BIOCHEMICAL PROFILE OR BIOMARKERS OF ANXIETY:

Biomarkers are defined as anatomical, biochemical or physiological traits that are specific to certain disorders or syndromes.

1. 5-HT (5-hydroxytryptamine) also known as serotonin:

A hormone and neurotransmitter, 5-hydroxytryptamine (5-HT), found in many tissues, including blood platelets, intestinal mucosa, pineal body, and central nervous system; it has many physiologic properties, including inhibition of gastric secretion, stimulation of smooth muscles, and production of vasoconstriction.

Decreased serotonergic activity has been implicated in anxiety and major depression, and antidepressants directly or indirectly increase the long-term activity of the serotonin system. A key component of serotonin circuitry is the 5-HT1A autoreceptor, which functions as the major somatodendritic autoreceptor to negatively regulate the “gain” of the serotonin system. In addition, 5-HT1A heteroreceptors are abundantly expressed post-synaptically in the prefrontal cortex (PFC), amygdala, and hippocampus to mediate serotonin actions on fear, anxiety, stress, and cognition.Importantly, in the PFC 5-HT1A heteroreceptors are expressed on at least two antagonist neuronal populations: excitatory pyramidal neurons and inhibitory interneurons. Rodent models implicate the 5-HT1A receptor in anxiety- and depression-like phenotypes with distinct roles for pre- and post-synaptic 5-HT1A receptors.

1. C-reactive protein (CRP):

CRP is a pentameric protein synthesized by the liver, whose level rises in response to inflammation. CRP is an acute-phase reactant protein that is primarily induced by the IL-6 action on the gene responsible for the transcription of CRP during the acute phase of an inflammatory/infectious process.Two recent studies have correlated anxiety symptoms with increased cytokine levels, in particular C-reactive protein (CRP).With regard to anxiety disorders, research has mainly focused on posttraumatic stress disorder, in which high levels of inflammatory markers have been found.**CRP is**elevated in chronic stress and may be **the link between stress and low-grade inflammation-related diseases.** Scientists found that both psychological and social stress significantly impacts CRP. In a study that examined job stress and CRP levels among Chinese workers, effort, overcommitment, and effort-reward imbalance were all significantly correlated with higher CRP; while rewards were significantly related to lower CRP.A link was found between higher CRP levels, chronic stress, and burnout in women with psoriasis.A study suggests that people who engage and work on resolving their issues (positive engagement coping) may have lower CRP in the context of interpersonal stress (e.g., arguments with parents or siblings, conflicts between adults in the home, friendships ended).

1. **Interleukin-1β:**

Interleukin-1, an inflammatory cytokine, is considered to have diverse physiological functions and pathological significances and play an important role in health and disease.The cytokine interleukin-1β (IL-1β) is a key mediator of the inflammatory response. Essential for the host-response and resistance to pathogens, it also exacerbates damage during chronic disease and acute tissue injury.

The role of inflammatory cytokines in the pathophysiology of mood disturbances is increasingly recognized. For example, high levels of proinflammatory cytokines have been found in peripheral blood and in CSF of depressed patients, and blockade of cytokine signaling is associated with relevant mood-enhancing effects in humans.In addition, systemic administration of lipopolysaccharide, which induces the expression of interleukin-1β (IL-1β) and of other inflammatory cytokines in the brain causes depression and anxiety in healthy subjects. In line with this, the clinical use of IL-2, interferon-α, or interferon-β in chronic diseases has been convincingly associated with the development of anxious depression and suicidal ideation.Also, ample data show that proinflammatory cytokines in rodents are involved in mood regulation and, in fact, systemic or central administration of the major inflammatory cytokine IL-1β causes behavioral manifestations closely resembling anxious-depressive symptoms in humans, including anhedonia, reduced exploratory behaviors, social withdrawal, fatigue, and sleep disturbances. The mechanism by which inflammation exerts its mood-controlling effects is largely undetermined, although alterations of serotonin metabolism and of the hypotalamus–pituitary–adrenal (HPA) axis have been implicated.

