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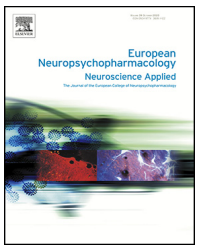

An increase in IL-6 levels at 6-month follow-up visit is associated with SSRI-emergent suicidality in high-risk children and adolescents treated with fluoxetine

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Abstract
Major depressive disorder (MDD) is associated with alterations in circulatory cytokines, in adults as well as in children and adolescents. Administration of selective serotonin reuptake inhibitors (SSRIs) to MDD pediatric patients modifies cytokine levels. However, most studies only assessed changes over a short time period. In this study, we evaluated long-term effects of the SSRI fluoxetine (FLX) in children and adolescents treated for anxiety and/or MDD, including a high-risk group with pre-treatment suicidality. The study group included ninety-two patients (35 boys and

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57 girls) with MDD and/or anxiety disorders, aged 13.90 ± 2.41 years. All patients were treated with FLX and followed for 6 months. The study group included children with pretreatment suicidality (high-risk group;$N = 62$) and without pretreatment suicidality ($N = 30$) according to the Columbia Suicide Severity Rating Scale. Plasma concentrations of TNFα, IL-6, and IL-1β were measured by enzyme linked immunosorbent assays before and after six months of treatment. IL-6 and IL-1β significantly increased as a factor of time after 6 months of treatment. The elevation was statistically significant confined to children with pretreatment suicidality. Within the children with pretreatment suicidality, IL-6 levels increased significantly after 6 months only in the children who developed SSRI-associated suicidality. To summarize, an increase in IL-6 levels after 6 months of treatment may be associated with SSRI-emergent suicidality in children with pretreatment suicidality. Further studies are needed to clarify the role and mechanism(s) of IL-6 in the pathogenesis of this life-threatening adverse event.

1. Introduction

Immune dysregulation has been implicated in the pathophysiology of major depressive disorder (MDD) (Dantzer et al., 2008; Schmidt et al., 2016). Pro-inflammatory cytokines may contribute to the behavioral and immune disturbances observed in these patients, in adults as well as in children and adolescents (Dowlati et al., 2010; Mills et al., 2013; Mitchell and Goldstein, 2014). Moreover, several studies have shown that abnormalities in cytokine levels comprise risk factors for suicidal behaviors (SBs) beyond their association with depressive symptoms (Keaton et al., 2019). Inflammatory factors can reach the central nervous system through several mechanisms and within the brain may play a role in triggering affective symptoms.

Selective serotonin reuptake inhibitors (SSRIs) are commonly used to treat depression and/or anxiety in the pediatric population. Several studies have shown that neuroinflammation may be influenced by pharmacologic interventions, and specifically by SSRIs (for a comprehensive review, see Wang et al. (2019)). Most studies have indicated that SSRI treatment may cause a reduction in peripheral inflammatory markers (Hannestad et al., 2011; Hiles et al., 2012). However, most investigations only examined the short-term influence of SSRIs and only a paucity of studies assessed long-term effect of SSRIs on inflammatory markers and the relevance to behavior. One study showed an increment in IL-2 and IL-1β after 52 weeks of SSRI treatment (Hernández et al., 2008). To date, no study has evaluated the long-term effects of SSRIs in pediatric population. Moreover, no study has assessed inflammatory markers in SSRI-induced adverse events (AEs) and specifically in SSRI-associated aggravation of SBs, a known AE of SSRIs in the pediatric population (Hammad et al., 2006). This phenomenon is especially worrisome in high-risk children with pretreatment suicidality (Wilkinson et al., 2011).

In a previous study, our group showed that an increase in IL-6 levels during 8 weeks of fluoxetine (FLX) treatment is a risk factor for the emergence of SSRI-associated suicidality (Amitai et al., 2019). In the current study, we assessed pro-inflammatory cytokines after 6 months of follow-up. We hypothesized that a persistent neuroinflammatory process, as indicated by continuously rising levels of pro-inflammatory cytokines, may be detected in children suffering from MDD and/or anxiety, despite adequate SSRI treatment. Moreover, we hypothesized that such neuroinflammatory process may be more pronounced in a high-risk group of children and adolescents with pretreatment SBs who are treated with FLX, and specifically in children who develop SSRI-associated suicidality.

