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ORIGINAL RESEARCH

# Evaluation of Inflammatory Response System (IRS) and Compensatory Immune Response System (CIRS) in Adolescent Major Depression

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**Purpose:** Nowadays, the role of two tightly interconnected systems, the inflammatory response system (IRS) and the compensatory immune response system (CIRS) in depression, is increasingly discussed. Various studies indicate pro-inflammatory activity in adolescent depression; however, there is an almost complete lack of findings about IRS and CIRS balance. Thus, we aimed to assess different IRS and CIRS indices, profiles, and IRS/CIRS ratios in drug-naïve MDD patients at adolescent age, with respect to sex.

**Patients and Methods:** One hundred MDD adolescents (40 boys, average age: 15.4±1.2 yrs.) and 60 controls (28 boys, average age: 15.3±1.5 yrs.) were examined. Evaluated parameters were 1. plasma levels of interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, interferon gamma, tumor necrosis factor alpha (TNF-α), soluble receptor of IL-6 (sIL-6R), soluble receptors of TNF-α (sTNF-R1, sTNF-R2); 2. profiles: IL-6 trans-signaling, M1 macrophage signaling, helper T lymphocytes (Th) 1 profile, regulatory T lymphocytes (Treg)+Th2, allIRS, and allCIRS; 3. IRS vs CIRS activity ratios: TNF-α/TNF-R1, TNF-α/TNF-R2, TNF-α/sTNF-Rs (ie sTNF-R1 +sTNF-R2), Th1/Th2, Th1/Treg, Th1/Th2+Treg, M1/Th2, M1/Treg, M1/Treg+Th2, allIRS/allCIRS.

**Results:** MDD patients showed increased IL-4, IL-10, TNF-α, sIL-6R, Treg+Th2, allIRS, allCIRS, and TNF-α/sTNF-Rs, and decreased Th1/Th2+Treg. MDD females showed increased IL-10 and TNF-α compared to control females. MDD males showed increased IL-4, IL-10, sIL-6R, Treg+Th2, and TNF-α/TNF-R1 compared to control males. Increased sTNF-R1 was found in MDD males compared to MDD females. Positive correlations were found between CDI score and sIL-6R and IL-10 in the total group and between CDI score and IL-10 in adolescent males.

**Conclusion:** Our study for the first time extensively evaluated IRS and CIRS interactions revealing enhanced pro-inflammatory TNF-α signaling and IL-6 trans-signaling in association with increased IL-10- and IL-4-mediated anti-inflammatory activity in first-episode depression at the adolescent age. Moreover, results reflect the sex-specific simultaneous activation of IRS and CIRS pathways in adolescent depression.

**Keywords:** major depressive disorder, adolescent age, comprehensive cytokine analysis, neuroimmune interactions, sex differences

## Introduction

Major depressive disorder (MDD) represents a common mood disorder affecting approximately 300 million people worldwide[1] with increasing incidence, particularly at adolescent age.[2,3] Moreover, MDD is the largest mental health contributor to the disease burden in adolescents.[4] However, the response rates to the psycho- and pharmaco-therapy in adolescent depression are only modest,[5] thus a better understanding of the underlying pathophysiological mechanisms in MDD can contribute to personalized assessment and treatment of depression. Although various risk factors and etiological models in depression have been proposed, in the last few decades, the role of immune dysregulation as one of the most robust mechanisms contributing to MDD pathophysiology has been increasingly discussed.[6,7] The presence

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of the inflammatory process in depressive patients can be easily revealed by complete blood cells count reflecting cell blood composition including red blood cells (RBC), RBC distribution width (RDW), platelets, mean platelet volume, and white blood cells (WBC) with differential (eg neutrophils and lymphocytes) as one of the most widespread and frequently used laboratory tests in the clinical setting. More specifically, increased WBC as indicators of the overall immune system activity associated with neutrophilia have been repeatedly reported in adult and adolescent depressive patients compared to controls.[8–11] Further, measures of complete blood cells count can be used for the estimation of the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and RDW-to-platelet ratio – other parameters determined as markers of conditions associated with inflammation including depression.[11,12] Specifically, increased NLR and PLR have been the most replicated finding in adult and also adolescent MDD studies.[10–15] In summary, this hematological test can represent a helpful method for identifying the subgroup of depressive patients with "inflamed" depression.

Nowadays, the role of two tightly interconnected systems – inflammatory response system (IRS) and compensatory immune response system (CIRS) – is at the center of scientific interest in the inflammation–depression relationship according to their relevance for the activity of the specific parts of the immune system. However, the precise understanding of the IRS and/or CIRS dysregulation in MDD pathogenesis is just in the beginning.[16,17]

## Inflammatory Response System and Compensatory Immune Response System – Focus on the Adolescent Depression

Various immune cells including macrophages and T lymphocytes, and their effector cytokines seem to play a crucial role in IRS and CIRS pathways.[16] Macrophages can be through the macrophage polarization process discriminated into M1 macrophages whose activation leads to the secretion of pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-alpha (TNF-α), or into M2 macrophages releasing anti-inflammatory cytokines (eg IL-10).[18] T lymphocytes express on their surface T cell receptor and CD4 (ie helper T lymphocytes) or CD8 (ie cytotoxic T lymphocytes) glycoprotein. The helper T lymphocytes (Th) differentiate into multiple subsets characterized by distinct immune functions (eg Th1 cells release pro-inflammatory IL-2 and interferon-gamma (IFN-γ), Th2 cells release anti-inflammatory IL-4, Th17 release pro-inflammatory IL-17, and T regulatory (Treg) cells release anti-inflammatory IL-10).[19] Additionally, macrophages and T lymphocytes are bi-directionally modulated. While M1 macrophages promote Th1 responses, M2 macrophages drive the differentiation and recruitment of Th2 and Treg lymphocytes. On the other hand, cytokines released by T lymphocytes influence the macrophage polarization; the exposure of naïve monocytes to Th1 cytokines drives the macrophage polarization towards the M1 phenotype, while Th2 and Treg cytokines promote macrophage polarization towards the M2 phenotype.[20,21]

With respect to adult depression, IRS is characterized by mild chronic inflammation caused by M1 macrophages activation leading to increased production of TNF-α, IL-1β, IL-6, and soluble IL-6 receptor (sIL-6R) as well as enhanced Th1 and Th17 responses associated with increased production of IFN-γ, IL-2, and IL-17. Many meta-analyses confirmed that adult depression is accompanied by an immune-inflammatory response with increased levels of pro-inflammatory cytokines, acute phase proteins, and other components released by activated IRS.[22–27] On the other hand, MDD-linked CIRS components likely counter-regulating IRS effects involve the increased Th2 and Treg activity resulting in elevated production of anti-inflammatory cytokines IL-4 and IL-10, increased levels of soluble IL-1 receptor antagonist (sIL-1RA), soluble IL-2 receptor (sIL-2R), and soluble TNF receptors (sTNF-R1, sTNF-R2).[16,17,25] The main elements of IRS and CIRS pathways in MDD are presented in Figure 1.