1. Alkaline phosphatase (ALP)

Results showed a positive correlation 0.511 between the scores of anxiety and depression. It was also found out that most of the liver patients were experiencing anxiety (n=73, 71.6 %) and depression (n=72, 70.6 %). Negative correlation -0.335 was observed between years of education and depression while housewives were found to be more anxious than people doing jobs.

Hepatitis C is now the leading cause of end stage liver failure and leading indication for liver transplant in the developed world. Like many other medical illnesses, hepatitis C is also associated with an increased prevalence of psychiatric disorders particularly anxiety and depression3, 4.

The evidence about presence of psychiatric symptoms in hepatitis is important because they have an adverse effect upon the course of illness in form of amplification of physical symptoms, functional impairment, reduced treatment compliance, and reduced quality of life5 The association is of particular importance in hepatitis C because hepatic patients often come from population groups at risk of psychiatric disorders, such as injecting drug users.

Another study indicates that about 55% of all the liver cirrhosis patients developed diagnosable psychiatric morbidity. These psychiatric conditions include depressive episode, generalized anxiety disorder, delirium, and adjustment disorder. The same proportion (55%) of hemodialysis patients and a lesser proportion (30%) of Chronic Obstructive Pulmonary Disease (COPD) patients compared with cirrhosis patients were found to have psychiatric morbidity.

Patients with HCV face numerous emotional and psychosocial stressors that have a significant effect on well-being. These stressors include adjusting to and managing this chronic disease and making lifestyle changes [[8](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4196401/#R8)]. Consequently, mental health clinicians who work with HCV patients may find themselves unable to anticipate and unprepared to manage the diverse array of psychological issues that they will encounter

There was a significant decrease in mean values of Alkaline Phosphatase (ALP).

1. Creatinine:

A creatinine test is a measure of how well your kidneys are performing their job of filtering waste from your blood.Creatinine is a chemical compound left over from energy-producing processes in your muscles. Healthy kidneys filter creatinine out of the blood. Creatinine exits your body as a waste product in urine.

The endogenous creatinine clearance rate and psychosocial management ability were significantly higher in patients without anxiety and depression than in patients with anxiety and depression.

1. Dopamine

Dopamine is involved in reward-motivated behaviour and motor control. Findings on brain imaging and genetics of the dopamine system.

##### *PDA (*Panic disorder with or without Agoraphobia)

[Eriksson et al. (1991)](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5341771/#R153) reported no significant change in CSF levels of HVA, the major metabolite of dopamine in patients with PDA compared with healthy controls. Nevertheless, in another study in both PDA and SAD, low CSF HVA levels were observed ([Johnson et al. 1994](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5341771/#R240)).

##### *SAD (social anxiety disorder)*

In a study evaluating eye-blink response to administered levodopa, no dysfunction of the dopaminergic system was reported ([Tancer et al. 1994a](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5341771/#R469)). Another approach is to challenge with dopamine agents such as the antagonist sulpiride and the agonist pramipexole. [Hood et al. (2010)](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5341771/#R227) found that patients with SAD responded with increased anxiety to both drugs but that the effect of treatment with SSRIs was to attenuate the impact of pramipexole, suggesting a degree of dopamine D3 receptor desensitisation after SSRI treatment.

##### *OCD (*Obsessive-Compulsive Disorder)

Acute deep brain stimulation targeted at the nucleus accumbens of 15 OCD patients induced a decrease in binding potential to the dopamine D2/D3 receptor (measured via SPECT [123I]IBZM binding), and chronic stimulation induced an increase in HVA plasma levels, implying that deep brain stimulation induces striatal dopamine release in OCD patients ([Figee et al. 2014](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5341771/#R168)).

##### *PTSD (posttraumatic* stress disorder)

In the aforementioned study by [Geracioti et al. (2013)](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5341771/#R184), CSF HVA concentrations diminished significantly after a traumatic video. Compared with control subjects, PTSD subjects showed significantly higher mean 24-h urinary excretion of dopamine ([Spivak et al. 1999](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5341771/#R440))

7. Norepinephrine

(Noradrenaline; NE) has been connected to “emotional memory” and the consolidation and retrieval of the emotional arousal induced by particular behaviours ([van Praag et al. 1990](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5341771/#R500); [Goddard et al. 2010](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5341771/#R192)). NE neurons regulate vulnerability to social defeat through inhibitory control of ventral tegmental area DA neurons ([Isingrini et al. 2016](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5341771/#R236)).

