Biomedical and public health studies on susceptibility to post-traumatic stress disorder

BIOMEDICAL AND PUBLIC HEALTH STUDIES ON SUSCEPTIBILITY TO POST-TRAUMATIC STRESS DISORDER

GHAZI IBRAHIM A. AL JOWF

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by

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by

Ghazi Ibrahim A. Al Jowf

This thesis is dedicated to my family and friends, who have been an infinite source of love, support and inspiration throughout these last years.

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1. GENERAL INTRODUCTION

The need to include mental health among the first priorities of the public health agenda has been increasingly recognized worldwide over the past decades. This recognition is based on the growing evidence and awareness about the magnitude of mental health problems in many countries. Mental disorders, such as post-traumatic stress disorder (PTSD), are significant clinical, financial and disability burdens worldwide. In 2018, the total economic impact of PTSD in the United States was estimated to be $232.2 billion ($19,630 per PTSD patient). Direct health care and unemployment expenditures drove the excess burden on the civilian population. The burden on the military population was caused by disability and direct health-care expenses [1]. In Europe, higher figures for cost are present. The total healthcare cost of PTSD is estimated to be around 43,000 EUR per person, three times higher than the no-PTSD control group, with costs being twice as high every year after diagnosis. It is important to note that only 27% of the increased costs are due to PTSD, while other costs are mainly related to the other co-occurring mental disorders [2].

1.1. PTSD

Exposure to extremely traumatic occurrences is the trigger of the onset of PTSD [3]. Being a witness to or a participant in a violent accident, crime, military combat, or assault, being kidnapped, being involved in a natural disaster, being diagnosed with a life-threatening illness, or experiencing physical or sexual abuse are all examples of traumatic or stressful events. According to epidemiological research in the Netherlands, the lifetime prevalence of exposure to a shocking / potentially traumatic events (PTEs) ranges from 77.9 to 83.3 percent, while the lifetime rate of developing PTSD is about 7.4 percent, making it the fourth most common psychiatric disorder worldwide [4]. In general, prevalence is higher in women (10–12%) than in men (5–6%) [5]. Although PTSD can appear at any age, it is most prevalent in young adults, because they tend to be more exposed to precipitating situations [6, 7]. A familial pattern seems to exist for PTSD, among many other psychiatric disorders [8].

PTSD is a heterogenous and complex psychiatric disorder which main symptoms include re-experiencing the traumatic event through intrusive flashbacks or recurrent nightmares, avoidance being reminded of the trauma, hypervigilance and hyperarousal as well as alteration of cognition and mood [9]. The diagnostic and Statistical Manual (DSM-5) for mental disorders categorizes PTSD as a trauma and stress-related disorder that derives from a maladaptive response to an extreme stress leading to symptoms that persist over a month and seriously affect one's ability to daily function [9]. Long-term persistence of symptoms is characteristic of PTSD, however, the course of the illness varies with some people recovering within 6 months, while others having symptoms that last much

longer and become chronic. Patients are diagnosed with PTSD using symptom-based evaluation by clinicians that fullfil DSM-5 criteria. Because of the variety of symptoms and the patient's aversion to discussing past trauma, diagnosing PTSD can be difficult. Another complication for the diagnosis is the presence of comorbidities occurring with PTSD including depression, anxiety, alcohol use and substance use disorders [10]. The high complexity of the disorder, the symptoms-based diagnosis and the high level of co-morbidities frequently lead to underdiagnoses and misdiagnosis of PTSD [11].

Currently, the main treatments for PTSD are psychotherapy and pharmacotherapy. Psychotherapies, including both trauma-focused and non-trauma-focused psychotherapy, have been effectively employed in PTSD treatment, and are considered the first-line therapy. Due to the complex nature of the pathology and the differences in response among patients, both modalities might be combined, depending on the needs of each person. In terms of medications, antidepressants, in particular, serotonin reuptake inhibitors (SSRIs) can be used to reduce symptoms of PTSD as well as depressive symptoms that often accompany PTSD. Anti-anxiety medications are also prescribed and may help to decrease heightened physiological arousal and sleep disturbances. Early and effective treatment results in enhanced outcome and quality of life, and prevents complications of the disorder [12, 13]. Unfortunately, many patients with PTSD are refractory to current treatment and no cure for the disorder exists. The frequent misdiagnosis and the current lack of effective treatment for PTSD are placing patients at risk of long-term cognitive, social, and occupational impairments, thus imposing a considerable socioeconomic burden. As a result, there is a critical need to understand the biological processes underlying PTSD to develop more effective preventive strategies and improve the diagnosis and treatment options.

1.2. DIFFERENTIAL SUSCEPTIBILITY TO TRAUMATIC STRESS

Even though a considerable proportion of the general population is exposed to PTEs, roughly 80–90 percent of trauma-exposed individuals do not acquire PTSD, while a susceptible minority of the population will develop the disorder. The ability to withstand trauma without developing any stress symptoms or rapid recovery without progression to PTSD is referred to as resiliency [14], while susceptibility correspond to the inability to cope with the trauma-induced psychological and physiological symptoms and leads to PTSD. This differential susceptibility to traumatic stress is implying an inter-individual variance in PTSD risk [15]. A complex interaction of elements, including neurobiological predisposition and previously existent risk factors, (e.g., childhood adversity, coexistent psychiatric conditions, lack of support system, substance misuse and jobs with a high rate of traumatic stress exposure), will influence whether or not someone develops PTSD after being exposed to trauma.

The biological mechanisms underlying this differential susceptibility are poorly understood. Genetics, in combination with environmental factors, are suspected to play a role in an individual's susceptibility to PTSD. Over the past years, increasing efforts have been made to unravel the biological underpinnings of PTSD and new study discoveries are being published all the time. To date, dysfunction of several neurological and hormonal systems has been associated with PTSD. To understand this inter-personal susceptibility to traumatic stress, epigenetic research, which describes the interaction between the environment and gene expression, has become more pertinent to neurosciences and mental health in general. Therefore, epigenetic mechanisms have been proposed to underlie the relationship between exposure to traumatic stress and the susceptibility to develop PTSD.

1.3. PTSD DEVELOPMENT AND DISEASE MODELS

PTSD is a multifaceted pathological condition that grows over time. It is typically caused by extremely high amounts of physiological stress, which affect neuronal biochemical configurations and neuroplasticity in the central nervous system. Numerous studies have been conducted on the neurobiological changes underlying post-traumatic stress disorder (PTSD) and its associated phenotypes. These studies have revealed brain circuit disruption, neurotransmitter dysregulation, and dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis. Some studies have found that patients with PTSD have altered functioning of various neurotransmitter systems such as GABA, glutamate, serotonin, neuropeptide Y, and other endogenous opioids. PTSD has also been linked to changes in the structure of the brain. Individuals with PTSD have a smaller hippocampus and an overly reactive amygdala (which processes emotions and modulates fear response). In PTSD patients, the medial prefrontal cortex (inhibitory control over the amygdala's emotional reactivity) appears to be smaller and less responsive [10].

The onset and maintenance of PTSD vary between individuals with the same level of trauma and present with varying degrees of illness severity. For that, etiological models have been proposed to explain the interaction of biology, environment, and mind in illness manifestation. The diathesis-stress and biopsychosocial models are two examples of such models. According to the diathesis-stress model, the individual's pre-trauma state (risk factors) is a condition of susceptibility (diathesis) that can result in the disorder after a traumatic experience (stress), while the biopsychosocial model proposes that biological (e.g., genetics, chemical changes, and organ damage), psychological (e.g., stress, mental illness, behaviour, and personality), and social (e.g., peers, socioeconomic status, beliefs, and culture) factors interact in the expression of health and illness [16].

A significant scientific effort is being committed to unravelling the brain mechanisms behind PTSD in order to pave the way for the development of novel or improved

therapeutic techniques and medications to treat PTSD. The use of animal models of human diseases is an important scientific technique for gaining insight into the physiological and neurological mechanisms underlying diseases and for treatment development. The models can be classified as physical, social, or psychological depending on the type of stressor [17]. The development of human cell-based models to analyze the intricate neurobiological processes underlying PTSD and other stress-related psychiatric illnesses has also benefited recent improvements in diagnosis and treatment [18, 19]. Although these models may not entirely duplicate the human state, they do simulate the symptoms and neurobiology of PTSD, allowing for the assessment of behavioural changes, neurobiological and epigenetic modifications, and the development of biomarkers and treatments [18, 19]. The generation of novel insights into disease mechanisms, diagnostic and prognostic markers has significant potential to be achieved through integrated multi-omic research. High-throughput omics data from genomics, transcriptomics, proteomics, methylomics, lipidomics, and metabolomics have been used in these investigations. Multi-omic data sets offer the potential to comprehend the underlying biological processes connected to disease networks by use of a systems biology framework [20].

1.4. EPIGENETIC MECHANISMS

Despite all cells within an organism containing the same genomic sequences, it is possible to distinguish over 200 different cellular phenotypes that constitute the various tissues and organs in the human body. Thus, there must be strict regulation of gene expression that accounts for each cell-specific transcriptional identity. Such sophisticated regulation can be accomplished by the cooperation of multiple levels of control that influence both transcriptional and translational mechanisms. These cellular processes are collectively called epigenetics, a term coined by Conrad Waddington in 1942, and include direct chemical modifications on top of the DNA sequences or on histone proteins and interactions of noncoding RNAs (ncRNAs) with messenger RNAs (mRNAs). Over the last few decades, many definitions have been adopted to embrace all the aspects underlying this complex system of regulation [21]. However, the general and contemporary perspective of epigenetics refers to the branch of science that studies heritable changes in gene expression that do not involve alterations of the DNA sequence [22].

While epigenetics does not change the DNA code, it may play a role in long-term phenotypic changes [23]. These epigenetic changes can be achieved by a variety of mechanisms including histone modifications, DNA methylation and noncoding RNAs (ncRNAs). Histones are octamer protein (comprising two copies of each of the core histones H2A, H2B, H3 and H4) around which DNA basepairs are wrapped, which can affect chromatic structure and gene expression. Histone modification (namely acetylation and deacetylation) alters how much a gene is expressed by modifying chromatin architecture to be either coiled or uncoiled for RNA polymerase to work upon [24]. This process has been linked to a variety

of brain functions related to emotion regulation, including traumatic memory encoding and fear extinction, which are an important processes in the pathophysiology of PTSD [25]. DNA methylation is the attachment of a methyl group directly to a cytosine nucleotide to form 5-methlcytsoine (5-mC). This occurs at a cytosine-guanine dinucleotide (CpG), which is commonly surrounded by other CpGs, forming a CpG island, and is catalyzed by DNA methyltransferase enzymes. A promoter region's methylated cytosines draw in gene suppressor proteins and lessen the interaction of the DNA with transcription factors. As a result, DNA methylation in promoter regions silences genes. Furthermore, cytosine hydroxymethylation to 5-hydroxymethylcytosine (5-hmC), which, besides an intermediate in the demethylation process, is now known to have its own function, mainly in the brain, and rather relates to activation [26]. Both hydroxymethylation and methylation were found to be involved in conditioned fear learning and extinction [27, 28]; moreover, methylation of the glucocorticoid receptor (GR) binding site in the *FKBP5* gene was found to disrupt its role as a regulator of the HPA axis [29]. Noncoding RNAs, which are DNA transcripts that are not translated into polypeptides or protein sequences, have also been found to be involved in gene expression related to PTSD [30], and some studies reported their potential to become useful biomarkers for PTSD [31].

Epigenetic processes do not act independently but form a multilayered network with joint activities of different mechanisms contributing to the same transcriptional regulation [32]. Longitudinal investigations have found links between the establishment of PTSD symptoms and changes in DNA methylation levels. Rutten *et al*. (2018) investigated post-deployment methylation profiles related with PTSD status while controlling for pre-deployment parameters [33]. The researchers discovered 17 loci and 12 chromosomal regions that had methylation alterations that were linked to PTSD symptoms. Three genes, *HIST1H2APS2*, *ring finger protein 39 (RNF39*), and *ZFP57*, were shown to have nominal replication in a group of 98 US Marine personnel. Reduced DNA methylation levels at these locations were linked to an increase in PTSD symptoms. However, future research should address some limitations including sample sizes and power estimates, cross-sectional methodologies, clinical heterogeneity (e.g., comorbidities and medications) and the sex difference of combat-related trauma (as many males included in these studies have past histories of combat). Of interest is the association found for *RNF39*, which was a novel finding [33]. RNF39 is hypothesized to play a role in the initial stages of synaptic plasticity [34]. It was found to be upregulated in the amygdala after fear conditioning and in the dentate gyrus after corticosterone challenge [34, 35]. However, the literature is lacking of studies on RNF39 and its relation to susceptibility to PTSD, both in animal and human studies. Collectively, these studies may have implications for identifying objective diagnostic biomarkers, disease mechanisms, and therapeutic interventions in immune disturbances for PTSD.

1.5. PREVENTION OF PTSD

Despite its crucial necessity, PTSD-prevention is under-researched and poorly implemented. Identifying the causes and triggers of PTSD should be considered as an essential step in approaching the disorder, followed by prevention at various phases of the disease's progression, with the goal of reducing the disorder burden at all levels. As mentioned earlier, the majority of the population is exposed to traumatic events, and although many develop symptoms of the disease, only a minority have persistent symptoms. For this nature of the disorder, a two-goal approach for prevention might be adopted, with the first goal being to reduce the intensity of the symptoms developing shortly after trauma, and the second, to induce remission in those who have persistence of symptoms [36].

In particular, many disease-preventive models have been developed for systematic intervention early on in the course of the disease. A widely accepted model is the Social-ecological model, which applies prevention at different levels, starting with the individual level (the core), the relationship level, the community level, and lastly the societal level [37]. At these levels, it tries to discover the factors that contribute to disease development and poor health outcomes. The goal of such models is early detection of individuals at risk, followed by the implementation of measures that halt disease progression at different intervals, *e.g.*, before trauma exposure, after trauma exposure, after PTSD diagnosis), with the first two relating to primary, and the latter to secondary and tertiary prevention. Primary prevention aims to prevent disease development in individuals at risk. Secondary prevention aims for early intervention in the course of the disease, to achieve a cure if possible, or rather to control the disease. Tertiary prevention aims to prevent complications and disabilities and to maintain a better quality of life. Applying these types of prevention at the different levels of preventive models could further improve results [37].

A variety of interventions have been tested for the early prevention of PTSD with different modalities. Cognitive Behavioural Therapy (CBT) has been demonstrated to reduce PTSD symptoms in randomized controlled trials [38, 39]. However, heterogeneity in trauma survivors needs to be considered when implementing CBT, as different modalities, including trauma-focused cognitive behavioural therapy or Prolonged Exposure (PE), have been shown to be effective in different settings. Many pharmacological agents have been tested for prevention, including propranolol [40], hydrocortisone [41], oxytocin [42], 3,4-methylenedioxymethamphetamine (MDMA) [43], and benzodiazepines [44], among others. However, a collaborative approach, combining the different modalities according to the need of the patient seems to be more effective according to evidence [45]. Future research will focus on predictors and physiological indicators to identify effective

prevention methods for PTSD, thereby reducing the prevalence of PTSD and preventing more individuals and families to suffer from this disorder.

2. OUTLINE OF THE THESIS

AIM OF THE PRESENT THESIS

The overall scope of my studies described in this thesis is to investigate differential susceptibility to the effects of exposure to traumatic stress linked to the expression of PTSD and in relation to mental and public health measures. In particular, this thesis aims (i) to further address the gaps of knowledge in the understanding of the association between epigenetic mechanisms, gene activity, and differential susceptibility to PTSD, and (ii) to identify the public health measures that may be effectuated for the prevention of PTSD and how biomarkers can support these measures. The specific aims of the chapters included in this thesis are described below.

In order to provide an overview of the current knowledge with respect to certain important aspects and highlights of the neurobiology of susceptibility to PTSD, **Chapter 2** provides a summary of, and reflection on, the findings from various molecular, biological, biochemical and physiological alterations in PTSD, focusing on changes at the genomics and epigenomics levels.

The ability to use blood samples and other markers to clearly distinguish the differential expression of psychopathology is still in its infancy. Therefore, **Chapter 3** describes the study of differential gene expression profiles at the mRNA levels (transcriptome) in Dutch soldiers that were deployed to Afghanistan and who were clinically characterized. The results showed significantly different mRNA levels for a number of genes in soldiers that developed PTSD after exposure to traumatic stress during their deployment period, with a few candidate genes being upregulated (e.g., *FOSB*, *NPR3* and *SLC13A4*), while others were downregulated (i.e., *TBC1D16*, *FMN1*). These results propose a potential involvement of these genes in regulation of stress, and may also serve as initial findings for biomarker discovery, potentially providing markers for early identifying and following people with a diagnosis of PTSD.

In order to expand that knowledge base and elucidate how traumatic stress may impact biology in distinct brain regions, **Chapter 4** zooms into the expression patterns of RNF39 in key brain regions of animals from an experimental stress model. Prior studies from our group identified *RNF39* as a differentially methylated candidate gene for PTSD susceptibility in the blood of human subjects. Given the lack/paucity of knowledge on regional and cell-type specific expression of RNF39 protein in the brain and the lack of

information on how its brain expression may be affected by exposure to stress, this chapter comprises an immunohistochemical investigation of the expression patterns of RNF39 in the hippocampus of mice exposed to severe and chronic stress or to a control condition (from an earlier conducted study in our group). In addition, the expression of RNF39 was linked to differences in physiological and behavioural variables that had been recorded before. Stereological analysis revealed higher expression of RNF39 through higher total fibre lengths in the social defeat (SD) group, as well as positive correlation between RNF39 and stress anhedonic behaviour.

Next, **Chapter 5** provides an overview of the current evidence on traumatic stress, PTSD and the differences in traumatic stress with respect to various important epidemiological aspects. Morover, it provides an overview of key public health measures of prevention and intervention, and the importance of such measures in reducing the disease burden, both at the medical and economic levels.

Moving back from epidemiology and public health measure of traumatic stress and PTSD towards the biological knowledge basis of susceptibility and PTSD, **Chapter 6** provides a comprehensive summary of the up-to-date knowledge on the neurobiology underlying PTSD. The chapter discusses the known neurobiological disturbances, and how other factors (e.g., social) affect the development of the disease. Following that, the recent advances in the field of PTSD biomarkers that aid in prevention, diagnosis and treatment are highlighted. Finally, the current state of evidence-based PTSD interventions are discussed.

Chapter 7 and **8** thereafter provide an overall discussion and conclusions drawn from the integrated thesis as well as the summary. **Chapter 9** includes and explores the social and scientific impact of the thesis, describing its contribution to the fields of public health and pathophysiology of PTSD.

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THE MOLECULAR BIOLOGY OF SUSCEPTIBILITY TO POST-TRAUMATIC STRESS DISORDER: HIGHLIGHTS OF EPIGENETICS AND EPIGENOMICS

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ABSTRACT

Exposure to trauma is one of the most important and prevalent risk factors for mental and physical ill-health. Excessive or prolonged stress exposure increases the risk of a wide variety of mental and physical symptoms. However, people differ strikingly in their susceptibility to develop signs and symptoms of mental illness after traumatic stress. Post-traumatic stress disorder (PTSD) is a debilitating disorder affecting approximately 8% of the world's population during their lifetime, and typically develops after exposure to a traumatic event. Despite that exposure to potentially traumatizing events occurs in a large proportion of the general population, about 80–90% of trauma-exposed individuals do not develop PTSD, suggesting an inter-individual difference in vulnerability to PTSD. While the biological mechanisms underlying this differential susceptibility are unknown, epigenetic changes have been proposed to underlie the relationship between exposure to traumatic stress and the susceptibility to develop PTSD. Epigenetic mechanisms refer to environmentally sensitive modifications to DNA and RNA molecules that regulate gene transcription without altering the genetic sequence itself. In this review, we provide an overview of various molecular biological, biochemical and physiological alterations in PTSD, focusing on changes at the genomic and epigenomic level. Finally, we will discuss how current knowledge may aid us in early detection and improved management of PTSD patients.

KEYWORDS

Epigenetics; DNA methylation; PTSD; traumatic stress; neurobiology; trauma; resilience.

1. INTRODUCTION

Mental disorders have a high economic burden and are known to impact one's productivity and quality of life [1]. Among them, stress-related mental disorders are common in various societies and can develop after exposure to environmental stressors. Trauma can be defined as a response to a deeply distressing or disturbing event that overwhelms an individual's ability to cope, causes feelings of helplessness, and diminishes their sense of self along with their ability to feel the full range of emotions and experiences [2]. Traumatic stress typically relates to events that are shocking and emotionally overwhelming, and that may involve actual or threatened death, serious injury, or a threat to physical integrity.

Exposure to traumatic stress is highly prevalent; it has been reported that approximately 40–90% of the general population are exposed to one or more traumatic stressors during their lifetime, with distinct populations being at ultra-high risk for exposure to multiple traumatic stressors (e.g., military and police personnel) [3, 4]. It is one of the most important and prevalent risk factors for various mental disorders [5, 6], including post-traumatic stress disorder (PTSD), addiction, schizophrenia, chronic fatigue, and depression. Amidst the aforementioned mental disorders, PTSD unarguably remains among the most well-known, due to its uniqueness in having an etiological factor (i.e., the exposure) incorporated into the diagnostic description and possibly also due to its high prevalence in certain populations, such as in the combat veteran population [7]. On a societal level, the economic costs of trauma-related psychiatric disorders in Europe are enormous. For example, the costs for anxiety disorders have been estimated at an approximate EUR 74 million, including over EUR 8 million for PTSD in 2010 [8]. In addition, the annual productivity loss of PTSD patients is estimated at approximately USD 3 billion [4]. As a result, stress-related disorders pose an enormous burden on affected individuals, families and society as a whole. These figures are expected to rise further when projected to the year 2030, making these mental disorders among the top contributors with regard to the worldwide disease burden [9]. Additionally, there currently are no preventive measures to minimize the impact of traumatic stress on health, and PTSD treatment options are limited.

At the clinical level, PTSD has a range of symptoms and/or behavioural alterations such as intrusions, avoidance/numbing, hyperarousal, sensitization to stressors, and negative alterations in cognitions and mood [10]. While the lifetime prevalence for stress-related psychiatric disorders exceeds 40%, the lifetime prevalence for PTSD is approximately 2–12% and is significantly higher in high-risk populations [4, 8]. Moreover, there is great variation in individual susceptibility to develop PTSD symptoms after exposure to a traumatic stressor [11]. Indeed, more than 80% of trauma-exposed individuals will remain symptom-free [11]. Evidence accumulates that differential susceptibility to traumatic

stress may be related to context-dependent functional and transcriptional “epigenetic” alterations in distinct neuronal circuitries involving the hippocampus and the amygdala, as well as in brain–body interactions including the hypothalamic–pituitary–adrenal axis (HPA axis) and the immune system, amongst others [5, 12].

Clinically, the cornerstone of PTSD treatment strategies is formed by exposure-based therapies, such as prolonged imaginary exposure, Eye Movement Desensitization and Reprocessing (EMDR) or narrative exposure therapy (NET) [13], with fear extinction learning as one of the assumed mechanisms mediating a reduction in symptoms, while also more classical forms of cognitive therapies are being applied [13]. Moreover, selective serotonin reuptake inhibitors (SSRIs) are administered, with sertraline and paroxetine as FDA-approved agents, and several other pharmaceuticals applied off-label, including atypical antipsychotic agents [14]. Neuromodulation strategies, including electroconvulsive therapy, transcranial stimulation, cranial nerve stimulation and deep brain stimulation, have been applied experimentally and await additional studies before clinical recommendations can be made for their potential inclusion as additional modes of treatment for patients with (distinct forms of) PTSD [15].

As with many other diseases, both genetic and environmental factors affect the onset, severity, and manifestations of PTSD. By studying epigenetic factors, one can potentially obtain a measure of the impact of environmental insults on genetic expression. Epigenetics refers to DNA modifications caused by environmental changes that regulate gene transcription without altering the genetic sequence itself. Because of the brain’s central role in a person’s dynamic adaptations to environmental exposures, epigenetic research is pertinent in neurosciences and mental health [16–18]. Hence, gaining a better understanding of epigenetic modifications caused by traumatic stress would be of great benefit for patients, clinicians and society as a whole. DNA methylation, or the methylation of cytosine residues to 5-methylcytosine (5-mC), is one of the best-characterized epigenetic mechanisms in the mammalian genome and is involved in long-term persistent alterations along with more volatile changes induced by environmental exposures [19, 20]. DNA methylation not only programs the identity of cells, but also contributes to the adaptive capacity of the transcriptional response to dynamic alterations in environmental factors throughout life. Other epigenetic mechanisms are also being increasingly studied, including histone modifications and miRNAs. For all of these, in recent years, approaches have moved from targeted gene or genomic region-based ones to genome-wide explorations looking for integrated regulatory patterns or mechanisms in the field of epigenomics.

Therefore, the aim of this narrative review paper is to provide an overview of the current knowledge with respect to certain important aspects and highlights in the fields of

neurobiology, epigenetics and epigenomics in relation to PTSD. Figure 1 provides a general overview of the information about PTSD discussed in this review.

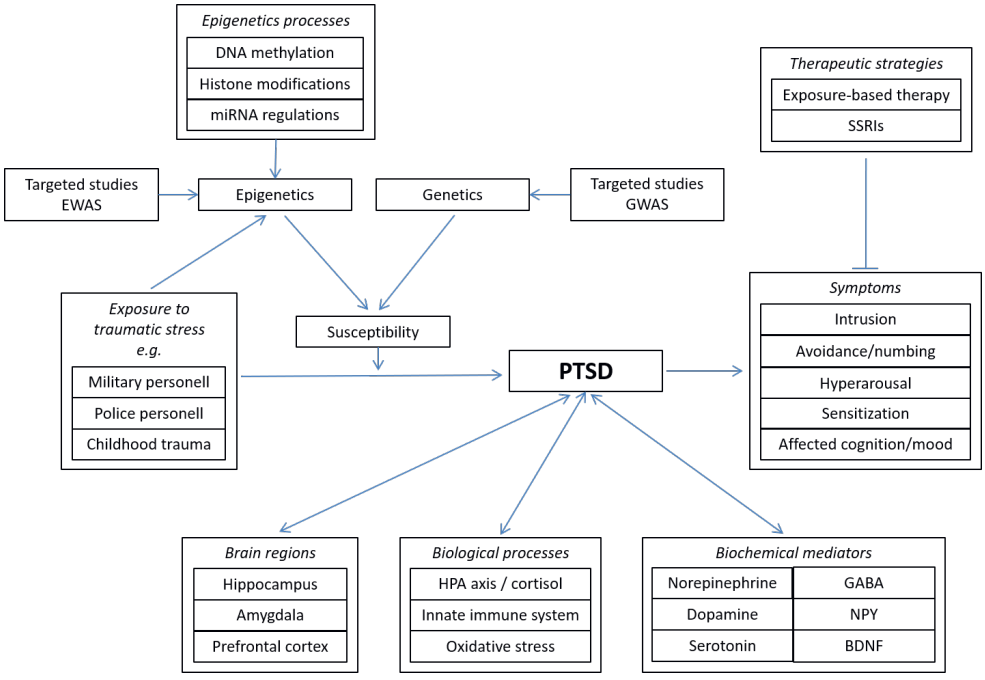

Figure 1. Concept map giving an overview of the information about PTSD discussed in this review.

2. NEUROBIOLOGY OF PTSD

PTSD is a complex pathological entity that evolves over time. It is usually triggered by abnormally high levels of physiological stress that impact neuronal biochemical configurations and neuroplasticity within the central nervous system [21]. In the following sections, we will elaborate on the neurobiological characteristics of PTSD.

2.1. NEUROANATOMICAL CHANGES ASSOCIATED WITH PTSD

In recent decades, imaging techniques have been used to visualize the brains of people exposed to trauma. Several studies have reported structural changes in two specific brain areas, i.e., the hippocampus and the anterior cingulate cortex (ACC) [22]. While the hippocampus is involved in memory and learning, the ACC plays a role in reasoning and decision-making [23]. Moreover, functional magnetic resonance imaging (fMRI) has revealed reduced prefrontal cortex activity when people with PTSD are reminded of the

trauma, and increased activity of the amygdala, which processes fear and emotion [24]. This contrasts with increased activity in the prefrontal cortex of people who experience trauma without developing PTSD [25–27]. Recent research has furthermore indicated that functional differences might also play a role in the development and persistence of stress-induced mental disorders. Research performed by Ressler and colleagues on highly traumatized individuals showed that resilient individuals have better connections between the ACC and the hippocampus in contrast to those suffering from PTSD [28]. This suggests that susceptibility to stress may relate to disturbed communication between the cortical reasoning circuitry and the emotional circuitry of our limbic system.