Despite multiple studies evaluating the relationship between adolescent depression and pro-inflammatory/anti-inflammatory cytokines, the precise IRS- and CIRS-related knowledge is severely limited by the inconsistency of individual studies. More specifically, pro-inflammatory IL-2 and IFN-γ, as the cytokines showing case–control significant differences in most studies, have been increased,[28,29] decreased[30] or unchanged[31] in adolescent depressive patients compared to healthy controls. Further, pro-inflammatory IL-1β, IL-6, and TNF-α as the most frequently evaluated cytokines in multiple studies[30–38] have shown significant differences between depressive adolescents and controls (ie increased levels of these cytokines in depression) only in few of them.[33,37] Regarding anti-inflammatory

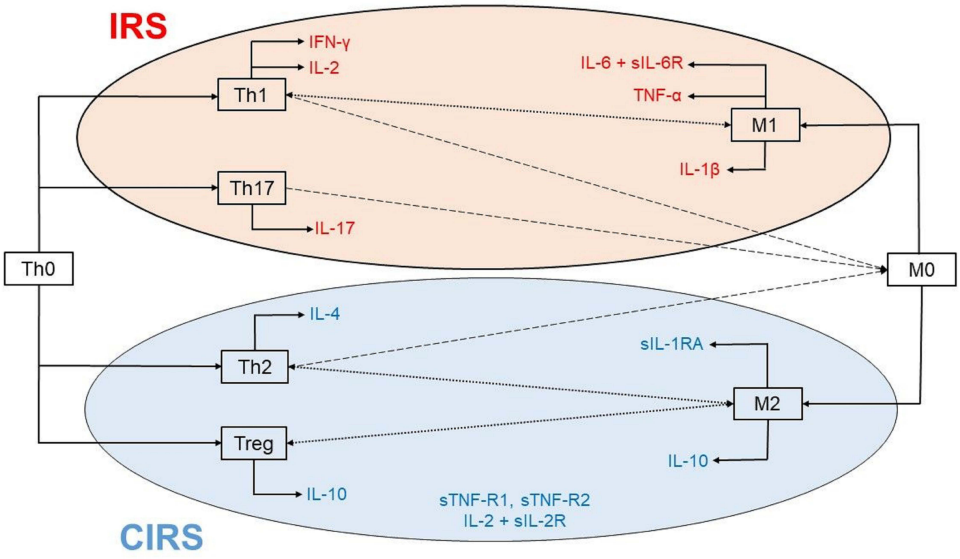


**Figure 1** Simplified schematic diagram of crucial immune cells and mediators involved in inflammatory response system (IRS) and compensatory immune response system (CIRS) pathways in major depression. IRS pathway includes: 1. activated M1 macrophages releasing interleukin (IL)-1β, IL-6 associated with soluble IL-6 receptor (sIL-6R), tumor necrosis factor α (TNF-α); 2. activated T helper 1 cells (Th1) releasing IFN-γ; and 3. activated Th17 cells releasing IL-17. CIRS pathway consists of: 1. activated M2 macrophages releasing IL-10; 2. activated Th2 cells releasing IL-4; and 3. activated T regulatory (Treg) cells releasing IL-10. Other markers involved in CIRS pathway include soluble cytokine receptors (sIL-2R, sTNF-R1, sTNF-R2) and soluble IL-1 receptor antagonist (sIL-1RA). Macrophages and T-lymphocytes interplay with each other and can drive their polarization. More specifically, Th cells (Th1, Th2, Th17) drive the polarization of M0 to M1 and/or M2 macrophages. Moreover, Th1 cells bi-directionally interact with M1, while Th2 and Treg cells with M2.

cytokines IL-4 and IL-10, no significant differences in IL-4 and/or IL-10,[28,31,39] increased levels of IL-4 and/or IL-10,[29,33] or decreased levels of IL-10[30] were found in adolescent depression compared to healthy controls. Similarly, regarding meta-analyses, D´Acunto et al[40] reported significantly increased levels of TNF-α with no differences in other included cytokines (ie, IL-1 β, IL-4, IL-6, IL-8, IL-10, and IFN-γ) in adolescent MDD patients, while more recent meta-analysis revealed significantly higher levels of IL-1β with no differences in the levels of the other cytokines (IL-2, IL-6, IFN-γ, and TNF-α) in depressive adolescents compared to healthy controls.[41] Moreover, a meta-analysis by Colasanto et al[42] reported positive associations between depression and IL-6 in adolescents, with IL-6 as a predictor of future depression. Lastly, no relationship between IL-1β, IL-2, IL-6, IL-10, IFN-γ, and TNF-α and pediatric internalizing disorders was observed in the meta-analysis by Howe and Lynch.[43] The heterogeneous findings in the relation to depression and inflammation can be influenced by various factors including genetics, medication use, and sex. Thus, further research in this area is needed to reveal the exact depression-linked immune alterations.

## Genetic Contribution in the Relationship Between Inflammation and Depression

The substantial heritable link between depression and inflammation[44] has determined a potential genetic substrate for "inflamed" depression. In this context, the most replicated and relevant gene variants involved in both depression and immune activation (according to the review by Barnes et al[45]) include polymorphisms in the genes for interleukin IL-1β,[46–48] IL-6,[48–50] IL-10,[51,52] TNF-α,[51,53] CRP,[54,55] monocyte chemoattractant protein-1,[56,57] and phospholipase A2.[58] However, it is important to note that even for the most replicated findings there are discrepant results in the immune effects of the genetic variants and the resulting effects on depression. For example, a polymorphism in the IL-1β promoter at position 511 has been associated with higher depressive symptoms severity whether the polymorphism is associated with increased IL-1β production (allele 511T) or decreased IL-1β production (allele 511C).[59–61] Similar findings have been reported with polymorphisms in TNF-α and CRP promoters.[62] Further, the genome-wide association analyses identified the 44 risk gene variants in MDD with 4 of them (namely *LACC1, OLFM4, TIAF1*, and *NR4A2*) being associated with immune responses.[63] Besides, a recent weighted gene co-expression network analysis (WGCNA) identified three genes related to immune responses (*IL1RAP, UBE2W, and UBE2D1*) as hub genes of adolescent

MDD.[64] Further research involving genome-wide association studies, transcriptomics and WGCNA is needed to exactly shed light on the genetic contributions of inflammation-related depression.

## The Effect of Treatment in the Relation Between Depression and Inflammation – the Focus on Antidepressants and Anti-Cytokines

Antidepressant treatment seems to complexly interact with IRS and CIRS pathways; the recent largest meta-analysis has reported significantly decreased levels of IL-6, TNF-α, and IL-10 in adult MDD patients after conventional antidepressant treatment.[25] More specifically, treatment by selective serotonin reuptake inhibitors (SSRIs) led to the reduction in levels of pro-inflammatory cytokines TNF-α, IL-6, and IL-1β[65,66] but also to the reduction in levels of anti-inflammatory cytokines IL-4 and IL-10.[67] Contrary, treatment by serotonin and noradrenaline reuptake inhibitors (SNRIs such as venlafaxine and duloxetine) seems to be associated with an increase in TNF-α[68] and IL-6 levels.[69] This difference between SSRIs and SNRIs can be explained by the known pro-inflammatory effect of noradrenaline.[70] Further, ketamine (a non-competitive N-methyl-D-aspartate glutamate antagonist) infusions as a "novel" antidepressant[71] have reduced the levels of pro-inflammatory cytokines IL-6 and TNF-α in MDD.[72] However, a simultaneous decrease in the levels of pro-inflammatory (IL-1β, IL-6, TNF-α) as well as anti-inflammatory cytokines (IL-4 and IL-10) in MDD after ketamine treatment has also been reported.[73] Regarding adolescent age, evaluation of the treatment effect on inflammation-related depression using antidepressants (SSRIs) has shown mixed results. Specifically, SSRIs have been repeatedly shown to reduce depressive symptoms and influence (increase or decrease) the levels of at least one cytokine.[33,74,75] However, the findings did not always align with the pro-inflammatory or anti-inflammatory nature of each cytokine. For example, while pro-inflammatory cytokine TNF-α has been reported to decrease post-treatment,[33,75] another pro-inflammatory cytokine IL-2 has been observed to increase.[30]