NE is a catecholamine produced mainly in the locus coeruleus in the pons. It is an important neurotransmitter in the autonomic nervous system. The metabolism and functions of norepinephrine have been studied extensively in depression and anxiety disorders. Hypofunction is postulated for the former, and hyperfunction for the latter.

##### *PDA*

Stimulation of noradrenergic systems produces abnormal changes in measures of anxiety, somatic symptoms, blood pressure and plasma NE metabolite and cortisol levels in patients with PDA but not in patients with GAD, OCD, depression or schizophrenia, indicating specificity of abnormality in the regulation of the NE system in patients with PDA ([Boulenger & Uhde 1982](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5341771/#R56); [Heninger & Charney 1988](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5341771/#R219)).

There is a body of evidence for NE involvement in anxiety in humans; e.g., anxiety can be induced using NE neuronal activators such as piperoxane and yohimbine ([Redmond & Huang 1979](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5341771/#R392)). In patients with PDA, peripheral markers, including platelet aggregation to NE and to 5-HT, platelet a2-receptor density, lymphocyte β-receptor density, [3H] ketanserin binding to platelet 5-HT2 receptors and [3H] 5-HTT uptake into platelets, largely remained abnormal during 6 months treatment with either clomipramine or lofepramine, despite clinical improvement ([Butler et al. 1992](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5341771/#R72)). Therefore, these peripheral markers have been suggested to be potential trait markers in patients with PDA. Adrenergic receptor function has been measured in several clinical studies. Platelet α2-adrenoceptors have been studied in PDA patients using clonidine and yohimbine binding assays and correlated to symptom ratings and measurement of lying and standing plasma adrenaline and NE levels ([Cameron et al. 1996](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5341771/#R76); [Nutt & Fraser 1987](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5341771/#R346)). Tritiated clonidine binding was decreased and resting heart rate was increased in PDA patients before treatment (fluoxetine, tricyclics or alprazolam). The magnitude of decrease in receptor binding was correlated with symptom severity and standing plasma NE ([Cameron et al. 1996](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5341771/#R76)). In a similar approach, [Gurguis et al. (1999)](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5341771/#R210) showed that patients with PDA had high α2-adrenoceptor density in both conformational states.

#### γ -Aminobutyric acid

There is ample evidence that the pathogenesis of anxiety disorders is in part linked to a dysfunction of central inhibitory mechanisms. With regard to neurotransmission, the γ-aminobutyric acid (GABA) system serves as the most important inhibitory neurotransmitter system ([Domschke & Zwanzger 2008](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5341771/#R145)). According to both preclinical and clinical studies, this system has been suggested to be strongly involved in the pathophysiology of anxiety and anxiety disorders. For example, benzodiazepines, which act at the GABA system, are used to treat anxiety. GABA is synthesised by a specific enzyme – glutamate acid decarboxylase – from glutamate. Released in the synaptic cleft, it either binds on GABA receptors or is removed by the main degradative enzyme GABA-transaminase (GABA-T)

1. Lipids:

Negative correlations between anxiety and high-density lipoprotein (HDL) levels were observed, while higher triglyceride levels were observed in patients with depression and comorbid anxiety compared to depressive patients without anxiety. Furthermore, serum triglycerides, very-low-density lipoprotein (VLDL)-cholesterol and free-cholesterol were higher in patients with anxiety disorders as compared to healthy controls, whereas the opposite was observed for esterified cholesterol. A study conducted in menopausal women observed no correlation between lipid profiles (total cholesterol, HDL, VLDL, low-density lipoproteins (LDL), triglycerides) and anxiety. In young women, on the other hand, low lipid and lipoprotein levels (cholesterol, LDL, total cholesterol, ratio of total cholesterol to HDL) were inversely correlated with anxiety scores. Huang et al. observed differences in HDL cholesterol and the ratio of total cholesterol to HDL with regard to an anxious state in men. In healthy men, levels of total cholesterol and LDL cholesterol were higher in those who scored higher on an anxiety inventory. Thus, several studies support the role of lipids in anxiety disorders, although differences with respect to gender and hormonal status likely exist.

