Biochemical Mechanisms and Methodologies Applied to the Study of Posttraumatic Stress Disorder (PTSD)

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Abstract
PTSD has been considered as a syndrome with multiple faces due to the complexity of its pathophysiology. There is a need to elucidate the biochemical mechanisms involved in the disease in order to improve its diagnosis, prognosis, and treatment. Believing that the university has the responsibility to help the community, and hoping for the development of research devoted to this matter, the purpose of the present study was to present a brief review about the main biochemical mechanisms involved in PTSD and the methodologies applied to assess the disease. The review was done based on recent literature. According to the studies PTSD presents pre-exposure vulnerability factors, besides trauma-induced alterations. The disease was found associated with hypothalamic-pituitary-adrenal axis and hypothalamus-pituitary-thyroid axis dysfunctions. Alterations of sympathetic nervous system activity play a role in PTSD by releasing norepinephrine and epinephrine. The release of cortisol from the adrenal cortex amplifies the SNS response, reducing it later through negative feedback mechanisms. This response leads to a decreased level of cortisol in patients with PTSD. The negative feedback contributes to neuroendocrine alterations, promoting structural brain changes that culminate in PTSD. Abnormal levels of serotonin and dopamine have been found in the disease. Mechanisms such as the induction of neuroinflammation and alterations of mitochondrial energy handling were also associated with PTSD. Controversies can be found regarding to which biomarkers would be possible for the disease. Therefore, there is a need for studies in order to find biomarkers for PTSD.

Keywords: PTSD; Stress; Trauma; Homeostasis; Biomarkers.

Introduction
The post-traumatic stress disorder (PTSD) is a severe anxiety disorder caused by exposure to an event with actual, threatened, or perceived death or serious injury, or a threat to the physical integrity of oneself or others that results in significant psychological trauma.1
In Iraq, the possibility of having an increased incidence of PTSD should be taken into consideration due to frequent extremes adversities faced, especially wars and internal conflicts. These events make the disease a relevant mental health problem among the population. In 2017, the “International Organization for Migration” identified over 3 million Iraqis internally displaced by violence.2
The Yazidi’s ancestral homeland in northwestern Iraq was attacked by the Islamic State of Iraq and Syria (ISIS), in 2014.3 During that time the Yazidis living in Sinjar found refuge in different regions of Kurdistan Independent Region. As a result of this context, there are millions of people living in internally displaced people (IDP) camps and villages in many different areas of Kurdistan, including its capital Erbil, and the city of Dohuk. Among other atrocities, ISIS abducted thousands of women and girls and traded many of them into sexual slavery.3 To make it worse is estimated that the prevalence of PTSD following trauma is higher in women (10-13%)4,5 than in the general population (5 and 10%)6. In many IDP families, there are individuals who have been rescued from ISIS captivity. In this dramatic context, PTSD represents a mental
health problem that urges to be better understood and treated. Therefore, the purpose of the present article was to do a brief review of the main biochemical mechanisms and methodologies applied to assess PTSD. This review was done based on data collected from recent literature.

**Literature Review**

The peripheral biologic correlates of PTSD to date encompass genes, epigenetic regulation, neuroendocrine factors, inflammatory markers, autonomic risk and resilience, and sleep disturbances. Some biologic features constitute preexposure vulnerability factors such as a polymorphism in the *FKBP5* gene, environmental exposure, socio-cultural beliefs and heart-rate variability, pre-conditioning, among others. However others biologic factors might reflect trauma-induced alterations such as immune changes, neuroinflammation, and postexposure epigenetic regulation.

**Orchestrate participation of SNS, HPA and HPT in the development of PTSD**

Neurobiological findings showed that PTSD is associated with hypothalamic-pituitary-adrenal axis (HPA) dysfunctions and other brain-related structures such as prefrontal cortex, hippocampus, and amygdala, which are related to appropriate contextual processing. The hypothalamus-pituitary-thyroid (HPT) axis was found also dis-regulated in the presence of the disease.