On the other hand, as currently available monoaminergic antidepressants are ineffective for over a third of MDD patients,[76] targeting inflammation may represent a promising way to develop novel antidepressants.[77] Monoclonal antibodies against pro-inflammatory cytokines as the most intriguing anti-inflammatory treatment for depression allow precise targeting of specific immune pathways and offer a way to personalized medicine. Regarding adult age, the treatment by cytokine inhibitors has been shown to improve depressive symptoms.[7] Specifically, TNF inhibitors such as etanercept or adalimumab and anti-IL-6 antibody sirukumab have been more efficacious compared to placebo in the reduction of depressive symptoms.[78–80] Further, the large meta-analysis by Kappelmann et al[81] reported that anti-cytokine treatments (adalimumab, etanercept and infliximab – TNF inhibitors, and tocilizumab – IL-6 inhibitor) significantly improved depressive symptom severity in patients with chronic inflammatory conditions resistant to antidepressant treatment. Regarding adolescent age, the effect of anti-cytokine agents in MDD patients has not been evaluated yet, however, the effect of the anti-inflammatory agent rosuvastatin influencing the cytokine levels has been estimated in adolescent MDD patients with no efficacy in lowering depressive symptoms or cytokine levels.[82] In summary, the effect of antidepressants on immune dysregulation as well as of anti-cytokines on depressive symptoms remains to be further explored, particularly at the adolescent age.

## Sex Differences in the Relationship Between Depression and Inflammation

Depression is twice as common in females compared to males.[83] This sex gap emerges around age 13 with increasing magnitude across adolescence and persisting through the rest of the lifespan.[84] Moreover, females are also more susceptible to autoimmune disorders.[85] Given mounting evidence that females are at a greater risk for depression and show increased inflammatory activity, it is critical to examine sex differences in the inflammation-depression relationship. However, the findings regarding the role of sex in the relationship between inflammation and depression in adults are mixed. For example, a stronger relationship between inflammatory markers (eg IL-6) and depressive symptoms was found in males compared to females.[86] Conversely, females receiving interferon-alpha showed greater increases in depressive mood compared to males.[87] Besides, no sex differences were found in the relationship between inflammation and depression[88,89] Notably, although already adolescent females are identified as particularly vulnerable to

inflammation-linked depression,[29] to the best of our knowledge, the findings reflecting the role of sex in the relationship between inflammation and first-episode depression prior to pharmacotherapy at the adolescent age are missing.

This study aimed to 1) evaluate IRS and CIRS pathways by assessment of various cytokines and soluble IL receptors in association with cytokine profiles during the acute phase of the first-episode drug-naïve MDD patients at adolescent age, 2) assess IRS/CIRS ratios as there is an almost complete lack of information on IRS versus CIRS activity in adolescent depression, 3) evaluate IRS and CIRS indices in adolescent females (MDD vs controls), in adolescent males (MDD vs controls), and in adolescent MDD females vs adolescent MDD males. To the best of our knowledge, this is the first study comprehensively assessing IRS versus CIRS balance in adolescent depression, with respect to sex.

# Materials and Methods

## Subjects

We have examined 100 MDD adolescent patients (average age: 15.4±1.2 years, BMI: 20.5±0.3 kg/m$^2$, 40 males – average age: 15.6±0.2 years, BMI: 20.2±0.4 kg/m$^2$; 60 females – average age: 15.4±0.2 years, BMI: 20.6±0.32 kg/m$^2$) and 60 controls (average age: 15.3±1.5 years, BMI: 19.9±0.3 kg/m$^2$, 28 males – average age: 15.1±0.3 years, BMI: 19.8 ±0.4 kg/m$^2$; 32 females – average age: 15.4±0.3 years, BMI: 19.9±0.4 kg/m$^2$). The patients suffering from first-episode MDD were recruited from the inpatients admitted to the Psychiatric Clinic of Jessenius Faculty of Medicine and University Hospital in Martin and were examined prior to the pharmacotherapy during the first days of hospitalization. The diagnosis of MDD without other comorbid psychiatric disorders (eg attention-deficit/hyperactivity disorder, anxiety disorders) was classified by a thorough clinical investigation based on an unstructured diagnostic interview by a staff child/adolescent psychiatrist according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5[90]). The control participants have never been treated for any mental disorder. Participants included in both groups were free of acute and chronic infections, allergies, endocrine- or immune-related diseases, cancer, and systemic diseases, as determined by self-report or doctor's report. Patients and controls with a history of antidepressant or antipsychotic treatment, immunomodulatory treatment, analgesic/anti-inflammatory use, antibiotic therapy, or substance abuse (alcohol, marijuana, heroin, cocaine, or methamphetamine) were also excluded. All participants filled the Children's Depression Inventory (CDI) to detect the presence and severity of depressive symptoms.[91]

## Blood Analysis

Fasting peripheral venous blood samples (5 mL) were collected into EDTA test tubes by venipuncture in the morning. Consequently, the blood samples were centrifuged for 15 minutes at 2500 rpm and 4°C (refrigerated centrifugation, Hettich Universal 320R, Tuttlingen, Germany), and the obtained plasma was kept frozen at −80°C until the analysis. Selected cytokines (interleukins - IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IFN-γ, TNF-α – analytes involved in Cytokine array I), soluble cytokine receptors (sIL-6R, sTNF-R1, sTNF-R2) – analytes involved in Cytokine array IV were analyzed and quantified using a biochip array technology (Evidence Investigator, Randox, United Kingdom). Biochip array technology is a precision multiplex immunoassay-based testing platform allowing for the simultaneous quantitative detection of a wide range of analytes from a single sample. Plasma assays were provided in a separate kit from Randox Laboratories Ltd. (United Kingdom). Each kit (Cytokine array I – CTK I and Cytokine array IV – CTK IV) contains test-biochip cartridges, calibrators, assay diluent, conjugate, wash buffer, and signal reagent (luminol and peroxide, 1:1). Quality Controls (CTK I controls and CTK IV controls) were used for each run validation. There are 3 levels of quality control – low (level 1), medium (level 2), and high (level 3). A nine-point calibration was performed for each assay series as recommended. After the addition of 100 µL of plasma samples to the biochip, analytes present in the sample bind to the specific biochip-bound ligands. The degree of binding is determined using a chemiluminescent light source and quantified using a Charge Coupled Device camera and imaging system.