2.2. BIOCHEMICAL MEDIATORS IN PTSD PATIENTS

2.2.1. NOREPINEPHRINE

Norepinephrine is a pivotal transmitter in the peripheral and central nervous system. The locus coeruleus, in which the cell somata produce norepinephrine reside, is also targeted by antianxiety drugs, including benzodiazepines and alcohol [29]. Some phenotypes and symptoms of PTSD, including irritability, hyperarousal, and sleep disturbance, have been specifically related to altered noradrenergic function [30], and it has been proposed that the sympathetic function in PTSD individuals may suf fer from an uncoupling between the central and peripheral autonomic fields [31]. The α2-norepinephrine receptors have been documented to show particular alterations in stress conditions, although this is likely not specifically linked to PTSD [32]. It has been documented that deficiency of deficient α2-adrenoreceptors is associated with increased vulnerability to stressors in mice [33, 34].

2.2.2. DOPAMINE

Dopamine is another abundant neurotransmitter in the brain, mainly produced in the midbrain region and transported via axons to other brain regions [35]. Evidence for its role in PTSD can be viewed from three different aspects: a molecular aspect, including genetic, ligand–receptor and metabolic alterations, regional alterations, and specific clinical symptoms. While several studies trying to find associations between specific dopamine receptor genes (DRD2) and PTSD showed associations [36, 37], others did not [38]. Metabolic alterations of dopamine have been confirmed by finding increased dopamine concentrations in the plasma or urine of PTSD patients [39, 40], and an increase in their dopamine beta-hydroxylase activity [41]. Regionally, the medial prefrontal cortex shows a high release of dopamine from axonal projections after stressful conditions [42, 43]. Interestingly, cognitive impairment associated with the stress response has been linked to dopamine release in the prefrontal cortex in conjunction with other neurotransmitters [44]. These studies suggest an important role of the dopamine system in the pathophysiology of PTSD.

2.2.3. SEROTONIN

Serotonin is a neurotransmitter which has widespread actions on behavioural and physiological functions [35]. Human studies show that decreased serotonergic function is associated with PTSD-related symptoms and behaviour such as impulsivity [45–47], aggression [46], depression and suicide [48, 49]. Genetic associations between PTSD and specific serotonin transporter molecules have been reported. For example, PTSD is associated with the SS (short–short) allele genotype, which leads to lower expression of the serotonin transporter 5-HTT and as such lower serotonin reuptake from the synapse [50–54]. In contrast, the LL (long–long) genotype of the serotonin transporter in PTSD is associated with enhanced response to SSRIs, while the SS allele is associated with non-responsiveness [54–56]. The SS genotype in PTSD is also associated with a poor response to cognitive behavioural treatment [57]. These findings highlight the genetic precursors of susceptibility to PTSD associated with serotonin.

2.2.4. GAMMA-AMINOBUTYRIC ACID (GABA)

GABA is an inhibitory neurotransmitter which is ubiquitously present throughout the brain. Its receptors have been classified in three main classes (GABA-A, GABA-B, and GABA-C). Benzodiazepines are the most important agonists binding to GABA-A receptors. GABA-A receptors show altered (decreased) binding with ligands in the frontal cortex following specific types of stressors [58]. GABA benzodiazepine receptors also showed decreased expression in the hippocampus after prenatal stress [59] and in a fearful strain of rats [60]. Human studies also showed decreased GABA-A binding capacity in Vietnam combat veterans with PTSD [61]. These findings show that alterations in GABA receptor expression or binding capacity may have effects on stress-related mental disorders, with a possible role in PTSD.

2.2.5. NEUROPEPTIDE Y (NPY)

NPY is a neuropeptide expressed in various parts of the brain, including the forebrain, limbic system, and brainstem, which regulate emotional and stress behaviours [62]. Interest in NPY took off in 2000 following a study on healthy US Army soldiers [63]. These soldiers participated in a survival course, which was designed to simulate the conditions experienced by prisoners of war [63]. Their serum NPY levels went up within a few hours after exposure to military interrogations during the survival course. Furthermore, most of the soldiers of the Special Forces who had trained to be resilient had significantly increased NPY levels relative to the non-Special Forces or the typical ones [63]. This supports the idea that NPY enhances stress resilience, which is generally seen in humans. Other preclinical and clinical studies corroborate these data, suggesting that reduced NPY in the central nervous system is associated with PTSD [62]. The most common SNP studied for NPY is rs16147 (−399T > C) polymorphism, which is associated with low concentrations of NPY, resulting in hyperarousal in the brainstem, stress activation alterations in the HPA axis and

re-experiencing in the hippocampus [62]. Other studies, in addition, found other factors caused by trauma (TNFa) increase dysregulating the HPA axis [64]. The −1002T > G loci are also associated with low NPY concentration in the CSF and amygdala, associated with increased levels of anxiety, arousal, addictive behaviours, and reduced stress resiliency [62].

2.2.6. BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF)

BDNF, which regulates neuronal survival, growth differentiation, and synapse formation, is known to be involved in PTSD as well as depression [65]. Some studies have shown that BDNF is associated with PTSD risk and exaggerated startle reaction (a major arousal manifestation of PTSD) in the United States military service members who were deployed during the wars in both Iraq and Afghanistan [65]. Furthermore, subjects with PTSD showed higher levels of BDNF in their peripheral blood plasma than the non-PTSD controls. Increased BDNF levels were observed in both blood plasma and hippocampal tissue in the inescapable tail shock rat model of PTSD [65]. This highlights the importance of BDNF as a potential biomarker and its possible roles in the onset of PTSD [65]. Fundamental research shows that stress decreases the expression of BDNF in the hippocampus [66]. This, along with another finding on the effect of stress on inducing neuronal loss and damage in the CA3 region of the hippocampus [67], and the pivotal role of the hippocampus in handling stressful conditions, may point to the fact that stress initially decreases BDNF and subsequently results in hippocampal damage, leading to PTSD.

2.2.7. OXIDATIVE STRESS IN PTSD

Many studies have reported that increases in oxidative stress might contribute to the development of psychiatric disorders, including PTSD. Stress is considered to be a risk factor for PTSD development and triggers a sustained growth in nitric oxide synthase (NOS) activity, which might generate extreme amounts of nitric oxide. In general, the oxidation of nitric oxide produces a substance called “peroxynitrite”, which is extremely toxic to all nerve cells. Observations of high levels of peroxynitrite and its predecessor nitric oxide have been reported in patients with PTSD [68].

2.3. NEUROENDOCRINE CHANGES: ALTERATIONS IN THE HPA AXIS

The HPA axis is a hierarchical system comprising the hypothalamic, pituitary and adrenal glands which orchestrates the homeostatic balance of the organism’s response to environmental stress. The effective arm of this axis is cortisol and its analogues, which are stress hormones. Stress hormones modulate the physiological, immunological, and treatment responses in PTSD [69]. There are gender differences in response to traumatic events. Women show a higher incidence of trauma-related psychiatric disorders, including PTSD, than their male counterparts [69]. Animal studies also show a strain-specific difference in fear behaviour in mice, related to the difference in their

glucocorticoid response to stressful events [70]. In parallel, human foetuses experiencing severe pregnancy stress will have an altered HPA axis and will have a higher risk of neuropsychiatric disorders later in life [71].

Several studies have aimed to demonstrate a relationship between increases in stress hormone levels and the intensity of an encountered traumatic event. A captivity survival program in the Canadian Armed Forces showed that salivary cortisol and dehydroepiandrosterone (DHEA) increased the most in scenarios with higher-intensity interrogations [72]. Data from adolescents exposed to the 1988 earthquake in Armenia showed a significant relation between adrenocorticotropic hormone (ACTH) response to exercise challenges, and severity of PTSD [73].

Other studies showed a relationship between initial hormonal levels immediately after a stressful event and the risk of subsequent development of PTSD. Higher initial urinary epinephrine and cortisol levels immediately following a traumatic event are associated with an increased risk of developing PTSD later in life in child trauma victims [74]. In contrast, urinary cortisol levels immediately following admission to hospital in motor vehicle accident victims were lower in those who subsequently developed PTSD [75].

Therapeutic approaches can modulate the HPA axis in PTSD patients. A study on PTSD patients showed an increase in serum cortisol and DHEA levels after brief eclectic psychotherapy in those who responded to this treatment modality [76].

3. HERITABILITY AND GENETIC FINDINGS OF PTSD

Though it is predicted that up to 90% of individuals may suffer from a significant traumatic experience in their lifetime, PTSD is only found to develop in 20–30% of exposed individuals [77]. Benerjee *et al*. (2017) indicate that a major contributing factor to this difference could be genetic heritability along with early childhood trauma. This is reflected by twin studies showing 30–40% genetic heritability estimates for PTSD [78]. Genome-wide association studies (GWAS) have addressed some of these genetic factors and identified associations with single-nucleotide polymorphisms (SNP) in certain genes, including the retinoid-related orphan receptor gene (RORA), Neuroligin gene (NLGN1) and Tolloid-like 1 gene (TLL-1) [19]. The RORA gene protein has the potential to modulate neurons to respond to steroid hormone changes, oxidative stress, and inflammation. This modulatory effect is severed by trauma-induced changes of RORA. Furthermore, common variants within the FKBP5 gene have been shown to increase the risk of developing PTSD and major depressive disorder (MDD) [79, 80]. A recent work further studied the genetic underpinning of PTSD in relation to MDD, by applying a multi-omics integrative approach

using genomics and transcriptomics data. They identified 13 potential driver genes for PTSD symptoms, with ESR1, RUNX1, PPARA, and WWOX also driving MDD symptoms and connected to biological pathways which have been linked previously to the regulation of stress and trauma response [81].

Additionally, the NLGN1 gene is also found to be linked with PTSD and other psychiatric disorders [82]. This gene encodes for synaptic adhesion molecules and thereby has a role in synaptogenesis and synaptic maintenance. NLGN1 depletion in the amygdala in a mouse model of PTSD shows a depletion of fear-associated memory storage, indicating a prominent role in PTSD [82]. Moreover, the TLL-1 gene that functions in remodelling the extracellular matrix has recently been found to be a contributing gene for the development of PTSD [19]. It is important to consider, however, that there is a conflict in considering GWAS methods while trying to move away from false positives and heterogeneity across samples [83]. Hence, despite the major contribution of GWAS in this field, GWAS approaches to date have not (yet) produced many replicable findings [83].

In 2019, Nievergelt *et al*. conducted a meta-analysis of PTSD genome-wide association studies, using a sample size of about 200,000 individuals consisting of more than 30,000 PTSD cases and 170,000 controls. The main aim of their study was to demonstrate the genetic risk for PTSD by estimating heritability based on molecular genetic data, to discover genome-wide significant hits linked to PTSD. They found heritability estimates in the range of 5–20% and identified contributing genes, including PARK2, PODXL, SH3RF3, ZDHHC14, KAZN, as well as several non-coding RNAs [84].

4. EPIGENETIC CHANGES

Epigenetic mechanisms regulate gene expression levels without altering the DNA sequence [85, 86]. These mechanisms include histone modifications, DNA methylation, and post-transcriptional regulation by non-coding RNAs (RNA-associated silencing) such as microRNAs (miRNAs). Epigenetic changes can be acquired over the lifespan and mediate environmental effects on gene expression. In the brain, epigenetic regulation is vital for basic cellular processes involved in multiple aspects of neuronal function, such as synaptic plasticity, and for complex behaviours, such as those involved in learning and memory [87]. Epigenetic mechanisms are affected by various factors and processes, including development (both in utero and during childhood), environmental chemicals, drugs/pharmaceuticals, aging, and diet.

An increasing number of studies show the relevance of epigenetic changes in response to traumatic stress across the life span. These provide a possible link between the

environment and gene expression, since stress could have an influence on gene expression by inducing specific changes in epigenetic regulation [88–90]. These changes might be different for some individuals who develop PTSD and those who do not. By finding specific epigenetic patterns, scientists could discover diagnostic and/or prognostic biomarkers, and even enhance therapeutic opportunities. In addition, many animal studies have been instrumental in delivering clues for a causal relationship between the early life environment, epigenomic changes, and subsequent behavioural changes.

Although available evidence is limited, transgenerational epigenetic research performed in animals indicates that the epigenetic effects of trauma may be transmitted to multiple generations. Therefore, traumatic stress could already affect the epigenetic background of said offspring during pregnancy [90–94]. If confirmed, this could be predictive for the possible future development of PTSD, especially in light of at-risk jobs where trauma exposure is more prevalent. Below, we describe the roles of the different epigenetic mechanisms, pharmacological aspects, and relationships with other factors.

4.1. HISTONE MODIFICATION

Histone proteins can be altered by the addition of one or more chemical groups to one or more residues in the chain, including acetyl, citrulline, methyl, ubiquitin, a small ubiquitin-like modifier, phosphorus, and ribose [95]. The effect of histone modification on transcription is quite diverse: histone acetylation opens up chromatin conformation to allow transcription, histone phosphorylation often indicates DNA damage, whereas the transcriptional impact of mono-, di-, or tri-methylation is dependent on the modified histone protein and residue [96].

Animal studies that measured brain histone acetylation after fear conditioning [96, 97] showed that histone H4 lysine 5 acetylation (H4K5ac) and histone H3 lysine 9 (H3K9ac) were increased significantly in the lateral, basal, and centrolateral amygdala. H3K9ac and H4K5ac were also shown to be increased in the centromedial amygdala and the prelimbic-prefrontal cortex (PL-PFC), but this only occurred after fear learning. Similarly, after fear learning, differential H4K5ac levels were observed in the prefrontal cortex, with a significant decrease in the infralimbic-prefrontal cortex (IL-PFC) and an increase in the PL-PFC. Furthermore, histone acetylation also showed variations after fear extinction in which rat IL-PFC showed significantly higher levels of H3K9ac after delayed extinction compared to no (or immediate) extinction [96, 98].

In humans, histone trimethylation variances have been noted in PTSD subjects at various lysine sites in peripheral blood monocytes [96, 99]. It is worth noting that several histone modifications are associated with DNA methylation changes in certain genes, as well as

expression changes of miRNAs, especially relating to the expression of proinflammatory cytokines [96].

4.2. DNA METHYLATION

DNA methylation, mediated by DNA methyltransferases (DNMT), typically reduces or blocks gene transcription and occurs most frequently at the cytosine of a CpG site (a DNA sequence where cytosine is 5-prime to guanine, connected via a phosphate group) [96, 100].

A study taking account of genome-wide gene expression and DNA methylation in 12 PTSD subjects as well as 12 trauma-exposed healthy control subjects discovered 3989 genes that were significantly upregulated in those with PTSD and three downregulated ($p < 0.05$), which was adjusted for multiple comparisons. However, there was no significant difference in DNA methylation [96, 101]. The same study observed upregulation in olfactory function and immune system gene expression. Other studies also noted downregulation in immune system genes [96]. Other methylome-wide studies have discovered that DNA methylation variation at certain loci, genes and biological processed showed significant association with PTSD, as shown in the epigenome-wide association studies (EWAS) mentioned in Section 5.

4.3. MICRORNA

RNA-associated silencing is a process whereby non-coding RNAs (nc-RNAs) negatively impact gene expression. Long nc-RNAs (lncRNA) are transcripts that are larger than 200 nucleotides. Short nc-RNAs (sncRNA) appear in a very large range of molecules, and include miRNA, piwi-interacting RNA (piRNA), and small interfering RNA (siRNA) [96].

Hundreds of different miRNAs can be found in humans, which together regulate the activity of 30–60% of all protein-coding genes [102]. miRNAs are transcribed from DNA into primary miRNAs, which are processed further into precursor miRNAs, and finally mature miRNAs [103]. Mature miRNAs most often bind to the 30 untranslated region (UTR) of target messenger RNAs (mRNAs), thereby affecting gene regulation by silencing and/or suppressing gene expression [102]. By targeting a large number of mRNAs, miRNAs are involved in a wide variety of critical processes, including development, metabolism, growth and differentiation [104]. As such, aberrant expression of miRNA has been associated with several human diseases, including cancers, autoimmune diseases, and inflammatory diseases, as well as in some psychiatric disorders, such as MDD and schizophrenia [104]. Various studies based on animal models of PTSD or clinical patients demonstrate alterations in circulating miRNAs in PTSD subjects [105].

In a mouse model for PTSD, the prefrontal cortex (PFC) miRNA profiles demonstrated that traumatic stress on its own did not influence long-term miRNA expression, when compared to control subjects [96, 106]. However, the use of fluoxetine treatment in traumatized mice significantly reduced some miRNAs—especially mmu-miR-1971—in comparison to untreated traumatized mice. This finding in particular is relevant for future studies on the subject of RNA-associated epigenetic mechanisms in PTSD sufferers with comorbid depressive/anxiety disorders, as these kinds of comorbidities are often treated with selective serotonin reuptake inhibitors (SSRIs)—for instance, fluoxetine [96]. Additionally, serotonin has been shown to alter the levels of an miRNA that modulates the expression levels of the serotonin transporter [107].

4.4. EPIGENETICS BASED PHARMACOLOGY

In recent years, drugs that have been used clinically, such as the anticonvulsant and mood-stabilizing agent valproic acid (VPA), have been shown to act on different aspects of the epigenetic machinery. Epigenetic mechanisms of VPA include regulation through transcription factors, DNA methylation and direct inhibition of histone deacetylation [108]. VPA was also shown to affect DNA methylation along with quetiapine when used in bipolar disorder [109]. Though conventionally used drugs for neurological or psychiatric disorders are classified based on their receptor binding, many of them also have additional epigenetic mechanisms of action [110]. The extent of the contribution of these epigenetic mechanisms relative to their neurotransmitter receptor binding capacity is unknown [111]. Amitriptyline alters DNA methylation and is used to treat PTSD [112]. Imipramine, a histone deacetylase (HDAC) inhibitor, and many other psychiatric drugs, such as paroxetine, citalopram, haloperidol, and clozapine, have been shown to have epigenetic mechanisms [107, 110–112]. Here, we describe some recent insights into the role of HDAC inhibitors and DNMT inhibitors in more detail.

4.4.1. HDAC INHIBITORS

Histone acetyltransferases (HAT) add an acetyl group to the histone proteins, causing the chromatin to relax and allow transcription to take place, leading to the formation of memory. HDAC removes acetyl groups, causing the chromatin to tighten, and hinders transcription [113]. Although both HDAC and HAT can be targeted using distinct compounds, only HDAC inhibitors have thus far been tested in relation to fear processing [114]. The inhibition of HDAC has demonstrated a role in enhancing a number of putatively protective genes and has been linked with neuroprotection and memory formation [115]. When injected before the fear conditioning session, HDAC inhibitors boosted the long-term fear memory formation, but when injected into the IL-PFC during extinction training in mice, they have been shown to boost new memory formation during extinction [116]. This highlights the potential use of HDAC inhibitors in the treatment of PTSD.

VPA, trichostatin A, and sodium or phenylbutyrate are clinically used HDAC inhibitors [113]. VPA in particular is currently used for its role as an anti-convulsant and mood stabilizer. Long-term treatment with VPA has been linked to enhancement of GABAergic signalling [114]. VPA and Vorinostat, also known as suberanilohydroxamic acid, which inhibits histone deacetylase, have both shown to be promising new avenues in treating PTSD and mood symptoms. Nevertheless, a randomized control trial (RCT) should be the next step in order to fully comprehend their efficacy [117, 118].

Histone-modifying complexes can further include HAT and HDACs [119]. The delayed onset of the effect of many antidepressant drugs is an example of the possible indirect epigenetic modification effects of these pharmacotherapies. Some psychiatric drugs have a direct effect on enzymes catalysing epigenetic changes. Among them are VPA [120] and the antidepressant tranylcypromine [121].

4.4.2. DNA METHYLTRANSFERASE (DNMT) INHIBITORS

DNMT catalyse the transfer of a methyl group to the genome, generally hindering transcription when occurring in CpG islands. DNMT inhibitors, such as HDAC inhibitors, have shown promising effects on fear extinction by inducing deficits in learning in relation to fear. However, they work in much the opposite way as HDAC inhibitors. Infusion of DNMT inhibitors to the lateral amygdala in rodents promotes the extinction of memory rather than the formation of new memories [113]. 5-aza-20′-deoxycytidine (5-AZA) is one of the few DNMT inhibitors that have been tested in this regard, and it has demonstrated a preventative effect on fear memory formation by increasing acetylation of histones [122].

4.5. THE IMPACT OF LIFESTYLE, PSYCHOTHERAPY, AND BEHAVIOURAL THERAPY ON EPIGENETIC MECHANISMS

There is accumulating evidence that epigenetics affect the stress, health, and behaviour of an individual, but more importantly, this also works vice versa [123]. Recent studies indicate that behavioural approaches used to treat PTSD such as cognitive behavioural therapy and/or lifestyle modifications such as long-term meditation can alter neurophysiological traits and transcriptional profiles [123]. The study conducted by Kaliman *et al*. (2014) depicted the dynamic role of behavioural change on epigenetics and its benefits in some PTSD symptoms [124]. This study included 8 h of meditation and within those hours, experienced meditators indicated lower expression of HDAC genes (HDAC2, 3, and 9). These histone changes could be beneficial to PTSD patients as the downregulation of HDAC2 is associated with an improved cortisol recovery after stress. Additionally, the intervention group showed a significant reduction in the pro-inflammatory gene COX-2, as this gene is dependent on HDAC expression changes as well [124].

5. EPIGENOMIC TECHNOLOGY MODERN APPROACH

In contrast to earlier epigenetic research, which was more aimed at demonstrating the existence of epigenetic alterations in disease, generally focussing on individual genes or genomic regions, novel aims are to apply genome-wide screening methods and obtain an integrated understanding of regulatory changes. Here, we describe some avenues of state-of-the-art research approaches.

5.1. RECENT DEVELOPMENTS IN EPIGENETICS OF STRESS DISORDERS RESEARCH

5.1.1. MODERN TECHNOLOGIES USED IN EPIGENOMICS PTSD RESEARCH

This section will elaborate on the techniques most commonly used to study epigenetic underpinnings of stress-related disorders such as PTSD. Of note, it does not aim to provide a comprehensive overview of the available techniques used to study epigenetic mechanisms. DNA methylation analysis has been among the most popular methods to investigate epigenetic variations [125]. The most conventional method includes the sequencing of PCR-amplified and bisulphite-modified DNA [126].

The most comprehensive method to analyse bisulphite-converted DNA is whole-genome bisulphite sequencing (WGBS). Although this approach allows for an unbiased exploration of methylation patterns across the whole genome, its main limitations are a relatively high cost and high complexity of the generated data. Moreover, since full coverage is usually not required, techniques such as reduced representation bisulphite sequencing (RRBS), in which only specific CpG-rich regions are sequenced, are an interesting and less challenging alternative.

Another widely used alternative includes DNA methylation arrays. For example, the latest microarrays from Illumina allow researchers to interrogate over 850,000 CpG sites using the Infinium Methylation EPIC kit, while previous versions were limited to 27 K and, subsequently, 450 K sites. The genome-wide coverage and high-throughput nature of the EPIC BeadChips make them ideal for EWAS [127].

5.1.2. EPIGENOME-WIDE ASSOCIATION STUDIES OPEN NEW PERSPECTIVES

EWAS allow for a hypothesis-free investigation of DNA methylation patterns across the genome that are associated with a particular phenotype. To date, several main studies have identified methylation profiles associated with PTSD (Table 1).

Longitudinal studies highlight associations between the development of PTSD symptoms and the emergence of alterations in DNA methylation profiles [128, 129]. Studies using longitudinal designs can reveal specific genomic regions in which DNA methylation

patterns dynamically change across a period of stress exposure, and potentially mark susceptibility for PTSD [128].

Using such a design on a cohort of Dutch military members (the Prospective Research In Stress-related Military Operations; PRISMO, N = 93), a study conducted by Rutten *et al*. (2018) assessed post-deployment methylation profiles associated with PTSD status while correcting for pre-deployment measures [128]. Using Illumina HumanMethylation450 BeadChips, the authors identified 17 loci and 12 genomic regions which underwent methylation changes that were associated with PTSD symptoms [128]. Replication analyses in a cohort of US Marine soldiers (N = 98) showed nominal replication for three genes, i.e., HIST1H2APS2, RNF39, and ZFP57. Decreasing DNA methylation levels at these sites were related to increasing levels of PTSD symptoms [128]. Interestingly, when comparing the epigenetic alterations between the first dataset (N = 93) of the Dutch military and the second dataset (N = 98) of the US Marines, the gene region HIST1H2APS2 was one of the only mutual gene positions that had a decrease in methylation in both group sets and increased symptoms of PTSD [128].

Many recent studies have examined global DNA methylation on a genome-wide scale on different groups who have experienced similar traumas (wars, combat, child abuse, veterans, etc.) [64, 128–132]. Based on this, one could assume that due to these major commonalities in the studied populations, some CpG sites should be inconsistent throughout all of the studies. This is especially true if these studies claim that epigenetic changes in certain genes could be a biomarker of PTSD development [130–132]. However, the DOCK2 gene site was one of the only CpG site that has been found to have significant methylation changes across three studies [130, 132, 133].

Logue *et al*. (2020) recently examined a cohort of veterans from the Translational Research Center for TBI and Stress Disorders (TRACTS) and aimed to replicate their findings using data collected from several military cohorts [130]. One genome-wide significant association with PTSD was identified at cg19534438, located within the G0S2 gene. Locus methylation was shown to be associated with PTSD, as supported by previous evidence. This finding was replicated in their replication cohort. Other genes that showed association include AHRR gene, in which decreased methylation was observed upon smoking [130].

Interestingly, genes related to immune response show alterations in PTSD patients [64, 129–132]. Additionally, several studies examining traumatic stress exposure in patients found high associations between epigenetic changes and immune system dysregulation and/or PTSD development [64, 131, 132]. According to Mehta *et al*. (2017), who assessed 211 veterans, methylation changes of the DOCK2 gene that connect to immune dysregulation and development of PTSD symptoms were found [132]. Furthermore, the hypomethylation

of several CpG sites located within TPR and AHHR related to traumatic stress and PTSD were directly linked with immune system complications such as increased inflammation [64, 131]. Interestingly, when assessing the cytokine levels in relation to these epigenetic changes, results indicated that the cytokine changes (IL4, IL2, and TNFa) differ according to the subject's condition (PTSD, child abuse, or total life stress) [64].

This does not necessarily define a causality but signifies the potential for the influence of immune function on PTSD symptoms or alteration of general immune function in reaction to stressful exposures. Among other genes with correlation to PTSD symptoms is PARK2, which is involved in dopamine regulation and is associated with Parkinson's disease [84]. Further studies on DNA methylation patterns in the brain tissue of PTSD patients, in parallel with their blood samples, show that peripheral epigenetic alterations can mirror epigenomic alterations in the brain [130]. This highlights the possibility of finding PTSD biomarkers based on peripheral tissues [130].

Surprisingly, the study by Mehta and colleagues conducted in 2019 was one of the few studies that investigated transgenerational inheritance of epigenetics, studying 299 veterans diagnosed with PTSD. They tested the FKBP5 gene that has been previously identified in the literature as a transgenerational transmitted gene (parent to child). Their results in sperm showed four significant CpG sites within this gene that were correlated with PTSD [133].

More recently, Snijders and Maihofer *et al*. (2020) conducted one of the largest EWAS using longitudinal DNA methylation data of three male military cohorts, i.e., Marine Resiliency Study (MRS), Army STARRS and PRISMO (N = 266, 123 with PTSD) [129]. Their combined results point to three differentially methylated positions (DMP) and twelve differentially methylated regions (DMR), four of which were located within the human leukocyte antigen (HLA) region. Genes located within this region are known to have immune-related functions, and dysregulations within the immune system are not uncommon in PTSD [129]. Interestingly, one DMP and one DMR were located within MAD1L1, a gene previously associated with PTSD [134]. Along with the study conducted by Rutten *et al*. (2018), this study encourages the use of longitudinally collected DNA methylation data. Interestingly, it has been found that these epigenetic changes can be reversible once established. Treatment of PTSD seemingly reverses some of the DNA changes that are related to the development of PTSD, as demonstrated by Vinkers and colleagues. They found that successful treatment resulted in methylation changes in 12 different DMRs, of which ZFP57-increased methylation was the most consistent for PTSD symptom improvement [135].

