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Review

Molecular Biomarkers for Pediatric Depressive Disorders: A Narrative Review

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Abstract: Depressive disorder in childhood and adolescence is a highly prevalent mood disorder that tends to recur throughout life. Untreated mood disorders can adversely impact a patient's quality of life and cause socioeconomic loss. Thus, an accurate diagnosis and appropriate treatment is crucial. However, until now, diagnoses and treatments are conducted according to clinical symptoms. Objective and biological validation are lacking. This may result in a poor outcome for patients with depressive disorder. Research has been conducted to identify the biomarkers that are related to depressive disorder. Cumulative evidence has revealed that certain immunologic biomarkers including brain-derived neurotrophic factor (BDNF) and cytokines, gastrointestinal biomarkers, hormones, oxidative stress, and certain hypothalamus-pituitary axis biomarkers are associated with depressive disorder. This article reviews the biomarkers related to the diagnosis and treatment of pediatric depressive disorders. To date, clinical biomarker tests are not yet available for diagnosis or for the prediction of treatment prognosis. However, cytokines such as Interleukin-2, interferon-gamma, tumor necrosis factor-alpha, and BDNF have shown significant results in previous studies of pediatric depressive disorder. These biomarkers have the potential to be used for diagnosis, prognostic assessment, and group screening for those at high risk.

Keywords: pediatric, depression, biomarker, BDNF, cytokines

1. Introduction

Internationally, there are an increasing number of individuals with major depressive disorder. More than 300 million people suffer from depressive disorder worldwide [1]. Depressive disorder is associated with a high risk of recurrence. This not only worsens the individual's mental and physical health, but also results in socioeconomic loss [2, 3]. The diagnosis of depressive disorder is made through a clinician's evaluation according to the individual's clinical symptoms. An objective diagnostic evaluation has not yet been identified. In addition, biological predictors of responses to clinically available treatments have not been determined. More than half of patients do not achieve remission with their first course of treatment [4, 5]. Efforts have been made to explore risk factors and etiologies for depression and to improve objective diagnostic methods. These characteristics, which are objectively measured and evaluated as indicators of normal biological processes, pathogenic processes, or pharmacological responses to therapeutic interventions, are termed biomarkers [6]. Among these, the neuroimmune system has recently been investigated as a biomarker for depressive disorders.

Although no consistent results have been reported, several cytokines have been suggested to be associated with depressive disorders. There were relatively consistent findings showing an increase in several cytokines including interleukin-6 (IL-6), interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP) in patients with depressive disorder compared to the healthy control group [7-12]. In addition, it has been

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shown that an increase or decrease in certain cytokines also affects the treatment outcome with antidepressants [11, 13, 14]. In the association between the neuroimmune system and depressive disorder, interest in the role of brain-derived neurotrophic factor (BDNF) as well as cytokines has increased. BDNF is a neurotrophin that regulates neurogenesis, neuronal maturation, survival, and synaptic plasticity [15, 16]. Several previous studies have suggested that BDNF affects neuroimmune regulation in psychiatric disorders, including schizophrenia, mood disorders, and obsessive disorder [17-19].

Studies using various biomarkers have been conducted in adults with depression. In children and adolescents, the overall context is often similar. However, the study results of children and adolescents are not necessarily consistent with those of adults. These differences indicate the influence of maturation. Herein, we aim to review the biomarkers related to pediatric depressive disorders. It is challenging to rule out the impact of psychiatric drugs, such as antidepressants and antipsychotics, in adult depressive disorder. It is also problematic to clarify the changes in physical health and the effect this has on the biomarkers in adult depressive disorder. It is not clear whether the increased inflammation observed in major depressive disorder (MDD) is the cause or the result of this disorder [20]. The purpose of this study is to identify the characteristics of biomarkers in pediatric depressive disorders by comparing the results with results from biomarker studies in adults.

