th17 in the protein level.

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Conflict of interest: All authors declare that they have no conflicts of interest.

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