Table 1. Main epigenome-wide association studies in PTSD.

Study	Sample	Findings
Rutten et al. (2018) [128]	Dutch military members (N = 93) of the PRISMO cohort	Identified 17 loci and 12 genomic regions that underwent DNA methylation changes associated with PTSD
Rutten et al. (2018) [128]	US marine soldiers (N = 98) of the MRS cohort	Decreasing DNA methylation levels of the regions HIST1H2APS2, RNF39 and ZFP57 were associated with increasing levels of PTSD symptoms, with the first being the only mutual gene position between the two groups
Logue et al. (2020) [130]	Military veterans of the TRACTS cohort (N = 378)	Locus cg19534438 located within the G0S2 gene methylation was shown to be significantly associated with PTSD
Mehta et al. (2017) [132]	War veterans and males from Grady Trauma Project (N = 211)	Methylation changes of the DOCK2 gene that connect to immune dysregulation and development of PTSD symptoms
Mehta et al. (2019) [133]	Australian or New Zealand armed services in Vietnam (N = 299)	Four significant CpG sites (two CpGs within GR and two CpGs within FKBP5) within FKBP5 gene that were correlated with PTSD
Snijders and Maihofer et al. (2020) [129]	MRS, Army STARRS and PRISMO cohorts (N = 266)	Several differentially methylated positions (DMP) were found, four of which were within the (HLA) region, indicating the importance of immune dysregulation in PTSD

5.2. TRANSGENERATIONAL PASSING OF EPIGENETIC TRAITS

Epigenetic inheritance points to the transmission of epigenetic markers from parents to their offspring, hence from one generation to the next. The traits of the offspring are affected without alteration of the primary nucleotide sequence of their DNA. Experiments performed in the past showed that there is a reset of the patterns of DNA methylation in the whole mammalian genome in each generation. This suggests that epigenetic information would not necessarily be inherited [136, 137]. Nonetheless, the mammalian epigenetic information is usually not erased between generations and epigenetic modifications can be transmitted through the germline after its exposure [136, 138–141].

5.3. IDENTIFYING MIRNAS AS BIOMARKERS OF PTSD

The diagnosis of PTSD is based on reported symptoms and psychological history. However, there is no real diagnostic tool that can confirm the diagnosis. There are significant benefits accompanying the identification of a biomarker for the diagnosis of PTSD. Below, we will elaborate on the possible role of miRNAs as a potential biomarker.

Studies have shown that miRNA profiles could serve as ideal disease biomarker candidates due to their relative stability and presence in several peripheral biofluids [142]. The most common assays that are being used to investigate miRNA expression are microarrays,

high-throughput sequencing approaches, or quantitative PCR arrays [143, 144]. To date, eight studies have aimed to identify blood-based circulating miRNAs associated with PTSD in humans using one of these approaches [4, 99, 145–150]. Of these, one study performed a total RNA discovery study and found just one upregulated miRNA in PTSD cases (miR-21) [147]. Another study performed a targeted search for the implication of miR-15a following childhood trauma [145], while Wingo *et al*. (2015) found two downregulated miRNAs in PTSD with comorbid depression as compared to healthy controls (miR-212-3p and miR-3130-5p) [150]. The five remaining studies identified several miRNAs which were either up- or downregulated in military personnel [4, 99, 146, 148, 149]. However, comparing findings between these studies reveals very little overlap. This may, at least partly, be due to most studies using relatively small sample sizes and different study assays [151]. This further calls for a need for larger studies to replicate some of the current findings.

While circulating miRNAs are protected from degradation by being bound to Argonaute2 proteins or high-density lipoproteins [152, 153], a portion is also found packed within vesicles such as exosomes [154]. The finding that exosomes are involved in intercellular communication led to an increase in the number of studies researching the (miRNA) content of blood-based exosomes. Interestingly, exosomes have also been found to cross the blood–brain barrier and carry cell type-specific surface markers [155–157]. By specifically isolating peripheral exosomes exhibiting L1CAM (CD171), a transmembrane protein which is strongly enriched in brain tissue, researchers are now able to indirectly gain insights into (patho) physiological mechanisms occurring within the central nervous system [158]. Although, to the best of our knowledge, this method has not yet been applied to psychiatric disorders, the first studies examining the content of blood-based neuron-derived exosomes of patients with Alzheimer's disease [159, 160] show great promise in using these vesicles as biomarkers for cognitive impairment. This in turn opens up new avenues to explore the potential of delivering miRNA mimics or antagomirs to the brain through exosomes.

6. FUTURE PERSPECTIVES

With advances in technologies, reductions in cost, and developments and standardisation of analytical workflows, we foresee that genome-wide epigenetic screenings will be increasingly applied in PTSD research. The increased resolution of microarray-based screening (EPIC array) and the establishment of sequencing, WGBS, and RRBS in the field, will generate more precise maps of changes in DNA methylation that relate to the development or progression of PTSD. Some specific projected advances include the following: first of all, aside from DNA methylation, the study of other epigenetic marks will increase and substantially improve our knowledge on their implications in PTSD.

In contrast to methylation, 5-hmC markers in promoter regions have been associated with gene activity rather than repression [161]. The brain is the main organ where the probably deliberate and stable presence of DNA hydroxymethylation markers has been established [162]. With respect to measurement technologies, bisulphite sequencing cannot distinguish between cytosine methylation and hydroxymethylation. Modifications of the protocol, as used in oxidative bisulphite sequencing (oxBS) and Tet-assisted bisulphite sequencing (TAB-Seq), allow us to detect both markers separately [163, 164]. Specific investigation of DNA hydroxymethylation has already been applied to increase our understanding of other brain diseases, such as Alzheimer's disease [165]. Doing the same for PTSD will further increase our understanding of this disorder.

Furthermore, another layer can be added to our understanding of PTSD by the study of histone modifications. These marks can be determined in a genome-wide fashion using a technology called ChIP-seq, which stands for chromatin immunoprecipitation sequencing. In short, histones are fixed to the DNA that is fragmented, after which antibodies recognising a specific histone modification are used. Then, secondary antibodies with attached beads are used to separate the DNA fragments bound to the modified histones from the other DNA fragments. Sequencing of the bound fragments then tells us where the histone modifications were present. For PTSD, it has been applied to characterize epigenetic changes in white blood cells for H3 trimethylation at K4, K9, K27, and K36 [99]. Applying it to additional histone modifications, such as acetylation, and in larger cohorts, or even to generated neurons or organoids from PTSD patients as well could increase our understanding of the underlying regulatory mechanisms. Additionally, it could be used to study more individual epigenetic signatures, as has been performed to characterize H3–K4 trimethylation (H3K4me3) changes in cortical neurons in schizophrenia [166].

Another angle that warrants further study is the passing of traumatic stress-induced biological changes across generations. This has been studied in the offspring of war victims, for example by examining the offspring of holocaust survivors [167]. Differences were observed, but unravelling the underlying mechanisms warrants further investigations [167]. Stress-induced epigenetic changes have also been shown to be inherited by offspring and drive such inheritance, as shown for FKBP5 methylation [133, 168, 169]. However, this is mostly explained by direct exposure in utero or via exposed germ cells, called intergenerational epigenetic inheritance. As such, true epigenetic inheritance without exposure, called transgenerational, can only be studied in third-generation offspring in the female line and second-generation offspring in the male line. For now, experimental studies have not found any evidence for true epigenetic inheritance. A possible path towards studying this with respect to human PTSD would be to examine the grandchildren of holocaust survivors or victims of other wars. However, such studies need to be carefully designed to distinguish epigenetic inheritance from secondary

traumatization. Studies would need to collect rich phenotypic information to elucidate the mechanisms and prove or disprove true transgenerational epigenetic inheritance.

Among the many possible contributing factors to the development of PTSD, only a few of them had gained practical utility in the day-to-day care of the affected patients. Most conventional drugs currently used to treat PTSD have common actions among many other stress disorders, do not have enough disease specificity, and are symptom-based, such as the use of antidepressants. Importantly, none of these medications specifically target the underlying genetic or epigenetic pathologies. Proper management of patients merits due consideration of the epigenetic factors and novel research in drug development targeting these complex molecular changes. Increasing mechanistic understanding may thereby help identify subtypes of the disease. Additionally, novel biomarkers may pave the way for more and more targeted preventive measures. Additionally, current preventive measures do not consider possible transgenerational passing of psychiatric traits to the next generations.

In the longer future, elucidating the role of biological alterations, including epigenetic processes in susceptibility to traumatic stress exposure could promote breakthroughs in the development of new drug targets and biomarkers for psychiatric disorders. The identification of predictive biomarkers that may distinguish between individuals at high or low risk of developing PTSD following trauma exposure could enable more effective preventive strategies and early interventions.

7. CONCLUSIONS

Fundamental research has progressively given insight into the molecular mechanisms and cellular processes underlying the pathophysiology of PTSD.

Among many physiological alterations in PTSD, including the potential presence of blood biomarkers, PTSD is characterized by structural and functional brain changes. In addition, some of the underlying metabolic effects of conventional pharmacotherapies for PTSD have been linked to epigenetic changes, which also occur throughout disease progression. This points to the fact that future preventive, diagnostic, and therapeutic paradigms must take complex molecular and epigenetic alterations into consideration. This also promotes understanding of this disorder through a more organic rather than a purely psychological perspective.

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3

BLOOD-BASED TRANSCRIPTOMIC ANALYSIS OF SUSCEPTIBILITY TO PTSD IN DEPLOYED MILITARY SOLDIERS

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4

SOCIAL DEFEAT STRESS ALTERS RNF39 PROTEIN EXPRESSION IN THE HIPPOCAMPAL DENTATE GYRUS OF MICE

AL JOWF G.I., AHMED Z.T., ALOSAIMI F.M., WOLTERS A., REIJNDERS R.A., STEINBUSCH H., RUTTEN B.P.F., EIJSSEN L.M.T., DE NIJS L. *(TO BE SUBMITTED)*

5

A PUBLIC HEALTH PERSPECTIVE OF POST-TRAUMATIC STRESS DISORDER

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ABSTRACT

Trauma exposure is one of the most important and prevalent risk factors for mental and physical ill-health. Prolonged or excessive stress exposure increases the risk of a wide variety of mental and physical symptoms, resulting in a condition known as post-traumatic stress disorder (PTSD). The diagnosis might be challenging due to the complex pathophysiology and co-existence with other mental disorders. The prime factor for PTSD development is exposure to a stressor, which variably, along with peritraumatic conditions, affects disease progression and severity. Additionally, many factors are thought to influence the response to the stressor, and hence reshape the natural history and course of the disease. With sufficient knowledge about the disease, preventive and intervenient methods can be implemented to improve the quality of life of the patients and to limit both the medical and economic burden of the disease. This literature review provides a highlight of up-to-date literature on traumatic stress, with a focus on causes or triggers of stress, factors that influence response to stress, disease burden, and the application of the social-ecological public health model of disease prevention. In addition, it addresses therapeutic aspects, ethnic differences in traumatic stress, and future perspectives, including potential biomarkers.

KEYWORDS

Stress; traumatic stress; PTSD; prevention; public health; treatment; biomarkers; burden.

1. INTRODUCTION

According to the Diagnostic and Statistical Manual (DSM) and the International Classification of Diseases (ICD), a traumatic experience is an event that can pierce into the integrity of an individual or a group causing distress, feeling of helplessness, horror, or intense fear reaction [1]. As reported by Terr (1991), trauma is categorized into two levels/domains, type 1 trauma and type 2 trauma. Type 1 trauma usually originates in childhood following unanticipated single events, typical in inducing post-traumatic stress disorder (PTSD). On the other hand, type 2 trauma follows repeated exposure to long-standing external events [2]. Importantly, type 2 trauma can also lead to the development of PTSD and other trauma-related reactions. It is worth mentioning that, while as much as 90% of the general population experience traumatic stress, only 20–30% of them develop PTSD [3].

The lifetime prevalence of traumatic stress ranges from 0.56% to 6.67% in Europe, with high prevalence rates in the Netherlands, the UK, France, and Germany [4]. Exposure to traumatic events is recognized as the essential key to developing stress-related disorders [5]. The most frequent disorder resulting from traumatic stress is PTSD [6]. In general, PTSD is a severe, chronic, and disabling disorder, which develops after exposure to a traumatic event in susceptible individuals, involving actual or threatened injury to themselves or others [7, 8]. The most common stressor associated with PTSD is usually war and combat and witnessing, while in women, it appears to be sexual assault and rape [9]. Many models are developed aiming to predict the development of PTSD. A common aspect between these models is the interaction between the stressor, the peritraumatic condition, and the person's susceptibility [10–12].

In the DSM-1, PTSD was not categorized as it is today; rather, it was categorized as a gross stress reaction, one of the transient personality reactions (along with adult situational reaction, adjustment reaction of infancy, adjustment reaction of childhood, adjustment reaction of adolescents, and adjustment reaction of late-life). Subsequently in the DSM-3 (introduced in 1980), PTSD was then recognized (for the first time as post-traumatic stress disorder) as "war neurosis" or "shell shock" as it was commonly seen during times of war. As the requirement for a person's subjective negative response (defined as psychological distress or physiological reactivity to trauma-related cues) was eliminated as a diagnostic criterion, PTSD is no longer considered as fear- and anxiety-based disorder in the DSM-5 (introduced in 2013). Rather, it is categorized as a disorder arising in response to traumatic or stressful events preceding the emergence of its symptoms. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), PTSD has four components: intrusion symptoms, avoidance, alterations in cognitive function and mood, and arousal that impairs the individual's functioning [1].

There are two sets of diagnostic criteria in the DSM-5 for PTSD, one for those of six years of age and older, and the other for children under six years of age. This discrimination was based on evidence that children below six years of age have a lower diagnostic threshold [13, 14].

Differences in diagnostic definition and criteria of PTSD might hinder a precise and equal detection and diagnosis and can thereby limit effective and timely management [15]. In addition, it has been reported that declarative and short-term memory deficits may be susceptibility factors for PTSD [16]. Moreover, and as mentioned earlier, comorbidities (e.g., major depression disorder, anxiety disorders, and substance use disorders) further complicate the diagnosis and management of these comorbidities, demonstrating the need for the development of reliable diagnostic markers, such as biomarkers [17–21].

As part of management and patient care, prevention plays an important role in averting the disease before it develops or minimizing complications and morbidity if the disease already ensued. One way of doing so is by the implementation of prevention models, through which different levels of prevention (primary, secondary, and tertiary) can be enforced. Although out of the scope of this paper, it is worth mentioning that genetics and epigenetics have an important role in the interindividual differences in response to traumatic stress [22, 23].

This review provides an insight into the burden of traumatic stress and its complications, some of the known causes of traumatic stress, factors believed to influence the response to stress, and integrating with the public health prevention model applied for traumatic stress. A literature search was performed using keywords to finds papers in PubMed, EMBASE, and SCOPUS. The report of literature in this paper aims to provide a general perspective of public health that demonstrates the importance of the early recognition of traumatic stress.

2. THE BURDEN OF STRESS-RELATED MENTAL DISORDERS AND PTSD

As mentioned earlier, PTSD remains the most frequently encountered disorder as a result of traumatic stress. Due to the high lifetime prevalence and significant consequences, the burden of the disease, both on the patient and the community, is expected to be high. Besides the burden on the patient, an economic burden and medical burden also exist.

2.1. ECONOMIC BURDEN

Stress-related mental disorders often have their onset gradually and at an early age [24], and are expected to cost the world USD 16 trillion by 2030 [25, 26]. The economic

burden can be mainly categorized into direct healthcare costs, productivity loss, societal cost, and non-healthcare costs. The general cost of diseases is usually classified into three types: direct, indirect, and intangible cost [27]. Direct costs include healthcare costs (diagnosis, treatment, and rehabilitation) and non-healthcare costs related to consuming non-healthcare resources, such as transportation, household expenditures, relocating, property losses, and informal care [27]. Indirect cost is productivity losses due to morbidity and mortality, borne by the individual, family, society, or the employer [27, 28]. While intangible cost is not monetary but relates to function loss, increased pain, and reduced life quality, it can be regarded as an indirect economic burden and cost of illness [29].

The cost of psychiatric contact and outpatient treatment is surprisingly higher when compared to drug treatment and rehabilitation services [30]. The annual mean direct costs of PTSD per individual were much lower in South-Eastern European countries (USD PPP (purchasing power parities) 198–7110) compared to UK, Germany, and Northern Ireland (USD PPP 2337–26,991), probably due to the difference in healthcare spending between these countries. As expected, these numbers are negatively influenced by the severity of symptoms [30].

In Northern Ireland, the total economic burden in patients with PTSD as another example was GBP 172.8 million in 2008 values, including GBP 33.0 million in direct cost and GBP 139.8 million in indirect cost [31]. In Germany, the overall economic burden costs for PTSD account are EUR 43,000 per person. Mental disorders occupy 59%, which is the largest portion, and 18% of this value is for PTSD, with at least twofold more costs than a control group in 2013 [32].

Nearly all previous researchers found that indirect cost weighs similar or more in magnitude in the overall economic burden than direct costs [31, 33, 34].

2.2. MEDICAL BURDEN

The medical burden is the impact a disease has on a population, which can be measured by indicators such as morbidity, mortality, and cost. The medical burden includes health care burden, comorbidity, and substance abuse, which also needs further treatments [35]. The medical burden of illnesses can be quantitatively measured by a cumulative illness rating scale [36]. This scale is a tool prevalently used as a criterion to evaluate medical burden in older adults as well as veterans [37, 38].

Co-occurrence of mental as well as the general medical disorders are among the most common and disabling combinations, with approximately 30% of individuals with comorbidity having both a mental and a physical disorder [39]. In addition, 68% of adults

with mental disorders have physical medical conditions [40]. Patients with medical comorbidity are in greater need of medical services with the loading healthcare system. For example, PTSD is exacerbated by comorbid medical illness, accounting for cumulative service utilization.

Returning veterans with PTSD, as an example, have a higher medical burden than those without mental health conditions [41]. In addition, these medical burdens are conditional on gender. Women tend to have more medical burdens than men. The median number of medical conditions for women with PTSD was seven, while for men was five [41].

Substance abuse disorders and social disadvantages can contribute to comorbidity and exacerbate its effect [39]. For example, co-occurring substance use and mental disorders are common among adults with opioid use disorder [42]. Thus, the soaring medical burden is associated with treatment resistance, medical comorbidity, and related substance abuse.

3. TYPES OF STRESSORS

A stressor, or a stressful event, is the prime causative factor of PTSD, and is one of the criteria for diagnosing PTSD [43, 44]. There are numerous types of stressors that may give rise to PTSD, including sexual assault, war and combat, child abuse and neglect, medical illnesses and disasters, in addition to others (Table 1).

3.1. SEXUAL ASSAULT

Sexual assault is defined as “any form of sexual contact without voluntary consent, and that violates a person’s sense of autonomy, control and mastery over their body” [45]. The prevalence of PTSD due to sexual assault is 50%, making it the most common trauma resulting in PTSD in women [46]. Among those, rape is the most common form of sexual assault, but other forms also contribute to the trauma [46]. The dilemma of sexual assault is that it is a personal, individualized challenge to overcome and has a societal aspect that complicates the recovery process [46, 47].

3.2. WAR AND COMBAT

PTSD in military personnel is a common subject of psychiatric and psychological research, and tends to be correlated to the severity of the injury experienced [48]. Hoge *et al*. surveyed PTSD in US soldiers returning from Iraq months after returning from deployment. Their study showed that the prevalence of PTSD increased over the months, but interestingly, the severity of the physical injury was correlated to the earlier development of disease [49]. Additionally, some factors can affect disease development and progression. Of

importance is deployment with combat experience, childhood adversity (adversity relating to family relationships and childhood antisocial behaviour), leaving service, and serious accidents, as demonstrated by a study on British military personnel [50]. Important to note is that the effect of war is not only on soldiers that are deployed to the battlefield; the civilians are affected too. A systematic review on mental health outcomes among populations exposed to mass conflict and displacement found an overall prevalence of PTSD of 30.6% across all included studies (15.7% in studies > 1000 participants only). The main contributing factors retrieved were reported torture, cumulative exposure to potentially traumatic events, time since conflict, and assessed level of political terror [51]. Children are also strongly affected, not only by war trauma, but also by the effects this has on socio-ecological factors at family (parenting) and community levels [52]. This can also be seen in the 2022 Ukraine invasion, where multiple traumatic exposures have a critical impact on mental health [53, 54].

3.3. CHILD ABUSE AND NEGLECT

In a prospective cohort study, children were followed up until their young adulthood, and both physical and sexual abuse increased the risk of developing PTSD. Another significant finding was that childhood neglect similarly increased the risk, and these factors independently contributed to the risk of the disease [55]. In 2009, Koenen *et al*. investigated whether there is a sex difference regarding these risk factors. They found a twofold difference in the risk of developing PTSD after childhood trauma, as women were found to have the highest increased risk among all the risk groups [56]. Additionally, those who experienced childhood trauma showed greater somatic symptoms, affect dysregulation, and suicidal behaviour as compared with those without PTSD [57].

3.4. MEDICAL ILLNESS

It is becoming more evident than before that severe medical illnesses contribute to the risk of developing PTSD. Studies have been conducted on individuals with a specific disease to assess the degree of this risk. In a study on patients with Acute Coronary Syndrome (ACS), the prevalence rate of PTSD among 24 individuals was 12%, giving it the name ACS-induced PTSD [58]. Furthermore, patients with symptoms of PTSD that presented to the emergency room (ER) were more likely to be more worried about future stroke and have worries about medications, while not being adherent to medications [59]. Lastly, patients who experienced an intensive care unit stay showed a 24% PTSD prevalence 1–6 months after, as demonstrated in a meta-analysis [60].

3.5. DISASTERS

Disasters are traumatic events experienced by individuals that commonly lead to physical and mental consequences [61]. There are different types of disasters: natural disasters, such as tornados, floods, and hurricanes, and human-made/technology disasters, such

as the Chernobyl disaster, terrorism, and torture [62]. The prevalence of PTSD was found to be higher in studies that focused on individual victims of disasters than on the general population [63]. Being female is considered to be a risk factor for the initiation of PTSD after disasters [64]. Other factors that found to be correlated with the initiation of PTSD after disasters included weak coping skills, external locus of control, history of previous trauma, low social support, media exposure, and others [65–68]. Furthermore, the degree of individual exposure to disaster is associated with PTSD likelihood [65]. The prevalence of PTSD is higher among the individuals who were directly exposed to the disaster and lower among individuals who rescued disaster victims [62].

Table 1. Main types of stressors related to PTSD.

Stressor Related to PTSD	Study	Findings
Sexual assault	Creamer et al. (2001) [46]	Most common traumatic stressor resulting in PTSD, accounting for 50% of cases; among these, rape was the most common form of sexual assault
War and combat	Hoge et al. (2008) [49]	Severity of physical injury is correlated to the earlier development of PTSD in soldiers returning from deployment
Child abuse and neglect	Koenen, Widom (2009) [56]	Childhood physical and sexual abuse, as well as neglect significantly increase the risk of developing PTSD. Females tend to have an increased risk
Medical illness	Edmondson et al. (2012) [58]	The rate of PTSD among ACS patients was 12%, while it was 24% for those who stayed in the ICU. Additionally, PTSD patients were more likely to not adhere to their medications
Disasters	Ahern et al. (2002) [68]	Female gender, low social support, history of previous trauma, and direct exposure to the disaster were all factors that correlated with PTSD initiation after the disaster

3.6. OTHER FACTORS

While the causes mentioned above represent the main and most important ones, it must be acknowledged that the causes of traumatic stress are diverse. Furthermore, it is worth mentioning that some factors are considered risk factors, and they include a low social-economic status, female gender, family history of mental illness, and prior mental disorders [69]. The COVID-19 pandemic deserves a mention here, as some authors attempted the assessment of its impact on mental health. A meta-analysis was conducted on the prevalence of post-traumatic stress symptoms and psychological stress with a pooled prevalence of 23.88% and 24.84%, respectively [70]. Bridgland *et al*. found that participants in their study had PTSD symptoms when directly (COVID-19 diagnosed) or indirectly (e.g., media coverage and lockdown) exposed [71]. Surprisingly, patients had

symptoms in response to anticipated events, giving a new view that traumatic stress could be to anticipated future rather than only impact of past events [71]. Additionally, the sequential exposure to multiple stressors, such as the Ukrainian war trauma with the stress from the global pandemic of COVID-19 not completely over, can have disastrous effects [53].

4. FACTORS MODERATING THE IMPACT OF THE STRESSOR

4.1. EMOTIONAL CARE

Children during infancy should receive consistent and constant emotional care because it is a critical period of emotional, social, and cognitive development. The opposite of emotional care can be classified into two subtypes: one is active emotional abuse, which receives much attention, and the other is passive emotional neglect [72]. If emotional neglect occurred early in life, social and emotional rehabilitation deficiencies could be seen after being inflicted with traumatic stress [73–75]. A four-year longitudinal research on adopted children who experienced emotional neglect showed that children were in the clinical or borderline ranges for symptoms of post-traumatic stress arousal (19%), avoidance (14%), and intrusion (8%) [76]. Figure 1 provides a general overview of the information about emotional care discussed in this section.

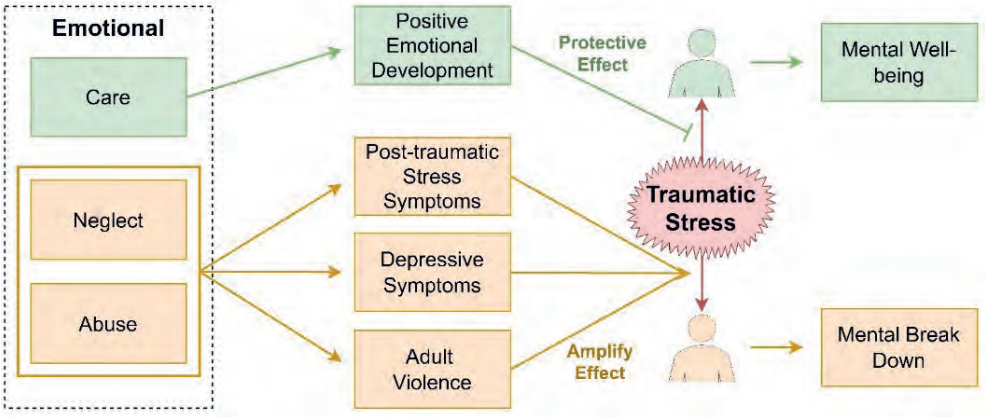

Figure 1. The impact of emotional care, emotional neglect, or abuse in early life on mental health in response to traumatic stress later in life.

Of all types of abuse, including childhood emotional abuse, physical abuse, and sexual abuse, only emotional abuse was independently associated with depressive symptoms, emotion dysregulation, and interpersonal problems in a cross-sectional study of 276 female college students [77]. In contrast, an emotionally responsive environment is found

to possibly protect from severe traumatic stress even in those with genetic vulnerabilities [73, 78].

Trauma-experienced youths tend to have emotional problems. A systematic review indicated that traumatized youths showed emotional regulation difficulties, including affect dysregulation, mood swings, affective and mood instability, or lability [79].

4.2. AGE

An earlier age of trauma exposure is associated with an increased risk of PTSD development [73, 80]. Within PTSD, youth age (9–17) is positively associated with the volumes of brain structures (amygdala), but this is not observed in the non-PTSD youth controls [81]. Thus, severe stress may influence age-related variation in brain structures. Furthermore, a study on combat veterans showed age-accelerated shrinking of the cortical surface area in some regions when combat-related mild traumatic brain injury and PTSD are present, a pattern that was not consistently found in those with mild traumatic brain injury only [82].