2. Experimental procedures

2.1. Study design

For study design, see Amitai et al. (2019).Briefly, children with depression and/or anxiety disorders according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) (American Psychiatric Association, 2013) were treated with FLX 20–40 mg. The study was approved by the Schneider Children's Medical Center of Israel Review Board (Petach Tikva, Israel) and informed consent and assent were obtained from subjects and their parents. After a confirmatory diagnostic assessment, all subjects received fluoxetine. The starting dosage for all patients was 10 mg/d for one week, and then increased to 20 mg/d through week 4. Response was measured with the Clinical Global Impressions-Improvement (CGI-I) scale (Guy, 1976). If the degree of improvement was minimal (CGI-$I \geq 3$) then the dosage was increased from 20 to 40 mg/day on week 5. A follow-up visit was held every two weeks, assessing improvement of symptoms and AEs. At the end of week eight, a child was considered a responder or a non-responder according to CGI-I (CGI-$I = 1/2$ vs. CGI-$I \geq 3$). The trial was extended as an open clinical treatment for another 4 months, with a follow-up visit every month. At the end of the 6-month follow-up, long-term response was measured with the CGI-I score.

For the evaluation of AEs, see Amitai et al. (2019). For this study, suicidality was evaluated using the Columbia Suicide Severity Rating Scale (C-SSRS). This scale is designed to standardize the assessment of a broad range of suicide-relevant behaviors, including the severity and intensity of suicidal ideation (SI), suicidal attempt (SA), and SA lethality. Suicidal events during the follow-up period were classified into three categories: (1) actual SA - all self-injurious behaviors or harmful acts with intent to die; (2) self-harm behaviors with no intent to die; and (3) new SI or worsening of SI (Posner et al., 2011). Category 2 coincides with the recent proposal to include non-suicidal self-injury (NSSI) as a nosological entity in the DSM Research Criteria (DSM-5). Pretreatment suicidality was determined according to the ideation subscale. The ideation subscale is a good predictor for future SAs and has a high sensitivity and specificity for SBs (Posner et al., 2011). SSRI-associated suicidality was assessed according to evidence for a clinically meaningful suicidality that

developed during the whole follow-up period (i.e., suicidality that demanded urgent medical intervention).

2.2. Plasma cytokine assessment

Blood samples were collected from all subjects prior to starting treatment and after 6 months of follow-up. From each participant, blood samples (20 mL) were collected by venipuncture between 9 a.m. to 11 a.m. Blood samples were centrifuged immediately (200 g) at 4 °C for 10 min to obtain plasma. Plasma samples were separated into aliquots and stored at −80 °C until assayed. Plasma was used for determination of the levels of the human pro-inflammatory cytokines TNF-α, IL-6 and IL-1β. Cytokines were measured in plasma according to the manufacturer's instructions (R&D Systems, Minneapolis, MN). Measurements of the cytokines for all participants were conducted in duplicates in the same run to avoid inter-assay variability. Cytokines were assayed on seven 96-well plates with samples from all groups distributed evenly. All cytokines were assessed with a sandwich enzyme linked immunosorbent assay (ELISA) based on a monoclonal-monoclonal antibody pair and a biotin-streptavidin amplification system (Siemens Medical Solutions Diagnostics, Los Angeles, CA), following the manufacturer's protocol. The laboratory team was blinded to the clinical data and the clinical team was blinded to the laboratory data. Only children with available cytokine measurements were included in the analysis.

2.3. Statistics

The Statistical Package for the Social Sciences (SPSS) was used to create a database and to conduct the statistical analyses (version 17 for Windows, SPPS Inc., Chicago, IL). Data for all three cytokines were transformed into normal distribution using natural logarithms for statistical analyses. To avoid batch-to-batch variation, all cytokine levels were normalized using z-scores. Samples with cytokine levels below detection limit were assigned a value corresponding to the lowest detectable value in the assay. Paired and unpaired *t*-tests were used as appropriate.

3. Results

3.1. The whole group

3.1.1. Subjects

Over 300 children and adolescents were screened, of which 96 were eligible for participation in the study and gave their assent. One child withdrew his assent prior to commencement of therapy. All in all, 95 children comprised the intent-to-treat population. Three children dropped out before finishing the 8 week follow up (one due to panic attacks and the other two due to unknown reasons) thus, 92 subjects completed the 8 weeks of the follow-up. Seventy-eight patients completed a 6 month follow-up. At the end of the study, 50 children (54% of the study sample) were still on FLX at last follow-up visit. All others had been switched to a different SSRI (sertraline or escitalopram).