increasing evidence suggests a crucial role for membrane lipids and lipid oxidation in the pathogenesis of anxiety disorders. Membrane lipids play a pivotal role in the barrier and signaling function of membranes. As dysfunctions in neuronal proteins and peptide activities are considered as a primary cause of anxiety disorders, brain lipids are essential for transmitter signaling. Lipids essential for membrane formation, i.e., n-3 polyunsaturated fatty acids, phospholipids, glycerolipids, and sphingolipids, are assumed to be involved in the pathogenesis of anxiety disorders, especially. The lipid composition of neuronal membranes is highly dynamic and likely affects the assembly of signaling proteins and, thus, neuronal signaling and function.

Omega-3 fatty acids serve as precursors for the synthesis of eicosanoids, which might induce perturbations of the system of inflammatory mediators. Anxiety disorders have been linked to inflammation. Thus, the consumption of specific fatty acids or leukotriene receptor antagonists might also contribute to the maintenance of the anxiety symptoms.

1. nitro-oxidative stress(NOx):

Studies also indicate a role of nitro-oxidative stress driving lipid oxidation and lowered lipid-antioxidant defenses in anxiety disorders. More specifically, increased superoxide dismutase, lipid hydroperoxides, nitric oxide metabolites (NOx), and uric acid were measured in individuals with general anxiety disorders than in those without anxiety disorders. Those changes were accompanied by a decrease in HDL and paraoxonase-1. It is suggested that the inflammation due to the overproduction of NOx is involved in the pathology of anxiety disorders. However, while studies focusing on NOx levels in acute stress models observed associations between anxiety and NOx, a study analyzing salivary NOx in daily psychological stress in humans and anxiety observed only correlations between stress and anxiety, but not between salivary NOx and anxiety.

1. lipid hydroperoxide :

Several studies in animals and humans have demonstrated a potential link of anxiety disorders with oxidative stress and lipid peroxidation, as neurochemical causes of anxiety disorders. Lipid peroxidation was enhanced in children with anxiety disorders as compared to a control group, as indicated by increased serum levels of lipid hydroperoxide. Thus, lipid hydroperoxide has been speculated as a potential biomarker for anxiety disorders. Oxidative stress as indicated by elevated levels of lipid hydroperoxide and lower paraoxonase activity (an HDL associated enzyme protecting lipids from peroxidation have been observed in individuals with generalized anxiety disorder (GAD) without any comorbid psychiatric disorder, further supporting the role of lipid peroxidation and oxidative stress in the etiopathogenesis of GAD. Thus, lipid hydroperoxide has been speculated as a potential biomarker for anxiety disorders.

1. salivary cortisone:

As many studies highlight the association between stress and anxiety disorders, salivary cortisone was suggested not only as a stress biomarker but also as a marker of state anxiety. Salivary alpha-amylase—a maker of sympathetic nervous system activity —was observed to be higher in adults with a higher dental anxiety score, thus showing potential to serve as a biomarker of dental anxiety [[71](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7369790/#B71-ijms-21-04784)]. However, a study conducted in children with and without temporomandibular disorders observed higher anxiety symptoms in children with the disorder, but no difference in salivary alpha-amylase and also salivary cortisol. However, elevated hair cortisol was found to predict later development of anxious behavior in response to a major life stressor in infant monkeys, thus showing some potential as a biomarker for stress-related mental problems. In healthy volunteers exposed to a psychosocial stressor, the anxiety score was associated with salivary alpha-amylase, but not to salivary cortisol or chromogranin-A. Therefore, further studies are needed to clarify whether cortisol, cortisone, and alpha-amylase show potential as biomarkers for anxiety disorders.

1. The neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP)

The neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) is assumed to be involved in stress response and has been suggested as a biomarker for the severity of stress-related psychiatric disorders. Serum PACAP analysis in male and female individuals diagnosed with GAD compared to healthy controls revealed no overall association between circulating PACAP and GAD, but an association in women, supporting prior work suggesting potential sex differences in PACAP effects, likely due to estrogen-dependent regulation of this pathway. The neurotrophin fibroblast growth factor-2 (FGF2)—a protein involved in stress regulation and neurogeneration —is also considered as an endogenous regulator of fear expression. Thus, FGF2 might also serve as a potential biomarker for anxiety disorders; however, further research is required to elucidate the potential of FGF2 to identify vulnerable individuals and to establish preventative interventions.

