

Physiological biomarkers of chronic stress: A systematic review

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ABSTRACT

Objective: The basic objective of this systematic review was to identify potential biomarkers for chronic stress.

Methods: A systematic review of studies linking biomarkers in people with chronic stress was conducted using PRISMA guidelines. The last 40 years' studies were included in the systematic review with no age restrictions; animal studies were excluded from the study. Electronic databases including PubMed, Embase, and Google Scholar were searched for the study purpose. The studies were searched using the combinations of search terms that comprised chronic stress together with the keywords hypothalamic-pituitary-adrenal axis (HPA axis), autonomic nervous system (ANS), immune system, metabolic biomarkers, cortisol, hair cortisol, salivary cortisol, urinary cortisol, epinephrine, norepinephrine, adrenocorticotropic hormone (ACTH), brain-derived neurotrophic factor (BDNF), metabolic biomarkers, antioxidants, glucose, hemoglobin, C-reactive protein (CRP), cytokines, pro-inflammatory cytokines, anti-inflammatory cytokines, and tumor necrosis factor (TNF).

Results: A total of 37 studies out of 671 studies met the eligibility criteria and were included in this review. Potential diagnostic biomarkers of chronic stress included cortisol, ACTH, BDNF, catecholamines, glucose, HbA1c, triglycerides, cholesterol, prolactin, oxytocin, dehydroepiandrosterone sulfate (DHEA-S), CRP, and interleukin - 6 and 8. While the others including antioxidants and natural killer (NK) cells require further validation. Taken together, addition, these stress biomarkers have critical prognostic capacities for stress-associated diseases and therapeutic guidance.

Conclusion: This systematic review provides an update to the literature by highlighting the role of physiological biomarkers in chronic stress and describing their prognostic and therapeutic values.

Keywords: Chronic stress, physiological biomarkers, stress, diagnosis, prognosis

Introduction

Stress is a usual psychophysiological response generated by the body due to the undesirable, challenging, and difficult circumstances or stressors.^[1-3] The stress effects on the nervous system are known for more than 50 years;^[4] and literature confirms the adverse effects of stress on the human brain.^[5] The structural changes, like that in brain atrophy due to chronic stress,^[6] lead to differential response and its impact on cognition and memory is also reported.^[5] The intensity of effects varies as per the duration of stress, and it causes long-term structural defects to the brain, leading to psychological alterations.^[5,7] Any traumatic event can serve as the trigger, for instance, being part of a road accident and getting a lifetime disability after it. Sometimes even witnessing any horrible event can cast a lifelong impact on mental health and well-

being.^[6] Furthermore, maternal separation is also determined as a powerful stressor that affects the individual in the postnatal period, ultimately extending till adulthood.^[5]

Acute stress refers to a short-term and adaptive state. In contrast, chronic stress is a long-lasting condition known to be related to maladaptive response, implying harmful effects on bodily mechanisms.^[3,8] Persistent or prolonged stress results in the secretion of certain hormones or chemicals from the body, which indicates the body's constant stressful condition and affects the vital organs such as the brain, heart, or liver, in various aspects that might not favor the subject's health. There are many systems in the body that either individually or collectively regulate the level of stress,^[9,10] including the hypothalamic-pituitary-adrenal axis (HPA-Axis), Autonomic Nervous System (ANS), and immune system.^[11-14] HPA axis

reacts to stressors by secreting cortisol, a chemical that prepares the body for fight and flight conditions and is a significant biomarker for stress.^[9] Cortisol is mostly concerned with psychological stress making HPA axis a system that mainly responds to psychosocial stress and interacts with ANS and the immune system.^[9,10] Due to this interaction, the HPA axis is considered the mediating system and used to determine the effects of stress on disease processes, playing an essential role in cognition, metabolism, behavior, and immune reactivity.^[9,15] Although usually, the cortisol level remains high in the body at specific times of the day, for instance, in the morning, if it is persistent throughout the day, it is an alarming situation. Cortisol levels are detectable through various biological media such as saliva, blood, urine, and hair sample.^[9]