The demographic details of the 92 subjects were previously described (Amitai et al., 2019). The study sample was comprised as follows: 35 (38%) boys and 57 (62%) girls aged 13.90 ± 2.41 years, 7 (8%) had a diagnosis of MDD alone, 67 (72.8%) had combined diagnoses of MDD and anxiety disorders, and 18 (20%) had a diagnosis of anxiety disorders alone (general anxiety disorder, social anxiety disorder, panic disorder with or without agoraphobia, separation anxiety disorder, specific phobia or a combination of these disorders). The mean number of anxiety disorders was 1.85 ± 1.13 per patient. Of subjects with MDD, 17 (19%) had a diagnosis of double depression as main diagnosis, 15 (16%) children had obsessive-compulsive disorder (OCD), 14 (15.2%) had eating disorders, and 47 (51%) had attention deficit/hyperactivity disorder (ADHD). The average pretreatment BMI was 20.62 ± 4.07 Kg/m^2. Six (7%) children had a previous trial with an SSRI, 59 (67%) had a first-degree relative with unipolar MDD, and 9 (10%) had a second-degree relative with unipolar MDD. Four children (4%) had a first-degree relative with bipolar disorder (BPD), and 10 (11%) had a second-degree relative with BPD.

The whole study group ($N = 92$) was divided to two groups: those with pretreatment suicidality ("the high-risk group", $N = 62$) and patients without pretreatment suicidality ("the low-risk group", $N = 30$). The high-risk group was further divided into two groups: children who developed SSRI-associated suicidality during the follow-up ("the SSRI-associated suicidality group", $N = 16$) and children who did not develop SSRI-associated suicidality ("the no-SSRI-associated suicidality group", $N = 46$). See Fig. 1 for a summary of the different groups in the study. Sixty-six (72%) children were rated as good responders in the long-term follow-up.

3.2. Cytokine levels in the whole group

TNF-α levels did not change significantly after 6 months of treatment (paired *t*-test, *p*=NS); both transformed IL-6 and IL-1β significantly increased as a factor of time after 6 months of treatment (paired *t*-test: $t=-2.74$, df=66, $p = 0.008$, after FDR correction $p = 0.012$, and $t=-3.26$, df=65, $p = 0.002$, after FDR correction $p = 0.006$, respectively). See Fig. 2 and Supplementary table S1 for the raw levels of the measured cytokines. No difference in immunological outcome was observed between those patients who continued with fluoxetine vs those who were switched to other SSRIs.

The total study group ($N = 92$) was divided into two groups according to pretreatment suicidality: sixty-two subjects had a non-zero grade in the ideation subscale of the C-SSRS (the high-risk group, $N = 62$). In this group, TNF-α levels did not change significantly after 6 months of treatment (paired *t*-test, *p*=NS); but both transformed IL-6 and IL-1-β levels significantly increased as a factor of time after 6 months of treatment (paired *t*-test: $t=-2.49$, df=45, $p = 0.017$, after FDR correction $p = 0.025$, and $t=-3.47$, df=44, $p = 0.001$, after FDR correction $p = 0.003$, respectively). In the group without pretreatment suicidality (the low-risk group, $N = 30$) no significant differences in cytokine levels were found between baseline and 6 months of treatment (paired *t*-test, data not shown).

See Table 1 and Figs. 2 and 3 for the Z scores of transformed levels of the measured cytokines.

3.3. The "pretreatment suicidality group" (high-risk group)

Further analysis was performed on the "pretreatment suicidality group" (the high-risk group): this group included 23