2. Neuroimmune system and pediatric depressive disorder

2.1. The cytokines in neuroinflammation

Neuroinflammation is defined as the reactive state of astrocytes and microglia, induced by pathological conditions [21]. These cells mediate the immune response in the brain. They produce and secrete pro-inflammatory cytokines which are known to be associated with depressive disorder [22, 23]. Secretion of peripheral cytokines increases in pathologic states, such as infection, and affects hypothalamic-pituitary-adrenal (HPA) axis activation. Cytokines can access the brain by passing through leaky regions in the blood-brain barrier. They have functions such as neuroprotection and neurodegeneration in the central nervous system (CNS). They also have significant effects on neurotransmitters such as dopamine and serotonin [24, 25]. Mae et al. showed that the activity of IL-6 was higher in patients with depressive disorder than in the control group. In the same study, increased IL-6 activity was shown to be related to hyperactivity of the HPA axis as well as to high levels of plasma cortisol [13, 26]. Since then, the number of studies on the relationship between cytokines and depressive disorder has increased. These studies have investigated several markers including IL-1, IL-1β, IL-6, IL-8, IL-10, TNF-α, interferon-γ (IFN-γ), and CRP [27] kines are generally divided into pro-inflammatory and anti-inflammatory cytokines. Pro-inflammatory cytokines, including IL-1, IL-1β, IL-6, IL-12, TNF-α, and IFN-γ, have been suggested to be associated with depressive disorders [8, 11, 12, 14, 25, 28-30]. Additionally, IL-6 and TNF-α negatively impact serotonin production and integrity [31], which may increase the risk of depression. Furthermore, the release of TNF-α, as well as IL-1β, is thought to induce synaptic pruning. This leads to impaired neuroplasticity and structural brain changes that negatively impact cognition [31, 32]. CRP is an acute-phase protein that is released in response to inflammation and increases cytokine levels [33, 34]. Evidence for an association between inflammatory cytokines and the pathophysiology of depressive disorder has been reported; however, the direction of this association remains unclear.

Cross-sectional and longitudinal studies have been conducted on the relationship between pediatric depression and inflammatory markers [7-11, 14, 35, 36]. The cytokines that have been studied in pediatric depression are IL-1, IL-1β, IL-2, IL-6, IL-10, TNF-α, and IFN-γ. Gabbay et al. measured the plasma levels of IL-1β, IL-6, IL-10, TNF-α, and IFN-γ in adolescents with depressive disorder as well as in healthy controls [7]. In this study, they showed that plasma IFN-γ levels were significantly higher in the patient group than in the healthy control group. The same results were also found in non-

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medicated MDD patients compared to healthy controls. Increased plasma IL-6 levels are one of the most consistent findings in depression [30, 37, 38]. Similar results were found in a study of 42 female adolescents with depression, conducted by Blom et al. [8]. Plasma levels of IL-6 and IFN-γ were associated with the severity of depression and anxiety symptoms. IL-6 levels were higher in the non-medicated patient group than in the medicated patient group. In addition to IL-6 and IFN-γ, IL-2, IL-10, and IL1-β levels were significantly higher in the patient group. In a meta-analysis of 22 studies (20,791 participants) on the association between depressive symptoms/depressive disorder and inflammation in children and adolescents, IL-6 was correlated with depressive symptoms. Elevated IL-6 was evaluated as a predictor of future depression [38, 39]. Evidence has also been gathered on the association between IL-6 and internalizing disorder symptoms [32, 40]. In a study of 134 students (N= 76 with internalizing disorder and N= 58 without internalizing disorder) aged 10-17 years conducted by Cristiano Tschiedel Belem da Silva and colleagues in Brazil, students with internalizing disorders, including major depressive disorder, generalized anxiety disorder, separation anxiety disorder, social anxiety disorder, or panic disorder showed significantly higher levels of IL-6 than students without internalizing disorder [9]. Internalizing behaviors evaluated at 8 years of age were associated with elevated levels of IL-6 measured at 10 years of age in a large community cohort in England [40]. Although there were no consistent results in pediatric depression, IL-1β levels have been significantly associated with pediatric depression, and have shown higher levels in MDD patients than in healthy controls [8, 10, 30]. Pallavi et al. found that plasma levels of IL-1 β did not show a statistically significant difference in MDD patients compared to the control group. However, IL-1β was associated with the severity of anxiety symptoms in patients with MDD [30]. In addition, IL-1β has been suggested to be related to treatment-refractory depression. Amitai et al. evaluated the plasma levels of IL-1β, TNF-α, and IL-6 in 41 adolescents aged 9–12 years with depressive and/or anxiety disorders [14]. IL-1β, TNF-α, and IL-6 levels were high in the SSRI-refractory patient group. This suggests the possibility that resistance to fluoxetine treatment in children and adolescents may be predicted.