4.3. NUMBER AND IMPACT OF TRAUMATIC EVENTS

Besides age, other modifiable risk factors, such as earlier traumatic events, have been associated with increased perceived severity of current traumas [69, 73, 83, 84]. An investigation on 444 refugees from the 1994 Rwandan genocide showed that higher numbers of different lifetime traumatic event types were associated with a higher probability of lifetime PTSD [85]. One additional traumatic event experienced was associated with a 19% increase in the probability of developing lifetime PTSD [85]. This increase indicated an accumulative effect of traumatic events on the onset of PTSD. Besides, 314 college students were asked to rate the importance of different events, including interpersonal or non-interpersonal ones. The results show that perceived importance was higher for interpersonal than non-interpersonal events [86]. Therefore, both the traumatic event number and event type impact mental health in response to stress.

4.4. EDUCATION

Lower levels of education render subjects at higher risk of PTSD in a study investigating emergency health care personnel in Italy [87]. Another study showed that a lower education level with other factors, such as race and age of combat exposure, predicts the current PTSD symptoms and symptom exacerbation in the longitudinal study on Vietnam veterans 40 years after the combat [88]. People with lower education levels have higher scores on the Kessler 10 scale, indicating more anxiety and depression than people with higher education in a cross-sectional study that included people who lived in Syria in different governorates [89].

4.5. GENDER

The lifetime prevalence of PTSD is different between genders with higher rates among females (10–12% vs. 5–6% among males). Explanations for this are both psychosocial (e.g., type of trauma, as women are exposed to high-impact trauma, e.g., sexual assault, as described earlier) and biological (e.g., lower oxytocin release, a hormone that has been shown to reduce PTSD development as discussed later) [90]. Women veterans reported the highest lifetime and past-year PTSD rates compared with women civilians, men veterans, and men civilians [91]. However, another study on US military personnel deployed in support of the operations in Iraq and Afghanistan showed no significant gender differences for the likelihood of developing PTSD or for PTSD severity scores [92].

4.6. RACE

Race has been reported as an impact factor mediating traumatic stress (race-based traumatic stress), largely due to race discrimination rather than biological reasons [93]. A study on 421 community-based adult respondents showed that race-based traumatic stress is significantly related to trauma symptoms, especially in people who consider negative race-based experiences stressful [93]. Empirical data in 2012–2017 suggests that, in the US, Latino Americans, African Americans, and Native Americans tend to present with the highest rates of PTSD, while Asian Americans tend to present with the lowest [94].

To our knowledge, African Americans have the highest prevalence rate of PTSD across all ethnicities [95–97]. Although not fully understood, this disparity between ethnicities might arise from a difference in traumatic exposure or the pre-exposure vulnerability [96, 98, 99]. Research has been conducted in this regard, but several factors are implicated in this difference. For example, higher PTSD among African Americans might be due to racism and verbal assault, stigmatization, and the discrimination perceived by themselves [100–102].

On the other hand, some factors might account for the lower PTSD prevalence among other ethnicities, such as better socioeconomic status, higher education, and higher income [69, 103]. Additionally, other psychiatric disorders, such as depression and anxiety, were associated with a higher risk of developing PTSD, and although these disorders are more prevalent in other populations, the risk of developing PTSD is found to be higher in African Americans [104, 105]. It also appears that sociopathy alcohol and drug abuse, which are seen to be lower in Asians, could contribute to the ethnic difference and might explain the lower PTSD prevalence in that group [106].

In 2017, Alexander and his colleagues investigated the ethnic difference in PTSD vulnerability following hurricane Katrina [107]. The two-fold higher odds of African Americans compared to other populations to screen positive for PTSD was related to

some factors, including worse prior mental distress, more stressful events, and less social support. Between these factors, only pre-hurricane mental distress has been shown to reduce this ethnic disparity. This might mean that hurricanes trigger the manifestation of PTSD of delayed onset, presumptively [107]. Overall, the retrospective nature of these studies might be seen as a limitation to the investigation of this ethnic association, and future prospective studies need to investigate the differences and arrive at a more solid conclusion.

Besides the likelihood of race discrimination exposure, ethnic origin can be a risk factor of traumatic stress mediated by culture. A study including Shanghai and Hong Kong residents and Americans showed the cultural differences in dialectical thinking, self-construal, and familyism in mediating resilience capacity. Dialectical thinking is the cognitive tendency toward attempts to reconcile two opposing perspectives or acceptance of contradiction [108]. Self-construal can be the independent self-construal common in the West or the interdependent self-construal common in East Asian countries. Similarly, tendency towards familyism (which means interests and gains are conceived at the level of the familial group rather than at the individual level) is present more in East Asian countries [94]. The study on three regions showed that independent self-construal, familyism, and dialectical thinking significantly mediated the relationship between culture and resilience capacity [108].

5. PUBLIC HEALTH MODEL FOR PREVENTION AND INTERVENTION

5.1. THE SOCIAL-ECOLOGICAL MODEL

From a public health perspective, approaching a disease starts by identifying the causes and triggers, after which prevention (rather than treatment) can be applied at different stages of the disease development, with the aim of decreasing the disease burden at all levels. One model is the social-ecological model, a four-level model that aims to identify the factors contributing to disease development and poor health outcome at these levels: individual, relationship, community, and society [109, 110] (Figure 2).

At the core of the model, the individual level is found, which includes personal characteristics, including biological and others (e.g., genetics, comorbidities, education level, and economic status). The next level, which is the relationship, includes one's close social connections that exert influence over the individual (parents, partners, family, close friends, etc.). The third level is the community, which explores one's contact with one's community, which happens at a wider social level (e.g., schools, universities, meeting places, and workplace). The final level, the societal level, looks at how the society in which the individual lives can affect his health outcome, and is usually a cultural and political

level (e.g., cultural habits, norms, societal education, economy, and policies). At each one of these levels, prevention is feasible, and if suitable, intervention can also be applicable [109].

The prevention applied at each level can be primary, secondary, or tertiary. Primary prevention focuses on recognizing individuals at risk and preventing disease development in a disease-free individual. Secondary prevention aims at intervening early after disease occurrence, to achieve cure if possible, or to control disease progression. Tertiary prevention aims at reducing disabilities resulting from the disease to maintain a better quality of life [111]. While it is easier to define a clear onset in medical (or physical) diseases, disorders resulting from traumatic stress are diagnosed based on specific criteria, which are sets of symptoms with duration. This might present a challenge to differentiate between primary and secondary prevention, as asymptomatic disease might be the case in many individuals [43].

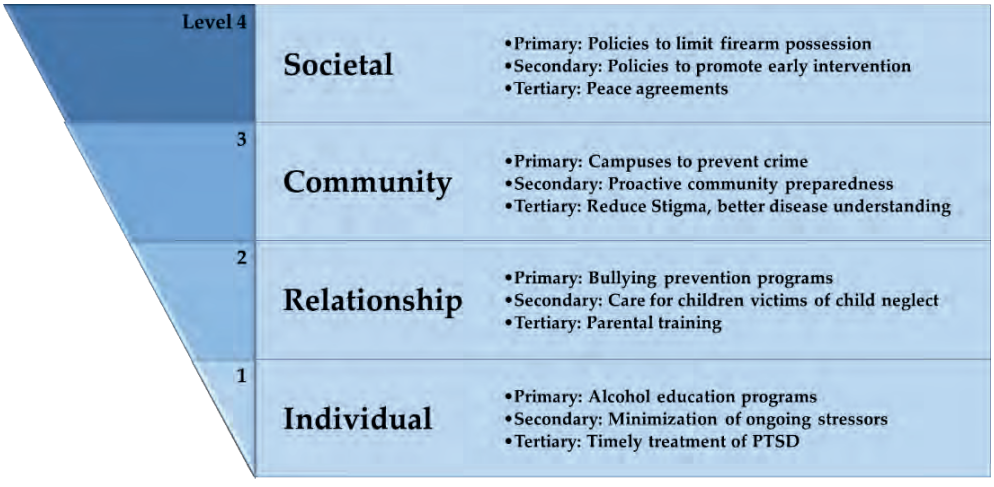

Figure 2. The social-ecological model of disease prevention as applied to PTSD. For each of the four levels, examples of primary, secondary, and tertiary prevention are given.

5.2. EXAMPLES OF PREVENTIVE MEASURES AT THE MULTILEVEL SOCIAL-ECOLOGICAL MODEL

As primary prevention aims at preventing disease occurrence, in a trauma situation, it aims at preventing exposure to trauma. As discussed earlier, there are many causes of trauma, and specific interventions can be directed at these causes. At the individual level, interventions can be in the form of educational programs on the risk of alcohol drinking and firearm acquisition for the youth and parental guidance for young children, as well as college programs to educate young adults about traumatic experiences [112]. Additionally,

psychoeducation programs target military personnel prior to deployment on trauma reaction [113]. At the relationship level, parental and caregiver guidance and education can aim at reducing traumatic experiences for the children, for example, bullying at school or programs to reduce assaultive violence. At the community level, interventions include community support services, neighborhoods, and streets surveillance campuses. At the societal level, policies can target reducing the acquisition of firearms and alcohol consumption, as well as recalling defective motor vehicles and street maintenance [114] (Figure 2).

Secondary prevention is implemented after exposure to trauma and disease development, but the key is early intervention to prevent disease progression. At the individual level, and for trauma-exposed individuals, preventing ongoing exposure to stressors can halt disease progression [115]. Additionally, building prediction tools based on the susceptibility to develop PTSD after trauma exposure can identify high-risk patients, and hence, provide opportunities for intervention [43]. In addition, early psychological interventions can be effective [116]. At the relationship level, caring for relatives of domestic violence or children who are victims of child neglect can be useful. Medical intervention (e.g., cortisol and adrenergic medication administration) immediately after trauma exposure can take part in the secondary intervention, but weighing the harms and benefits should be determined carefully [117]. At the community level, providing shelters in the aftermath of disasters and making rehabilitation programs are strategies taken. Not only these, but also preparedness and measures in anticipation of disasters and traumatic experiences can be considered secondary prevention [118]. At the societal level, policies addressing early medical intervention, as well as screening campuses can limit disease progression (Figure 2).

When interventions are aimed at preventing the progression of disease and development of disabilities, they are considered part of the standard care and treatment of the disease. At the individual level in tertiary prevention, seeking medical care and compliance with treatment, as well as better knowledge of the disease and complications helps to prevent disabilities. At the relationship level, special training for care from parents, relatives, and friends to patients undergoing therapy can be helpful. As demonstrated by Leve and colleagues, foster parents training for the care of trauma-exposed children with psychological illness halts disease progression [119]. Both community and societal levels fall under political influence, and measurements to promote community understanding of the disease, reducing stigma, and ensuring peace and fighting violence all play an important role in preventing disability [118-120] (Figure 2).

5.3. SECONDARY PREVENTION AND TREATMENT AND MODALITIES

While the absolute avoidance of traumatic stress is often not a matter of control, the prevention of its complications can be pursued. After exposure to traumatic stress, individuals become vulnerable to complications, with PTSD being the most common. Several interventions are currently either implemented or under research to prevent such complications. These include behavioural therapy (e.g., cognitive behavioural therapy (CBT), prolonged exposure (PE), and eye movement desensitization and reprocessing (EMDR)) as well as pharmacological agents.

Being an effective and important therapeutic modality, CBT aims to give patients a sense of control over their fears, using different methods, such as exposure, to achieve fear extinction and support cognition to change the patient's perception about the trauma [121]. Several clinical studies have been conducted on the effect of CBT early after trauma exposure to reduce complications and the appearance of symptoms. In 2021, Rothbaum and her colleagues investigated the effect of prolonged exposure CBT on patients who experienced traumatic stress, such as rape and motor vehicle accidents. It appeared that exposure-based CBT reduced PTSD symptoms at the assessment, especially for victims of sexual assault [122]. In a population of cardioverter defibrillator patients, CBT in less than two months for eight weeks significantly lowered the development of symptoms and promoted patient improvement in the CBT group [123]. Besides CBT, other therapies, such as PE in which the patient processes the traumatic event, as to decrease distress at further recall [124], and EMDR in which eye movements can reduce the intensity of traumatic memories [125], were the most utilized therapies in evidence [126, 127]. The new consolidation/reconsolidation therapy is based on memory processing and the modulation of this process by pharmacologically interfering with this process during the recall of the disturbing memory, and to date, it has shown promise [128]. In this systematic review, Forneris *et al*. assessed the efficacy and comparative effectiveness of different psychological and pharmacological interventions in reducing the incidence or severity of PTSD symptoms. Interventions such as CBT reduced symptoms severity, while other interventions, such as debriefing, did not show evident benefit. The evidence for several other approaches appears to be limited and insufficient due to shortcomings in many included studies [129].

The other intervention modality is the pharmacological one, and many drugs have been tested to prevent complications. In addition, the findings from meta-syntheses showed that selective serotonin reuptake inhibitors (paroxetine and sertraline) and noradrenaline were effective and thus are highly recommended in the current guidelines [1, 130].

Interestingly, propranolol helps to reduce traumatic recall in patients, given that it is administered early within hours after the traumatic exposure to affect memory formation

and reduce traumatic recall [131]. A study on 64 trauma patients tested the effect of hydrocortisone compared to a placebo group. The symptom severity in the hydrocortisone group was lower than the placebo group, as the patients reported lower scores on the clinician-administered PTSD Scale (CAPS) [132].

The effects of oxytocin in the prevention of PTSD seem complex. A single administration had adverse effects on symptoms as it increased fear processing, while repeated administration reduced the development of PTSD symptoms [133]. The recreational drug 3,4-methylenedioxymethamphetamine (MDMA), when combined with psychotherapy, was associated with improved symptoms and lower functional compromise greater than psychotherapy and placebo [134]. Although trials showed controversial results, the alpha-blocker agent prazosin is used in clinical practice, as it appears to reduce symptoms [135].

The clinical implementation of the interventions above is harder than it seems, and the choice of a single standard therapy modality might not be effective. For that, a tailored and collaborative strategy for patients at risk is recommended. Zatzick *et al*. (2013) developed a randomized trial in which trauma survivors underwent stepped combined care management, psychopharmacology, and cognitive behavioural therapy compared to the usual care control condition. As expected, this therapy strategy reduced PTSD symptoms and improved functioning compared to the usual care group [136]. However, CBT is the mainstay of early prevention modality, but the challenging nature of implementation elicits the need to develop other modalities suitable for the needs of the particular subjects.

6. FUTURE PERSPECTIVES: BIOMEDICAL MARKERS

Although PTSD is often a highly debilitating psychiatric disorder, no medical tools are currently available to prevent or minimize the impact of traumatic stress on mental health. PTSD symptoms prevent suffering individuals from leading a healthy lifestyle and are debilitating on a personal, societal as well as a professional level. Moreover, the economic burden of PTSD is substantial. There is thus a pressing need to develop additional tools to help PTSD prevention and treatment, such as biomarkers. We focus on biomarkers that aid the processes of diagnosis as well as determining therapy and response to treatment, supporting stratified precision medicine.

Integrating biomarkers along with the clinical assessment would provide a powerful means of managing PTSD and other psychiatric disorders. In addition to the role of biomarkers in the diagnosis and prediction of the onset of disorder, first (relative small scale) studies on biomarkers related to response to treatment are under investigation for possible future

applicability [137]. For example, Felmingham *et al*. found reduced right amygdala activity and increased right anterior cingulate cortex activity in patients successfully treated with CBT [138]. Another example is the association between rostral anterior cingulate cortex (rACC) volume and the reduction in PTSD symptoms [139]. The same study demonstrated that activation of the ventral anterior cingulate and amygdala predict a better response to therapy [139]. On the side of pharmacotherapy, a promoter-region polymorphism, namely the LL 5HTTLPR genotype, was associated with a better responsiveness to sertraline (SSRI) [140]. Snijders *et al*. investigated the diagnostic potential of miRNA in a pilot study of patients with PTSD. In their pilot study, miR-138-5p was found to be significantly higher in PTSD patients as compared to controls. Additionally, only miR-1246 was significantly downregulated in PTSD cases compared to resilient subjects [141]. Although biomarkers showed promising initial results in predicting and diagnosing PTSD, further dedicated research is needed to determine the applicability of these biomarkers. Additionally, ethical considerations related to biomarkers for PTSD should be given attention: whereas prevention of avoidable harm and suffering can be considered a moral duty, the availability of such markers can also raise some concerns as to whether a test can be made obligatory and what the social and professional consequences of a susceptible or resilient status will be [142].

7. CONCLUSIONS

This paper provided a literature review of PTSD with the focus on traumatic stress prevention form a public health perspective. A traumatic experience is an event that can pierce into the integrity of an individual or a group causing distress, feelings of helplessness, horror, or intense fear reaction. The cause of this traumatic experience might range from war, terrorism, and disasters to sexual assault and child abuse. A well-recognized complication of such experience is PTSD. Current descriptive and empirical evidence showed race, gender, and age differences in the risk of developing PTSD, resulting in interindividual differences in disease manifestation. Early recognition and diagnosis help in the application of different levels of prevention (primary, secondary, and tertiary prevention), improving the course of the disease and limiting the complications, while reducing costs and burden of the disease.

Additionally, the implementation of preventive measures according to public health models of disease prevention can be seen as a means to achieve these goals effectively. A widely accepted model, the socio-ecological model, was implemented to study prevention at the levels of the individual, relationship, community, and society. While a variety of different prevention and treatment modalities exist for PTSD, including behavioural and

pharmacological interventions, the identification of the suitable strategies is important to avoid treatment failure and relapses.

The earlier described differences in PTSD definition and lack of definitive diagnostic tools may be a contributing limitation to research on PTSD as well, which as such leads to some differences among studies and their conclusions and, thereby, our reporting thereof. A more detailed mapping of the definitions used across the several studies and possible inclusion of such criteria in a meta-analysis or systematic review could be a valuable future endeavor. The finding of biomarkers may help to synchronize diagnostic criteria, but also depends on the definition of disease classes for their discovery.

Although biomarkers showed promising initial results in predicting, diagnosing, and treating PTSD, further dedicated research is needed to replicate and validate these and (if successful) test for the clinical applicability of these biomarkers. Integrating biomarkers along with the clinical assessment may provide added value for diagnosing PTSD and prediction its course.


5

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6

TO PREDICT, PREVENT AND MANAGE POST-TRAUMATIC STRESS DISORDER (PTSD): A REVIEW OF PATHOPHYSIOLOGY, TREATMENT, AND BIOMARKERS

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ABSTRACT

Post-traumatic stress disorder (PTSD) can become a chronic and severely disabling condition resulting in a reduced quality of life and increased economic burden. The disorder is directly related to exposure to a traumatic event, e.g., a real or threatened injury, death, or sexual assault. Extensive research has been done on the neurobiological alterations underlying the disorder and its related phenotypes, revealing brain circuit disruption, neurotransmitter dysregulation, and hypothalamic–pituitary–adrenal (HPA) axis dysfunction. Psychotherapy remains the first-line treatment option for PTSD given its good efficacy, although pharmacotherapy can also be used as a stand-alone or in combination with psychotherapy. In order to reduce the prevalence and burden of the disorder, multilevel models of prevention have been developed to detect the disorder as early as possible and to reduce morbidity in those with established diseases. Despite the clinical grounds of diagnosis, attention is increasing to the discovery of reliable biomarkers that can predict susceptibility, aid diagnosis, or monitor treatment. Several potential biomarkers have been linked with pathophysiological changes related to PTSD, encouraging further research to identify actionable targets. This review highlights the current literature regarding the pathophysiology, disease development models, treatment modalities, and preventive models from a public health perspective, and discusses the current state of biomarker research.

KEYWORDS

Stress; traumatic stress; PTSD; behaviour changes; pathophysiology; public health; biomarkers; prevention; treatment.

1. INTRODUCTION

Post-traumatic stress disorder (PTSD) is a chronic mental disorder resulting in a reduced quality of life and increased economic burden. Exposure to a traumatic stressor is the trigger for PTSD development [1]. For that, a distinction between ordinary and traumatic stressors (those that have the potential to result in PTSD) is necessary. PTSD was first introduced in the Diagnostic and Statistical Manual of Mental Disorders (DSM-III), and further updates to the diagnostic criteria have been introduced in subsequent versions. Traumatic stress relates to the exposure to real or threatened injury, death, or sexual assault. Intrusion symptoms, avoidance/numbing, hyperarousal, sensitisation to stressors, and detrimental cognitive and affective changes are all symptoms of PTSD [1].

Although exposure to stressors is common in the general population, only a small proportion of susceptible individuals develop PTSD [2]; however, the underlying mechanism of susceptibility and resilience is still unclear. In the last decade, etiological models have been developed to explain the interplay between biology, environment, and mind in manifesting the disease. Examples of those models include the diathesis–stress and the biopsychosocial models [3]. In parallel, extensive research aiming to identify the pathophysiological mechanism of PTSD has found the association between genetic variants and an increased risk of PTSD, hypothalamic–pituitary–adrenal (HPA) axis dysfunction, neurotransmitter dysregulation, and alterations in brain circuits [4]. Research has advanced over the last years in aiming to connect the alterations at the genetic, molecular, chemical, cellular, and circuitry levels into a biological systemic view, while also aiming to discover diagnostic and prognostic biomarkers.

Psychotherapies and pharmacotherapies are two effective PTSD treatments. However, significant subsets of individuals who do seek treatment have symptoms that are difficult to treat. Changing to another treatment modality or combining treatment modalities (combining psychotherapy with pharmacotherapy) is frequently required. Trauma-focused psychotherapy is the first line of treatment for most individuals with PTSD, as opposed to other therapies or pharmacological medication. Cognitive behavioural therapy (CBT), prolonged exposure therapy (PET), and eye movement desensitization and reprocessing (EMDR) therapy are trauma-focused psychotherapies that have been shown to be useful in the treatment of PTSD [5, 6].

This review provides an introductory and overall overview of the current concept of findings on the etiology and disease models of PTSD, pathophysiology, treatment, prevention, and lastly biomarkers, including diagnostic and prognostic biomarkers. A literature search was performed using keywords to find papers in PubMed, Cochrane Library, Scopus, and Embase. The literature was then summarized with the aim to provide

a comprehensive overview of these topics, and representative examples of research findings were selected. The preferred studies were meta-analyses, systematic reviews, and randomised controlled trials (RCTs), as well as the most recent studies relevant to the presented perspective. Abstracts were then screened for their relevance. Once selected, the limitations of the studies were assessed for their impact on informed clinical decisions. Original studies were preferred for models and concepts. The literature was then summarised with the aim to provide an up-to-date, comprehensive overview of the available data (a graphical summary of the main findings is given in Figure 1).

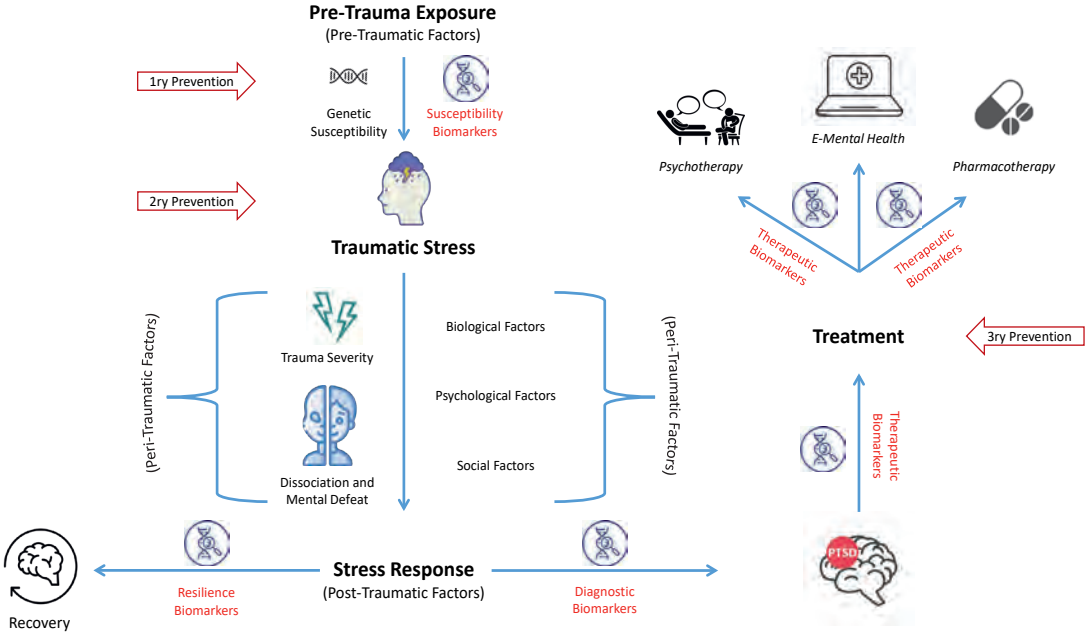

Figure 1. A graphical summary of the main findings of the paper. The entirety of the pre-, peri- and post-traumatic factors can be biological, psychological, or social, according to the biopsychosocial model.

2. EPIDEMIOLOGY AND MODELS OF PTSD DEVELOPMENT

2.1. EPIDEMIOLOGY OF PTSD

The public health perspective of traumatic stress takes a population-based approach and formulates policies based on it. Epidemiological studies concern the distribution and determinants of traumatic stress and stress-related mental disorders in specified populations. They have shown that traumatic stress often occurs among the war-

surviving population, refugees, especially females, public health workers, and indigenous populations [7].

The war-surviving population has a high prevalence of PTSD and depression. This statement is supported by a systematic literature search and meta-analysis of interview-based epidemiological surveys including samples from 43 war-ridden countries with a recent war history (1989–2019) [8]. Specifically, these war survivors diagnosed with PTSD or depression are primarily living in low/middle-income countries [8]. Therefore, income or social status may have an impact on the response to traumatic stress. A meta-analysis and the Millennium Cohort study support the association between low socioeconomic status and PTSD [9, 10]. Sex/gender has also been linked to risk, e.g., in a refugee population, females who experienced sexual trauma had a higher prevalence of PTSD than males [7].

Differences between groups of people have been reported. For example, indigenous populations in various countries show a higher prevalence of traumatic stress-related mental health problems than others. The standardized prevalence of 12-month PTSD in the Australian indigenous population was three times the Australian rates [11]. The independent predictors (determinants) of PTSD among Australian indigenous populations are female gender, rural residence, trauma under age 10, and sexual and/or physical violence [11]. Such findings might be attributed to the fact that the Australian indigenous population is disadvantaged in different aspects [12].

Public health workers have been experiencing huge traumatic stress during the COVID-19 pandemic [13]. Among 26,174 surveyed public health workers in the US, 53.0% reported symptoms of at least one mental health condition in the previous two weeks, especially those unable to take time off or those experiencing overwork [13]. This result indicates that overworking is associated with the negative impact of traumatic stress.

The studies above demonstrate that specific populations with certain characteristics are more likely to suffer from exposure to traumatic events. These characteristics can be environmental factors like traumatic events including war/violence and workload, social factors like social status, or biological factors like female sex. A dual influence of social and biological factors on females is suggested. Females are more likely to experience physical and sexual violence. Additionally, clinical evidence points to the possibility that cyclical oestrogen discharges throughout the reproductive cycle may contribute to women's greater susceptibility to and severity of PTSD symptoms following psychological stress [14, 15].

2.2. MODELS OF DISEASE DEVELOPMENT

2.2.1. DIATHESIS–STRESS MODEL FOR PTSD

The initiation and maintenance of the disorder are heterogeneous, differing between individuals with the same level of trauma and presenting with varying degrees of disease severity. An explanation for this obvious variation is the diathesis–stress model, tested for PTSD in different studies. According to the model, the pre-trauma state of the individual (risk factors) constitutes a condition of susceptibility (diathesis) that can produce the disorder after a traumatic experience (stress).

In PTSD, the trauma represents the stressor that activates certain processes in an individual with pre-traumatic vulnerability, thereby leading to the expression of psychopathology and social dysfunction. Vulnerability factors may involve aspects like genetic predisposition, psychiatric history, a history of child abuse, a stressful and unhealthy lifestyle, and others. The individual's diathesis represents a hypothetical threshold, and the impact of a stressor on the individual depends on the diathesis; the less favourable the diathesis, the less severe the stressor needs to be to initiate the disorder.

Not only pre-trauma (e.g., a risk of developing PTSD, genetic, and biological factors), but also peri-trauma (e.g., emotional distress), and post-trauma factors should be considered. McKeever and Huff state that the peri-traumatic perception of trauma and post-traumatic conditions can affect the severity and symptoms. Furthermore, different types of vulnerabilities (e.g., biological and psychological) may interact with one another [3].