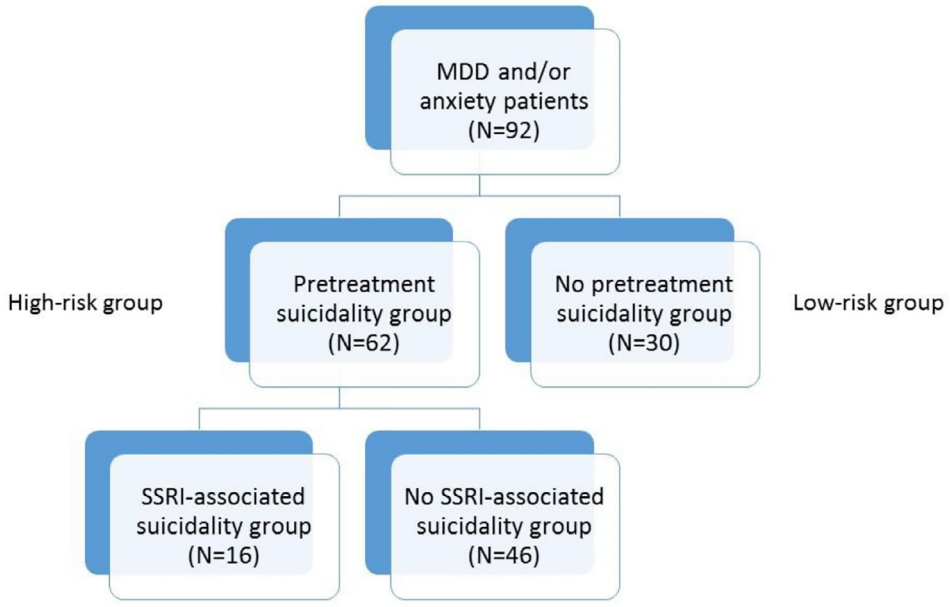

Fig. 1 The clinical groups analyzed in the study.

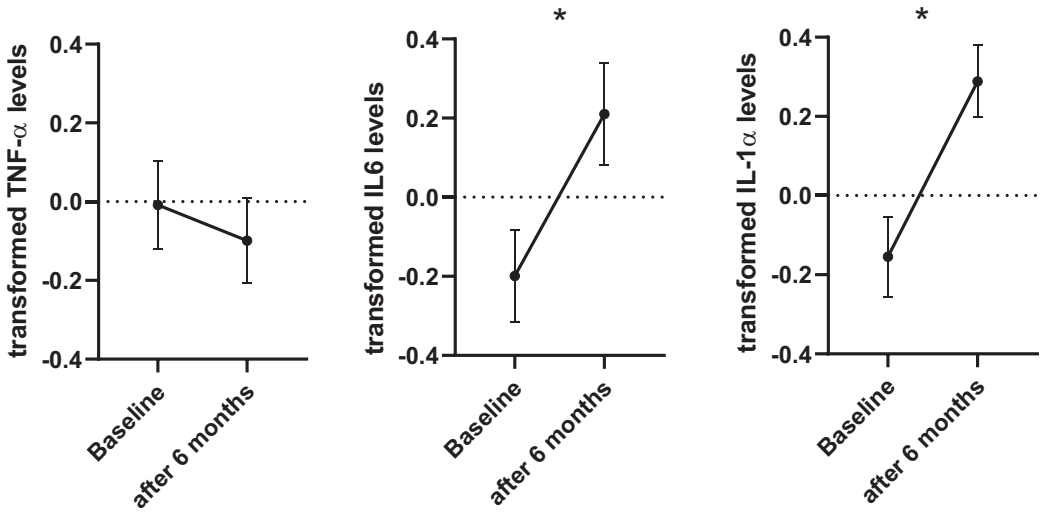

Fig 2 Z scores of the log levels of the three measured cytokines at baseline and after 6-month of SSRI treatment in the whole group ($N = 67$) $^{*}p$ (IL-6)=0.012, p(IL-1β)= 0.006.

Table 1 Cytokine levels (Z scores of transformed values) before and after 6 months of treatment in the whole group ($N = 92$).

Cytokine	Pretreatment	After 6 months	Paired t-test
Transformed TNF-α levels	-0.008 ± 1.05	-0.1 ± 0.91	$t = 2.45$ df =44 p = NS
Transformed IL-6 levels	-2.00 ± 1.10	0.21 ± 1.08	t=−2.49, df=45, $p = 0.017$, after FDR correction $p = 0.025$
Transformed IL-1-β levels	-0.15 ± 0.96	0.29 ± 0.76	t=−3.47, df=44, $p = 0.001$, after FDR correction $p = 0.003$