It has been reported that TNF-α is also highly relevant in pediatric depression. In previous studies of adult and pediatric depression, plasma levels of TNF-α have shown mixed results. However, treatment with cytokines, including TNF-α, has been reported to induce depressive symptoms [41-43]. In a comparison of patients with MDD and healthy controls, plasma levels of TNF-α showed no significant difference nor were they increased in the patient group [7, 8, 10, 11, 35, 36]. On the other hand, a decrease in plasma TNF-α levels after antidepressant treatment in MDD patients has been reported [10, 14]. In these studies, a statistically significant decrease in the plasma level of TNF-α was reported 4 and 8 weeks after antidepressant drug treatment, respectively. Studies have been conducted on the relationship between TNF-α and childhood trauma. It has been reported that the relationship between childhood trauma and blood levels of TNF-α is not clear [44, 45]. Peters et al. found that TNF-α was associated with reduced inhibitory control performance in adolescents with depression and childhood trauma [35]. Rengasamy and colleagues suggested that higher baseline levels of TNFα are associated with a higher severity of depression and anhedonia symptoms. They found that a higher baseline level of TNF-α will affect the depressive trajectory [36]. In a meta-analysis conducted by D'Acunto and colleagues on pediatric depression (although it was a small number of studies), TNF-α was higher in the MDD patient group than in the healthy control group [46]. On the other hand, Gabbay et al. reported that suicidal adolescents with MDD showed lower plasma TNF-α levels than non-suicidal adolescents with MDD. Consequently, further research on TNF-α is required [29].

The relationship between CRP and pediatric depression has not been clearly identified, and contradictory results have been reported. Chaiton et al. did not find a significant association between depressive symptoms and serum CRP levels after adjustment for variables, including body mass index, smoking, and blood pressure [47].

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In a study conducted by Copeland et al., CRP levels did not predict later depression. However, it was suggested that cumulative depressive episodes had an effect on later CRP levels [34]. On the other hand, Miller and Cole reported that the transition to depression in adolescents previously exposed to childhood adversity was accompanied by an increase in CRP levels [48]. In this study, CRP levels remained high in depressive adolescents with childhood adversity even 6 months after the depressive symptoms improved. This suggests a relationship between childhood adversity and the neuroinflammatory system. In a study of Iranian female adolescents, Tabatabaeizadeh et al. showed a higher level of CRP in the depressive disorder group, and serum CRP levels were positively associated with the severity of depressive symptoms [49]. In a meta-analysis conducted by Colasanto et al., a significant association between CRP levels and depression was observed, although causality could not be inferred [38].

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Table 1. Previous studies of cytokines in pediatric depressive disorder

Study	Objective	Design	Inflammatory markers	Findings
Gabbay et al., 2009	To examine the immune system in adolescents with MDD	N: 45, age 12-19 years; 13 psychotropic-free MDD Pts, 17 MDD Pts with medication, and 15 HCs	Plasma IL-6, IFN-γ, TNF-α, IL-4, and IL-1β	Significantly higher levels of plasma IFN-γ in MDD Pts and trend for IL-6 to be elevated in MDD group; Significantly increased level of IFN-γ in the un-medicated MDD group compared to HCs
Henje Blom et al., 2011	To investigate the effects of antidepressants on cytokines in adolescent females with anxiety disorder and/or depressive disorder	N: 102, age 14.5-18.4 years; 42 Pts (26 un-medicated Pts and 16 SSRI Pts) and 60 HCs	Plasma IL-1β, IL-2, IL-6, IL-10, TNF-α, and IFN-γ	Significantly higher values of IL-2, IL-10, and IL1-β in patient group; higher level of IL-6 in the non-medicated subgroup compared to the medicated subgroup; higher levels of IL-6 and IFN-γ were significantly related to more severe self-assessed symptoms of anxiety and depression,
Copeland et al., 2012	To test 1) the effect of CRP levels on later depression status; 2) the effect of depression status on later CRP levels; and 3) the effect of cumulative depressive episodes on later CRP levels.	N: 1420, age 9, 11 and 13 years at intake; longitudinal study with annual assessment to age 16 and again at 19 and 21 years	CRP (dried blood spot)	CRP levels were not associated with later depression status; Cumulative depressive episodes predicted later CRP levels
Rengasamy et al., 2012	To examine the associations of IL-6 and TNF-α with depression severity and anhedonia severity	N: 36, age 12-18 years; 36 adolescents with depressive disorder, cross-sectional and longitudinal study	TNF-α, and IL-6	Baseline TNFα was positively associated with baseline and follow-up SHAPS anhedonia scores, and follow-up CDRS-R
Amitai et al., 2016	To determine whether plasma levels of proinflammatory cytokines can predict response to treatment and/or are altered post fluoxetine treatment in children and adolescents.	N: 41, age 7-18 years; children and adolescents with depression and/or anxiety disorders.	Plasma IL-1β, IL-6, and, TNF-α	Significantly higher levels of proinflammatory cytokines in SSRI-refractory than in SSRI-responsive Pts; TNF-α levels significantly reduced after 8 weeks of antidepressant treatment.