Here, we provide a few examples of research findings in line with the diathesis–stress model. As the diathesis–stress model contends that the interplay of hereditary, biological predisposition, and environmental stress results in the development of mental disorders, researchers have hypothesized that during military service the onset of major psychopathology may be precipitated by psychosocial stress, leading to an increase in psychiatric hospitalisations during the first months of the military service period for those with greater sensitivity or a lower stress tolerance [16]. In a sample of 118 hospitalised subjects starting their military service assessed for the expression of psychopathology, 59.3% of the subjects were diagnosed with an anxiety disorder, especially PTSD, out of the total sample due to traumatic stress exposure, implicating the nature of warfare stress in the increased risk of anxiety and stress disorders. Intriguingly, the risk of disorder onset within the first two months and hospitalisation was also higher for psychotic spectrum disorders. As the sample was exposed to similar stress levels, these findings suggest that individuals with psychotic spectrum disorders have increased stress sensitivity [16].

Another study for testing the model in the emergency department (ED) was carried out by Edmondson in 2014. A sample of 189 acute coronary syndrome patients was observed

for the effect of ED crowding, depression status, and their interaction on the subsequent development of PTSD. ED crowding significantly affected the 1-month development of PTSD symptoms, as patients treated during ED crowding times scored significantly higher than those treated during times with medium or little ED crowding. Similarly, depression status and the interaction between ED crowding and depression status significantly affected the subsequent development of PTSD [17].

Another example of findings supporting the diathesis–stress model of PTSD comes from Elwood and colleagues, who investigated the connection between cognitive abilities/vulnerabilities and exposure to sexual assault. Negative attributional style (NAS) and anxiety sensitivity (AS) were used as cognitive vulnerabilities and sexual assault as the negative life event. NAS is the individual's inference that current negative events will have negative effects, and that a negative event reflects one's worthlessness, while AS is the extent of fear of the harmful consequences that can result from anxiety and anxiety symptoms [18, 19]. In line with the diathesis–stress model, the authors hypothesized that people who had both high levels of cognitive vulnerability and high levels of negative life experiences would have the highest levels of symptoms. The relation between these cognitive vulnerabilities and negative life events was examined for PTSD symptom clusters [20]. As expected, negative life events significantly predicted changes in avoidance, numbness, and dysphoria symptoms when both NAS and AS were present. The biggest symptom increase was recorded by participants who exhibited high levels of cognitive vulnerability and more traumatic life events. Correspondingly, a low frequency of bad life events and high levels of cognitive insensitivity, however, were linked to reductions in symptoms [20]. Findings from this study support the role of cognitive vulnerabilities as predictors of the development of PTSD symptoms after exposure to traumatic stressors.

2.2.2. BIOPSYCHOSOCIAL MODEL

Formulated by Engel in 1977, the biopsychosocial model emerged from the view of the insufficiency of the biomedical model alone in explaining illnesses [21]. Engel explained a need for a new medical model to extend the biomedicine model to account for all the factors influencing the patient's condition. The biopsychosocial model poses that biological (e.g., genetics, chemical changes, and organ damage), psychological (e.g., stress, mental illness, behaviour, and personality), and social factors (e.g., peers, socioeconomic status, beliefs, and culture) interact with each other in the expression of health and illness. Engel's justification for his criticism was made in several points:

- Biological disturbance alone is insufficient to cause the disease, as disease appearance results from multi-factor interaction.

- Vulnerability is better accounted for by psychological and social factors than by biological changes.

- The effectiveness of biological treatments is influenced by the psychological status of a "placebo effect."

- Health outcomes are affected by the doctor–patient relationship to a great extent.

The biopsychosocial model would consider these factors altogether, not only in terms of the expression of illness but also at the level of the social functioning of the individual. It also considers the cultural perception of illness, circumstances in which the patient does not acknowledge their illness, and other circumstances in which the patient admits their illness, as marked by their entry into the healthcare system [22].

2.2.3. ANIMAL MODELS OF PTSD

In 1993, Yehuda and Antelman proposed a list to systematically evaluate stress models of stress in animals for their relevance to PTSD [23]. To determine how applicable a model is to PTSD, at least five distinct factors might be applied, according to this list:

(1) Even very brief stressors should cause biological or behavioural symptoms of PTSD;

(2) The stressor should be able to produce symptoms in a dose-dependent manner;

(3) Produced biological alterations should persist or become more pronounced over time;

(4) Alterations should have the potential to express biobehavioural changes in both directions;

(5) Interindividual variability in response is present as a function of experience and/or genetics.

A sixth criterion has been proposed by Whitaker *et al*. which is the model's capacity to generate co-morbid states, such as an increase in alcohol consumption, an increase in compulsive drinking, and hyperalgesia [24]. Although these models do not fully replicate the human condition, they simulate the symptoms and neurobiology of PTSD, allowing the evaluation of behavioural changes, neurobiological and epigenetic alterations, and the development of biomarkers and treatment. According to the type of stressor, the models can be categorised as physical, social, or psychological. Table 1, provides a summary for the commonly used animal models in PTSD, which are mostly conducted in rodents.

Table 1. Summary of the commonly used preclinical animal model in PTSD.

Model	Description	Aim
Physical:		
Foot shock stress (FSS)	A metal-rod floor is used to deliver electrical shocks, which are coupled with non-harmful cues (usually auditory) to elicit post-stress fear recall using sound in novel environments, according to the fear conditioning model	Modelling the response to inescapable stress [25]
Single prolonged stress (SPS)	Intends to result in the development of PTSD from a single traumatic experience. Rats are restrained for 2 h, subsequently forced to swim for 20 min, and then 15 min later are subjected to the ether until unconsciousness	Inducing PTSD symptoms by combining multiple, severe, and different stressors [26]
Stress-enhanced fear learning (SEFL)	Exposure to repeated foot shocks in one environment produces conditional freezing (used to assess learned fear) in response to cues associated with foot shock in a second environment	Modelling the lasting effects of traumatic stress [27]
Restraint stress (RS)	Prolonged restraint between 15 min to 2 h on a wooden board or a plastic tube	Modelling an inescapable severe psychological trauma with chronic behavioural and neurochemical alterations [28]
Underwater trauma (UWT)	Being distinct from the forced-swim test, animals are placed in deep water and are forced to swim for 30 s, before being submersed for another 30 s	Modelling an inescapable severe psychological trauma [29]
Social:		
Social defeat (SD)	Subjects are categorised as either susceptible or resilient and are exposed to and suppressed by an aggressor animal for several days. Only susceptible subjects will develop behavioural avoidance	Modelling prolonged and chronic stress as a risk of PTSD [30]
Early life stress (ELS)	From postnatal days 2–14, new-born mice are separated from their mother 1 h daily	Modelling the ability of childhood trauma to influence the development of PTSD [31]
Housing instability (HI)	Animals are frequently paired with different cohorts, after being exposed to their natural predators (e.g., cats)	Modelling the effects of housing instability in PTSD patients [32]
Psychological:		
Predator scent stress (PSS)	Animals are confronted with the scents of their natural predators (cat litter, urine, etc.)	Modelling and simulating traumatising events and trauma-related stimulus response in humans [33, 34]

3. PATHOPHYSIOLOGY

While much about the pathophysiology of PTSD is unknown, research into the pathophysiological aspects of PTSD is in rapid development. Preclinical investigations of animal models of stress and evaluations of biological variables in populations with the condition have all contributed to the identification of biological factors and mechanisms involved in PTSD, which can be described on the levels of brain circuits, neurochemical factors, and HPA axis, as discussed in this section.

3.1. BRAIN CIRCUITS

The core features of PTSD are fear and worry in conjunction with other features and symptoms, including arousal, avoidance, sleep disturbance, and intrusion symptoms (e.g., flashbacks and nightmares) [35]. Neural circuitries and biological processes underlying these features involve brain structures such as (i) the amygdala, anterior cingulate cortex, and the insula in dysfunctional threat detection; (ii) frontoparietal regions (the dorsolateral prefrontal cortex, ventrolateral prefrontal cortex, and medial prefrontal cortex) in emotional regulation; (iii) the medial prefrontal cortex and the hippocampus in contextual processing [35].

A range of structural magnetic resonance imaging (MRI) studies has reported structural abnormalities in the hippocampus and anterior cingulate cortex (ACC) in patients with PTSD [36]. Additionally, functional magnetic resonance imaging (fMRI) studies have reported increased activity of the amygdala, which processes fear and emotion, and decreased prefrontal cortex activity when completing tasks that use either trauma-related or unrelated stimuli (script-driven recollections of trauma-related and unrelated stressful events) [37, 38].

While hippocampal alterations have been observed in patients with PTSD, including lower volumes and lower levels of activation [39], impaired connectivity in the frontoparietal areas, both inside and between executive function networks, has also been observed in patients with PTSD [40]. The aforementioned findings suggest that a disrupted connection within these circuits may reflect a vulnerability factor for PTSD. In contrast, people who were exposed to traumatic events but who do not develop PTSD were reported to exhibit higher prefrontal cortex activity during extinction recall [41, 42], and stronger connections between the ACC and the hippocampus, compared to patients with PTSD [43].

The novel findings by Borgomaneri *et al*. support the idea that the dorsolateral prefrontal cortex (dlPFC) plays a crucial role in the neural network that mediates the reconsolidation of fear memories in humans by showing that non-invasive repetitive transcranial magnetic stimulation (rTMS) of the prefrontal cortex after memory reactivation interferes with the

expression of fear towards a previously conditioned threatening stimulus. These results enhance our understanding of the processes behind fear memory reconsolidation, and also have potential therapeutic applications in treating fear memories [44]. Identifying the brain regions involved in the reconsolidation of emotional memories and their particular interactions within the overall fear-processing network remains a challenge for non-invasive brain stimulation (NIBS) and reconsolidation-based interventions, which are increasingly applied to conditions like PTSD [45].

3.2. NEUROCHEMICAL FACTORS

3.2.1. DYSREGULATION OF THE NORADRENERGIC SYSTEM

Catecholamine noradrenaline is a critical transmitter in the autonomic nervous system, and has been linked with the development of the autonomic symptoms associated with PTSD. Noradrenaline is found in the central nervous system's (CNS) cell nuclei and certain noradrenergic pathways that are implicated in the pathophysiology of the illness. One area with a high concentration of noradrenaline is the locus coeruleus (LC), which is located in the rostral pons and serves as the hub of the neurochemical activity associated with PTSD [46]. Clinical and preclinical evidence suggests that the dysregulation of noradrenergic signalling is involved in the pathophysiology of PTSD. The increased noradrenergic tone in PTSD arises from increased central and peripheral sympathetic activity leading to increased resting heart rates and systolic blood pressure [47]. In addition, noradrenaline levels are higher in the urine of individuals with PTSD than in healthy individuals, but recent studies have failed to establish such findings in the cerebrospinal fluid (CSF) [48, 49].

Many of the symptoms of PTSD emerge from an increased CNS noradrenergic tone [49, 50]. Increased noradrenaline activity has been linked with dysfunction of the medial prefrontal cortex (through impairing prefrontal signalling via α1- and β-AR in the prelimbic (PL) and infralimbic (IL) subdivisions of the medial prefrontal cortex) and disturbed fear extinction, which may underlie the increases in behavioural measures of anxiety and PTSD symptom severity [51, 52]. Excessive noradrenaline release was found to be increased in the hyperactive amygdala and LC, resulting in intrusion symptoms and autonomic hyperactivity [53].

Altered noradrenergic function is also associated with night-time and sleep symptoms in PTSD. Increased sympathetic activity during sleep, e.g., an increased heart rate, is found in individuals with PTSD [54]. Additionally, during rapid eye movement (REM) sleep, people with high levels of PTSD-like symptoms showed an increase in the ratio of low-frequency to high-frequency heart rate variability, which is associated with an elevated sympathetic tone [55].

3.2.2. DYSREGULATION OF SEROTONIN SIGNALLING

Serotonin (5-HT) is a monoamine neurotransmitter with multiple biological functions related to mood, cognition, memory, and behavioural regulation [56]. The 5-HT signalling in the amygdala has been linked to fear regulation and threat responsiveness. Several 5-HT receptors, including 5-HT1A, 5-HT1B, 5-HT2A, and 5-HT2C have been linked to PTSD and anxiety [57, 58].

According to reports from pharmacological studies, blocking the serotonin 5-HT2C receptor in rodents increases locomotion and reduces anxiety [59, 60], and in addition, the 5- HT1A receptor agonist induces anxiogenic responses to the elevated plus maze (EPM) test in mice [61]. A recent study found that both 5-HT1A and 5-HT2A in the hippocampus mediate anxiety-like behaviour in a mouse model of PTSD via the ERK pathway [62]. Clinical evidence showed higher 5-HT1A binding potential in people with PTSD, particularly in those with comorbid MDD [63].

3.2.3. DOPAMINE

Another prevalent neurotransmitter in the brain is dopamine, primarily synthesised in midbrain areas [64]. Dopamine is a neurotransmitter that is involved in the regulation of motor activity, limbic functions, attention, and cognition, particularly executive function and reward processing [65, 66]. It makes a significant contribution to the anticipatory processes required for planning voluntary action after intention as well as behavioural adaptability [67]. A range of studies have investigated links between the dopaminergic system and PTSD. For example, several studies have attempted to link PTSD with genetic variants in certain dopamine receptor genes (e.g., DRD2) [68-70]. Other studies have focused on dopamine-beta-hydroxylase (DBH), which catalyses the conversion of dopamine to noradrenaline, and have reported that high-plasma DBH levels may be linked to the development of intrusion symptoms [71, 72], yet other studies have focussed on the dopamine transporter SLC6A3 (solute carrier family 6, member 3), a member of the sodium- and chloride-dependent neurotransmitter transporter family which mediates the transport of dopamine from the synaptic cleft. The 9R allele of the SLC6A3 locus has been identified as a risk allele for PTSD [73]. Additionally, the epigenetic state of the promoter region of SLC6A3 has been identified as a potential risk factor for/indicator of PTSD [74].

3.2.4. GAMMA-AMINOBUTYRIC ACID (GABA)

The inhibitory neurotransmitter GABA is widely distributed throughout the entire brain. A complex pattern of results has been found in studies comparing GABA levels in numerous brain areas between those with and without PTSD. A proton magnetic resonance spectroscopy study reported lower GABA-levels in the temporal cortex, parieto-occipital cortex, and insula and higher GABA levels in the dorsolateral prefrontal cortex in people with PTSD compared to trauma-exposed healthy controls [75]. In times of extreme

stress, low plasma levels of GABA are related to PTSD and may lead to the overload of hyperadrenergic response regulation [76].

There are three primary classes of GABA receptors: GABA-A, GABA-B, and GABA-C. Human research has revealed that Vietnam War combat veterans with PTSD had lower GABA-A benzodiazepine binding ability. According to these findings, changes in the GABA receptor's expression or binding ability may have an impact on mental diseases linked to stress, including PTSD [77, 78].

3.2.5. NEUROPEPTIDE Y (NPY)

NPY is a neuropeptide that is expressed throughout the brain, including the forebrain, limbic system, and brainstem. It is involved in several physiological processes including the regulation of emotional and stress-related behaviours [79].

Early research developed a concept that NPY counteracts the actions of the corticotropin-releasing factor (CRF), terminating the stress response and countering the HPA axis [79-81]. Studies have demonstrated that plasma NPY levels rise in response to stress, and that higher NPY levels are associated with better behavioural performance under stress [82, 83].

Plasma NPY levels were assessed in soldiers who took part in a survival course meant to mimic the conditions that prisoners of war would encounter. Within a few hours of being exposed to military interrogations during the survival course, their serum NPY levels increased. Furthermore, compared to the non-Special Forces or regular soldiers, the majority of Special Forces members who had received resilience training had much higher NPY levels [84].

Several preclinical and clinical research reports point to an association between PTSD and decreased NPY in the CNS [79, 85]. Moreover, NPY levels appear to increase after PTSD remission, suggesting that NPY may act as a biomarker of PTSD or at the very least as a resilience element [86].

Together, these investigations show that NPY levels in PTSD patients closely mirror the disease course and that NPY can operate as a stress buffer in response to stressful experiences by lowering noradrenergic hyperactivity [87]. The most prevalent SNP for NPY investigated is the rs16147 (399T > C) polymorphism, which is linked to low levels of NPY and has been linked to hyperarousal, changes in the HPA axis response to stress, and the activation of the hippocampus and amygdala [79]. Another NPY SNP is 1002T > G, associated with low NPY content in the CSF and amygdala, which is linked to higher levels of anxiety, arousal, addictive behaviours, and decreased stress resilience [79].

3.2.6. BRAIN-DERIVED NEUROTROPIC FACTOR (BDNF)

BDNF, the richest neurotrophin in the brain, was first characterised for its involvement in the formation of the CNS. It has the ability to participate in neural activities such as survival, differentiation, development, and neuronal plasticity. It preserves synaptic plasticity, which is necessary for extinction learning and fear memory storage [88]. Variations in BDNF expression or in genetic background have been linked with a risk of various psychiatric disorders such as anxiety, depression, and PTSD [89].

In US military personnel deployed throughout the conflicts in both Iraq and Afghanistan, PTSD patients had higher BDNF protein levels in their peripheral blood plasma than non-PTSD controls. In the inescapable tail shock rat model of PTSD, increased BDNF levels were found in both blood plasma and hippocampus tissue. Furthermore, the polymorphism Val66Met in BDNF has been linked with an increased risk of PTSD, exaggerated startle response, and alterations in fear extinction [90, 91]. The same polymorphism was also found to affect hippocampal volume and memory [92]. Together, these findings emphasise that BDNF and related molecules may be interesting candidates for biomarker studies and for more fundamental studies aiming to identify actionable biological targets related to the onset or course of PTSD [89, 93].

3.2.7. CANNABINOID AND OPIOID RECEPTORS

Endogenous cannabinoids, including anandamide (AEA) and 2-arachidonolyflycerol (2-AG), work via cannabinoid (CB) receptors (CB1R, CB2R), which are implicated in the pathogenesis of PTSD [94]. Preclinical data showed that AEA levels are decreased in the brain in chronic stress models [95]. This is in agreement with the human data showing that endocannabinoid plasma levels are reduced in PTSD patients [57, 96]. In addition, defective endocannabinoid signalling is correlated with glucocorticoid dysregulation associated with PTSD [96].The CB1 receptors are the most abundant G-protein-coupled receptors in the CNS and are highly expressed in a fear circuit of the cortical and subcortical brain regions associated with PTSD [97]. Interestingly, the disruption of the CB1 receptor gene (knockout models) was found to increase anxiety, whereas a pharmacological blockade of the receptor had anxiolytic effects [98, 99]. Animal stress studies also showed that CB1 receptor expression was increased in female but not male animals [100, 101].

3.2.8. OXYTOCIN

Oxytocin is a neuropeptide produced in hypothalamic periventricular and supraoptic nuclei. It emerges from the posterior pituitary and enters the bloodstream. Oxytocin is transported to different parts of the brain through neuronal projections from the hypothalamus. The amygdala, brainstem, olfactory nucleus, and anterior cingulate cortex are among the human brain areas that express the oxytocin receptor and are therefore likely to be impacted by oxytocin [102]. As it acts on brain areas involved in PTSD, and

as oxytocin appears to have anti-stress and anxiolytic effects, oxytocin is thought to be involved in the constellation of dysregulations found in PTSD [103, 104].

3.3. DYSFUNCTIONAL HPA AXIS

The hypothalamus, pituitary, and adrenal glands make up the HPA axis, a hierarchical system that controls how the body responds to stress from the environment while maintaining homeostasis [105, 106]. As the axis is one of the main stress response systems that controls the release of cortisol and stress hormones, it has received a lot of attention. Exposure to stress causes increased corticotropin-releasing hormone (CRH) production from the hypothalamus [107, 108]. The HPA axis is first activated by the production of CRH, which travels through the infundibular stalk's hypophyseal portal arteries to the anterior pituitary, where it binds to CRH type 1 receptors (CRF1) to trigger the release of adrenocorticotropin (ACTH) into the bloodstream. Cortisol, the main HPA axis effector chemical, is released when ACTH binds to melanocortin 2 receptors in the zona fasciculata of the adrenal cortex. In order to promote the stress response, cortisol has a number of physiological impacts throughout the body, including blocking insulin signalling and increasing glucose availability, controlling immune system operations, and altering electrolyte balance [109-111].

Upon CRH administration, rodents exhibit PTSD-associated behaviours [112]. In addition, CRF-1 knockout mice showed impaired responses to stress and reduced anxiety [113, 114]. At the same time, CRF-2 knockout mice showed hypersensitivity to stress and augmented anxiety [113, 115]. PTSD patients have high CSF levels of CRH and a dysfunctional HPA axis [107, 116, 117]. Studies indicate CRH hyperactivity with subsequent glucocorticoid receptor (GR) hypersensitivity, resulting in higher negative feedback inhibition of cortisol and CRH release [118, 119]. In a meta-analysis, Morris *et al*. reported significantly lower basal cortisol levels in PTSD and trauma-exposed controls without PTSD compared to non-traumatised individuals. Additionally, individuals who had experienced childhood trauma had significantly lower morning cortisol levels compared to those exposed to adulthood trauma [120]. Figure 2 provides a general overview of the information about the HPA axis discussed in this section.

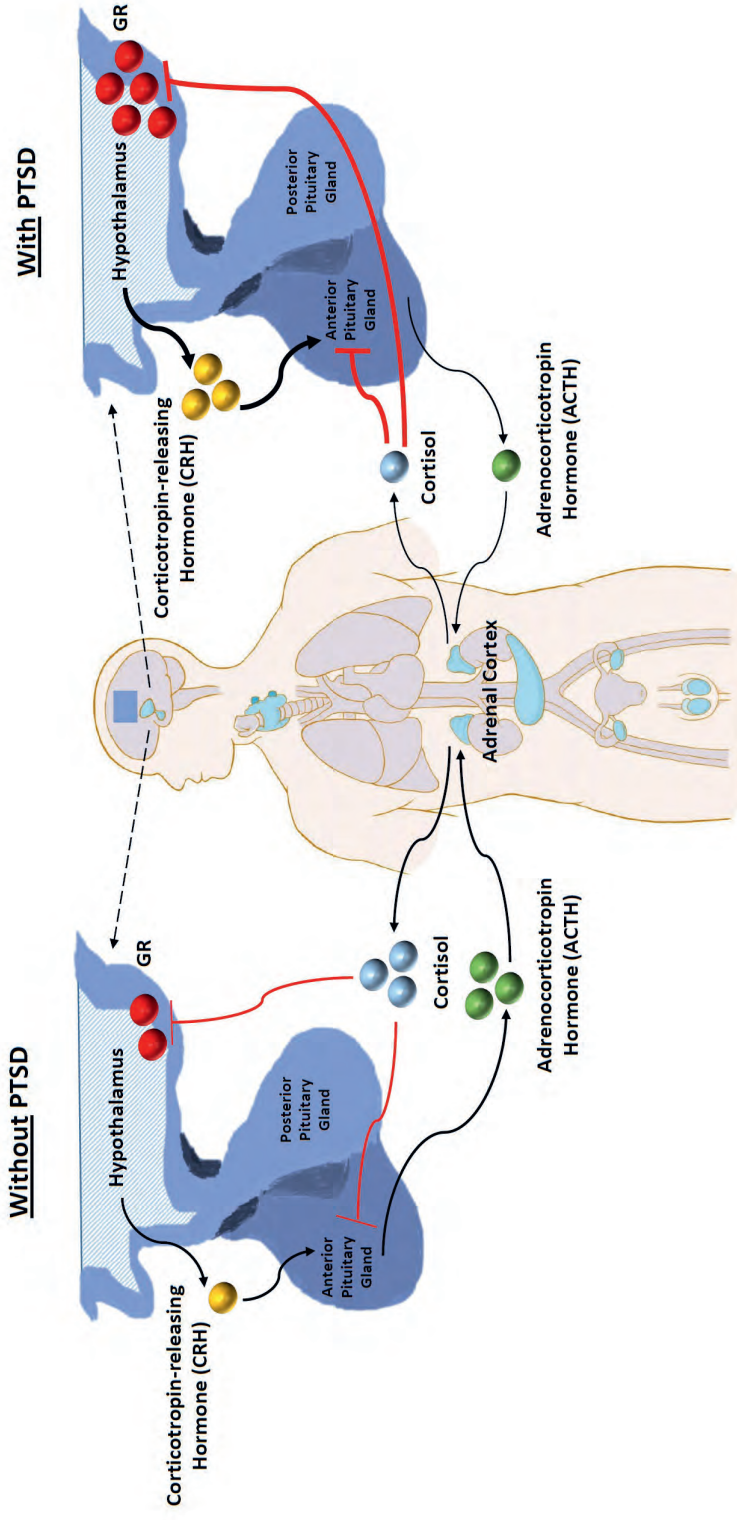

Figure 2. Basal activity of the HPA axis with or without PTSD. CRH secretion from the hypothalamus increases in PTSD (represented by a thicker black line). The release of ACTH from the anterior pituitary, and hence cortisol from the adrenal cortex, is decreased in PTSD (represented by a thinner black line). Cortisol's negative feedback inhibition of the HPA axis is increased in PTSD (represented by thicker red lines).

3.4. CONCLUSIVE REMARKS

Despite the extensive discoveries, the current understanding of the neurochemical factors in PTSD is still limited and requires more research. There is a number of understudied yet significant subjects in the discipline, such as variables that affect susceptibility and resiliency. For instance, one such subject is whether or not the exogenous administration of oxytocin and NPY, two neurobiological components that protect against stress, can foster resilience. Additionally, determining relationships between heritable variables (genetics and epigenetics) and trauma exposure is crucial to understanding PTSD risk, and predicting treatment response. It is important to thoroughly evaluate how trauma affects gene expression, neural plasticity (across the CNS), circuit remodelling, and neurotrophic factors. Future studies should focus on the characterisation of proteomic and transcriptomic abnormalities in PTSD, with the integration of GWAS and EWAS studies, in order to map out novel networks, and allow the development of reliable biomarkers.

Likewise, current controversies and conflictions in the studies assessing HPA axis function and diurnal cortisol levels can be a result of the methodological heterogeneity and limitations of the studies (e.g., different methods of cortisol measurement, different timings of cortisol measurement, and different methods in establishing PTSD in addition to other statistical limitations). Further studies with greater homogeneity are required to draw definite conclusions.

4. PREVENTION MODEL OF PTSD

PTSD has a predictable development pattern and follows a specific triggering event, unlike other mental diseases. Early PTSD symptoms appear days after exposure to stress. Emergency care providers and helpers are made aware of a lot of traumatised people. These circumstances present exceptional chances for identifying those who are in danger and offering preventive measures. Despite these benefits, the effective prevention of PTSD remains challenging, and the disorder's incidence in both military members and civilians over the past decades has been relatively steady [121]. With enough understanding of the condition, preventive and interventional measures can be used to enhance quality of life and reduce the disease's financial and medical burdens. This is supported by the development of prevention models, but also by the modern digital support of their implementation by e-health approaches (the latter will be explained in the treatment section).