Consequently, z-unit weighted composite scores were calculated from raw cytokines and soluble cytokine receptors data (in logarithmic transformation according to)[92] by using formula: $Z = (x - M)/SD$,[93] where x=proband´s raw cytokine or soluble cytokine receptor level, M=mean cytokine or soluble cytokine receptor level in combined group (ie depressive and control group together), and SD=standard deviation of cytokine or soluble cytokine receptor level in combined

group. These standardized data were used to assess indices reflecting individual IRS and/or CIRS activation profiles and ratios,[16,94] namely: IL-6 trans-signaling = zIL-6+zsIL-6R, M1 signaling = zIL-1β+zIL-6+zTNF-α, Th1 profile = zIL-2 +zIFN-γ, Treg+Th2 profile = zIL-10+zIL-4, allIRS profile (ie indicants of adolescent depression-linked immune-inflammatory activation[41]) = zIL-1β+zIL-2+zIL-6+zsIL-6R+zTNF-α+zIFN-γ, allCIRS profile (ie all indicants of immune-regulatory activation) = zsTNF-R1+zsTNF-R2+zIL-4+zIL-10, TNF-α/sTNF-R1 ratio = zTNF-α–zsTNF-R1, TNF-α/sTNF-R2 ratio = zTNF-α–zsTNF-R2, TNF-α/sTNF-Rs ratio = zTNF-α–z(zsTNF-R1+zsTNF-R2), Th1/Th2 ratio = z(zIFN-γ+zIL-2)–zIL-4, Th1/Treg ratio = z(zIFN-γ+zIL-2)–zIL-10, Th1/Th2+Treg ratio = z(zIFN-γ+zIL-2)–z (zIL-4+zIL-10), M1/Th2 ratio = z(zIL-1β+zIL-6+zTNF-α)–zIL-4, M1/Treg ratio = z(zIL-1β+zIL-6+zTNF-α)–zIL-10, M1/Treg+Th2 ratio = z(zIL-1β+zIL-6+zTNF-α)–z(zIL-10+zIL-4), allIRS/allCIRS ratio = z(zIL-1β+zIL-2+zIL-6+zsIL-6R+zTNF-α+zIFN-γ)–z(zsTNF-R1+zsTNF-R2+zIL-4+zIL-10) - a subtraction was performed as these are log-transformed values.[16,94]

## Statistical Analysis

The data were explored and analyzed in Jamovi version 1.2.27 (Sydney, Australia). The Shapiro–Wilk normality test was used for the evaluation of data distributions (Gaussian/non-Gaussian). The analysis of variance (ANOVA) was used to test the effects of group and sex of Gaussian-distributed IRS and CIRS-related data (IL-6 trans-signaling, Th1 profile, allIRS, allCIRS, TNF-α/sTNF-R2, TNF-α/sTNF-Rs, Th1/Treg, M1/Th2, M1/Treg, M1/Treg+Th2, allIRS/allCIRS) and CDI. Next, the statistical test of the main effect was followed by post hoc pairwise comparisons between the groups and sexes, which was corrected by the Bonferroni method used to adjust probability (p) values when performing multiple statistical tests in any context. The non-parametric (Kruskal–Wallis) ANOVA was used for the between-groups and between-sexes comparisons for non-Gaussian distributed IRS and CIRS-related data (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IFN-γ, TNF-α, sIL-6R, sTNF-R1, sTNF-R2, TNF-α/sTNF-R1, Treg+Th2 profile, Th1/Th2, Th1/Treg+Th2, M1 signaling). Gaussian distributed data were expressed as mean ± SEM and non-Gaussian distributed data as median (interquartile ranges). Besides, the associations between CDI score and IRS and CIRS indices were analyzed using Spearman´s rank-order correlation test. A value of $p \leq 0.05$ (two-tailed) was considered statistically significant.

# Results

## Evaluation of Selected Cytokines, Soluble Cytokine Receptors, Profiles, and Ratios

The main effect of group evaluated by ANOVA was significant for allIRS profile ($F_{[4]} = 48.60$, p=0.045), allCIRS profile ($F_{[10]}$=24.40, p=0.002), and TNF/TNFRs ratio ($F_{[4]} = 9.68$, p=0.029).

A post hoc pairwise comparison with the Bonferroni adjusted p revealed significantly higher allIRS and allCIRS profiles and TNF/TNFRs ratio in MDD adolescents compared to controls (p=0.045, p=0.002, p=0.029, respectively). The main effect of group was without significant changes for IL-6 trans-signaling, Th1 profile, TNF/TNF-R2, M1/Treg, M1/Th2, M1/Treg+Th2, Th1/Treg, and allIRS/allCIRS ratios (p=0.304, p=0.800, p=0.523, p=0.710, p=0.268, p=0.973, p=0.051, and p=0.908, respectively).

The non-parametric ANOVA revealed significantly increased IL-4, IL-10, TNF-α, sIL-6R, and Treg+Th2 profile in adolescent MDD patients compared to controls (p=0.046, p<0.001, p=0.002, p=0.004, and p<0.001; respectively), and significantly decreased Th1/Treg+Th2 ratio in MDD adolescents compared to controls (p=0.011). No significant between-groups changes were found for IL-1α, IL-1β, IL-2, IL-6, IL-8, IFN-γ, sTNF-R1, sTNF-R2, M1 signaling, TNF-α/sTNF-R1 ratio, and Th1/Th2 ratio (p=0.774, p=0.237, p=0.880, p=0.319, p=0.087, p=0.161, p=0.400, p=0.065, p=0.054, p=0.159, and p=0.496, respectively). All results are summarized in Table 1.

## Evaluation of Sex Differences in IRS and CIRS Activity

The main effect of sex evaluated by ANOVA was significant for Th1/Treg ($F_{[4]}$=8.42, p=0.035) and allIRS/allCIRS ($F_{[7]}$ =51.17, p=0.009) ratios. The main effect of sex was without significant changes for IL-6 trans-signaling, Th1 profile, allIRS profile, allCIRS profile, TNF/TNF-Rs ratio, TNF/TNF-R2 ratio, M1/Treg ratio, M1/Th2 ratio, and M1/Treg+Th2

**Table 1** Evaluated Cytokines, Soluble Cytokine Receptors, IRS and CIRS-Related Profiles, and IRS vs CIRS Activity Ratios