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da Silva et al., 2017	To compare serum levels of IL-6 and IL-10 between non-medicated adolescents with internalizing disorders and a comparison group of adolescents without internalizing disorders	N: 134, age 10-17 years; 76 adolescents with internalizing disorder and 58 adolescents without internalizing disorder	Plasma IL-6, and IL-10	Adolescents with internalizing disorders had significantly higher levels of IL-6 as compared to those without internalizing disorders
Perez-Sanchez et al., 2018	To detect 1) the alterations in the cytokine profiles of adolescents during 8 weeks of treatment with fluoxetine and 2) the correlation between symptomatology and inflammatory profiles	N: 40, age 14-19 years; 22 adolescents with first episode of MDD and 18 HCs, cross-sectional and longitudinal study	Plasma IL-2, IFN-γ, IL-1β, TNF-α, IL-6, , IL-15, , IL-10, IL-5, IL-13, IL-1Ra, and IL-12p70	Significantly increased levels of proinflammatory cytokines (IL-2, IFN-γ, IL-1β, TNF-α, IL-6, IL-12p70, and IL-15) and, anti-inflammatory cytokines (IL-4, IL-5, and IL-13) in MDD Pts; IFN-γ, IL-1β, TNF-α, IL-6, IL-12; decreased IL-15 only at week 4; increased IL-2 only at week 8; increased anti-inflammatory cytokines IL-4 and IL-5 at week 8
Peters et al., 2019	1. To compare groups with inflammation 2. To evaluate associations between inflammation and inhibitory control	N: , age 12-17 years; 22 depressive adolescents with childhood trauma (DEP-T), 18 depressive adolescents (DEP) and 40 HCs	Plasma IL-1β, TNF-α, IL-6	Significantly elevated levels of IL-6 in both DEP and DEP-T relative to HCs and significantly elevated levels of TNF-α in DEP; No group differences were detected in Il-1β; TNF-α was associated with behavior-based and observer-rated inhibitory control deficits.
Lee et al., 2020	1. To examine 1) the difference between inflammatory markers in MDD Pts and HCs and 2) whether these changes would be altered following antidepressant treatment 2. To investigate the relationship between cytokines level with the severity of depression	N: 50, age 13-18 years; 25 medication-naïve MDD Pts and 25 HCs, cross-sectional and longitudinal study	Plasma IL-1β, IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ	MDD Pts had significantly decreased level of plasm IL-2, IFN-γ, TNF-α and IL-10 compared to healthy controls; IL-2, IFN-γ and IL-10 showed significant increases after 12 weeks treatment compared to before treatment. IFN-γ level was negatively correlated with the CDI ($r = - 0.377$, $p < 0.01$) and HDRS score ($r = -0.457$, $p < 0.01$)

self-report Snaith-Hamilton Pleasure Scale (SHAPS), Children's Depression Rating Scale-Revised (CDRS-R), Healthy controls (HCs), Patients (Pts)

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2.2. BDNF in the neuroimmune system

BDNF is a protein of the neurotrophin family, encoded by the BDNF gene. Previous studies have suggested a link between BDNF and depressive disorders [50-53]. BDNF is synthesized in the brain and is widely distributed in the CNS [54]. BDNF plays an important role in neuronal development, neuroprotection, and modulation of synaptic interactions [55]. BDNF influences both neuronal structure and functional plasticity [17, 55]. Dysfunction of neural plasticity is related to the pathophysiology of depression. The BDNF hypothesis postulates that loss of BDNF plays a major role in the pathophysiology of depression [53, 56, 57]. In addition, changes in BDNF levels of patients with depressive disorder were observed after they received appropriate treatment. This treatment included antidepressant medication, repetitive transcranial magnetic stimulation, and electroconvulsive therapy (ECT) [58-61]. In a meta-analysis, patients with depressive disorder showed increased BDNF levels after ECT. BDNF has been suggested to be a potential indicator of ECT response [60]. Low levels of BDNF have been suggested to be associated with suicidality in patients with depressive disorders [62, 63]. The results of these changes in BDNF were relatively consistent in adults with MDD. However, in children and adolescents, the results were inconsistent [50-52, 64].