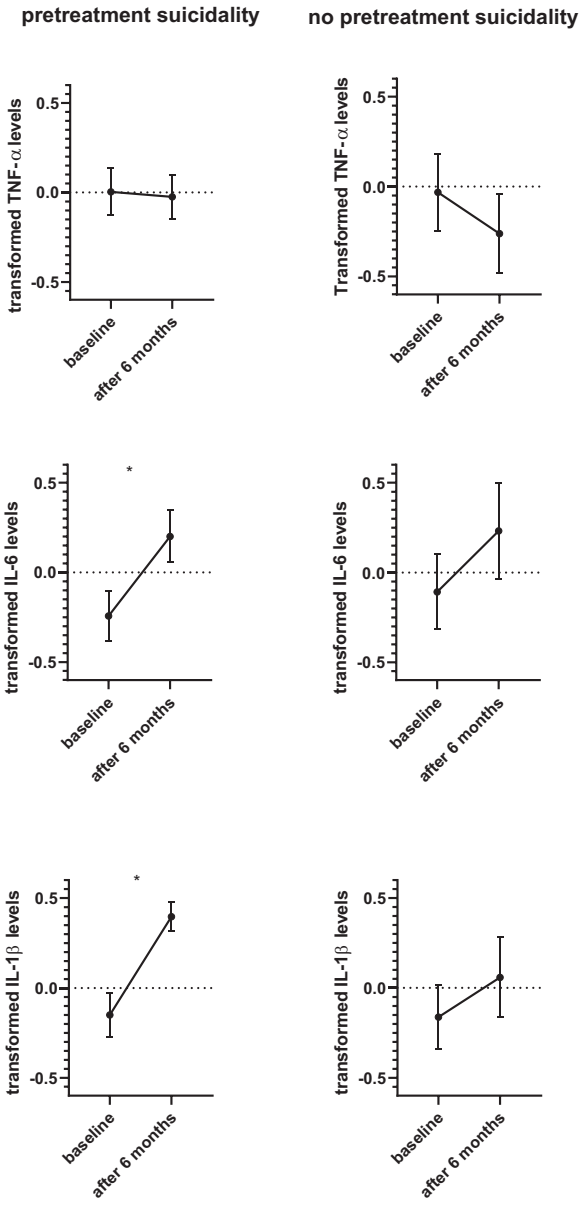

Fig. 3 Z scores of the log levels of the three measured cytokines at baseline and after 6-month of SSRI treatment in the patients with pre-treatment suicidality (high-risk patients; $N = 46$) and in the patients without pre-treatment suicidality (low-risk; $N = 21$) $^{*}p$ (IL-6)=0.025, p(IL-1β)= 0. 003.

(37%) boys and 39 (63%) girls aged 14.27 $\pm$ 2.33 years. Seven children (11%) were treated for depression, 7 (11%) for anxiety disorders and 48 (77%) for a combination of depression and anxiety. Sixteen children (26%) developed clinically meaningful SBs during the whole period of follow-up. Out of these, 7 had made a SA, 5 had worsening or new-onset NSSI and 4 had a worsening of SI. Within the high-risk group, no differences were observed regarding age, gender, diagnosis and all the tested clinical parameters between those who developed suicidality during the 6-monts treatment ($N = 16$) and those who did not develop such behaviors ($N = 46$). For the demographic data of the children, see Table 2.

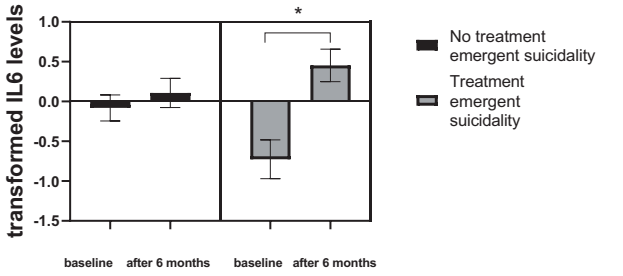

Fig. 4 Z scores of the log levels of IL-6 at baseline and after 6-months of SSRI treatment in the patients with (right) SSRI-associated suicidality ($N = 16$) and (left) in the patients without SSRI-associated suicidality ($N = 46$) $P = 0.006$.

The relationship between lifetime SAs (assessed by the C-SSRS at baseline) and SSRI-associated suicidality during the study was examined. Patients with histories of SA, interrupted attempts and aborted attempts ($N = 22$) were not more likely to exhibit SSRI-associated suicidality during the treatment compared to those without histories of SA, interrupted attempts and aborted attempts (chi-square: $p>0.05$, data not shown).