| Evaluated Indices | IRS, CIRS, IRS vs CIRS Function | Controls | | | MDD | | | p-value | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total[a] (N=60) | Females[b] (N=32) | Males[c] (N=28) | Total[d] (N=100) | Females[e] (N=60) | Males[f] (N=40) | a vs d | b vs e | c vs f | e vs f |
| **Cytokines** | | | | | | | | | | | |
| **IL-1α (pg/mL)** | IRS | 0.26 (0.19–0.35) | 0.27 (0.20–0.35) | 0.25 (0.18–0.33) | 0.26 (0.19–0.40) | 0.25 (0.19–0.33) | 0.26 (0.21–0.51) | 0.774 | 0.531 | 0.278 | 0.229 |
| **IL-1β (pg/mL)** | IRS | 1.17 (0.87–1.93) | 1.39 (0.96–2.12) | 1.09 (0.80–1.81) | 1.37 (1.00–2.04) | 1.41 (1.02–2.17) | 1.21 (0.98–1.69) | 0.237 | 0.517 | 0.428 | 0.216 |
| **IL-2 (pg/mL)** | IRS | 2.50 (1.50–5.22) | 2.88 (1.73–5.26) | 2.22 (1.41–3.31) | 2.34 (1.60–4.06) | 2.60 (1.82–4.06) | 1.98 (1.49–3.93) | 0.880 | 0.612 | 0.945 | 0.195 |
| **IL-4 (pg/mL)** | CIRS | 2.08 (1.84–2.73) | 2.29 (1.87–2.79) | 1.98 (1.80–2.41) | 2.34 (2.02–2.67) | 2.33 (2.01–2.82) | 2.36 (2.07–2.63) | **0.046** | 0.354 | **0.044** | 0.827 |
| **IL-6 (pg/mL)** | IRS | 0.79 (0.63–1.24) | 0.84 (0.69–1.29) | 0.69 (0.57–1.14) | 0.92 (0.69–1.19) | 0.98 (0.76–1.31) | 0.88 (0.63–1.07) | 0.319 | 0.594 | 0.345 | 0.132 |
| **IL-8 (pg/mL)** | IRS | 3.99 (3.04–5.91) | 3.89 (2.83–5.91) | 4.05 (3.59–5.58) | 3.31 (2.58–5.21) | 3.47 (2.51–5.10) | 3.22 (2.63–5.38) | 0.087 | 0.319 | 0.126 | 0.872 |
| **IL-10 (pg/mL)** | CIRS | 0.77 (0.58–0.99) | 0.79 (0.64–0.94) | 0.70 (0.54–1.02) | 0.95 (0.70–1.44) | 0.99 (0.68–1.49) | 0.94 (0.73–1.31) | **<0.001** | **0.017** | **0.007** | 0.952 |
| **IFN-γ (pg/mL)** | IRS | 0.42 (0.28–0.56) | 0.46 (0.31–0.66) | 0.34 (0.21–0.53) | 0.45 (0.30–0.79) | 0.44 (0.28–0.82) | 0.45 (0.31–0.76) | 0.161 | 0.993 | 0.052 | 0.759 |
| **TNF-α (pg/mL)** | IRS | 2.46 (2.00–2.96) | 2.29 (1.78–2.83) | 2.74 (2.21–3.14) | 2.89 (2.29–3.42) | 2.84 (2.16–3.19) | 3.12 (2.63–3.47) | **0.002** | **0.006** | 0.074 | 0.064 |
| **Soluble cytokine receptors** | | | | | | | | | | | |
| **sIL-6R (ng/mL)** | IRS | 0.91 (0.52–1.42) | 0.98 (0.57–1.58) | 0.90 (0.46–1.36) | 1.33 (0.87–1.80) | 1.34 (0.91–1.81) | 1.32 (0.65–1.79) | **0.004** | 0.062 | **0.035** | 0.616 |
| **sTNF-R1 (ng/mL)** | CIRS | 0.48 (0.39–0.59) | 0.42 (0.37–0.55) | 0.54 (0.44–0.69) | 0.49 (0.44–0.57) | 0.48 (0.42–0.55) | 0.51 (0.47–0.63) | 0.400 | 0.198 | 0.956 | **0.015** |
| **sTNF-R2 (ng/mL)** | CIRS | 0.44 (0.23–0.69) | 0.38 (0.22–0.69) | 0.48 (0.27–0.69) | 0.54 (0.38–0.73) | 0.52 (0.37–0.70) | 0.59 (0.47–0.77) | 0.065 | 0.178 | 0.177 | 0.156 |
| **Profiles** | | | | | | | | | | | |
| **IL-6 trans signaling** | IRS | −0.20±0.23 | −0.27±0.21 | −0.10±0.49 | 0.23±0.20 | 0.43±0.27 | −0.08±0.28 | 0.304 | 0.656 | 0.998 | 0.997 |
| **M1 signaling** | IRS | −0.52 (−2.17–1.36) | −0.42 (−1.76–1.59) | −1.80 (−2.32–0.22) | 0.06 (−1.52–1.43) | 0.24 (−1.35–1.85) | −0.11 (−1.51–1.09) | 0.054 | 0.309 | 0.086 | 0.421 |
| **Th1 profile** | IRS | −0.03±0.23 | 0.39±0.30 | −0.47±0.34 | 0.04±0.16 | 0.07±0.21 | −0.01±0.24 | 0.800 | 0.998 | 0.997 | 0.996 |

(*Continued*)


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**Table 1** (Continued).

| Evaluated Indices | IRS, CIRS, IRS vs CIRS Function | Controls | | | MDD | | | p-value | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total[a] (N=60) | Females[b] (N=32) | Males[c] (N=28) | Total[d] (N=100) | Females[e] (N=60) | Males[f] (N=40) | a vs d | b vs e | c vs f | e vs f |
| **Treg+Th2** | CIRS | −0.36 (−1.34–0.24) | −0.17 (−1.09–0.53) | −0.74 (−1.74– − 0.23) | 0.28 (−0.80–1.21) | 0.22 (−0.97–1.33) | 0.28 (−0.54–0.80) | **<0.001** | 0.074 | **<0.001** | 0.549 |
| **allIRS** | IRS | −1.04±0.46 | −0.38±0.56 | −1.80±0.74 | 0.17±0.36 | 0.43±0.48 | −0.21±0.55 | **0.045** | 0.996 | 0.449 | 0.998 |
| **allCIRS** | CIRS | −0.57±0.25 | −0.56±0.35 | −0.58±0.37 | 0.30±0.15 | 0.22±0.20 | 0.41±0.23 | **0.002** | 0.230 | 0.111 | 0.997 |
| **Ratios** | | | | | | | | | | | |
| **TNF-α/ sTNF-Rs** | IRS vs CIRS | −0.53±0.24 | −0.46±0.33 | −0.62±0.34 | 0.05±0.14 | 0.12±0.17 | −0.05±0.23 | **0.029** | 0.598 | 0.852 | 0.998 |
| **TNF-α/ sTNF-R1** | IRS vs CIRS | −0.19 (−0.58–0.38) | 0.03 (−0.45–0.72) | −0.47 (−0.75– −0.09) | 0.04 (−0.48–0.48) | −0.01 (−0.36−0.49) | 0.07 (−0.58–0.48) | 0.159 | 0.970 | **0.047** | 0.823 |
| **TNF-α/ sTNF-R2** | IRS vs CIRS | 0.06±0.17 | 0.03±0.25 | 0.09±0.23 | 0.16±0.09 | 0.12±0.12 | 0.23±0.14 | 0.523 | 0.979 | 0.958 | 0.950 |
| **Th1/Th2** | IRS vs CIRS | −0.15 (−1.05–0.99) | 0.47 (−1.03–1.23) | −0.23 (−1.18–0.68) | −0.36 (−1.23–0.69) | −0.27 (−0.94–0.70) | −0.44 (−1.23–0.67) | 0.496 | 0.369 | 0.958 | 0.998 |
| **Th1/Treg** | IRS vs CIRS | 0.28±0.21 | 0.67±0.32 | −0.15±0.25 | −0.18±0.13 | −0.11±0.17 | −0.28±0.22 | 0.051 | 0.085 | 0.996 | 0.998 |
| **Th1/Treg +Th2** | IRS vs CIRS | 0.10 (−0.73–1.40) | 0.57 (−0.77–1.51) | −0.03 (−0.69–0.74) | −0.49 (−0.73–1.40) | −0.48 (−1.25–0.60) | −0.61 (−1.24–0.78) | **0.011** | 0.053 | 0.076 | 0.784 |
| **M1/Th2** | IRS vs CIRS | −0.52±0.31 | −0.42±0.37 | −0.63±0.52 | −0.04±0.21 | 0.24±0.27 | −0.47±0.34 | 0.268 | 0.997 | 0.998 | 0.729 |
| **M1/Treg** | IRS vs CIRS | 0.03±0.30 | 0.18±0.35 | −0.17±0.53 | 0.18±0.22 | 0.30±0.27 | −0.01±0.37 | 0.710 | 0.995 | 0.992 | 0.998 |
| **M1/Treg +Th2** | IRS vs CIRS | 0.11±0.30 | 0.00±0.32 | −0.22±0.48 | 0.01±0.20 | 0.09±0.26 | −0.28±0.28 | 0.973 | 0.998 | 0.997 | 0.998 |
| **allIRS/ allCIRS** | IRS vs CIRS | −0.48±0.38 | 0.04±0.50 | −1.13±0.54 | −0.35±0.33 | 0.30±0.43 | −1.27±0.47 | 0.908 | 0.998 | 0.998 | 0.072 |

**Notes**: Values are expressed as mean±SEM for Gaussian-distributed data and as median (interquartile ranges) for non-Gaussian distributed data. p-value ≤ 0.05 (in bold) is considered statistically significant. [a]Control group; [b]Control adolescent females; [c]Control adolescent males; [d]Depressive group; [e]Depressive adolescent females; [f]Depressive adolescent males.