Research examining acute biological risk factors for PTSD has implicated HPA hypoactivity in addition to SNS hyperactivity. These two stress response systems may have independent effects on the development of PTSD symptoms, with lower cortisol levels associated with avoidance behaviors and higher catecholamines associated with reexperiencing and hyperarousal symptoms. A hypo-responsive hypothalamic-pituitary axis and hyper-responsive catecholamine system (persistently elevated blood norepinephrine levels and lower than appropriate glucocorticoid levels) have been understood as the main responses to PTSD. Taken together these responses indicate that the HPA axis has grown resistant to the effects of cortisol.

Meta-analytic findings regarding peripheral indicators of noradrenergic activity indicate that PTSD is associated with elevated resting heart rate, skin conductance, and blood pressure (both systolic and diastolic). Long-term hair cortisol levels and lower cortisol stress reactivity were considered predictive of a greater increase in PTSD symptomatology. According to Yehuda two-factor model of acute biological risk for PTSD, either SNS hypersecretion or cortisol hyposecretion during trauma exposure are capable of triggering PTSD symptoms. The decreased levels of cortisol, the increased responsiveness of glucocorticoid receptors, the increased sensitivity of the HPA negative feedback inhibition and its progressive sensitization are the neuroendocrine alterations specifically associated with the development of PTSD.

In addition to having inappropriately low serum cortisol levels and high epinephrine and norepinephrine levels, patients with PTSD also have mitochondrial dysfunctions and other hormonal abnormalities. These include subclinical hypothyroidism or hyperthyroidism and higher levels of brain natural opioids. Biochemical and sustained neurohormonal abnormalities are likely to influence the structural brain changes, particularly in the amygdala and hippocampus, which are characteristics of patients with PTSD.

Since SNS activation has been suggested to moderate cortisol’s effect on memory two factors can be analyzed. First, salivary alpha-amylase (sAA) and its interaction with cortisol in a synergistic fashion to predict the number and vividness of intrusive memories. Second, the cardiac defense response (CDR) which is a heart rate (HR) response to a sudden onset of loud noise. Baseline assessment of the psychological diathesis (i.e. psychiatric history and peritraumatic distress and dissociation), and the biological diathesis (i.e. cortisol, norepinephrine, epinephrine, C-reactive protein, total cholesterol, HDL cholesterol, glycosylated haemoglobin, waist-to-hip ratio (WHR), body mass index, diastolic and systolic
blood pressure and heart rate during the first week and at 1, 4, and 12 months post-trauma has shown the psychological diathesis as a better predictor of short-term dysfunction whereas biological diathesis has also been predictive of development and maintenance of PTSD. Furthermore, several lines of evidence point toward accelerated age-related processes in PTSD, reflected for instance in shortened telomere length, enhanced DNA damage or an altered N-glycosylation profile.

Recent work has shown that patients diagnosed with PTSD besides the lower plasma cortisol, have lower prolactin and TSH levels compared to the comparison group. The neuropeptides oxytocin (OT) and arginine vasopressin (AVP) have been associated with both regulating fear and neuroendocrine stress responsiveness and social behaviour. Basal salivary OT and AVP levels were measured in trauma-exposed male and female with and without PTSD. Saliva samples were collected during rest and OT and AVP levels were determined by radioimmunoassay. The findings indicate potential dysfunctioning of the OT system in male PTSD patients.

Oxidative stress in PTSD pathophysiology

Oxidative stress has been shown as paying an important role in PTSD. The brain is highly sensitive to oxidative stress as it consumes about 20–30% of inspired oxygen, contains high levels of both polyunsaturated fatty acids (PUFA) and redox transition metals and has lower antioxidant defenses compared to other organs. All these factors make the brain an ideal target for free radical attack. Of all the brain cells, neurons are particularly vulnerable to oxidative insults due to low levels of reduced glutathione.