THE SOCIAL-ECOLOGICAL MODEL FOR PTSD PREVENTION

A social-ecological model as a framework for prevention is proposed by the US Centers for Disease Control and Prevention, which emphasises risk factors at multiple levels,

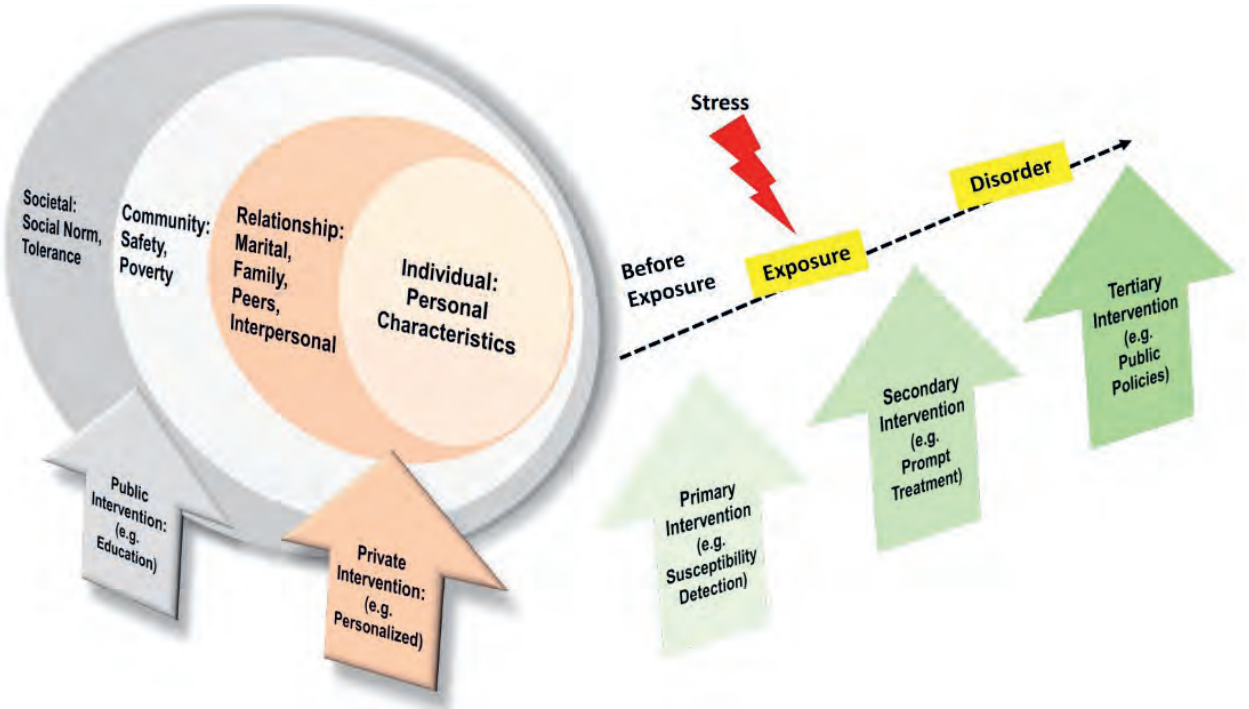

Figure 3. Social-ecological model for traumatic stress and related preventive interventions.

including the individual level, relationship level, community level, and societal level [122] (Figure 3). Risk factors on the individual level are about personal characteristics, which are the model's core. Furthermore, the risk factors on the relationship level are about the quality of relationships for the individual in the family, friends, or other interpersonal interactions. In addition, the risk factors on the community level could be the feeling of safety and economical status. Lastly, the risk factors on the societal level could be social norms, cultural background, and tolerance [123].

Therefore, preventive measures on the individual and relationship level should be individualised and personal. For instance, emergency hotlines and psychological counselling services should be available in the way that is easiest to reach. Accordingly, preventive measures on the community and societal level are public, legitimacy-related, and educational. For instance, the community or public health sector should increase the awareness of the impact of traumatic stress, and it could organise lectures, campaign movements, or give out brochures about traumatic stress, first-aid help information, and preventive measures (Figure 3). Community-based interventions to improve mental health for people in low- and middle-income countries usually use lay community members as intervention deliverers, and apply transdiagnostic approaches and customized outcome assessment tools [124]. Furthermore, the public sector should formulate laws, legislation, and policies to prevent discrimination and racism to protect specific populations [125].

Additionally, preventive measures should also be taken during different stages of traumatic exposure [123]. Before exposure, the primary prevention is of the actual occurrence of disease or illness. After stress exposure, the secondary form of prevention is to intervene early in the disease process for a cure, and for the reversal of illness or for optimal outcomes. When a disorder occurs, tertiary prevention steps are taken to prevent the disability that often accompanies an illness or disease [123] (Figure 3). For optimal outcomes, primary, secondary, and tertiary prevention measures should be evidence-based as they are part of disease management [126].

5. Treatment Modalities for PTSD and E-Mental Health

PTSD is often a chronic and disabling disorder. Many patients fail to seek medical care, and others have symptoms resistant to treatment. Early treatment as soon as the diagnosis is made is recommended to prevent chronicity and disability [127]. The main goal of treatment is to improve quality of life, maintain patient and others' safety, reduce distressing symptoms, and reduce hyperarousal and avoidant behaviours. The first-line intervention for PTSD patients is psychotherapy, either trauma-based psychotherapy (CBT, exposure therapy (ET), or EMDR) or non-trauma-focused psychotherapy (present-centred

therapy, interpersonal therapy, or mindfulness therapy). In the case of psychotherapy failure, pharmacotherapeutic treatment options are the next choice. Additionally, if the patient has a disability that impairs the success of trauma-focused psychotherapy, pharmacotherapy is considered the appropriate choice until psychotherapy can be initiated [5, 6]. In addition to current therapies, e-health is gaining attention as it has interesting potential for providing training, assessment, prevention, and the treatment of negative effects after trauma exposure on a global scale [128].

5.1. TRAUMA-BASED PSYCHOTHERAPY

As mentioned earlier, trauma-based psychotherapy includes CBT, ET, and EMDR. CBT has a cognitive and behavioural component. The cognitive component is mainly focused on the cognitive reconstruction of the effects of a traumatic event on one's life, by addressing all maladaptive beliefs and thoughts about safety, power, trust, and control, while the behavioural component is about learning how to deal with and challenge these thoughts through thinking or real experience, in order to achieve symptom reduction [129].

ET can be imaginal exposure, in vivo exposure, or virtual reality exposure. All of these focus on putting the patient in confrontation with their traumatic event and memory for these to become less distressing. In PET, multiple sessions of education on reactions to trauma, processing traumatic material, and breathing training are undertaken. It was shown to be effective in patients with comorbid conditions such as psychosis, personality disorders, and substance use [130, 131]. In written ET, the patient writes about their traumatic events in response to certain stimulations and discusses them with the therapist to pay attention to the thoughts and events that evoke the patient's symptoms, with exposure being imaginal [132].

EMDR is a combination between cognitive behavioural therapy and ET in addition to saccadic eye movements during the therapy. The patient remembers the traumatic event, and while focusing on the cognition aspects simultaneously, the therapist moves their fingers in front of the patient and asks the patient to follow them repeatedly until the anxiety subsides [133].

5.2. NON-TRAUMA FOCUSED PSYCHOTHERAPY

This approach includes present-centred therapy, interpersonal therapy, and mindfulness-based stress reduction. Present-centred therapy focuses on the current life stressors and how to cope with them [134]. Interpersonal therapy focuses on a specific symptom and impairment in the context of interpersonal relationships [135]. Mindfulness-based stress reduction mainly teaches the patient how to be fully focused on the current moment, not think about the traumatic event, and attend to the present in a non-judgmental manner [136].

5.3. PHARMACOLOGICAL THERAPY

The pharmacotherapy treatment is preferred in the case of psychotherapy treatment resistance, different patient preferences, or a patient's inability to participate in the former, and mainly comprises selective serotonin reuptake inhibitors (SSRI) and serotonin-noradrenaline reuptake inhibitors (SNRI). Occasionally, second-generation antipsychotics (SGAs) such as risperidone or olanzapine can be used. If effective, pharmacotherapy should be continued for at least six to twelve months to prevent relapse [137].

Due to their efficacy at reducing symptoms of PTSD, SSRIs and SNRIs are the first-line agents in the pharmacotherapy of PTSD. Treatment with SSRIs resulted in a higher reduction in the Clinician-Administered PTSD Scale (CAPS) score than treatment with a placebo in a meta-analysis of 12 studies including 1909 PTSD patients, with paroxetine and sertraline being most effective among SSRIs [138]. The approach to the usage of SSRIs is to «start low and go slow» until the response is achieved, in order to avoid unwanted side effects. However, failure cannot be determined until the maximum therapeutic dose is given and a period of 6–8 weeks is completed, with at least two different agents being used before documenting failure [139-141]. Although few studies have compared SSRIs to SNRIs, randomised trials have indicated that venlafaxine was superior to a placebo in decreasing PTSD symptoms [142]. Second-generation antipsychotics can be used as a monotherapy or as augmentation therapy in the case of concomitant psychosis or in the case of failed SSRI/SNRI [127-144]. In a trial involving 247 United States military veterans from the US who did not respond to two or more trials of SSRI and SNRI, participants received either 4 mg of risperidone or a placebo. However, no significant difference was observed between the two groups between the CAPS scores [145]. In another study, eighty United States military veterans with persistent PTSD were given quetiapine monotherapy as opposed to a placebo in a randomised clinical study. After 12 weeks, the individuals who received quetiapine had higher mean reductions in their CAPS total score than those who received a placebo [146]. RCTs and systematic reviews conducted on other SGAs (i.e., olanzapine, aripiprazole) show that these agents are reasonable either as monotherapies or augmentation therapies [147, 148].

The alpha-1 blocker prazosin is mainly used for symptom relief, especially during sleeping. It is the preferred agent in patients experiencing nightmares or sleeping disorders. It can be used as an adjunct to SSRI/SSNRI [149]. There are some preliminary clinical studies on riluzole (a glutamatergic modulator), 3,4-methylenedioxymethamphetamine (MDMA), and ketamine. The results of these studies are promising, but these agents are not yet approved [150-152]. As there is no clear benefit, benzodiazepines are not recommended, as they have been shown to decrease the effectiveness of psychotherapy by diminishing the extinction effect, and they should be avoided, especially in patients with substance use disorders [153, 154]. The effectiveness of anticonvulsant drugs with mood-stabilising

effects to lessen PTSD symptoms has not been adequately explored in clinical studies. Few sufficiently powered, randomised trials have been released, and the results have largely been unfavourable [155-158]. On the other hand, intranasal oxytocin administration has been found to be a promising approach to preventing and reducing PTSD symptoms. Frijiling *et al*. found that repeated intranasal oxytocin administration in the early post-trauma period reduced the development of PTSD symptoms [159]. Additionally, intranasal oxytocin was found to reduce symptom severity in females with PTSD upon a trauma-script challenge [160]. Oxytocin has also been suggested to enhance the outcomes of psychotherapy, although adequately powered RCTs are still needed to assess this use of oxytocin [161].

A novel method of treating PTSD involves the disruption of memory reconsolidation [162]. Pharmacologically, this could be done through trauma memory reactivation with the administration of an amnesic drug, resulting in the disruption of memory consolidation. One drug showing promise in such an approach is propranolol, as it has been shown to disrupt fear memory reconsolidation in the amygdala in rodents [163]. Despite the limitations, such as short-term follow-up and the conflicting results of the recent studies, propranolol shows promise as an early preventative measure for PTSD.

5.4. E-MENTAL HEALTH AND VIRTUAL REALITY (VR)

To integrate our understanding of traumatic stress as a public health problem, interdisciplinary and modern approaches can facilitate the mission, including health service research [164], internet-based digital approaches like e-health [165], and artificial intelligence applications like virtual reality (VR) [166].

In the area of traumatic stress, e-mental health, defined by Riper *et al*. as «the use of information and communication technology to support and improve mental health conditions and mental health care», has enormous potential to provide instruction, assessment, prevention, and treatment for negative effects following trauma exposure globally [167]. It is possible and effective to give intensive, trauma-focused treatment for severe or complicated PTSD via home-based telehealth. This can be a substitute for trauma-focused treatment that is delivered in person [168]. E-health being offered in times of pandemics (such as with COVID-19) has shown to be an efficient way to support prevention and possibly intervention at times of shortage [169]. This suggests that also in global PTSD care, this could be effective. However, several challenges arise in convincing patients to undergo such a new method of care. Bakker *et al*. propose three ways in which the application of e-health can be accelerated. First, optimising adherence and the engagement of users (including patients, clinicians, and relatives) can be achieved by designing approaches that meet the requirements of the users and implementing a holistic approach instead of focusing on a single disorder, in addition to featuring designs

that engage the patients, such as real-time engagement, rewarding systems, and the involvement of VR and augmented reality (AR) programs. Second, increasing the field's research with clinical and high-quality studies to help test evidence-based medicine for effective interventions. Lastly, the wide implementation of such interventions could be of great benefit, especially when local expert clinicians and clinics are unavailable. However, such a wide implementation must overcome several dilemmas, including physician and patient avoidance of internet interventions and patients' preference of therapist-guided interventions [165].

VR for ET or psychophysiological assessment and resilience training could reduce negative impacts or enhance well-being in response to traumatic stress in public health [166]. Studies show the promising and wide usage of VR in trauma management from combat scenarios to the COVID-19 pandemic [166].

5.5. CONCLUSIVE REMARKS

Although recent developments utilising methodologically sound designs have increased confidence in the effectiveness of PTSD therapy, a sizable proportion of patients fail to respond to treatment, stop receiving it, or never receive it. Research on agents that can target disrupted circuits in PTSD can improve both prevention and treatment. Therefore, there is definitely room for more research on PTSD therapies and delivery methods. With the wide range of available modalities of psychotherapy and pharmacotherapy, the scheme of individualising treatment, according to the severity and personal symptom profiles of PTSD, comorbid conditions, and the use of predictive therapeutic biomarkers, can greatly enhance the efficacy of treatment. With the rapid development of new technology, research in the field of e-mental health is advancing. In light of these quick advancements, future research should concentrate on preserving a high standard of assessment of the effectiveness and acceptability of new technologies, while the evaluation of side effects and hazards should not be disregarded.

6. PTSD BIOMARKERS

A biomarker is a measurable characteristic, which can be a substance (molecular or histological), response (physiological), or structure (radiographic); it is an indicator of biological or pathological processes, or responses to exposures or interventions [170]. Recently, the identification of biomarkers for PTSD has received increasing focus [171]. PTSD biomarkers are currently used for research purposes, but they might soon assist in screening and supporting the early detection of the disorder, resulting in timely intervention and better outcomes [172]. These biomarkers could be structural changes,

substances, and responses which can help assess the disease risk, diagnosis, prognosis, and response to treatment. Here, we explore the different biomarkers of PTSD.

6.1. SUSCEPTIBILITY BIOMARKERS

Susceptibility markers comprise those that assess the risk of developing the disorder, and are assessed before and after trauma exposure in individuals at risk [173]. Perhaps a person's vulnerability to developing PTSD is hard to measure, and many models have been developed to explain the complexity of its evolution [174]. As discussed earlier, both pre-traumatic, peri-traumatic, and post-traumatic factors can affect disease development and progression. In terms of these, researchers have investigated several susceptibility biomarkers in the pre-traumatic and post-traumatic periods as predictors of disease. Table 2 provides a summary of the susceptibility biomarkers implicated in PTSD.

Table 2. Summary of the susceptibility biomarkers implicated in PTSD.

Susceptibility Biomarker	Findings
Number of GR in lymphocytes and monocytes	A higher number pre-trauma of GR is associated with a high PTSD symptoms in soldiers after deployment [175].
Sensitivity of T cells to dexamethasone before deployment	High sensitivity pre-trauma is associated with a high amount of PTSD symptoms without comorbid depressive symptoms. Different sensitivity patterns are associated with different symptomatology [176].
mRNA levels of *FKBP5*	Low levels after deployment are associated with a high amount of PTSD symptoms [176].
Glucocorticoid-induced leucine zipper mRNA	High levels pre-trauma are associated with a high amount of PTSD symptoms post-deployment [177].
Corticotropin-releasing hormone type 1 receptor gene	Polymorphisms were associated with PTSD development [178].
Heart rate	Increased heart rates in the post-traumatic period were associated with PTSD development [179].
Occurrence of Nightmares	Higher occurrence of nightmares pre-trauma was associated with disease susceptibility in Dutch combat soldiers [180].
Increased skin conductance	Skin conductance response (SCR) within hours of trauma exposure was a predictor of chronic PTSD development [181].

A study, the Prospective Research in Stress-Related Military Operations (PRISMO) study, investigated vulnerability markers in Dutch Armed Forces soldiers. The study included a cohort of Dutch military members deployed to Afghanistan for 4 months who experienced trauma and developed PTSD, those who did not develop PTSD, and healthy (unexposed) individuals. It was found that the number of GR in lymphocytes and monocytes before military deployment was significantly higher in soldiers who developed high amounts of PTSD symptoms after deployment which remained high for several months after

deployment [175]. Moreover, T-cells' high glucocorticoid sensitivity (GCs) (dexamethasone) before deployment was associated with high amounts of PTSD symptoms without comorbid depressive symptoms. Additionally, different patterns of GR sensitivity were associated with different presentations (e.g., severe fatigue and depressive symptoms) [176]. Furthermore, the study explored the roles of GR pathway components in disease prediction. It was found that low mRNA levels of FKBP5 (a cochaperone modulator of receptor sensitivity to cortisol) before deployment were associated with high amounts of PTSD symptoms after deployment [177]. High glucocorticoid-induced leucine zipper mRNA levels before deployment were also associated with high amounts of PTSD symptoms post-deployment [177].

Other studies report the findings of HPA axis involvement, including one on 103 children in the USA, which concluded that polymorphisms in the CRH type 1 receptor gene were associated with PTSD development in these subjects [178]. These findings elaborate on the importance of the HPA axis, and dysregulation, and how biomarkers related to the axis can be used as disease predictors. As mentioned before, the BDNF polymorphism Val66Met also appears to increase the risk of PTSD, as it results in a decrease in BDNF expression, which obtunds conditioned fear extinction [91, 182]. Studies have shown an increased frequency of the Met allele in those who develop PTSD compared to controls [93, 183]. However, recent meta-analyses have found that there is no significant correlation between the Met allele and PTSD symptomatology [90, 184, 185].

The markers mentioned above comprise molecular ones, but other non-molecular susceptibility markers have also been investigated, especially in the post-traumatic period. Heart rate has been considered a secondary risk marker of the disease, as increased heart rates in the post-traumatic period have been associated with PTSD development [179]. Additionally, nightmares in the pre-traumatic period were associated with disease susceptibility in Dutch combat soldiers [180]. Additionally, increased skin conductance, a psychophysical marker of hyperarousal, in the immediate aftermath of trauma, was also associated with the subsequent development of PTSD [181]. As patients with PTSD tend to have higher rates of hypertension, as well as higher resting systolic and diastolic blood pressure, blood pressure is considered a candidate risk marker of PTSD [186, 187]. However, a recent meta-analysis showed no association between elevated blood pressure and the subsequent development of PTSD symptoms [188].

6.2. DIAGNOSTIC BIOMARKERS

Diagnostic biomarkers are those used to assess and classify people that are already exposed to traumatic experiences, and are identified in PTSD patients in comparison to those exposed to trauma but who are disorder-free [189]. Currently, the diagnosis of PTSD is clinical, and it does not depend on its pathophysiology and underlying biological

changes. Rather, it depends on the disease's manifestation and the fulfilment of certain criteria. This is because PTSD, like all mental disorders, is complex, and phenotype manifestation differs greatly between individuals with the same level of traumatic experience [190] and even between those with similar biological and neurological activity changes. Consequently, this discrepancy might contribute to the limited applicability of such biomarkers. Therefore, biomedical biomarkers are not yet clinically used to diagnose the disorder [172], but based on scientific advances they may provide diagnostic evidence soon. In the literature, these biomarkers are classified as structural (e.g., neuroanatomical changes), peptides (e.g., monoamines and cortisol), neuroendocrine (e.g., HPA axis activity), responses (e.g., arousal, startle response), genetic and epigenetic biomarkers, and other classifiers [173, 191, 192]. Table 3 provides a summary of the diagnostic biomarkers implicated in PTSD.

Table 3. Summary of the diagnostic biomarkers implicated in PTSD.

Diagnostic Biomarker	Findings
Noradrenaline levels	Increased urinary noradrenaline levels were associated with PTSD development in men [193].
FKBP5 levels	Reduced FKBP5 expression in blood was found in PTSD patients [194].
Amygdala activity	Amygdala over-activation is found in PTSD patients [42, 195].
Hippocampus volume	Hippocampal loss is a common anatomical change in patients with PTSD [196].
miR-138-5p overexpression miR-1246 downregulation	Plasma isolated miR-138-5p was significantly overexpressed in subjects with PTSD compared to controls, and miR-1246 was significantly downregulated in subjects with PTSD compared to resilient subjects [197].
Plasma levels of NPY	Plasma baseline levels are lower in individuals with traumatic stress exposure and PTSD [198, 199].
CSF levels of NPY	Levels of NPY were lower in combat veterans with PTSD compared to veterans without PTSD and healthy controls [200, 201].
Plasma BDNF level	Patients with PTSD have higher plasma levels of BDNF [202].
Oxytocin receptor mRNA levels	Blood mRNA levels of OXTR were lower in patients with hyporeactive HPA axis subtype at baseline, which increased during stress testing [203].
Others	A rise in inflammatory markers, increased startle response, symptoms of hyperarousal, and impaired cognitive function were found in PTSD patients [173, 191].

Several studies have been conducted on the levels of monoamines in individuals with PTSD, which have indicated a rise in their levels, specifically in the case of noradrenaline, both peripherally and centrally. Hawk *et al*. examined the rise in catecholamines and cortisol in the urine of 55 participants who experienced serious motor vehicle accidents. They found that increased NE levels were associated with PTSD development. However, these findings were in men only, which might indicate gender differences for this biomarker [193]. Studies point to increased monoamine levels also being found in other anxiety disorders and that they are not specific to PTSD [204].

The HPA axis is the main neuroendocrine regulator in the body and is dysregulated in PTSD [205]. Several studies have concentrated on the difference in cortisol levels in individuals with PTSD, but the results were considerably variable. Meewisse *et al*. conducted a meta-analysis and indicated that cortisol levels are inconsistent and insignificant in patients with PTSD [206]. Interest has increased in FKBP5 and cortisol levels in response to stress. Yehuda *et al*. explored gene expression alterations in 35 participants who experienced the 9/11 attack on New York City. It was found that patients with PTSD had reduced levels of FKBP5 compared to controls [194]. As BDNF regulates synaptic plasticity, which is essential for fear learning and extinction, studies have suggested its role as a potential biomarker in PTSD. A systematic review and meta-analysis compared the peripheral blood levels of BDNF in patients with PTSD compared to controls without PTSD. Plasma BDNF levels were significantly higher in PTSD groups when compared to controls [202]. However, this increase in BDNF levels appears to follow a descending pattern, as BDNF levels tend to fall again in the long-term [207].

In a pilot study by Snijders *et al*. participants from the PRISMO study were divided into PTSD subjects, resilient subjects (trauma-exposed with no PTSD diagnosis), and non-exposed healthy controls. In this work, several miRNAs were identified as candidate diagnostic biomarkers. Five miRNAs (miR-221-3p, miR-335-5p, miR-138-5p, miR-222-3p, and miR-146-5p) were able to perfectly separate PTSD subjects from controls after adjusting for confounders [197]. Furthermore, the downregulation of miR-1246 was shown to be significant in PTSD patients compared to resilient subjects, suggesting its potential as a diagnostic biomarker [197]. Another study also suggested miRNAs as potential biomarkers, identifying a panel of nine stress-responsive miRNAs (miR-142-5p, miR-19b, miR-1928, miR-223-3p, miR-322*, miR-324, miR-421-3p, miR-463*, and miR-674*) [208].

As mentioned earlier, circuits involving the amygdala are dysregulated in PTSD. One important neuroanatomical finding in PTSD patients is amygdala over-activation [42, 195]. Several studies reported increased amygdala activity in PTSD patients responding to fearful and traumatic stimuli during functional neuroimaging [209]. This hyperactivity might be due to decreased control and inhibitory signals from regulatory structures,

such as the medial prefrontal cortex and the hippocampus [210]. In mentioning the hippocampus, hippocampal volume loss is a common anatomical change in patients with PTSD [196]. However, using hippocampal volume loss as a biomarker is unreliable, as it might be a direct consequence of exposure to trauma itself [211]. NPY, which is implicated in the pathophysiology of PTSD, could also serve as a diagnostic marker. Studies have shown lower NPY levels in the CSF of combat-exposed subjects with PTSD when compared to combat exposed subjects that did not develop PTSD [200, 201]. Additionally, trauma exposure and PTSD are associated with diminished baseline plasma levels of NPY [198, 199].

Of new interest is the role of oxytocin and its receptor expression patterns in patients with PTSD. Hofmann *et al*. compared serum oxytocin and oxytocin receptor mRNA (OXTR mRNA) levels at the baseline and during a Trier Social Stress Test (TSST). Serum oxytocin was found to be higher, while OXTR mRNA levels were found to be lower in the PTSD patients at the baseline compared to the healthy controls. During TSST, an increase in OXTR mRNA was markedly correlated with PTSD symptoms. It should be noted, however, that these findings apply only to the HPA axis hyporesponsive subtype of PTSD in the study [203]. However, due to the small sample size and opposing findings in other studies [212], it is still early to consider oxytocin a reliable biomarker for PTSD. Future studies should clarify its role in PTSD pathophysiology and its reliability in aiding the diagnosis.

Some psychophysical markers in patients with PTSD were also investigated. One promising candidate is skin conductance (SC), which was found to be increased in patients with PTSD [213, 214]. Although resting blood pressure was found to be elevated in PTSD patients [186, 187], further studies are needed to assess its feasibility as a biophysical marker. Other biomarkers suggested to help predict and diagnose PTSD include the rise in inflammatory markers and mediators (CRP, IL-2, IL-6, etc.), an increased startle response, symptoms of hyperarousal, and impaired cognitive function, among others [173,191].

6.3. THERAPEUTIC BIOMARKERS

Therapeutic biomarkers are those that allow the prediction/monitoring of the response to the delivered treatment, and are assessed throughout the treatment process [173]. Both diagnostic and susceptibility biomarkers can contribute to the treatment process. However, a set of biomarkers that can specifically monitor treatment effectiveness and others that can predict responses to the different modalities of “stratification” have also been investigated [215, 216]. Establishing a reliable and cost-effective biomarker for treatment monitoring can lead to significant improvement in PTSD management [172]. Table 4 provides a summary of the therapeutic biomarkers implicated in PTSD.

Table 4. Summary of the therapeutic biomarkers implicated in PTSD.

Therapeutic Biomarker	Findings
Amygdala and anterior cingulate cortex activity	Successful cognitive behavioural therapy was observed to decrease right amygdala activity while increasing right anterior cingulate cortex activity [217].
Cerebral blood flow to the medial temporal cortex	A normalisation of the difference in cerebral blood flow to the medial temporal cortex after EMDR [218].
Amygdala and ventral anterior cingulate activity	The greater activation of the bilateral amygdala and ventral anterior cingulate was associated with poorer response to CBT [219].
Rostral anterior cingulate (rACC) volume	A larger rostral anterior cingulate (rACC) volume was also found in responders to CBT [219].
LL genotype of serotonin transporter gene promoter (5HTTLPR)	A polymorphism in the LL genotype 5HTTLPR was found to be associated with a better response to sertraline [220].
BDNF levels	Lower serum levels of BDNF were associated with a decrease in PTSD symptoms in chronic patients on escitalopram [221].

Many studies have been conducted on biomarkers predicting the response to treatment and progression. In PTSD patients, successful cognitive behavioural therapy was observed to decrease right amygdala activity while increasing right anterior cingulate cortex activity [217]. Additionally, a cerebral blood flow alteration, made evident by a difference in 99mTc-HMPAO uptake was observed between responders and non-responders to EMDR treatment. Compared to controls, patients had increased uptake in the medial temporal cortex, temporal pole, and orbitofrontal cortex. After treatment with EMDR, the uptake difference in the medial temporal cortex was not present anymore but extended to the lateral temporal cortex and the hypothalamus [218]. The medial temporal cortex is involved in memory encoding, consolidation and retrieval, and re-experiencing symptoms [222, 223]. A larger rostral anterior cingulate cortex (rACC) volume was also found with a reduction in PTSD symptoms [219]. In the same study, Bryant *et al*. additionally found that a larger volume of rACC was present in those who responded to CBT. They also found that a greater activation of the bilateral amygdala and ventral anterior cingulate was associated with a poorer response to treatment [219]. A polymorphism in the serotonin transporter gene promoter LL 5HTTLPR was found to be associated with a better response rate to sertraline compared to the other genotypes (SS and SL) [220]. Interestingly, lower serum levels of BDNF were associated with a decrease in PTSD symptoms in chronic PTSD patients on the SSRI escitalopram [221].

As described, a significant advancement in therapeutic care will be the discovery of accurate PTSD biomarkers. Nevertheless, the ultimate therapeutic utility of biomarkers as a component of precision medicine will augment rather than replace current decision-making procedures since specific treatment needs are established through a collaborative process between patient and physician.