**Abbreviations**: IRS, inflammatory response system; CIRS, compensatory immune response system; MDD, major depressive disorder; IL, interleukin; IFN-γ, interferon-gamma; TNF-α, tumor necrosis factor-alpha; sIL-6R, soluble interleukin-6 receptor; sTNF-R1, soluble tumor necrosis factor receptor 1; sTNF-R2, soluble tumor necrosis factor receptor 2; M1, macrophages M1; Th1, T helper cells 1; Treg, T regulatory cells; Th2, T helper cells 2; allIRS, all indices involved in the adolescent depression-linked inflammatory response system; allCIRS, all indices involved in the compensatory immune response system; sTNF-Rs, soluble tumor necrosis factor receptors (ie sTNF-R1+sTNF-R2).

ratio (p=0.617, p=0.082, p=0.084, p=0.769, p=0.522, p=0.613, p=0.381, p=0.214, and p=0.377, respectively). The post hoc pairwise comparison revealed no significant changes in any of the above-mentioned indices between adolescent females and males.

The non-parametric ANOVA revealed significantly increased IL-10 and TNF-α in adolescent MDD females compared to control females (p=0.017 and p=0.006, respectively). On the other hand, adolescent MDD males had significantly increased IL-4, IL-10, sIL-6R, Treg+Th2 profile, and TNF-α/TNF-R1 ratio compared to control males (p=0.044, p=0.007, p=0.035, p<0.001, and p=0.047; respectively). Lastly, significantly increased sTNF-R1 was found in adolescent MDD males compared to adolescent MDD females (p=0.015). All results are summarized in Table 1.

## CDI Analysis and Correlation Between Depressive Symptoms and IRS and CIRS Indices

The main effect of the group evaluated by ANOVA was significant for the CDI score ($F_{[364]}$ = 16,093.70, p<0.001). The main effect of sex evaluated by ANOVA was not significant for CDI score (females vs males = 16.50±1.34 vs 16.10 ±1.64, p=0.300). The post hoc pairwise comparison revealed significantly increased CDI score in adolescent MDD patients compared to controls (25.10±0.89 vs 3.75±0.45, p<0.001), significantly increased CDI score in adolescent MDD females compared to adolescent control females (24.30±1.21 vs 3.78±0.62, p<0.001), and significantly increased CDI score in adolescent MDD males compared to adolescent control males (26.50±1.24 vs 3.71±0.66, p<0.001).

Correlation analysis in the total group (depressive group and control group) revealed significant positive correlations between CDI score and IL-10 and sIL-6R (r=0.167, p=0.041; r=0.163, p=0.050; respectively, Figure 2A). Further, significant positive correlations were found between CDI score and IL-10 (r=0.306, p=0.017) in adolescent males (Figure 2B). No significant correlations were found between the CDI score and adolescent females.

# Discussion

This study for the first time such extensively investigated IRS and CIRS indices, profiles, and ratios in depressive adolescents. The main significant results can be summarized as follows: 1) increased levels of TNF-α and sIL-6R associated with increased allIRS profile indicating IRS activation, 2) increased TNF-α/sTNF-Rs ratio reflecting enhanced IRS-linked TNF-α signaling, 3) increased levels of IL-10 and IL-4 associated with increased Treg+Th2 and allCIRS profiles indicating CIRS activation, 4) decreased Th1/Treg+Th2 ratio reflecting enhanced CIRS activity, 5) increased IL-10 and TNF-α in adolescent MDD females compared to adolescent control females, 6) increased IL-4, IL-10, sIL-6R, Treg+Th2 profile, and TNF-α/TNF-R1 ratio in adolescent MDD males compared to adolescent control males, 7) increased sTNF-R1 in adolescent MDD males compared to adolescent MDD females, and 8) positive correlations between CDI score and sIL-6R and IL-10 indicating that more severe depressive symptoms are associated with enhanced

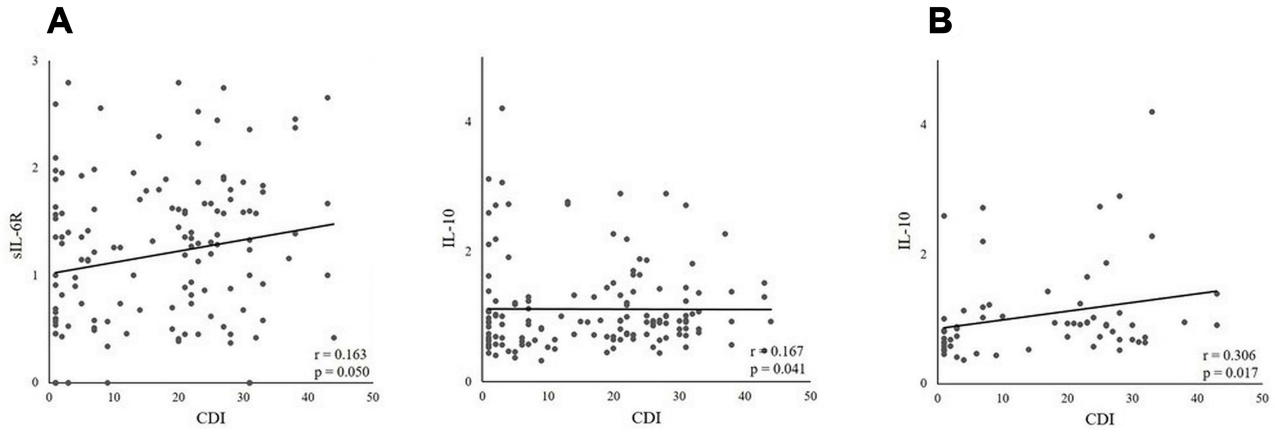


**Figure 2** Correlation analysis. (**A**) Positive correlations between depressive symptoms and sIL-6R and IL-10 in the total group; (**B**) Positive correlations between depressive symptoms and IL-10 in adolescent males.

pro-inflammatory IL-6 trans-signaling and anti-inflammatory IL-10 signaling in adolescent depression. Importantly, adolescent males showed positive correlations between CDI score and IL-10, while no correlations between CDI score and evaluated indices were found in adolescent females. Our results indicate discrete abnormalities in both, IRS and CIRS, pathways associated with sex-specific differences in drug-naïve depressive patients already at adolescent age. Several mechanisms are supposed.