Several studies on the relationship between pediatric depressive disorder and serum BDNF levels have suggested that the MDD group showed decreased levels of BDNF compared to the healthy control group. This is similar to the results observed in adult depressive disorder. Pandey and colleagues observed decreased gene expression of BDNF in lymphocytes and decreased protein expression of BDNF in the platelets of adult and pediatric depressed patients compared to healthy controls [18]. In this study, they did not find a significant relationship between BDNF and the severity of depressive symptoms. This suggests that reduced BDNF may be associated with the diagnosis of depressive disorder. Sun et al. observed lower BDNF levels in patients with depressive disorder [64]. In this study, the group receiving comprehensive treatment showed higher serum BDNF levels than the group receiving routine treatment. The quality-of-life index showed greater improvement in the group with comprehensive treatment. Studies have reported that the role of BDNF differs according to sex. In a Japanese study of adolescents aged 8 to 15 years, Sasaki et al. confirmed that the serum level of BDNF differed according to sex in patients with MDD [50]. Male pediatric patients with MDD showed significantly decreased serum levels of BDNF compared to male healthy controls; however, female pediatric patients with MDD did not. Furthermore, there was a significant negative correlation between serum BDNF levels and the duration of illness only in males. In this study, there was no correlation between serum levels of BDNF and the Children's Depression Rating Scale-Revised (CDRS-R) scores in pediatric patients with MDD. They suggested that decreased serum levels of BDNF may play an important role in the pathophysiology of male pediatric depressive disorder [50]. Similar results of alterations in BDNF serum levels in adolescents were also confirmed in a study by Pallavi et al. [51]. Both male and female adolescents with depressive disorder showed a lower level of BDNF than the healthy controls. However, BDNF levels showed a negative correlation with the severity of depressive symptoms only in male patients, and not in female patients. BDNF expression and activity are affected by female hormones such as estrogen [65, 66]. For this reason, the authors suggested that BDNF may have a different role in depressive disorders depending on the sex [51].

There was one study that showed conflicting results with respect to higher levels of serum BDNF in adolescent depressive disorder. Bilgiç et al. compared 70 treatment-free MDD patients to a healthy control group aged 11 to 19 years [52]. Serum BDNF levels were significantly higher in adolescents with depressive disorder, and there was no correlation between BDNF levels and depressive symptoms or suicidality. In a study comparing healthy individuals with a family history of MDD and those without, the healthy individuals with a family history of MDD showed higher serum BDNF levels,

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and elevated BDNF levels were suggested as a risk factor for MDD [67]. In a longitudinal study, patients with MDD had a sharper decrease in BDNF over time compared to healthy controls [68]. Similarly, it has been reported that changes in BDNF are related to treatment response. Lee and colleagues found no significant differences in serum BDNF levels between drug-naïve depressive adolescents and healthy controls. However, they confirmed that the change in BDNF at 2 weeks of treatment was related to the SSRI response [69]. Early BDNF reduction (baseline-Week 2) was evaluated as a predictor of SSRI response at week 8. Taken together, an elevated serum BDNF level may be a finding observed in early stage adolescent depressive disorder. Further research is required to determine the exact mechanism of BDNF.

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Table 2. Previous studies of BDNF in pediatric depressive disorder