6.4. CONCLUSIVE REMARKS

There are now a variety of biomarkers linked to the risks, symptoms, and course of PTSD. Despite this relationship, there is a limited prospect of employing a single marker either diagnostically or prognostically, due to the prevalent comorbidity with other mental illnesses and the limitations of studies. For instance, it is possible that decreased hippocampus volume is linked to both PTSD and comorbid depression and can act as a biomarker of the constellation of symptoms connected to both conditions. For this, biomarker panels (as opposed to the use of a single biomarker) are needed in order to maximise the specificity, sensitivity, and repeatability of diagnostic tools. Future research must examine the biological and psychological aspects of PTSD in more detail in order to meaningfully identify a combination of biomarkers that may cluster around symptoms and symptom development, for example by the use of (multi-)omics data and machine learning approaches.

7. LIMITATIONS AND FUTURE DIRECTIONS

Since the introduction of PTSD as a diagnosis four decades ago, our understanding of the disorder has grown tremendously. However, the ability to aid recovery and enhance the quality of life of PTSD patients is still lagging behind. A lot of patients are not diagnosed timely, if at all. Although efficacious treatment regimens have been developed, many patients do not receive their treatment, and others fail to optimally respond.

Recent research on neurobiological models has given unparalleled insight into the potential underlying causes of PTSD. Yet, it is crucial to emphasise that a collection of condensed working models is unlikely to adequately describe the full intricacy of the illness. These neural models' ability to pave the way from new pathophysiological understanding to ground-breaking treatments for PTSD may be their most important contribution. Limitations to current studies are mostly concerned with study designs and methodology, as mentioned earlier. For example, the mixed results observed in studies evaluating the HPA axis function can largely be due to traumatic exposure in the control groups being a confounder in some studies. As a result, further studies evaluating the association between trauma exposure (as opposed to PTSD) and HPA axis dysfunction are needed. The standardisation of study designs, techniques, and protocols for obtaining diurnal cortisol should be another main goal of future research. For instance, Ryan *et al*. suggested measuring the salivary diurnal rhythm of cortisol over a period of at least two days before and after the given intervention, as this can characterise the function of the HPA axis and the relationship between diurnal cortisol and PTSD in greater detail [224].

With the introduction of DSM-5 and ICD11, considerable modifications have been made to the diagnostic criteria and categorisation of illnesses linked to trauma and stressors. In addition to highlighting the enormous research that has gone into understanding these phenomena, the repeated revisions in diagnostic criteria and categorisation also draw attention to the difficulties that occur when evaluating disorders that are caused by traumatic experiences. Given the current understanding, there are a number of necessary next steps to comprehend how to best classify trauma- and stressor-related disorders, including, but not limited to: (1) further clarifying partial PTSD phenotypic expression and determining whether categorisation under a dimensional vs. a categorical approach would be beneficial; (2) continuing to refine assessments to ensure that traumatic experiences are thoroughly assessed and symptoms after these experiences are best captured; (3) examining possible phenotypes of stress- and trauma-related diseases in further detail. In addition, research needs to take into consideration the possible biological subtypes of HPA axis responsiveness in PTSD, as they have been found to differ significantly in symptom intensity and comorbid anxiety symptoms [225].

Because PTSD has no cure and exposure to trauma is unpredictable, it is crucial to reveal susceptibility in order to find effective resilience-building techniques and avoid PTSD from ever occurring. As only a small portion of the trauma-exposed population has PTSD, susceptibility does exist. One of the promising fields to aid this goal is the genetics and epigenetics field. New discoveries in this field can greatly aid not only the detection of susceptible individuals but also diagnosis and new routes of targeted pharmacological treatment. In a review by Al Jowf *et al*. we highlight the recent advancements in epigenetics and epigenomics, drawn from EWAS and GWAS studies [106]. A major limitation of these studies is the fact that a lot of these studies are preclinical studies based on animal models of PTSD. Still, a number of human cohort EWAS and GWAS studies have also been conducted, leading to candidate (epi)genetic markers [226-229]. This highlights the need for translational studies in humans that can make clinical use of these markers, which can aid in the detection of susceptibility and early diagnosis.

Biological indicators cannot yet independently validate the evaluation of PTSD, drawing a clear contrast from other medical conditions like cancer, hypertension, and autoimmune diseases that have objective biological testing procedures for diagnosis, assessing the severity of illness, and response to therapy. Instead, self-report screening tests and clinical interviews are used to diagnose PTSD rather than an identification of the underlying pathology. The growing interest in PTSD biomarkers shows significant potential and promise; however, it is currently challenging to make inferences that can be used practically from the existing fundamental and translational research on PTSD biomarkers. Once enough data are collected, machine learning approaches can help

combine biomarkers in reliable, valid, and cost-effective integrated panels that can greatly enhance the prediction, diagnosis, and monitoring of the disorder.

Likewise, instead of addressing the biological etiology, treatment has mostly been restricted to symptom control and behavioural adaptation techniques. Drug development for PTSD has thus far been mostly opportunistic, based almost entirely on empirical findings using medications already licensed for other disorders. A single pharmaceutical therapy for PTSD has not yet been created as of the time of this writing. For now, future studies should pinpoint strategies for enhancing effective treatments, such as in specific populations (e.g., military personnel), for the further investigation of recommended and promising treatments, for developing strategies to individualize treatment, for maintaining patient engagement in treatment (i.e., preventing dropout), and for identifying individual factors predicting response/nonresponse. For instance, there are interventions that hold promise in improving PTSD symptoms, including repetitive transcranial magnetic stimulation (rTMS), biofeedback, exercise (e.g., yoga, and aerobic and resistance exercise) and deep brain stimulation (DBS) [230-233]. However, a lot of these studies are preclinical, while others are controversial and inadequately powered, creating a need for the further assessment for their efficacy in PTSD treatment. In the future, research on PTSD treatment should be guided by discoveries of the disruptions underpinning the development of the disorder, so that targeted and more effective interventions can be developed.

8. CONCLUSIONS AND PERSPECTIVES

PTSD is usually associated with chronicity and disability. Although the underlying neurobiology might be elusive, several established mechanisms have been studied, which have had significant implications on management. These dysregulations include brain circuit disruption through the dysregulated release of neurotransmitters, namely NE and serotonin among many others (e.g., dopamine, GABA, and NPY), a dysfunctional HPA axis, and disordered cannabinoid and opioid activity. Although PTSD is partly attributed to these dysregulations, models such as the biopsychosocial model and the diathesis–stress model have been developed to emphasise that underlying biology is not the only contributor to the disorder, yet there is an interplay between biological (e.g., genetics, chemical changes, and organ damage), psychological (e.g., stress, mental illness, behaviour, and personality), and social factors (e.g., peers, socioeconomic status, beliefs, and culture) in the manifestations of the disease. The current treatment for PTSD involves two main modalities, namely psychotherapy and pharmacotherapy. While psychotherapy is considered the treatment of first choice, when needed, pharmacotherapy can be used as an alternative or in conjunction with psychotherapy. Preventing the disease at different time points (primary, secondary, and tertiary prevention) can significantly reduce the

disease's burden on patients' quality of life and economic and medical burdens. Thus, the development and application of disease prevention models are of great importance. In the end, further research on susceptibility and resilience, pathophysiology, and possible targeted intervention is needed for better understanding and treatment. Over the past decade, the identification of disease biomarkers has gained more interest. Establishing reliable and cost-effective biomarkers can greatly enhance primary prevention, diagnosis, the monitoring of therapy, and the prevention of disability. None of the putative PTSD biomarkers reported so far are being used in clinical settings, which highlights the urgent need for additional studies on PTSD biomarkers with large sample sizes and for translational research strategies aiming to understand the underlying molecular causes of PTSD.

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7

GENERAL DISCUSSION AND CONCLUSION

In this chapter, the main findings of this thesis will be discussed, addressing its main aims and providing an overall conclusion.

THE FIRST MAIN AIM OF THE THESIS WAS TO FURTHER ADDRESS THE GAPS OF KNOWLEDGE IN THE UNDERSTANDING OF THE ASSOCIATION BETWEEN EPIGENETIC MECHANISMS, GENE ACTIVITY, AND DIFFERENTIAL SUSCEPTIBILITY TO PTSD.

The onset, intensity, and manifestations of PTSD are influenced by both hereditary and environmental variables, as is the case with many other diseases. One may be able to gauge how environmental insults affect the expression of genes by researching epigenetic factors. The term "epigenetics" describes DNA alterations that control gene transcription without changing the genetic sequence itself and are brought on in response to environmental factors. Therefore, it would be very beneficial for patients, doctors, and society at large to have a better knowledge of the epigenetic changes induced by traumatic stress. Thus, in **Chapter 2**, latest advancements in PTSD epigenetics were reviewed. One of the well-studied epigenetic mechanisms, DNA methylation of cytosines next to guanines (CpGs), is involved in both more volatile modifications brought on by environmental exposures and long-term stable alterations. In addition, to determining a cell's identity, DNA methylation also aids in the transcriptional response's capacity to adapt to changing environmental conditions over the course of a lifetime. Histone modifications and miRNAs are two other epigenetic processes that are involved in the control of DNA accessibility and mRNA translation, respectively and being investigated more and more [1]. To find integrated regulatory patterns or mechanisms in the field of epigenomics, screening techniques have changed for all of these modifications in recent years, shifting from targeted gene or genomic region-based approaches to genome-wide research [2].

In a study by Chen *et al*. accounting for genome-wide gene expression and DNA methylation in 12 PTSD patients and 12 trauma-exposed healthy controls, it was found that 3989 genes were significantly upregulated in those with PTSD and three genes were significantly downregulated ($p < 0.05$), after multiple comparisons were taken into account [3]. However, DNA methylation showed no discernible variation. Histone H4 lysine 5 acetylation (H4K5ac) and histone H3 lysine 9 acetylation (H3K9ac) were considerably enhanced in the lateral, basal, and centrolateral amygdala and prefrontal cortex, according to animal studies that examined brain histone acetylation after fear conditioning [4-6]. When compared to control individuals, the prefrontal cortex miRNA profiles in a mouse model for PTSD showed that severe stress did not affect long-term miRNA expression on its own. However, fluoxetine treatment significantly decreased several miRNAs in traumatized mice compared to untreated traumatized mice, particularly mmu-miR-1971 [7]. Future research on miRNA-associated epigenetic pathways in PTSD patients with co-

occurring depressive and anxiety disorders will be impacted by this finding in particular, because these comorbidities are frequently treated with SSRIs like fluoxetine, which then may influence the results.

Epigenome-Wide Association Studies (EWAS) allow for the exploration of DNA methylation patterns across the genome that are linked to a specific phenotype. Rutten *et al*. discovered 12 chromosomal areas and 17 specific locations where there were PTSD-related alterations in DNA methylation. Among others, they found that lower levels of DNA methylation in the areas *HIST1H2APS2*, *RNF39*, and *ZFP57* were linked to higher levels of PTSD symptoms, with the first being the single gene location being replicated in a cohort of US Marine soldiers [2]. Logue *et al*. demonstrated that methylation of the *G0S2* gene's locus cg19534438 has been demonstrated to be strongly linked with PTSD [8]. Mehta *et al*. found that immune dysregulation and the emergence of PTSD symptoms are related to methylation alterations of the *DOCK2* gene. Additionally, they discovered two CpGs inside *GR* and two CpGs within *FKBP5* to be associated with PTSD [9, 10]. Furthermore, Snijders and Maihofer *et al*. identified multiple differentially methylated positions (DMP), four of which were in the (HLA) region, highlighting the significance of immunological dysregulation in PTSD [11]. Genome-wide epigenetic screening is anticipated to be used more frequently in PTSD research as a result of technological advancements, reduced operational costs, and the development and standardization of analytical procedures.

It has also been demonstrated that stress-related epigenetic alterations influence and are driven by inheritance in offspring. However, intergenerational epigenetic inheritance, as a result of direct exposure in utero or exposed germ cells, best accounts for this. As a result, only third-generation offspring in the female line and second-generation offspring in the male line can be investigated for real transgenerational epigenetic inheritance without exposure. Experimental studies to yet have not discovered any proof of true epigenetic inheritance, which calls for further investigation. Additionally, the scope of epigenetic studies in PTSD should address the possibility of developing novel therapies that directly target epigenetic changes. Moreover, genetically and epigenetically subtyping the disorder may aid in proper diagnosis and management. In the longer future, the use of these epigenetic changes as disease biomarkers could promote breakthroughs in the management of the disorder.

In light of these findings, and to further expand the known genetic susceptibility profiles of PTSD, in **Chapter 3** we performed a transcriptome study of the blood mRNA expression levels of two groups of military personnel in the PRISMO cohort (those who experienced PTSD symptoms vs. controls), after using the Self-Rating Inventory for PTSD (SRIP) to assess susceptibility. RNA from whole blood mononuclear cells (PBMCs), was analyzed by RNA-sequencing. After false discovery rate (FDR) correction, no genes were determined

to be significantly different between susceptible and resilient groups. However, 21 genes were nominally expressed differently with a fold change of at least 1.5 in either direction. Of those, *SLC13A4*, *NPR3* and *FOSB* were upregulated, while *TBC1D16* was downregulated.

Of them, *FOSB*, has been extensively researched in connection to vulnerability to PTSD and is widely acknowledged as a marker for chronic stress activation [12, 13]. It functions as a common interaction element with the GR across the genome by heterodimerizing with proteins from the JUN family to generate a transcription factor AP-1 complex. The GR is recognized to have a role in PTSD as an element of the HPA axis, where GR hypersensitivity causes a greater level of negative feedback inhibition of cortisol and corticotropin-releasing hormone production (CRH), which contributes to the HPA axis dysfunction [14]. *SLC13A4* has been proposed as a potential stress-associated candidate gene. Consensus sequences for GATA-1, AP-1, and AP-2 are present in potential transcription factor binding sites on *SLC13A4* [15]. *NPR3*, ecoding the receptor for natriuretic peptide hormones, which among others function as neuromodulators, has been linked to mental illnesses in a number of animal and human studies [16-18]. Finally, *TBC1D16* is relevant to PTSD vulnerability. In our study, a decrease in *TBC1D16* expression levels potentially driven by eQTL variants (TBC1D16 - rs7222531 and TBC1D16 - rs62076639) was linked to a higher risk of developing PTSD. *TBC1D16* is connected to sadness and suicidal thoughts, even if its role in the pathophysiology of PTSD susceptibility is uncertain [19]. Controlling the epidermal growth factor receptor (EGFR) signaling and transferrin receptor recycling is the main function that TBC1D16 is known to do [20]. EGFR is involved in cellular development, differentiation, and apoptosis, and several investigations have revealed a relationship between the EGFR genotype and depression or MDD [21].

In conclusion, our study done in **Chapter 3** contributes to the body of evidence connecting transcriptomic, genetic, and epigenetic variables to biological processes of vulnerability to PTSD by combining various layers of molecular data gathered from a PTSD cohort. The findings are in line with past studies linking PTSD susceptibility to the immune system and signalling cascades, particularly AP-1 and EGFR. We showed that a transcriptome profile of PTSD susceptibility at a post-traumatic time point might provide novel insights for further follow-up study and should be targeted with more precision. This large dataset is a valuable resource and a big step forward in understanding the genomic architecture of the PTSD brain by finding molecular pathways that play a substantial role in mediating the consequences of trauma. Deeper sequencing to better study very lowly expressed transcripts,and to replicate the current study's conclusions is a future path for this research. Integrating brain transcriptome profiles with those of peripheral tissue databases to find biomarkers of PTSD, as well as neuroimaging to correlate cortical differences in PTSD should be investigated in the future.

As addressed by the research discussed in this thesis, the study done by Rutten *et al*. identified differential DNA methylation patterns of the *RNF39* gene in the blood of soldiers who developed PTSD compared to those who were resilient, which makes *RNF39* a candidate gene potentially linked to the susceptibility to PTSD. The precise function and location of RNF39 protein are yet unclear, which is a crucial first step in understanding its role in illness etiology. Thus, in **Chapter 4** of this thesis, the RNF39 protein expression pattern was assessed with immunohistochemistry (IHC) in the hippocampus of control as well as social defeat (SD) mice. Based on the control group, RNF39 was found to be expressed in the hippocampal subfields CA1, CA2, CA3 and DG. We found that SD mice had a significantly increased length of RNF39-positive immunoreactive fibres in the DG compared to controls. Additionally, behavioural correlation revealed a strong association between the social interaction ratio (SIR), and the RNF39 expression in the DG. These findings support the expression of RNF39 in stress-related brain regions and a role of RNF39 in stress-response. However, future research with larger sample sizes and translational methods should be done to futher understand the role of RNF39 in the stress-response and to assess whether RNF39 could be a biomarker for PTSD susceptibility. Indeed, RNF39's blood-based DNA methylation levels may serve as brain-relevant peripheral biomarkers indicative of trauma-induced vulnerability for PTSD, although the relationship between RNF39's DNA hypomethylation in blood and its regulation in the brain is not yet clear.

THE SECOND MAIN AIM OF THE THESIS WAS TO IDENTIFY THE PUBLIC HEALTH MEASURES THAT CAN BE EFFECTUATED FOR THE PREVENTION OF PTSD AND HOW BIOMARKERS MAY SUPPORT THESE MEASURES.

With enough understanding of PTSD, how it develops, and who is at higher risk, guided partly by the previous studies, preventive and interventional treatments and strategies can hopefully be used in the future to enhance patients' quality of life and reduce the disease's financial and medical burden, as discussed in **Chapter 5**. From the public health standpoint, tackling a disease begins with identifying the causes and triggers. Then, at various phases of the disease's development, prevention (rather than therapy) can be used with the goal of reducing the disease burden at all levels. The social-ecological model supports such endeavours. This model has four levels at which it seeks to uncover the causes underlying illness development and poor health outcomes: individual, relationship, community, and society [22]. Each level's preventative strategy may be primary, secondary, or tertiary. Primary prevention is concerned with identifying those who are at risk and stopping the onset of illness in a healthy person. The goal of secondary prevention is to intervene quickly after the onset of the disease in order to slow its progression or, if feasible, to cure it. Reduced disease-related disabilities are the goal of tertiary prevention in order to preserve a higher standard of living [23].

For primary prevention at the individual level of the model, interventions can be in the form of educational programs on the risk of alcohol drinking and firearm acquisition, as well as military stress coping training. At the relationship level, parental and caregiver supervision and education can work to lessen traumatic events for the kids, such as programs to stop assaultive violence or bullying at school. Neighbourhoods, street surveillance campuses, and community support services are a few examples of initiatives at the community level. Policies can be implemented at the societal level to limit access to firearms and alcohol, as well as to recall faulty automobiles and maintain public infrastructure.

For secondary prevention, at the individual level, for those who have experienced trauma, limiting continued stressor exposure, such as temporary military exemption, can slow the onset of illness. Taking care of family members who have experienced domestic abuse or kids who have been neglected can be beneficial on a relationship level. At the community level, solutions include creating rehabilitation programs and offering refuge following disasters. Policies targeting early medical intervention and campus screening can slow the progression of disease at the societal level.

Interventions are regarded as a standard aspect of the care and treatment of the disease when they work to stop the advancement of the illness and the emergence of disability. Individuals can prevent disabilities at the tertiary preventive level by getting medical attention, adhering to therapy, and learning more about the illness and its repercussions. Specialized training for parents, relatives, and friends who are caring for patients who are in therapy might be beneficial at the relational level. Measures to foster community knowledge of the illness, reduce stigma, uphold peace, and combat violence all play a crucial role in avoiding disability at both the community and societal levels [24].

There is still a pressing need for the development of more advanced measurements in the prevention of PTSD. A deeper knowledge of PTSD susceptibility is needed for more effective screening and earlier intervention. One means of achieving this goal are biomarkers. Although there is increasing interest in PTSD biomarkers research, results are still preliminary and are still far from producing panels for clinical utility. Accordingly, in **Chapter 6** we summarized the numerous possible biomarkers that have been associated with pathophysiological PTSD alterations, which motivates more investigation to find useful targets. The current usage of PTSD biomarkers is for research, but they may soon be utilized for screening and enabling early detection of the disorder, allowing for prompt intervention and better outcomes. These biomarkers may be structural alterations, substances, or responses that can be used to gauge the disorder's risk, diagnosis, prognosis, and therapeutic response.

Perhaps it is difficult to assess a person's susceptibility to acquiring PTSD, and numerous theories have been created to describe the complexities of such progression. Researchers looked into a number of susceptibility biomarkers as illness predictors during the pre-traumatic and post-traumatic phases to achieve this. A longitudinal study on Dutch military soldiers found a significantly higher number of GR in lymphocytes and monocytes, as well as T-cells' high glucocorticoid sensitivity to dexamethasone before deployment in soldiers who developed severe PTSD symptoms thereafter [25, 26]. Additionally, low mRNA levels of *FKBP5* and high *glucocorticoid-induced leucine zipper (GILZ)* mRNA levels before deployment had the same association [27].

Other investigations confirmed the HPA axis' participation and found that polymorphisms in the *CRH type 1 receptor* gene were related to the development of PTSD [28]. As discussed in **Chapter 6**, the *BDNF* polymorphism Val66Met also appears to raise the incidence of PTSD because it lowers BDNF expression, which impairs the extinction of conditioned fears. Studies have revealed that PTSD sufferers have a higher frequency of the Met allele than do controls [29]. Besides molecular markers, non-molecular markers are also investigated, especially in the post-traumatic period. These include increased heart rate and the occurrence of nightmares [30].

Studies on monoamines in PTSD patients observed an increase in the level of catecholamines in the urine [31]. Also, BDNF tends to be of higher levels in PTSD groups when compared to controls, while neuropeptide Y (NPY), has been shown to be of lower levels in plasma of combat-exposed subjects with PTSD, and both are implicated as potential biomarkers for PTSD [32, 33]. Molecular studies on the levels of serum miRNAs have also shown interesting findings. Five miRNAs (miR-221-3p, miR-335-5p, miR-138-5p, miR-222-3p, and miR-146-5p) were able to precisely distinguish PTSD individuals from controls. Additionally, miR-1246 was shown to be significantly downregulated in PTSD patients compared to resilient participants, suggesting that it may be useful as a diagnostic biomarker [34]. Other neuroanatomical markers implicated in the diagnosis include amygdala overactivation (upon exposure to traumatic stimuli) and loss of hippocampal volume [35].

Researchers have also looked into a group of biomarkers that can particularly track therapy efficacy and others that can forecast how patients will react to certain modalities of treatment. Successful cognitive behavioural therapy (CBT) was found to increase right anterior cingulate cortex (ACC) activity while decreasing right amygdala activity in PTSD patients [36]. Additionally, an increase in cerebral blood flow was found using 99mTc-HMPAO uptake in EMDR responders [37]. Additionally, a decrease in PTSD symptoms was associated with a bigger rostral anterior cingulate cortex (rACC) volume [38]. The same study also discovered that people who react to CBT have a greater volume of rACC.

When compared to the other genotypes (SS and SL), a polymorphism in the serotonin transporter gene promoter LL *5HTTLPR* was shown to be related with a higher response rate to sertraline [39]. Intriguingly, in chronic PTSD patients using the SSRI Escitalopram, reduced serum levels of BDNF were linked to a reduction in PTSD symptoms [40].

The current diagnosis of PTSD is clinical, disregarding its pathophysiology and underlying biological changes. Instead, it is based on how the disease manifests, and whether certain requirements are met. This is due to the complexity of PTSD, like all mental disorders, and the fact that different phenotype manifestations can occur in people who have had equal levels of traumatic experiences and even have similar changes in their biological and brain activity. Biological biomarkers are not currently used in clinical diagnosis of the condition, but due to recent scientific advancements, they may do so in the near future. Development of biomarker panels rather than single markers, in conjunction with symptom-based phenotyping of PTSD as mentioned earlier, can enhance their cost-effectiness and utility in clinical practice.

In order to better understand and treat the condition, further study is ultimately required on susceptibility and resilience to PTSD, its pathophysiology, and potential targeted interventions. Although the discovery of PTSD biomarkers has drawn greater attention over the past ten years, establishing trustworthy and affordable biomarkers is essential, which can significantly improve primary prevention, diagnosis, monitoring therapy, and disability prevention. None of the putative PTSD biomarkers that have been discovered so far has been used in clinical settings, which emphasizes the urgent need for more research on PTSD biomarkers with large sample sizes and translational research methods that aim to comprehend the underlying molecular causes of PTSD.

In conclusion, the findings of this thesis can help benefit those who are at risk or even suffer from PTSD. The findings presented in this thesis contribute to the discussion of susceptibility to PTSD at different levels, including early detection of vulnerability, early diagnosis following exposure to traumatic stress, monitoring of disease and therapy, and finally improving the outcome and quality of life. Moreover, the thesis provides expands on the current understanding of PTSD pathophysiology and new potential players in the development of the disorder. Concurrently, it provides a transcriptomic and epigenetic evidence to the susceptibility to PTSD, contributing to the much needed risk stratification of PTSD. Additionally, the thesis provides a first-time insight into a candidate gene, *RNF39*, that is probably involved in traumatic stress through the use of an animal model. A main objective of its contributions is to expand on the current efforts aimed at developing methods to identify those vulnerable to PTSD, so that early preventive measures can be taken. Such methods include mainly the use of blood biomarkers, which are more feasible or accessible than other tissue and imaging biomarkers. It also aims at describing different

strategies that can be implemented by the authorities in order to reduce exposure to traumatic stress, and subsequently the development of the disorder. Implementation of these findings can contribute to the existing body of efforts aiming to reduce the burden associated with PTSD. As a follow-up of this thesis work, larger cohort studies should replicate the findings we report here. Findings from GWAS and transcriptomic studies should be used to better identify the molecular basis of the disease, as well as develop or enhance new interventions that will result in a better outcome. These findings should also be interpreted in parallel with their counterparts from the other comorbid conditions of PTSD (e.g., depression), as the disorder is highly comorbid. Additionally, larger translational studies on RNF39 should be conductd to ascertain its role both in neurogenesis and traumatic stress. Moreover, systematic reviews and meta-analyses on the available transcriptomic studies should be conducted to aid clinical application. The next few years hold enormous promise for fusing genetic research with a thorough comprehension of the brain circuits that control the main PTSD-related behavioural traits. PTSD is a disorder that is prevalent in those who have experienced severe trauma, is commonly comorbid, and is linked to reduced quality of life. Large-scale longitudinal studies and new insights into the neural circuitry, physiology, intermediate phenotypes, and genetics of PTSD hold great promise for progress in the prediction, intervention, and perhaps prevention of this crippling psychiatric disorder.

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8

SUMMARY

Traumatic stress exposure can induce the development of mental disorders such as post-traumatic stress disorder (PTSD), as well as physical ill-health, resulting in a decreased quality of life and increased disease burden, both economic and medical. As timely and appropriate treatment can prevent chronicity and disability, and hence result in a better quality of life, it is necessary that the identification of those at increased risk and susceptible to develop PTSD are known. Additionally, early diagnosis and management should be the priority once a person is known to be exposed to traumatic stress. Over the past decade, the focus of research has become more on the identification of susceptibility factors, including epigenetics and biomarkers studies.

This thesis aims to address the gaps of knowledge in the understanding of the association between epigenetic mechanisms, gene activity, and differential susceptibility to PTSD. It also aims to identify the public health measures that can be effectuated for the prevention of PTSD and how biomarkers may support these measures. Accordingly, the thesis discusses the possible ways that can aid in the early identification and detection of PTSD resulting from traumatic stress, and the possibility of developing biomarkers of increased susceptibility. It also discusses how this early detection can help with early intervention, and if early enough, prevention. Application of such models, with findings from future research to establish cost-effective measures, can greatly enhance the patient's quality of life and decrease the disease burden.

Chapter 2 summarized the recent studies and their findings on epigenetic and epigenomic changes associated with PTSD development. The identification of such changes can help identify patients at increased risk and hence intervene early with the application of preventive measures. These epigenetic changes can be applied in real-life practice once proven reliable and cost-effective.

In **Chapter 3**, we conducted a transcriptomic analysis on participants of the Prospective Research in Stress-Related Military Operations (PRISMO), a cohort of Dutch military members deployed to Afghanistan for 4 months, who experienced trauma and developed PTSD, those who did not develop PTSD and healthy (unexposed) individuals. We aimed to replicate previous findings, as well as discover new differential expression patterns of blood mRNA for the detection of PTSD susceptibility. Among the important genes implicated in PTSD from our study, *FOSB*, *SLC13A4* and *NPR3* were upregulated, while *TBC1D16* was downregulated. We demonstrated that a transcriptome profile of PTSD vulnerability at a post-traumatic time point may offer new perspectives for additional follow-up research and should be addressed with more precision.