As mentioned, ANS also contributes to acute and chronic stress generation and regulation. It regulates bodily functions by autonomic reflexes in response to external (environmental, vision, smell, touch, etc.) and internal stimuli (maintenance of body homeostasis including temperature, blood sugar, removal of excess water, obesity etc.).^[12,16-19] Epinephrine, norepinephrine, and acetylcholine (ACh) are the prime neurotransmitters released by the sympathetic and parasympathetic ANS. During a stressful condition, catecholamines level increases while acetylcholine decreases, regulating the fight, and flight response.^[13,20] The HPA-axis and ANS regulate a series of non-immunological biomarkers, for example, arginine vasopressin (AVP) and dehydroepiandrosterone that are not involved in an immune response. Still, a few of the immunological biomarkers regulated by these are accountable for the immune system's response to stress. The body's immune system has fought against these stressors; initiation of such stressful events, for instance, any injury or infection, promotes immune cells' release into the bloodstream, which then induce fight and flight response.^[21] Increase inflammatory responses due to the immune system dysregulation among acute and chronic stress subjects, as the level of pro-inflammatory cytokines rapidly excels under both conditions increasing the likelihood of chronic diseases and associated frailty.^[22-24] Moreover, chronic stress's health consequences are severe compared to acute and lead to latent viral activation, ultimately affecting the immune system.^[25] These responses and their intensity may vary from person to person, as an exaggerated immune response to stress might be generated among some individuals.^[26,27]

These immunological biomarkers are mainly cytokines, that is, interleukin 6 (IL-6),^[9,28,29] IL1- beta,^[9] C-Reactive Protein (CRP),^[9,30] tumor necrosis factor-alpha (TNF-alpha)^[9,28] also termed as pro-inflammatory cytokines, and natural killer cells (NK).^[31-33] These immunological indicators and mediators, in particular cytokines and CRP, not only prepare the body against pathogens but also play an essential role in stimulating either acute or chronic stress concerning psychological stimuli or social interactions.^[34]

Along with these specified mediators, there are also other metabolic biomarkers such as Fasting glucose, glucose tolerance,^[35,36] glycosylated hemoglobin (HbA1c),^[37] triglycerides, and cholesterol levels,^[38,39] for examining the association of chronic stress with prolonged diseases. Other than these stress hormones, endocrine hormones such as prolactin,^[40,41] estradiol,^[42] oxytocin,^[40] Growth Factor (GF), and Dehydroepiandrosterone Sulfate (DHEA-S)^[43,44] are also studied to evaluate the level of chronic stress through blood samples. Hence, these neural endocrine-immune system interactions play an essential role in shaping an individual's reactivity to persistent stress. In addition to these hormones, the human body also has a complex array of enzymatic and non-enzymatic antioxidants such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GTPx), malondialdehyde (MDA) and Ascorbic Acid, which are involved in antioxidant defense system, that is one of the natural defense systems against the harmful effects of reactive oxygen species (ROS) inducing oxidative stress.^[45-47]

Therefore, it provides a useful link to understand the association between stress and diseases also specifies the significance of testing these biomarkers among stressed subjects in routine clinical practices.

Methodology

Study design and search strategy

A systematic search of articles was conducted using PubMed, Embase, and Google scholar, using the combinations of search terms such as chronic stress together with the keywords HPA-Axis, ANS, immune system, metabolic biomarkers, cortisol, hair cortisol, salivary cortisol, urinary cortisol, epinephrine, norepinephrine, Adrenocorticotrophic hormone (ACTH), BDNF, metabolic biomarkers, antioxidants, glucose, hemoglobin, CRP, cytokines, Pro-inflammatory cytokines, anti-inflammatory cytokines, and TNF.

Participants

The study population was human subjects. Since the aim of the study is to identify the potential biomarkers for chronic stress. Hence, all similar studies with the selected biomarkers, without restricting age and gender of the participants, were studied.

Data sources, studies selection, and data extraction

Studies of the last 40 years were included in the systematic review. All the articles fulfilling the eligibility criteria were read thoroughly for relevancy. The data were extracted, screened, and scrutinized for the robustness of the article and errors in the inclusion, to confirm the authenticity of the review. The primary information sources were the published research articles, while the reference lists of eligible articles were also searched and scrutinized to obtain all potential data.

Quality of studies

The quality of the included studies was assessed on the basis of selection bias, study design, confounders, and data collection methods.

Inclusion and exclusion criteria

Eligible studies reporting empirical findings on the association between physiological biomarkers and chronic stress were included in the study. Studies of the last 40 years were included in the systematic review with no age restrictions, only human studies published in English were included in the study. While all studies on non-human subjects, outside specified time duration, review articles, and studies with biomarkers other than those studied were excluded from this review.

Data analysis

A systematic summary was established through data synthesis including extraction, and management of the studies. The results were then screened, and combined according to the PRISMA guidelines.

Results

The systematic search and review yielded 628 studies from electronic database searching and 43 additional studies identified through other sources. A total of 616 non-human subject studies were excluded and remaining 55 full-text experimental studies were assessed for eligibility. Finally, 37 studies were included in the quantitative synthesis [Figure 1].