3.4. Cytokines levels within the high-risk group

No significant differences were found in baseline cytokine levels between children who developed suicidality during SSRI treatment ($N = 16$) vs. those who did not (unpaired t-test: $p>0.05$, data not shown). IL-6 levels increased significantly only in the children who developed SSRI-associated suicidality during treatment ("the SSRI-associated suicidality group", $N = 16$; paired t-test: t=−1.19, df=11, $p = 0.003$, after FDR $p = 0.006$) (Fig. 4). No alteration was detected in IL-6 levels in the group that did not develop SSRI-associated suicidality (paired t-test, p=NS). No differences were detected in the levels of TNF-α and IL-1β.

4. Discussion

A substantial amount of literature has formed around the role of inflammatory markers in mental disorders, and specifically in depression (Dowlati et al., 2010; Felger and Lotrich, 2013). Interaction between the nervous system and immunity plays an important role in the pathophysiology of MDD. Inflammation contributes to depressive symptoms in patients with MDD (Lichtblau et al., 2013; Maes et al., 2012), and high circulating proinflammatory cytokine levels are associated with poorer response to treatment (Eller et al., 2008; Strawbridge et al., 2015). Furthermore, anti-inflammatory agents may play an antidepressant role in patients with MDD (Bai et al., 2019). SSRIs affect levels of inflammatory markers in patients with MDD, but studies have reported inconsistent findings.

Inflammatory processes have also been implicated in suicidality. Numerous studies have shown that abnormalities in cytokine levels comprise risk factors for SBs beyond their role in promoting depressive symptoms (Bastos et al., 2017; Black and Miller, 2015; Coryell et al., 2018; Ducasse et al.,

Table 2 Comparison of demographic and clinical variables between the treatment emergent suicidality group and all others.

Characteristics	Treatment associated suicidality group ($N = 16$)	No Treatment associated suicidality group ($N = 46$)	p value unpaired t-test/ χ^2
Age [years]	14.19 ± 1.97	14.30 ± 2.47	$t = 0.17$ df=60 p=NS
Male/Female	4 / 12	19 / 27	$\chi^{2=}1.35$ p=NS
Diagnosis			
MDD	0	7	$\chi^{2=}2.75$
Anxiety	2	5	P=NS
MDD + Anxiety	14	34	
CGI-S	5.63 ± 0.50	5.45 ± 0.62	t=−1.05 df=60 P=NS
SA at baseline	7	15	$\chi^{2=}0.64$ P=NS
Response/non-response	3/13	21/25	$\chi^{2=}3.62$ P=NS
C-SSRS ideation score at baseline	0.44 ± 0.51	0.33 ± 0.47	t=−0.79 df=60 P=NS
C-SSRS ideation score after 8 weeks of treatment	0.44 ± 0.51	0.33 ± 0.47	t=−0.96 df=60 P=NS

C-SSRS, Columbia Suicide Severity Rating Scale; CGI-S, Clinical Global Impressions - Severity; SA - suicide attempt.

2015; Ganança et al., 2016; Pandey, 2015; Serafini et al., 2013). For instance, mRNA and protein levels of IL-1β, IL-6, TNF-α, and lymphotoxin A were significantly increased in the prefrontal cortex of depressed individuals who died by suicide compared with controls (Pandey et al., 2018).

In a previous study, our group has examined three pro-inflammatory cytokines after 8 weeks of FLX treatment in children and adolescents treated for anxiety and/or depressive disorders (Amitai et al., 2019). We studied 3 pro-inflammatory cytokines that have most often been studied in research on depressive disorders: IL-1β, IL-6 and TNF-α (Dowlati et al., 2010; Haapakoski et al., 2015; Miller et al., 2009). Our findings suggested that IL-6 levels increased after treatment but only in the group of children who developed FLX-associated suicidality, suggesting that an increase in IL-6 levels during treatment may be associated with the emergence of FLX-associated suicidality (Amitai et al., 2019). In the current study, we assessed the levels of the same cytokines in a long-term (6-month) follow up, and specifically in a high-risk group of children and adolescents with pretreatment suicidality. Risk-factors for SSRI-associated suicidality in the pediatric population include, among other factors, high suicidality at baseline (Wilkinson et al., 2011). Thus, it is likely that children with pretreatment suicidality comprise a high-risk group for this serious AE.

In this study, we evaluated the long-term effects of SSRIs on pro-inflammatory cytokines in children and adolescents treated for MDD and/or anxiety disorders, with a special emphasis on children with pretreatment suicidality (namely a high-risk group).