The damage to brain membrane lipids is an early event. In thirty minutes after trauma, higher levels of MDA and 4-HNE can be detected, whose levels are maintained elevated 72 h after the injury onset. The peroxidation of membrane lipids can change the membrane function by modifying its fluidity, permeability, metabolic processes, and ionic equilibrium. Damage to mitochondrial membranes can also increase the production of ROS, besides generating mitochondrial dysfunction. Most studies analyzing the oxidative damage to lipids in animal models find a correlation between this parameter in conjunction with cognitive damage, installation of edema, and volume of injury. These data suggest that the damage to lipids of biological membranes can be an important event in the pathology of PTSD.

In an animal model of PTSD, inflammation and oxidative stress were reported to play a critical role in the development and exacerbation of PTSD. Oxidative damage to lipids can be estimated with assays for compounds called isoprostanes that is derived from either enzymatic, by cyclooxygenase, or nonenzymatic oxidation of arachidonic acid. F2-Isoprostanes are widely used because they are chemically stable, specific products of peroxidation, present in detectable amounts in all normal tissues and bodily fluids, and unaffected by lipid content in the diet is considered an excellent marker of oxidative stress in vivo. Some clinical studies have observed a marked increase of cerebral neurotransmitter glutamate in PTSD. Stress induces glutamate release, which is recognized as an important mediator of excitotoxicity. Glutamatergic pathways may have an important role in stress-related hippocampal degenerative pathology, neuronal damage and cognitive deficits seen in patients with PTSD. A positive correlations between antioxidant enzymes activities such us SOD, GPx and MDA, with the severity of PTSD has been found. This correlation may support the involvement of mild oxidative stress in the pathogenesis of PTSD.

Inflammation in PTSD pathogenesis

Observational studies largely support an association of PTSD with increased peripheral inflammation. A large cross-sectional community-based study found that patients with PTSD had about twice the odds of those without this disorder of elevation in the inflammatory marker, C-reactive protein (CRP). In most such studies PTSD cohorts have had significantly greater
plasma levels of CRP or IL-6, alteration of Tumor necrosis α and IL1β among other inflammatory markers, than did controls.68-61 It is plausible that the observed association between PTSD and inflammation is due to PTSD-related stress hormone dysregulation leading to alterations in immune, and therefore inflammatory signaling.60,62-64 However, it remains possible that rather than PTSD promoting inflammation, inflammation places individuals at heightened risk for developing PTSD in the setting of trauma – in other words, the direction of causality runs from inflammation to PTSD rather than from PTSD to inflammation. A marker of peripheral inflammation, plasma CRP, may be prospectively associated with PTSD symptom emergence, suggesting that inflammation may predispose to PTSD.

Impact of PTSD on coming generations
Since 1918 has been demonstrated that 74% of 100 patients experiencing war neuroses showed a family history of psychoneurosis compared to none of 100 matched comparison subjects.65 The concept that familial contributions can improve the likelihood of developing PTSD has found reinforcement by studies showing a relationship between psychological responses in trauma survivors and family history of psychopathology in veterans, families of soldiers and traumatized civilians exposed to war or natural disaster.66-69 An interesting study proceeded in Holocaust survivors has suggested that their children constitute a high-risk group for PTSD since they were found to have a greater prevalence of lifetime PTSD compared to demographically similar persons.70 Additionally, adult children of Holocaust survivors also showed a greater prevalence of mood and other anxiety disorders.70 Therefore PTSD in children of Holocaust survivors appeared to be strongly related to parental PTSD.

According to the studies was concluded that parental rearing practices may be substantially affected by the presence of PTSD in one or both parents. The effect of maternal behavior has been shown to persist across multiple generations and to be associated with increased hippocampal glucocorticoid receptor expression.70 This effect has been understood as a nongenomic transmission of stress. In such situations as shown in the study with Holocaust survivors cortisol level of offspring with both parental PTSD and lifetime PTSD was significantly different from that of offspring with no parental PTSD and no lifetime PTSD and that of comparison.70 Therefore, parental PTSD, has been considered a putative risk factor for PTSD, and it appears to be associated with low cortisol levels in offspring, even in the absence of lifetime PTSD in the offspring.70 The findings suggest that low cortisol levels in PTSD may constitute a vulnerability marker related to parental PTSD.