## Adolescent Depression-Linked IRS and CIRS (Dys)regulation

Multiple immune cells and immune effector molecules ensure the maintenance of IRS/CIRS functional homeostasis (ie IRS and CIRS represent components of the integrated immune-signaling system with CIRS counter-regulating the IRS effects). While IRS exerting pro-inflammatory effects consists of activated M1 cells (IL-1 + IL-6 + TNF-α), Th1 cells (IL-2 + IL-12 + IFN-γ), and Th17 cells (IL-17 + IL-6), CIRS exerting anti-inflammatory effects constitutes from activated Th2 cells (IL-4), Treg cells (IL-10 + transforming growth factor beta), and cytokine receptors (eg sIL-1RA, sIL-2R, sTNF-Rs).[17] Disruption of the IRS/CIRS balance (eg M1/M2, Th1/Th2 balance) caused by exogenous adverse conditions such as psychosocial stress can lead to the chronic inflammatory response.[17,20] Dysregulated IRS/CIRS axes seem to play a crucial role in the complex adult MDD immunopathology influencing the onset of depression, number of depressive episodes as well as recovery.[17] Importantly, the depression rate dramatically increases from childhood into young adulthood,[3] thus, the knowledge about the IRS and CIRS interactions and depression at the adolescent age can be crucial.[95] However, the number of IRS and CIRS studies at this age period is limited.

In line with a recent systematic review and meta-analysis of cytokines in children and adolescent depression,[40] our results confirmed increased peripheral levels of TNF-α in depressive adolescents. The cytokine TNF-α plays a key role in the inflammatory response. Increased TNF-α levels may drive differentiation of monocytes, T-cells proliferation, activation of IL-1 and IL-6 production as well as acute phase response.[96] Moreover, besides its pro-inflammatory effects, TNF-α can also promote modifications of neuronal synaptic transmission.[97] These properties are mediated through two specific TNF receptors (TNF-R1 and TNF-R2) expressed on the cell surfaces.[98] The TNF receptors can also be found in the circulation as the soluble TNF receptors (sTNF-R1 and sTNF-R2) predominantly generated by proteolytic cleavage of TNF receptors by the TNF-α-converting enzyme (TACE, also called a disintegrin and metalloprotease 17 (ADAM 17)).[99] Multiple inflammatory signals (eg IL-2, IL-6, IL-10, TNF-α itself) may cause the shedding of TNF receptors. These soluble receptors acting as decoy receptors bind TNF-α and thus attenuate TNF-α signaling resulting in inhibition of TNF-α effects.[100] From this perspective, the meta-analysis by Köhler et al[25] reported elevated sTNF-R2 plasma levels in adult depression. In our study, there was a tendency to an increase in sTNF-R2 plasma levels in adolescent MDD compared to controls. These findings may contribute to the assumption that increased sTNF-R2 levels may counter-regulate enhanced TNF-α-mediated pro-inflammatory effects through the negative regulatory feedback.[16] Importantly, our study for the first time evaluated TNF-α/TNF-α receptors ratios (ie TNF-α/sTNF-R1, TNF-α/sTNF-R2, and TNF-α/sTNF-Rs) in depressive adolescents compared to controls with significantly increased TNF-α/sTNF-Rs indicating dominant pro-inflammatory TNF-α signaling during the acute phase of depression in adolescent drug-naïve patients.

Further, increased TNF-α may enhance the production of another important cytokine, IL-6, characterized by bimodal immune activity mediated through two separate pathways (classical signaling and trans-signaling). In the classical signaling pathway, IL-6 binds to membrane-bound receptor (IL-6R), the complex of IL-6 and IL-6R associates with a second protein (glycoprotein – gp130) inducing dimerization and initiation of intracellular signaling. Similar to TNF receptors, IL-6R can be proteolytically cleaved from the cell membrane by the metalloproteinase ADAM17 to generate a soluble form (sIL-6R).[101] IL-6 bound to sIL-6R forms a complex that can associate with gp130 to initiate signal transduction, this process is known as trans-signaling.[102] While IL-6 classical signaling contributes to anti-inflammatory properties of this cytokine, IL-6 trans-signaling is reported to be pro-inflammatory.[103] It is important to note that IL-6 signaling can be upregulated by increased sIL-6R levels alone, without a change in IL-6 levels.[104] In this way, our results of higher levels of sIL-6R are in accordance with the studies indicating enhanced IL-6 trans-signaling and thus pro-inflammatory activity in adult depression,[105–107] and extend them into adolescent age.

Contrary to pro-inflammatory responses, a predominant CIRS-linked anti-inflammatory effect in depression may occur when naïve T helper (Th0) cells differentiate into Treg and/or Th2 cells producing IL-10 and IL-4. The cytokine

IL-10 signals through a receptor complex (IL-10R alpha and two IL-10R beta subunits) and regulates the activity of monocytes, macrophages, and Th1 cells resulting in the release suppression of pro-inflammatory cytokines such as TNF-α, IFN-γ, IL-1β, IL-6.[108,109] Similarly, another cytokine with anti-inflammatory properties, IL-4, signals through two different receptor complexes (a type I receptor expressed on hematopoietic cells or a type II receptor expressed on non-hematopoietic cells). IL-4 mediates the alternative activation of M2 macrophages attenuating the pro-inflammatory activity by the release of transforming growth factor-beta, sIL-1RA, and IL-10.[16,110] Therefore, IL-10 and IL-4 seem to play a crucial role in the control of the inflammatory responses. Our results of increased IL-10 and IL-4 plasma levels are in accordance with the adult MDD studies,[25,111] and extend this finding into the adolescent age. Moreover, our study revealed increased Treg+Th2 and allCIRS profiles in association with decreased Th1/Treg+Th2 ratio confirming enhanced CIRS activity during the acute phase of depression in adolescent patients.

Taken together, abnormalities in the plasma levels of selected cytokines, soluble cytokine receptors in association with altered selected cytokine profiles and ratios provide the evidence for simultaneous signs of IRS and CIRS pathways activation in depression at adolescent age. In addition, contrary to an assumption that during the acute phase of mood disorder in adult patients the IRS is more active compared to CIRS,[112] our study revealed concurrent activation of both, IRS and CIRS confirmed by the unchanged IRS/CIRS ratio during the acute phase of depression in adolescent patients. Further, our study revealed the association between increased severity of depressive symptoms (based on the total CDI score) and IRS as well as CIRS biomarkers (ie increased sIL-6R and IL-10) in depressive adolescents. This finding is in accordance with adult MDD studies (eg[106,113]) and extends this knowledge into the adolescent age.

With regard to sex, this study identified different responses of IRS- and CIRS-related indices between females and males. Specifically, adolescent MDD females had increased levels of TNF-α and IL-10 compared to control females, reflecting enhanced TNF-α signaling counter-balanced by heightened anti-inflammatory IL-10 signaling. This result is partially in accordance with the finding of the positive association between TNF-α and depression in adult MDD females.[114] Similarly, increased TNF-α responses in females induced by a low-dose endotoxin administration were associated with mood disturbances.[115] In the same way, other pro-inflammatory markers such as CRP and IL-6 were associated with an increased risk of depression among females.[9,116–118] On the other hand, adolescent MDD males showed increased IL-4, IL-10, sIL-6R, Treg+Th2 profile, and TNF-α/sTNF-R1 ratio compared to control males, indicating increased pro-inflammatory, but particularly more enhanced anti-inflammatory activity. These findings are partially in accordance with increased pro-inflammatory activity in adult depressive males[119,120] and extend them into adolescent age. In contrast, other studies reported no association between inflammation and depression in adult males.[115,118] Moreover, sTNF-R1 was significantly increased in adolescent MDD males compared to adolescent MDD females and the correlation of IL-10 with the severity of depressive symptoms was found only in adolescent males. Thus, sex-specific differences found in this study could be related to immune variability across the lifespan from childhood through adolescence into adulthood.[121] Specifically for adolescence, this crucial developmental period is associated with a decline in lymphatic tissue accompanied by the reduction of circulating lymphocytes, partially initiated by the puberty-onset release of sex hormones. In this context, estrogen and androgen receptors are expressed on all major components of the innate and adaptive immune systems.[122,123] With respect to sex hormones-related immunomodulation, testosterone appears to attenuate inflammation as it decreases pro-inflammatory cytokines,[124] while estrogen may exert biphasic (ie pro-inflammatory and anti-inflammatory) effects.[125] Altogether, our findings are the first complexly exploring the sex-specific IRS and CIRS association in adolescent depression and can serve as preliminary evidence of the sex-dependent relationship among adolescent depression and cytokines, soluble cytokine receptors, cytokine profiles and IRS/CIRS activity ratios. However, the convergence impact of biological sex, sex hormones, and peripheral immunity on the mood in adolescents remains to be elucidated.