1. triglycerides

metabolic disturbances predict anxiety and, here, triglycerides are the significant biomarker features. This marker, amongst others, is indicative of metabolic syndrome, which is known as a cluster of conditions occurring together, increasing people’s risk of heart disease, stroke and type 2 diabetes. These conditions include increased excess body fat around the waist, and abnormal triglyceride levels. Higher triglyceride molecules are related to obesity and obesity that leads to chronic inflammation. People with a low body mass index (BMI) have adipose tissue that expresses M2 macrophages (stimulated by, e.g., cytokine interleukin 4 (Il-4)) whereas obese people recruit M1 macrophages (evoked by e.g. cytokine interferon-γ (IFNγ) and obese people shift the M1/M2 balance in favor of M1. These pro-inflammatory cytokines (such as IFNγ or TNFα) then reach the brain via the humoral, neural and cellular routes, where they cause the already mentioned shift in balance from M2 to M1 activation in microglia (or macrophages), thus, causing anxiety.

1. Glutamate:

Glutamate is an amino acid that functions as an excitatory neurotransmitter. It has also been associated with somatic and psychiatric distress and implicated in the pathophysiology of psychiatric disorders such as schizophrenia.

In addition to glutamate’s association with somatic distress such as pain, pain sensitivity, physical weakness, and fibromyalgia symptoms, glutamate has also been associated with psychiatric distress. Specifically, central system glutamate dysregulation has been associated with symptoms of anxiety, posttraumatic stress, obsessive-compulsive disorder, mania, depression, and psychosis, with the strongest evidence for glutamate’s role in schizophrenia . As outlined below, altered glutamate homeostasis across various psychiatric disorders suggests the potential utility for psychopharmacological interventions targeted at the glutamate system as well as dietary interventions.

In addition to anxiety, trauma, and obsessive-compulsive symptomatology and disorders, glutamatergic dysregulation has been demonstrated in mood disorders across both bipolar and depressive disorders (for a review, see Sanacora et al. ). Specifically, for bipolar disorder, characterized by periods of mania and depression , elevated glutamate neurotransmission has been demonstrated across multiple studies and converging methodologies, including post-mortem , neuroimaging in acute mania , specific brain regions as measured with MRS

While research on glutamatergic homeostasis in mood disorders has associated bipolar disorder with excessive levels of glutamate, depressive disorders are thought to show reduced glutamate neurotransmission , which has promoted much of the research in regards to ketamine’s use in depression, as ketamine is a known NMDAR antagonist which contributes to its antidepressant effect.

1. Cortisol

Cortisol is a steroid hormone that is produced by the adrenal glands, which sit on top of each kidney. When released into the bloodstream, cortisol can act on many different parts of the body and can help:

* the body respond to stress or danger
* increase the body’s metabolism of glucose
* control blood pressure
* reduce inflammation

Another approach is to use challenge tests to provoke anxiety/stress. In one, administration of 7.5% carbon dioxide did not significantly change salivary cortisol levels in medication-free GAD patients. Moreover, no difference in pre-sleep salivary cortisol level was found among children with GAD, despite the presence of sleep disturbances.On the other hand, both higher and lower cortisol awakening responses were observed among elderly GAD patients than with nonanxious controls, positively associated with symptomatic severity in one studyand irrespective of the duration of illness in another one. Furthermore, both untreated and venlafaxine-treated GAD patients demonstrated significantly higher cortisol levels than normal controls in a clonidine-challenge study. Nevertheless, some studies reported a significant reduction in post-treatment cortisol levels after successful psychological or pharmacological treatment of GAD. For example, elevated plasma cortisol levels decreased after successful CBT, and greater reductions in both peak and total salivary cortisol were shown in elderly GAD patients after escitalopram treatment than in placebo-treated patients. However, no association was reported between a positive therapeutic outcome to buspironeor alprazolam and post-treatment cortisol levels in GAD.