Study	Objective	Design	Findings
Pandey et., al, 2010	To examine the gene expression of BDNF of pediatric depressed Pts (drug naïve or unmedicated for a period of up to 2 weeks)	N: 28, 14 MDD Pts (age 14.9 ± 1.7) and 14 HCs (age 13.0 ± 1.7)	Significantly decreased gene expression of BDNF in the lymphocytes and the protein expression in the platelets of pediatric depressed Pts. compared with HCs
Sasaki et al., 2011	To investigate whether serum levels of BDNF are altered in pediatric Pts with depression	N: 52, age 8-15 years; 13 male and 17 female MDD Pts; 10 male and 12 female HCs	Significantly lower levels of serum BDNF only in male MDD Pts compared to male HCs, not in female MDD Pts; Significant negative correlation between the serum BDNF levels and the duration of illness in males, but not in females
Pallavi et al., 2013	1) To compare serum levels of BDNF in depression patients with healthy controls and 2) to investigate the correlation between clinical severity and serum BDNF levels	N: 148, age 13-18 years; 84 (56 males) MDD Pts, and 64 (39 males) HCs	Adolescents with depression had significantly lower levels of BDNF; BDI-II score showed a statistically significant negative correlation with BDNF in male patients, but not in female patients
Sun et al., 2017	To investigate 1) the correlation between serum BDNF and depression in children and 2) the change in BDNF after treatment	N: 178, age 7-16 years, 128 (55 males) MDD Pts, and 50 (25 males) HCs,	Significantly lower levels of serum BDNF in MDD Pts compared to HCs; MDD Pts with comprehensive nursing showed a significantly increase in BDNF expression
Bilgiç et al., 2020	To identify potential differences in serum BDNF levels in adolescents with MDD compared to HCs	N: 110, age 11-19 years; 70 treatment-free MDD Pts, and 40 HCs	Serum BDNF levels were significantly higher in adolescents with MDD than in HCs; No correlations between the levels of serum BDNF and the severity of depression or suicidality
Lee et al., 2020	To investigate whether pre-treatment BDNF levels and their early changes predict antidepressant response in MDD Pts	N: 135, age 12-17 years, 83 MDD Pts (46 responders and, 37 non-responders), 52 HCs; baseline, 2 weeks, 8 weeks follow-up	No significant findings of serum BDNF between the responders, non-responders, and HCs at baseline; Early decrease in BDNF levels of responders at week 2; Early BDNF decrease predicted later SSRI response at week 8

brain-derived neurotrophic factor (BDNF), self-report Snaith-Hamilton Pleasure Scale (SHAPS), Children's Depression Rating Scale-Revised (CDRS-R), healthy controls (HCs), patients (Pts), major depressive disorder (MDD), Columbia-Suicide Severity Rating Scale (CSSR-S)

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3. Neurohormonal system and pediatric depressive disorder

There are many known hormonal systems associated with pediatric depressive disorders. HPA, hypothalamus-pituitary-gonadal (HPG), hypothalamus-pituitary-somatotropic, and hypothalamic-pituitary-thyroid (HPT) axes are all known to be related to mood. The relationship between these neurohormonal axes and depressive disorders has been established through studies performed in adults. However, when we attempted to view studies with pediatric populations, the number of studies on the basis of evidence decreased.

The most studied neurohormonal system in depression is the HPA system. The main activator of the HPA axis is corticotropin-releasing hormone (CRH), which is released in the hypothalamus. CRH reaches the anterior pituitary and stimulates the release of adrenocorticotropic hormone (ACTH). ACTH stimulates cortisol secretion in the adrenal cortex. The HPA axis is known to be a stress regulating system. Depression has been linked to altered stress responses. When we consider the link between youthful depression and stress exposure, efforts to identify related biomarkers involve the HPA axis [70].

HPA axis dysregulation has been reported in several studies. Lopez-Duran et al. conducted a meta-analysis of depressed children and adolescents. They analyzed 17 published studies of the HPA axis response to the dexamethasone suppression test (DST) in depressed youth (total number: 926), 17 studies of basal HPA axis functioning (total number: 1332), four studies that compared cortisol levels post CRH infusion, and 3 studies that evaluated HPA axis reactivity to psychological stressors in children or adolescents with depression. The HPA axis system tends to be dysregulated in depressed youth, as evidenced by atypical responses to the DST and higher baseline cortisol values. When compared to non-depressed peers, depressed youths have a normative response to CRH infusion but an overactive response to psychological stressors [71]. The increased activity of the HPA axis is thought to be related, at least in part, to reduced feedback inhibition by endogenous glucocorticoids – cortisol in humans. It has been proposed that elevated cortisol in patients with depression is a compensatory mechanism in response to decreased glucocorticoid receptor function and expression in the brain. In addition, the disruption of glucocorticoid rhythms may also be related to depression [72].