Three main findings were observed. First, we found that two of the three measured cytokines were elevated in the whole group following 6 months, despite the continuous SSRI treatment. This finding is contrary to what has been described in the literature regarding the effect of SSRI on peripheral cytokines. Most of the studies show that SSRIs have anti-inflammatory properties. A recent meta-analysis by Wang et al. (Wang et al., 2019) indicated that SSRI treatment in adults decreased the levels of the pro-inflammatory markers IL-6, TNF-α and IL-1β and anti-inflammatory marker IL-10 in patients with MDD. They hypothesize that moderate immunomodulating effects of SSRI treatment for MDD may be relevant to some of the therapeutic effects. However, most of the studies evaluated short-term treatments. Very few studies have been conducted on the long-term influence of SSRI on cytokine levels. Hernandez et al. showed in adults that after 52 weeks of treatment there was an elevation in IL-1β and IFN-γ and a decrease in Th2 cytokines (Hernández et al., 2008). They concluded that depressed patients only reach a partial re-establishment of neurobiological functioning after the long-term administration of SSRIs.

Actually, in the same group of children reported on here, cytokine levels did not change after 8 weeks of treatment (Amitai et al., 2019). However, there was an elevation in two of the three measured cytokines (IL-6 and IL-1β) after 6 months of treatment. The continuous administration of SSRIs induces progressive changes in serotonin levels in MDD patients (Blardi et al., 2002). Thus, it is important to emphasize that serotonin effects on the immune response may be dose-dependent. Previous stud-

ies have shown that serotonin induces both the secretion of pro-inflammatory cytokines and cellular proliferation at physiological concentrations (0.15 a 1.5 μg/mL). However, at supra-physiological doses of serotonin (15 μg/mL), pro-inflammatory cytokine secretion is decreased (Kubera et al., 2005). According to Kubera et al., intracellular 5-HT is necessary for optimal synthesis of IL-6 and TNFα; serotonin in physiological concentrations may increase IL-6 and TNF-α production by stimulating 5-HT2 receptors (Kubera et al., 2005). Thus, it is possible that SSRI intake in the first weeks of treatment increases circulating levels of plasma serotonin to supra-physiological levels, which in turn suppresses production of pro-inflammatory cytokines. However, after long-term treatment, serotonin returns to normal physiological levels and consequently promotes the secretion of pro-inflammatory cytokine secretion, such as IL-1β, IFN-γ and TNF-α. Thus, our findings here are in accordance with the findings of Hernandez et al. in adult population (Hernández et al., 2008).

The second finding in our study suggests that the elevation in the cytokine levels was statistically significant only in the pretreatment suicidality group (the high-risk group). Both of the groups showed an elevation in IL-6 and IL-1β, but a significant persistent elevation was apparent only in the high-risk group of children with pretreatment suicidality. This is suggestive of an enhanced inflammatory process in these children despite the SSRI treatment. Immune system dysregulation has already been associated with suicidal symptomatology, in adults as well as in adolescents (Courtet et al., 2016). Moreover, proinflammatory cytokines have been implicated in the pathophysiology of SBs (Black and Miller, 2015; Ducasse et al., 2015; Erhardt et al., 2013; Ganança et al., 2016; Miná et al., 2015; Pandey, 2015; Serafini et al., 2013). There is growing evidence that inflammation, as manifested by increased levels of pro-inflammatory cytokines and inflammatory chemokines, is present in patients with SB and ideation. Recently, Keaton et al. (2019) showed a unique immunobiological profile linked to increased suicide risk (Keaton et al., 2019). The profile was different from that observed in patients with depressive symptoms. However, the results are inconclusive. Little research has been published regarding the inflammatory system in SSRI- associated suicidality, and especially in pediatric population. Our previous data indicate that IL-6 levels differ in children who developed SSRI-associated suicidality compared to children who do not have this AE (Amitai et al., 2019). Thus, the present study is in accordance with research that implicates immune activation in suicidality.

Our third finding suggests that within the group of pretreatment suicidality patients, namely the high-risk group, only children who developed SSRI-associated suicidality had a statistically significant elevation in IL-6 levels between baseline and 6 months of SSRI administration. Both of the groups showed an elevation in IL-6, but a significant elevation was only apparent in children with aggravation in suicidality.