## Communication Between Peripheral Cytokine Activity and the Brain – The Role of Neuroimmune Interactions in Depression

Some peripheral cytokines can cross the blood–brain barrier (BBB) to act on astrocytes, microglia, and neurons throughout the brain contributing to the normal neurophysiological processes (neuronal differentiation, astrocyte

proliferation, etc.).[126] Peripheral cytokines can reach the brain through three major pathways: 1) humoral – cytokines access the brain through leaky regions in BBB such as the circumventricular organs,[127] 2) neural – activation of cytokine receptors on afferent vagal nerve fibers transducing cytokine signals to the brain,[128] and 3) cellular – chemokines released by activated microglia and adhesion molecules expressed in the central nervous system (CNS) can attract activated peripheral cells (monocytes and T cells) to the meninges and brain parenchyma.[129]

Notably, persistent peripheral inflammation can disrupt BBB leading to enhanced BBB permeability.[130] In turn, peripheral pro-inflammatory cytokines can more easily cross BBB to reach the brain leading to the activation of microglia. Microglial activation can then lead to the production of glutamate, reactive oxygen species, nitric oxide, proteases, as well as pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α. Excess and/or prolonged inflammatory cytokine activity within the brain may result in disturbed neurotransmitter metabolism, neuroendocrine function, neurogenesis/apoptosis, mitochondrial biogenesis, and neural plasticity affecting cognitive, emotional, and behavioral attributes associated with depression.[131–136]

Further, peripheral pro-inflammatory cytokines activate the enzyme indoleamine-2,3-dioxygenase (IDO), an important immunoregulator, breaking down the tryptophan into kynurenine (KYN).[137,138] KYN produced at the periphery can be transported into the brain where it is through the immune-inducible enzyme, kynurenine monooxygenase, presented in microglia, metabolized into neurotoxic KYN metabolites including quinolinic acid. Quinolinic acid, together with glutamate released by activated microglia, activates N-methyl-D-aspartate (NMDA) receptors throughout the brain contributing to depressive symptoms.[139] This pathway can explain why ketamine-linked blockade of NMDA receptors can abrogate depression-like behavior induced by lipopolysaccharide in experimental studies[140] indicating the promising potential of ketamine as a future treatment for depression.[141] Importantly, this pathway does not discard the role of serotonin in inflammation-linked depression. From this view, the inflammation modulates serotonin signaling by increasing the expression of serotonin transporters re-uptaking the synaptic serotonin resulting in the decreased amount of serotonin available at the synapses.[142,143] This inflammation-linked serotonin depletion process and consequently diminished brain serotonin activity has been reported to be associated with MDD.[144,145]

In summary, disturbed interactions between the peripheral immune system and CNS may result in central immune dysregulation and subsequently neurobehavioral MDD-linked abnormalities.[139,145] However, elucidating whether neuroimmune dysregulation is an etiological element leading to depression or a depression's consequence remains a chicken-or-egg dilemma.

Several limitations should be considered in our study. Firstly, other important IRS (eg IL-17, acute phase proteins) and CIRS (eg sIL-2R, sIL-1RA, IL-6 classical signaling, haptoglobin) indices were not evaluated in this study. Secondly, the smoking status as a factor influencing the relationship between inflammation and depression was not exactly monitored. Further, all participants came from the same place and were of the same ethnicity, thus our findings might be limited in the general applicability. Lastly, we have compared MDD adolescents and controls to identify potential biomarkers helpful for MDD diagnosing; however, future studies should explore whether our potential biomarkers could be also used to distinguish MDD adolescents from adolescent patients suffering from other mental disorders.

## Conclusion

Adolescent depression is characterized by enhanced pro-inflammatory TNF-α signaling and IL-6 trans-signaling associated with increased IL-10- and IL-4-mediated anti-inflammatory activity. Importantly, the association between IRS and CIRS pathways and adolescent depression seems to be sex-specific. Our findings can contribute to a clearer understanding of IRS and CIRS interactions in the complex immunopathogenesis of depression already at the adolescent age. Since IRS and CIRS can influence the treatment outcomes, further research is needed to identify precise IRS and CIRS biomarkers that could serve as a new therapeutic target for MDD adolescents, also considering the sex.

## Abbreviations

BBB, blood–brain barrier; CDI, Children Depression Inventory; CIRS, compensatory immune response system; CNS, central nervous system; CRP, C-reactive protein; DSM-5, Diagnostic and Statistical Manual of Mental Disorders, fifth edition; gp130, glycoprotein 130; IDO, indoleamine-2,3-dioxygenase; IFN-γ, interferon-gamma; IL, interleukin; IRS,

inflammatory response system; KYN, kynurenine; M1, macrophages M1; M2, macrophages M2; MDD, major depressive disorder; NMDA, N-methyl-D-aspartate; NLR, neutrophil-to-lymphocyte ratio\; PLR, platelet-to-lymphocyte ratio; RBC, red blood cells; RDW, red blood cell distribution width; sIL-1RA, soluble IL-1 receptor antagonist; sIL-2R, soluble IL-2 receptor; sIL-6R, soluble IL-6 receptor; SNRIs, serotonin and noradrenaline reuptake inhibitors; SSRIs, selective serotonin reuptake inhibitors; sTNF-R1, soluble tumor necrosis factor receptor 1; sTNF-R2, soluble tumor necrosis factor receptor 2; sTNF-Rs, soluble tumor necrosis factor receptors (ie sTNF-R1 + sTNF-R2); TACE/ADAM17, metalloproteinase, TNF-α converting enzyme; Th, helper T lymphocytes; TNF, tumor necrosis factor; TNF-α, tumor necrosis factor-alpha; TNF-R1, tumor necrosis factor receptor 1; TNF-R2, tumor necrosis factor receptor 2; Treg, regulatory T lymphocytes; WGCNA, weighted gene co-expression network analysis WGCNA; WBC, white blood cells.

## Ethics Approval and Consent to Participate

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava (protocol code EK23/2021 of 29 June 2021). Informed consent was obtained from all subjects and their legal guardians involved in the study.

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## Author Contributions

All authors made a significant contribution to this work whether in the conception, study design, execution, or data acquisition, analysis and interpretation. All authors critically reviewed the article, gave final approval of the version to be published, agreed on the journal to which the article has been submitted; and agreed to be accountable for the contents of the article.

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## Disclosure

The authors declare no conflicts of interest in this work.

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