Methodologies applied to assess PTSD
Baseline assessment of the psychological and biological diathesis

1- Psychological diathesis:
Semistructured Diagnostic Interviews:
The Clinician-Administered PTSD Scale (CAPS) is one of the most widely used semistructured clinical interviews for the assessment of PTSD.71

2- Biological diathesis:
Evaluation of the following parameters: cortisol, norepinephrine, epinephrine, C-reactive protein, total cholesterol, HDL cholesterol, glycosylated haemoglobin, waist-to-hip ratio (WHR), body mass index, diastolic and systolic blood pressure and heart rate during the first week and at 1, 4, and 12 months post-trauma.36,37
Physiological differences distinguish between individuals with and without PTSD can be analyzed:\textsuperscript{71}
\begin{itemize}
  \item a) at rest,
  \item b) perceiving standardized trauma cues (e.g., Vietnam veterans viewing general images of Vietnam), or
  \item c) perceiving idiographic trauma cues (e.g., hearing a script describing the individual participant’s traumatic experience).
\end{itemize}

**Physiological arousal and reactivity are viewed as potential markers of PTSD to include:**\textsuperscript{71-73}
\begin{itemize}
  \item heart rate;
  \item skin conductance (sweat gland activity);
  \item blood pressure;
  \item cardiac defense response (CDR) and
  \item facial electromyography (a measure of muscle contractions in the face).
\end{itemize}
\* Heart rate and skin conductance have emerged as particularly reliable markers of PTSD status.

**Neuroendocrine markers using samples of blood or saliva:**
\begin{itemize}
  \item plasma and saliva cortisol\textsuperscript{17,26}
  \item plasma adrenocorticotropic (ACTH)\textsuperscript{17,26}
  \item prolactin and TSH levels\textsuperscript{20}
  \item leukocyte glucocorticoid receptor (GR) density\textsuperscript{17,19}
  \item corticotropin-releasing factor (CRF)\textsuperscript{17,19}
\end{itemize}

**Cortisol’s effect in memory:**
\begin{itemize}
  \item salivary alpha-amylase (sAA)\textsuperscript{74}
  \item cardiac defense response (CDR) which is a heart rate (HR) response to a sudden onset of loud noise\textsuperscript{34,35}
\end{itemize}

**Regulation of fear, neuroendocrine stress responsiveness and social behaviour:**
\begin{itemize}
  \item Basal salivary neuropeptides oxytocin and arginine vasopressin during rest analyzed by radioimmunoassay\textsuperscript{41}
\end{itemize}

**DNA damage and aging process:**
\begin{itemize}
  \item telomere length\textsuperscript{38}
  \item DNA damage\textsuperscript{39}
  \item N-glycosylation profile\textsuperscript{1}.
\end{itemize}

**Markers of oxidative stress:**
\begin{itemize}
  \item malondialdehyde (MDA)\textsuperscript{75-77}
  \item 4-hydroxy-2-nonenal (4-HNE)\textsuperscript{75-77}
  \item acrolein\textsuperscript{76,77}
  \item F2-isoprostanes\textsuperscript{51,52}
  \item glutamate\textsuperscript{53-56}
\end{itemize}

**Inflammatory markers:**
\begin{itemize}
  \item C-reactive protein (CRP)\textsuperscript{15}
  \item IL1\textbeta, IL-6, Tumor necrosis \alpha\textsuperscript{58-61}
\end{itemize}
Conclusion
Especially in places as Iraq where the population has been hardly affected by traumatic events there is an extreme calling for research initiatives what could bring new knowledge about the pathogenesis of PTSD and potential treatment targets. PTSD has very complex pathophysiology showing similarities with other psychiatric disorders, such as major depression, at some biochemical pathways, what can result in misdiagnose, and affecting the treatment. Therefore, there is an urgent need for studies that would allow a better understanding of the disease and perhaps lead to biomarkers for PTSD. The existence of specific biomarkers for PTSD could facilitate its diagnoses, prognoses and treatment. With this review, we hope to highlight this subject and encourage researchers to devote themselves to this challenging matter.

References
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