IL-6 is a pro-inflammatory cytokine that has previously been linked to suicidality in multiple studies. In fact, the most consistent finding in the literature of mood disorders is elevated IL-6 in subjects with suicidality, as compared to patients without suicidality or healthy controls: Isung et al. (2014) showed that violent suicide attempts tended to be associated with high plasma IL-6 levels (Isung et al., 2014). Janelidze et al. (2011) found increased levels of IL-6 and TNF-α as well as decreased IL-2 concentrations in suicide attempters compared to non-suicidal depressed patients and healthy controls (Janelidze et al., 2011). Lindqvist et al. (2009) showed that IL-6 in cerebrospinal fluid was significantly higher in suicide attempters than in healthy control subjects. Patients who performed violent suicide attempts displayed the highest IL-6 (Lindqvist et al., 2009). Mina et al. (2015) show that IL-6 is not only found to be elevated in the cerebrospinal fluid of suicide attempters, even its levels in the peripheral blood have been proposed as a biological suicide marker (Miná et al., 2015). Our current study is in accordance with these previous findings.

Thus, our study shows that children with depression and or/anxiety have immune system dysregulation that is not resolved despite SSRI treatment. This dysregulation is expressed in rising levels of IL-6 and IL-1β after 6 months of treatment and is significantly pronounced in suicidal children. Moreover, children at high-risk for suicidality (those with pretreatment suicidality) who develop SSRI-associated suicidality have higher IL-6 levels after 6 months of treatment compared to those who do not develop this phenomenon. It seems that in children prone to suicidality associated with SSRI treatment, there is an incapability of SSRI to suppress elevation in IL-6 synthesis, and persistent accumulation of the cytokine may lead to SBs. The elevated levels of IL-6 expose these children to an even higher risk of suicidality.

4.1. Limitations

For a comprehensive description of the limitations in the current study, shared by our previous study, please refer to Amitai et al. (2019). One limitation is the heterogenic nature of the sample and the lack of a healthy control group. An additional limitation in the current study is that we did not have clinical measures after 6 months of treatment, except CGI-I. It is possible that children with a more pronounced inflammatory process after 6 months have different clinical profile as compared to children with a resolution of the inflammatory process. Moreover, given the well-known fluctuations of immunological parameters over time, it would have been preferable to have some more measurement points. At present the current data can only indicate an association between IL-6 and suicidality at 6 month's time point. Thus, the clinical implication of the 6 month's time point measurement is currently unclear. None the less, this observation supports our previous findings regarding IL-6 as a potential inflammatory biomarker for SSRI-associated SB. Notably, when comparing clinical variables between patients with or without SSRI-emergent SB, gender ratio and response rates considerably differ between groups, but without reaching statistical significance (see Table 1). As gender and treatment response are two factors associated with pro-inflammatory cytokines levels, it is important to repeat this analysis in the future in large-scale studies in order to control for such possible confounders. Another limitation of the current study (and also our pre-

vious one) is that we measured only three cytokines. A cytokine network analysis approach may be more comprehensive than analyses focusing on only one or a few markers, as there are often synergistic and distinct effects, depending on what type of immunobiological network is active.

5. Conclusion

In this study, we assessed the cytokine profiles of children with mood and anxiety disorders after 6 months of SSRI treatment. We identified a unique biological profile in subjects with increased suicide risk. This finding is in accordance with our previous short-term (8-week) study (Amitai et al., 2019) and many other studies that have implicated IL-6 as a potential biomarker with clinical relevance to suicidality. Our study underscores circulatory IL-6 as a potential inflammatory biomarker of specific importance in the pathophysiology of SSRI-associated SB. Additional studies are needed to advance the understanding of the complex immunobiological processes underlying both clinical response to SSRI and SSRI-associated-suicide risk.

Role of the founding source

None.

Contributions

M Amitai designed the study and wrote the first draft, Michal Taler managed the laboratory work, Maya Lebow managed the literature searches and analyses, Reut Ben-Baruch helped with collecting the clinical data, Alan Apter helped with recruiting the patients and collecting the clinical data, Abraham Weizman helped with designing the study and edited the final draft, Silvana Fennig commented on the final draft, Alon Chen helped with designing the study. All authors contributed to and have approved the final manuscript.

Conflict of Interest

None.

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.euroneuro.2020.07.007.

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