In **Chapter 4**, we aimed to provide for the first-time an investigation of the involvement of RNF39 in traumatic stress and PTSD. Recently, RNF39 has been implicated as a potential

modulator in stress, however, no investigation to date has been done to ascertain its role. Through the social defeat animal model of PTSD, we demonstrated higher expression of RNF39 in dentate gyrus fibres of the hippocampus in animals exposed to traumatic stress, as well as a correlation between stress-induced anhedonic behaviour and RNF39 expression in these animals. Our study paves the way for translational studies with larger human cohorts to assess the exact role of RNF39 in PTSD and its applicability as a biomarker.

In **Chapter 5**, the identification of the most important and common stressors, and the main factors that can affect the response to traumatic stress were discussed together with the the socio-economic model for disease prevention, and the potential of its application in the prevention of PTSD. The model comprises four levels, with the individual level at its core, up to the relationship, community, and societal levels. From a practical point of view, the thesis discussed examples of preventive measures at each level of the model, the three types of prevention, namely primary, secondary and tertiary prevention, by applying real-life examples of applicable interventions and programs. For integration, the latest treatment modalities used for PTSD are inventoried, and how these interventions can contribute to enhanced quality of life and decreased disability.

To wrap up the thesis, **Chapter 6** reviewed the most recent advances and discoveries in PTSD development, treatment and prevention, and emphasising the current state of the art of biomarkers. We demonstrated the important involvement of psychological, environmental and social factors, in conjunction with biological factors in the induction and maintenance of the disorder. Of importance for diagnosis and treatment is the underlying disturbance in neurobiology, which includes neuroanatomical changes, neuroendocrine disturbances in molecules such as norepinephrine; serotonin; BDNF; NPY and oxytocin, in addition to the essential dysregulation in the HPA axis as a key player of stress regulation. The approach to treating the disorder involves both psychotherapeutic and pharmacological interventions. Although trauma-based psychotherapy is considered a first line of management, pharmacotherapy is also used in conjunction and in specific situations. Although the science of PTSD biomarkers still has a long way to go, we showed that most of the implicated biomarkers are actually reflective of the underlying neurobiological changes, substances, structural alterations, and responses associated with PTSD. For that, future research on PTSD should take into consideration all these aspects, starting from the pathophysiology, and how it can be translated into effective interventions and clinically meaningful biomarkers.

9

APPENDICES

IMPACT PARAGRAPH

ABBREVIATIONS

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LIST OF PUBLICATIONS,
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IMPACT PARAGRAPH

PTSD is a complex condition that develops after exposure to trauma. Although evidence-based treatments are successful in lowering symptoms, there is no known cure, and low retention rates present a hurdle. As not all people exposed to trauma develop PTSD, underlying interindividual differences in susceptibility are expected. Therefore, it is crucial to create strategies to lower the risk of acquiring PTSD in addition to looking for novel therapies. The aim of this research were to identify the main factors in determining susceptibility to PTSD, with a focus on epigenetics and the use of novel PTSD biomarkers to aid early detection, which can lead to early recognition of those at risk, aiding the process of prevention and early intervention to reduce the burden of the disease, both medical and economic. Accordingly, a number of biomedical studies included in this thesis were developed for this purpose. The thesis also provides a comprehensive starting point and review of the literature for researchers investigating the field of PTSD susceptibility.

In **Chapter 2**, we give a summary of the various molecular biology, biochemical, and physiological changes in PTSD with a focus on genomic and epigenomic abnormalities, and their role in susceptibility to develop PTSD after exposure to traumatic stress. Additionally, we discuss how modern studies on epigenetics and epigenomics could help us identify PTSD patients earlier and provide them with timely management. These results could accelerate advancements in the discovery of novel therapeutic targets and biomarkers for PTSD in the long term. The discovery of predictive biomarkers that may differentiate between people who are at a high or low risk of developing PTSD after experiencing trauma will enable or support preventive measures and early treatments.

In **Chapter 3**, we performed a transcriptomic analysis for susceptibility to PTSD in the PRISMO cohort. We demonstrated that a transcriptome profile of PTSD vulnerability at a post-traumatic time point may offer new perspectives for additional follow-up research into its pathophysiological processes and candidate genes and their regulatory mechanisms to be investigated with more precision. This study adds to the body of research connecting transcriptomic, genetic, and epigenetic factors to biological processes underpinning vulnerability to PTSD by combining multiple layers of molecular data collected from a PTSD cohort. Such integrated knowledge may support future preventive, diagnostic and therapeutic strategies.

In **Chapter 4**, tissue analysis was used to determine the pattern of the RNF39 expression, which is thought to be regulated by epigenetic mechanisms and involved in the pathogenesis of PTSD, among other neuro-epigenetic changes [1, 2]. Since no investigation has provided an insight into the regular brain expression of RNF39 and its changes in relation to traumatic stress yet, this study provides a valuable basis for future researchers

to compare and build upon. Consequently, a blood based biomarker for RNF39 can be investigated for clinical utility.

In **Chapter 5**, we give a summary of recent research on traumatic stress, focusing on disease burden, causes or triggers of stress, variables influencing stress response, and the use of the social-ecological public health paradigm of disease prevention. We focus on the different means to early detect and prevent the development of PTSD. It is supportive to see the adoption of such preventive measures in accordance with public health models of disease prevention as a way to successfully accomplish these objectives. Additionally, we discuss therapeutic considerations, ethnic variations in traumatic stress, and perspective views, including possible biomarkers, all of which can enhance the patient outcome, as well as decrease the burden of the disorder. Implementation of the measurements by the public health sector can accelerate the efforts of traumatic stress prevention and result in improved outcome and diminished burden.

Additionally, in **Chapter 6** recent discoveries in the area of PTSD are reviewed, including pathophysiology, treatment, and disease biomarkers. More dedicated attention is given to these biomarkers as a way to predict, diagnose, and follow-up treatment of PTSD. Susceptibility biomarkers could be especially of added value from a public health point of view, as they can detect those vulnerable to developing PTSD after current or future exposure to traumatic stress, allowing for earlier intervention and prevention of complications. Diagnostic biomarkers, although still not applicable clinically, show promising perspectives, but more research into more reliable and cost-effective biomarkers or biomarker panels that can aid diagnosis is needed. Therapeutic biomarkers appear to be the least developed, but researchers have been able to draw conclusions about some that can help monitor therapy and response to treatment, which can enhance the patient's quality of life and yield better treatment results. None of the potential PTSD biomarkers is reported as being used in therapeutic settings, highlighting the urgent need for further research on PTSD biomarkers with large sample sizes and for translational research methods aimed at understanding the underlying molecular origins of PTSD. Going forward, such development can greatly enhance the outcome of the disorder, both its impact on the patient's quality of life and its economical and societal impact.

The prevalence of trauma exposure and PTSD development is rising substantially, leading to an increased medical and economic burden, affecting both the patient and the community [3, 4]. This thesis contributes to mitigate this burden by addressing various aspects. Factors known to affect the response to trauma are reviewed and linked to prevention (e.g., emotional care, age, education, and gender). Of pivotal importance, examples of practically implementable methods of trauma prevention on various levels based on public health models of prevention are provided. Moreover, these preventive

measures are defined as either primary, secondary or tertiary for appropriate intervention. Additionally, modalities of intervention, including pharmacological and psychotherapeutic are summarized. As the burden of the disease is substantial, it is necessary to develop strategies through prevention and intervention [5]. Another important player to decrease the burden of PTSD is the development and utilization of biomarker panels that can detect susceptible subjects and enable to offer earlier interventions. To address that, and in addition to reviewing the recent advances in this field, we conducted a blood-based study to examine the presence of susceptibility gene expression markers, that expand on the current state of knowledge. Additionally, we provided a first-time insight into RNF39 protein expression, and concluded a possible involvement in the neurobiology of PTSD, and the possibility to utilize it as a biomarker.

The results provided in this thesis serve both researchers and public health authorities. The major initial contribution of this thesis is to the research field. To researchers, it provides multiple sources to be informed about the current up-to-date knowledge about PTSD susceptibility, while giving templates to compare with future studies, both preclinical and clinical ones. To public health agencies and health authorities, as well as researchers, we provide markers of disease susceptibility for timely intervention. However, more in-depth clinical research is needed to confirm the reliability and cost-effectiveness of such markers. This thesis supports such endeavours by providing a contextual discussion of susceptibility prediction or estimation, connecting biomedical and public health perspectives, and a comprehensive overview of methods and modalities for disease prevention, which when integrated can reduce disease burden.

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ABBREVIATIONS

5-AZA	5-aza-20′-deoxycytidine
5-hmC	5 hydroxymethylcytosine
5-mC	5-methylcytosine
ACC	Anterior Cingulate Cortex
ACTH	Adrenocorticotropic Hormone
BDNF	Brain-Derived Neurotrophic Factor
DHEA	Dehydroepiandrosterone
DMP	Differentially Methylated Positions
DMR	Differentially Methylated Regions
DNA	Desoxyribonucleic Acid
DNMT	DNA Methyltransferases
EMDR	Eye Movement Desensitization and Reprocessing
EWAS	Epigenome-Wide Association Studies
fMRI	Functional Magnetic Resonance Imaging
GABA	Gamma-Aminobutyric Acid
GWAS	Genome-Wide Association Studies
HAT	Histone Acetyltransferases
HDAC	Histone Deacetylase
HLA	Human Leukocyte Antigen
HPA	Hypothalamic-Pituitary-Adrenal
IL-PFC	Infralimbic-Prefrontal Cortex
lncRNA	Long nc-RNAs
MDD	Major Depressive Disorder
miRNAs	MicroRNAs
mRNAs	Messenger RNAs
MRS	Marine Resiliency Study
nc-RNAs	Non-coding RNAs
NET	Narrative Exposure Therapy
NLGN1	Neuroligin Gene
NOS	Nitric Oxide Synthase
NPY	Neuropeptide Y
PFC	Prefrontal Cortex
piRNA	Piwi-Interacting RNA
PL-PFC	Prelimbic-Prefrontal Cortex
PRISMO	Prospective Research In Stress-related Military Operations
PTSD	Post-Traumatic Stress Disease
RCT	Randomized Control Trial
RNAs	RNA-associated Silencing

RNF39	Ring Finger Protein 39
RORA	Retinoid-related Orphan Receptor Gene
RRBS	Reduced Representation Bisulphite Sequencing
siRNA	Small Interfering RNA
sncRNA	Short nc-RNAs
SNP	Single-Nucleotide Polymorphisms
SSRIs	Selective Serotonin Reuptake Inhibitors
TAB-Seq	Tet-Assisted Bisulphite Sequencing
TLL-1	Tolloid-Like 1 Gene
TRACTS	Translational Research Center for TBI and Stress Disorders
UTR	Untranslated Region
VPA	Valproic Acid
WGBS	Whole-Genome Bisulphite Sequencing

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Hold fast to dreams
For if dreams die
Life is a broken-winged bird
That cannot fly.

Hold fast to dreams
For when dreams go
Life is a barren field
Frozen with snow.

Langston Hughes

ABOUT THE AUTHOR

CURRICULUM VITAE

Ghazi Ibrahim A. Al Jowf was born on June 2, 1983, in the Eastern Province (Al Khobar), Saudi Arabia.

He started his Bachelor's degree in 2001 at King Faisal University in the Eastern Province (Dammam). Following that, he successfully did his medical training at different governmental hospitals in the same region as part of his internship program.

Between 2008 and 2011, he worked in different places, including as a senior medical employee at the Health Facility Administration (polyclinics) at King Faisal University, KSA, and Aramco Hospital, KSA.

In 2012, he started working as a demonstrator at the Public Health Department at King Faisal University, KSA. Then, he carried out his Master's research titled: "The Relationship between Mental Wellbeing and the Behaviour of Workers in the Mining Industry (Western Australia)" under the supervision of Dr. Kim Clark at the Public Health Department, School of Medical and Health Sciences at Edith Cowan Unversity in Perth, Western Australia, Australia.

Between 2016 and 2017, he became a lecturer at the Public Health Department at King Faisal University, KSA, a position he still holds. In 2023, he received University Teaching Qualification (UTQ) certificate from Maastricht University, for demonstrating the teaching skills of lecturers in academic education.

Dr. Al Jowf carried out his PhD research under the supervision of Prof. Bart Rutten at the Psychiatry and Neuropsychology Department at Maastricht University in the Netherlands. Dr. Al Jowf received an international award by the Ministry of Higher Education (KSA) for part of his research during his PhD program.

LIST OF PUBLICATIONS, ABSTRACTS AND PRESENTATIONS

PUBLICATIONS

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PUBLICATIONS AWAITING FOR ACCEPTANCE/IN PREPARATION

***Al Jowf et al.** "Blood-Based Transcriptomic Analysis of Susceptibility to PTSD in Deployed Military Soldiers". Submitted during 2023.*

***Al Jowf et al.** "Social Defeat Stress Alters RNF39 Protein Expression in the Hippocampal Dentate Gyrus of Mice". To be submitted during 2023.*

ABSTRACTS

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نظرًا لعدم إصابة جميع الأشخاص المعرَّضين للصدمات باضطراب الكرب التالي للصدمة، فمن المتوقّع وجود اختلاف في الحساسية بين الأفراد. لذلك، من الضروري إيجاد تقنيات لتقليل مخاطر الإصابة باضطراب الكرب التالي للصدمة، بالإضافة إلى البحث عن علاجات جديدة. الهدف من هذا البحث هو تحديد العوامل الرئيسة في تحديد القابلية للإصابة باضطراب الكرب التالي للصدمة، مع التركيز على الوراثة الفوق جينية، واستخدام المؤشرات الحيوية الجديدة لاضطراب الكرب التالي للصدمة للمساعدة في الكشف المبكِّر، التي يمكن أن تؤدي إلى التعرُّف المبكِّر على الأشخاص المعرَّضين للخطر، مما يساعد عملية الوقاية والتدخل المبكِّر لتخفيف عبء المرض بشقيه الطبي والاقتصادي. لذا، تم تطوير الدراسات المدرجة في هذه الأطروحة لهذا الغرض. يقدّم **الفصل الثاني** مراجعة للبيانات الحديثة المتاحة عن التغيرات الفوق جينية المرتبطة باضطراب الكرب التالي للصدمة، متبوعًا **بالفصل الثالث**، حيث أجرينا تحليلاً نصيًا للتأثر باضطراب الكرب التالي للصدمة في مجموعة (PRISMO). لقد أظهرنا أن الترنسكريبتوم الخاص باضطراب الكرب التالي للصدمة في نقطة زمنية ما بعد الصدمة، قد يقدّم وجهات نظر جديدة لأبحاث متابعة إضافية، ويجب معالجتها بمزيد من الدقة. تسهم هذه الدراسة في مجموعة الأدلة التي تربط المتغيرات الترانسكريبتومية والوراثية والجينية بالعمليات البيولوجية، للتعرض لاضطراب الكرب التالي للصدمة من خلال الجمع بين طبقات مختلفة من البيانات الجزيئية التي تم جمعها من المرضى. في **الفصل الرابع**، تم استخدام تحليل الأنسجة لتحديد نمط تعبير البروتين (RNF39) (التي تنظمها آليات الوراثة الفوق جينية)، التي يُعتقد أنها متورطة في التسبب في اضطراب الكرب التالي للصدمة من بين التغيرات العصبية الفوق جينية الأخرى. كما توفِّر هذه الدراسة أساساً قيّماً لتحليل أنسجة المخ الصحية للباحثين في المستقبل للمقارنة والبناء عليها. توفِّر الرسالة أيضًا نقطة انطلاق شاملة ومراجعة المصادر الحديثة للباحثين في مجال القابلية لاضطراب الكرب التالي للصدمة. يتزايد انتشار التعرُّض للصدمات واضطراب الكرب التالي للصدمة بشكل كبير، مما أدى إلى زيادة العبء على الأنظمة الاقتصادية والرعاية الصحية. كما تم تلخيص في **الفصل الخامس** من هذه الرسالة، بأن اضطراب الكرب التالي للصدمة يحمل عبئًا طبيًا واقتصاديًا يؤثر على كلٍّ من المريض والمجتمع. كما تمَّت مراجعة العوامل المعروفة بتأثيرها على الاستجابة للصدمات وربطها بالوقاية. من الأهمية بمكان، تقدم الرسالة أمثلة على طرق جاهزة لتنفيذ الوقاية من الصدمة على مستويات مختلفة بناءً على نماذج الصحة العامة للوقاية. علاوة على ذلك، يتم تعريف هذه التدابير الوقائية على أنها إما أولية وثانوية وثالثية للتدخل المناسب. بالإضافة إلى ذلك، تم تلخيص طرق الوقاية، بما في ذلك العلاج الدوائي والعلاج النفسي. نظراً لأن عبء المرض كبير، كان من الضروري تطوير استراتيجيات، من خلال كل من الوقاية والتدخل. بينما يلخص **الفصل السادس** الاكتشافات الحديثة في الفيزيولوجيا المرضية لاضطراب الكرب التالي للصدمة، والأهم من ذلك المؤشرات الحيوية الجديدة التي يمكن أن تساعد في اكتشاف المرض، وبالتالي القابلية للإصابة به وتشخيصه بالنسبة لأولئك الذين تعرَّضوا للصدمات، ويراقبون في النهاية للاستجابة للعلاج. من الضروري الكشف عن العوامل التي تحدِّد القابلية للتأثر من أجل إيجاد تقنيات فعَّالة لبناء المرونة ووقف اضطراب الكرب التالي للصدمة من الحدوث في المقام الأول.

النتائج الواردة في هذه الرسالة تخدم كلاً من الباحثين، وسلطات الصحة العامة. فبالنسبة للباحثين، توفِّر الرسالة مصادر لأحدث المعلومات حول قابلية الإصابة باضطراب الكرب التالي للصدمة، مع إعطاء قوالب للمقارنة مع الدراسات المستقبلية، سواء قبل السريرية أو السريرية. أما بالنسبة لوكالات الصحة العامة والسلطات الصحية، تقدّم الرسالة مؤشرات على قابلية الإصابة بالمرض للتدخل في الوقت المناسب. وعلى الرغم من ذلك، هناك حاجة إلى مزيد من البحث السريري المتعمق لتأكيد موثوقية هذه المؤشرات وفاعليتها من حيث التكلفة. من ناحية أخرى، تم توفير طرق ومنهجيات للوقاية من المرض بطريقة شاملة، التي عند دمجها مع قابلية الإصابة بالمرض، يمكن أن تقلل من عبء المرض على المريض والمجتمع والاقتصاد.

أخيراً في **الفصل السابع والثامن** من هذه الرسالة، قمت بعرض مناقشة عامة لأهم النتائج التي توصلت إليها هذه الرسالة البحثية ومن ثم تلخيص أهم ما جاء فيها.

نبذة مختصرة عن الرسالة

لقد تمَّ الاعتراف بشكل متزايد، بالحاجة إلى إدراج الصحة النفسية ضمن الأولويات الأولى، لأجندة الصحة العامة في العالم على مدى العقود الماضية. الاضطرابات أو الأمراض العقلية، فعلى سبيل المثال اضطراب الكرب التالي للصدمة (PTSD)، والمنتشرة بشكل كبير في العالم، وتفرض عبئًا كبيرًا على الأفراد والمجتمع والاقتصاد. لذلك، أصبح تعزيز الصحة النفسية مدعاة لاستجابة مهمة لهذه التحديات. أعباء الإعاقة والنفقات المجتمعية المرتبطة باضطراب الكرب التالي للصدمة مرتفعة. في عام ٢٠١٨، قُدِّر إجمالي الأثر الاقتصادي لاضطراب الكرب التالي للصدمة في الولايات المتحدة بـ ٢٣٢,٢ مليار دولار، (١٩,٦٣٠ دولاراً لكل مريض باضطراب الكرب التالي للصدمة).

اضطراب الكرب التالي للصدمة (PTSD)، هو حالة يمكن أن تحدث بعد أن يشاهد الأشخاص صدمة أو يمرّون بصدمة. والصدمة هي حدث شديد يتضمَّن الإصابة الخطيرة أو الوفاة، أو فرصة حدوث إصابة خطيرة أو الوفاة. وقد يشمل ذلك الأحداث الطبية، مثل النوبة القلبية أو الجراحة، أو العلاج في وحدة العناية المركزة في المستشفى ("ICU"). وقد يسبِّب اضطراب الكرب التالي للصدمة الكوابيس، والذكريات المزعجة، والقلق وأعراضاً أخرى. وليس كلّ من يشهد صدمة أو يمر بصدمة سيصاب باضطراب الكرب التالي للصدمة. ولا يعلم الأطباء لماذا يُصاب بعض الأشخاص باضطراب الكرب التالي للصدمة، بينما لا يصاب البعض الآخر، وقد يحدث اضطراب الكرب التالي للصدمة في أي عمر.

وفقًا للأبحاث الوبائية، يتراوح معدَّل انتشار التعرض لحادث صادم مدى الحياة، من ٤٠ إلى ٩٠ في المائة، بينما يتراوح معدَّل الإصابة باضطراب الكرب التالي للصدمة على مدى الحياة، من ١ إلى ٩ في المائة فقط، مما يجعله رابع أكثر الاضطرابات النفسية شيوعاً في جميع أنحاء العالم. بشكل عام، الانتشار أعلى بين النساء (١٠-١٢٪ عند النساء و٥-٦٪ عند الرجال).

تُناقش هذه الرسالة الطرق الممكنة، التي يمكن أن تساعد في الكشف المبكِّر عن الاضطرابات الناتجة عن الإجهاد الناتج عن الصدمة والكشف عنها، وإمكانية تطوير علامات زيادة القابلية للتأثر. كما تناقش الرسالة أيضًا كيف يمكن أن يساعد هذا الاكتشاف المبكِّر في التدخل المبكِّر، والوقاية في وقت مبكِّر بما فيه الكفاية. يمكن أن يؤدي تطبيق مثل هذه النماذج، مع نتائج الأبحاث المستقبلية لإنشاء وسائل فعَّالة لتحسين جودة حياة المريض بشكل كبير وتقليل عبء المرض.

تبدأ الرسالة بمناقشة التغيرات الفوق جينية المحتملة المرتبطة بتطور اضطراب الكرب التالي للصدمة. ثم تلخيص الدراسات الحديثة ونتائجها التي يمكن أن تساعد في تحديد مثل هذه التغييرات لمعرفة المرضى المعرَّضين لخطر متزايد، وبالتالي التدخل مبكِّراً في تطبيق التدابير الوقائية. يمكن تطبيق هذه التغييرات فوق جينية في الحياة العملية بمجرد إثبات موثوقيتها وفاعليتها من حيث التكلفة المشار إليها أعلاه. يتحوَّل التركيز إلى تحديد أهم عوامل الإجهاد الذي يعانيه المريض وأكثرها شيوعاً، والعوامل الرئيسة التي يمكن أن تؤثر على الاستجابة للإجهاد الناتج عن الصدمة. تتحوَّل المناقشة إلى النموذج الاجتماعي والاقتصادي للوقاية من الأمراض، وإمكانية تطبيقه للوقاية من اضطراب الكرب التالي للصدمة. يتألَّف النموذج من أربعة مستويات، بدءاً من المستوى الفردي في جوهره، ووصولاً إلى مستويات العلاقات والجماعة والمجتمع. من الناحية العملية، تناقش الرسالة أمثلة للتدابير الوقائية في كل مستوى من مستويات النماذج، عن طريق الأنواع الثلاثة للوقاية، وهي الوقاية الأولية والثانوية والثالثية، من خلال طرح أمثلة من الحياة الواقعية للتدخلات والبرامج القابلة للتطبيق. لإكمال الرسالة، يتم سرد أحدث طرق العلاج المستخدمة لاضطراب الكرب التالي للصدمة بطريقة مفصلة، وكيف يمكن أن تسهم هذه التدخلات في تحسين نوعية الحياة وتقليل الإعاقة، والأهم من ذلك، التخلص من الأعباء الأخرى.

شكرٌ وعرفانٌ

الحمد لله على ما أنعم به عليّ من فضله الكبير والعلم الوفير، وأعانني على إنجاز هذا العمل الذي أحتسبه عبادةً من العبادات جعلها الله خالصةً لوجهه الكريم.

لكل من يستحق، ولكل من ساعدنا في إنجاز وتحقيق هذه الأطروحة، فمن حسن أخلاق المسلم شكر كل شخص أحسن له قريباً كان أم غريباً، قال تعالى: ((ومن يشكر فإنما يشكر لنفسه))
وقال (صلى الله عليه وسلم): "من لم يشكر الناس لا يشكر الله"،
فحمدًا لله وثناءً كما يحب ربنا ويرضى.

ووراء كل نجاح وتقدُّم بعد فضل الله داعمون ومحبون، وزارعون وساقون لما نحصده من ثمار ونعم، وإني لأرجو أن أنْظم عقد شكرٍ ودررٍ لأقلِّدها عنق من أعانوني، كما قلَّدوني عقد فضلهم ودعمهم.
وأخص بالشكر منهم أمي وأبي، اللذَين غرسا فيَّ حُب العلم من الصغر، وقدما لي كل غالٍ ونفيس، وكان لهما الفضل من بعد الله فيما وصلت إليه الآن، فمهما قلت شكرًا فلن أوفِيهما حقهما فيما قدَّماه من سعيٍ ودعم ودعاء أنار لي طريقي وذلل لي كثيرًا من الصعاب.

ثم عقدٌ من درر الشكر لزوجتي ورفيقة دربي، التي تحمَّلت الغربة وصبرت عليَّ ودعمتني بما أوتيت من قوة، ثم لصغيري الحبيب الذي خفَّف عني بابتسامته كثيرًا من الأيام وجعلني أقوم مهما تعثرت وأصرُّ لأجله ولأجل ضحكته.

ثم كل التقدير والحُب والاحترام لأساتذتي المشرفين على رسالتي، على ماقدَّموه لي من علم نافع وعطاء متميز وإرشاد مستمر، وعلى ما بذلوه من جهد متواصلٍ ونصحٍ وتوجيهٍ من بداية مرحلة البحث حتى إتمام هذه الرسالة، وعلى جهدهم المبذول بحب كبير.

ثم الشكر لنجوم سمائي البرَّاقة التي لم يخفت بريقها أبدًا، زملائي الذين ساعدوني في إتمام عملي وسهروا معي وتواصلوا دائمًا بحب للخروج بأفضل صورةٍ ونتيجة.

والشكر موصولٌ إلى كل فرد أسدى لي مساعدةً أو معروفًا في إدارة جامعة ماستريخت (هولندا)، الذين كانوا خير عون، فعملهم معي معروفٌ دائم وجميلٌ محفوظٌ لا ينسى.

ويسرني أيضًا أن أتقدَّم بالشكر والعرفان لوطني الغالي وحكومتي الرشيدة، (المملكة العربية السعودية) على الدعم المتواصل خلال فترة دراساتي السابقة والحالية، كما أود أن أوجِّه شكراً خاصاً لمعالي مدير جامعة الملك فيصل وجميع الوكلاء والعمداء ورؤساء الأقسام والإداريين، وجميع الدكاترة والأساتذة والزملاء.

وأخيراً، لكل من مد لي يد العون، أو أسدى لي معروفاً، أو قدَّم لي نصيحةً، أو كان له إسهاماً صغيراً أو كبيراً في إنجاز هذا العمل فله مني خالص الشكر والتقدير.

لكل مبدعٍ أنجز معي، ولكل محبٍّ أعانني، قصيدة شكرٍ واجبه عند جني الثمر وتحقيق الهدف، فللأيام التي قضيتها معكم أريجٌ عالق بذهني وحلاوة اشعرها بقلبي، أهديكم أزهار المحبة وشكراً من الأعماق لا حد له.

والحمد لله رب العالمين أولاً وآخرًا، ظاهرًا وباطنًا، عدد خلقه ورضا نفسه وزنة عرشه ومداد كلماته، والصلاة والسلام على نبينا محمد وعلى آله وصحبه أجمعين.

دراسات الطب الحيوي والصحة العامة حول قابلية الإصابة باضطراب الكرب التالي للصدمة

غازي بن إبراهيم بن عبدالرحمن الجوف