The relationship between early childhood traumatic exposure and later development of depression in relation to the HPA axis has been reported [73]. Childhood trauma is related to the sensitization of the neuroendocrine stress response, glucocorticoid resistance – reduced feedback inhibition by endogenous glucocorticoids, increased CRH activity, HPA axis, and autonomic nervous system hyperreactivity. HPA-axis hyperreactivity was also observed in unaffected individuals at familial risk for depression and it predicted the onset of depression. This suggests that it may be a genetic vulnerability marker for depression [74]. Another study targeting adolescents who are at high risk for developing depression due to parental depression also showed elevated nocturnal urinary-free cortisol excretion at baseline. Elevated nocturnal urinary-free cortisol levels at baseline were associated with the development of depression over a 5-year follow-up period. Thus, we can conclude that these variables showing HPA activity might act as vulnerability biomarkers for depression [75].

In the case of the HPG axis, there is one meta-analysis of male depressive disorders. The participants' ages ranged from 18 to 97 years. Seventeen studies were included in the analysis. Depressed men showed diminished testosterone and marginally elevated estradiol levels ($p = .055$)[76]. Although the influence of the HPG axis in children and adolescents has not been studied thus far, the relationship between hypogonadism and depression has been suggested in certain studies. The physical development of puberty is accompanied by psychosocial and emotional changes. Disrupted puberty due to hypogonadism can be a psychological burden along with victimization and bullying that

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are associated with increased depression [77]. Symptoms of hypogonadism may also appear similar to symptoms of depression.

Dorn et al. reported that free thyroxine levels (free T4) [78] and triiodothyronine (T3) uptake [79] were lower in depressed adolescents than in controls. This shows the relationship between the HPT axis and depression. A previous study reported that cortisol levels and TSH levels were significantly elevated in comparison to controls ($p = <.001$, $d = 1.35$, large effect size, and $p = <.001$, $d = 0.79$, moderate effect size, respectively) in adolescent MDD. These results also show that HPT and HPA axis dysfunction are common in adolescents with MDD. However, no relationship between TSH and cortisol was found in depressed adolescents with elevated cortisol levels (cortisol levels at the 97.5th percentile). The interactive functions of both axes are loosely related and differ from the study results of adults. The relationship between the HPT and HPA axes in adolescents is influenced by age-related maturation [80].

4. Gastrointestinal (GI) biomarkers and pediatric depressive disorder

As depressive disorders have been shown to be associated with inflammation, the gastrointestinal tract has emerged as a possible source of such inflammatory activity. Intestinal permeability refers to the flow of material through the wall of the gastrointestinal tract into the rest of the body. This is how nutrients are absorbed and how potentially harmful substances are prevented from entering the body. Increased intestinal permeability is thought to be a factor in various diseases, including schizophrenia and autism **[81, 82]**. The concept of a 'leaky gut' is claimed to cause chronic inflammation throughout the body. However, solid evidence remains relatively scarce. Nevertheless, gut biomarkers are associated with depressive disorders; however, studies *on* pediatric patients are uncommon.

Ohlsson et al. proposed that zonulin and intestinal fatty acid binding protein (I-FABP) might be possible biomarkers for depressive disorders. Their results showed that I-FABP, a marker of enterocyte damage, was elevated in suicidal patients when compared to non-suicidal depression patients, whereas zonulin, a protein that modulates intestinal wall junctions, was decreased in suicidal patients **[83]**. These results suggest a possible relationship between intestinal permeability and severity of depressive symptoms. Zonulin levels have also been shown to be altered in pediatric patients with autism, attention deficit and hyperactivity disorders, or obsessive compulsive disorder; however, no studies have confirmed changes in children and adolescents with depression **[84]**.

A more recent study was conducted with unmedicated adolescents with major depressive disorder. Gut permeability was assessed using mannitol and lactulose. An increase in permeability was measured by a higher proportion of lactulose absorption compared to mannitol, since lactulose is a larger disaccharide. This lactulose-to-mannitol ratio (LMR) was shown to be significantly associated with the severity of depression, particularly with neurovegetative symptoms **[85]**.

Another important concept is the gut microbiome, which is the flora that live in the human digestive tract. Changes in the microbiome are related to an increase in pro-inflammatory cytokines, which in turn are associated with various psychiatric disorders. A recent review article summarized the effects of the gut microbiome on adolescent mental health. Changes in the microbiome impact the HPA axis and can induce depressive symptoms either through vagal nerve stimulation or by activating the kynurenine pathway **[86]**. Nevertheless, definite measurable biomarkers with high sensitivity and specificity are yet to be identified.

Research on the gut microbiome and relevant gastrointestinal biomarkers is increasing rapidly. Nevertheless, children and adolescents are not a central targeted group because of the natural difficulty of study design. Further in-depth research is crucial to establish a standard for the use of GI biomarkers in pediatric patients with depression.

5. Oxidative stress biomarkers and pediatric depressive disorder

Oxidative stress is the damage induced by reactive oxygen species as a result of insufficient detoxification of reactive intermediates by the biological system. Oxidative stress is thought to be associated with various psychiatric disorders, including autism, attention deficit hyperactivity disorder, and depression.

Relatively reliable markers of oxidative stress on DNA and lipids include 8-hydroxy-2′-deoxyguanosine (8-OHdG) and F2-isoprostanes. A meta-analysis by Black et al. reviewed the association between these biomarkers in depressed patients and reported that both were increased in depression **[87]**. It is interesting that 8-OHdG levels were strongly associated if measured from plasma or serum samples but not when measured from urine samples. Another study on inflammatory and oxidative stress markers and depression by Lindqvist et al. showed that F2-isoprostanes and 8-OHdG are both elevated in depressed patients. This study also showed that patients who did not respond to selective serotonin reuptake inhibitor treatment had higher levels of F2-isoprostanes before and after treatment and higher levels of 8-OHdG over the course of treatment **[88]**. Such studies may be small in number but they provide possible evidence for the use of oxidative stress biomarkers in depressive disorders.

Studies on oxidative stress in pediatric depression are scarce. A pilot study by Horn et al. with 50 adolescents suggested that elevated levels of F2-isoprostanes were associated with the severity of internalizing symptom scores and the existence of four or more adverse childhood events, such as abuse or neglect **[89]**. It should be noted that the clinical diagnosis of psychiatric disorders was not a part of this study. Guney et al. matched 40 children and adolescent patients with anxiety disorders with healthy controls. Oxidative stress was measured with a colorimetric method using a ferrous ioneo-dianisidine complex, resulting in an oxidation reaction with the venous blood sample. The reaction was expressed and measured as the total oxidative status, which was shown to be higher in children with anxiety disorders than in controls **[90]**. Another interesting review by Tobore suggested that cigarettes and electronic cigarettes could elevate oxidative stress. This in turn may be related to various social maladjustments, including aggressive behavior, impaired cognition, and depressed mood **[91]**. In an animal study, Moradi-Kor et al. worked with adolescent rats to investigate the effects of stress and various measures to nullify such effects. Stress in adolescence is associated with enhanced anxiety levels and depression in adulthood, as well as with elevated oxidative stress markers. Environmental enrichment, exercise, and treatment with *Spirulina platensis* tended to reduce oxidative damage induced by stress **[92]**.

Papers on oxidative stress are quite common. However, none of them are well designed studies with an adolescent patient group with clinically diagnosed depression. Further research is necessary to validate the use of oxidative stress markers for various purposes in pediatric patients with depression.

6. Summary and Conclusions

We have reviewed various biomarkers, including cytokines, BDNF, neurohormones, GI biomarkers, and oxidative stress indices specifically studied for childhood and adolescent depressive disorders. Although biomarker tests are not yet available as definitive diagnostic tools or to predict the prognosis of treatment in the clinical field, certain biomarkers have shown significant results in previous studies of pediatric depressive disorder. These help us to better understand the pathophysiology of pediatric depression. These biomarkers have the potential to be used for diagnosis and prognostic assessment, as well as for high-risk group screening. By integrating features from these biomarkers and using machine learning-driven biomarker discovery, we can expect to find additional advanced, clinically useful biomarkers.

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Abbreviations

ACTH, adrenocorticotropic hormone

BDNF, Brain-Derived neurotrophic factor

CDRS-R, Children's Depression Rating Scale-Revised

CNS, central nervous system

CRH, corticotropin-releasing hormone

CRP, C-reactive protein

CSSR-S, Columbia-Suicide Severity Rating Scale

DST, dexamethasone suppression test

ECT, Electroconvulsive therapy

GI, Gastrointestinal

HCs, Healthy controls

HPA, hypothalamic-pituitary-adrenal

HPG, hypothalamus-pituitary-gonadal

HPT, hypothalamic-pituitary-thyroid

MDD, Major depressive disorder

I-FABP, intestinal fatty acid binding protein

IFN-γ, Interferon-γ

IL, interleukin

LMR, lactulose to mannitol ratio

Pts, Patients

SHAPS, self-report Snaith-Hamilton Pleasure Scale

TNF, Tumor Necrosis Factor

8-OHdG, 8-hydroxy-2′-deoxyguanosine

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