Biomarkers of HPA-axis

Cortisol

Among the biomarkers of HPA-Axis in relation to chronic stress, cortisol, ACTH, and BDNF were studied. A total of nine studies supported cortisol measurement as a chronic stress biomarker through hair samples. HPA-axis starts from the hypothalamus by secreting the corticotropin-releasing hormone (CRH) is secreted that further induces the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary stimulating the secretion of cortisol.^[9] Cortisol is mainly synthesized from cholesterol as the main glucocorticoid in zona fasciculata of the human adrenal cortex secreted by the influence of biochemical stress.^[15,48,49] Cortisol is present as bound and unbound form; the unbound cortisol is of low molecular weight and is lipophilic. It enters the cell through passive diffusion, thus, easily determined in body fluids.^[50] The blood cortisol measurement timings have significant importance as it is increased in blood cortisol level in the early morning and decreases in the evening time and initial phase of sleep.^[50] Herein, we focused on those studies that helped us identify cortisol as a biomarker of chronic stress and cortisol quantification methods through hair samples, saliva, and urine. Rael *et al.* were the first who examined cortisol through hair samples.^[51]

Hair cortisol

According to Table 1, hair cortisol is demonstrated as a potential clinical biomarker for infants to address neonatal chronic stress better using a baseline measure

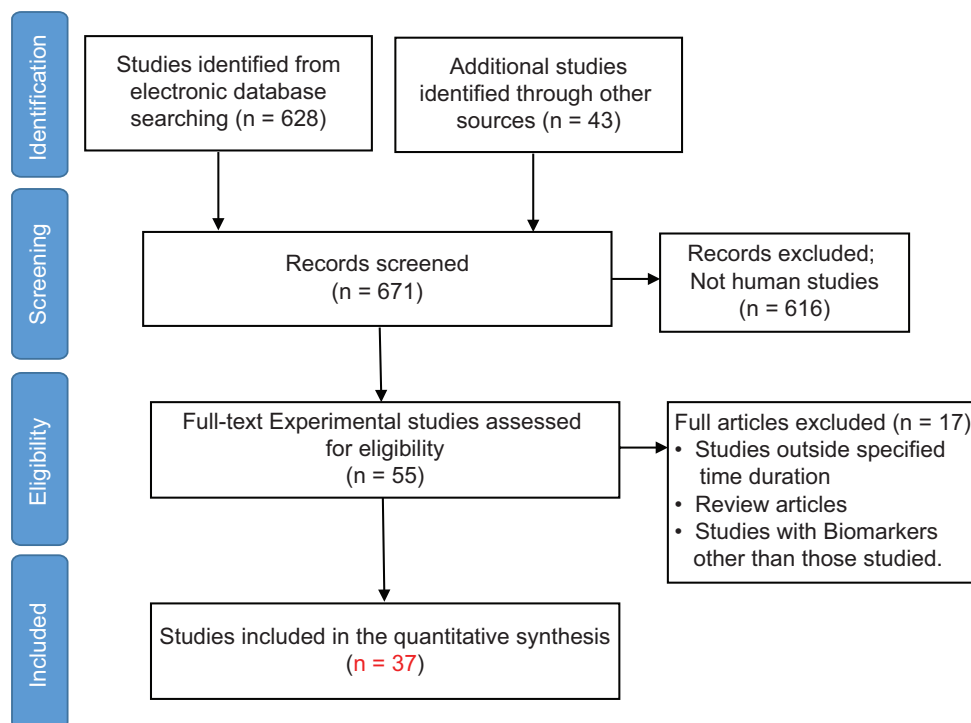


Figure 1: Flow diagram for study selection in this systematic review

comparison in which hair cortisol is influenced by day of ventilation in the neonatal intensive care unit (NICU).^[52] Moreover, another study assessed stress among mothers and their infants, using intra stability of hair, cortisol suggests its potential biomarker in detecting chronic stress experiences during the first year of life and the postpartum period.^[53] Furthermore, hair cortisol can be a good source for measuring chronic stress in pregnant women.^[54] This long-term biomarker provides a vital implication in clinical practices and research.^[54] Moreover, another study examined the role of cortisol in pregnant women's hair. This study suggested that hair cortisol is a potential biomarker of chronic stress. Hence, this long-term biological marker has a key association in clinical research practices.^[54-57]

The quantification of scalp hair cortisol in children seems to be a biomarker for long-term cortisol measurement and thereby a biomarker of stress. A study examined HCC in children in

the early phase of elementary school. It proposed a high-stress hormone level in fearful children at that stage.^[58] Furthermore, another study demonstrated that the chronic stress evaluated by increased cortisol levels in hair were positively associated with obesity measures among children with disabilities.^[59] Besides, Quellete *et al.* examined the validity of hair cortisol concentration (HCC) in mother and daughter characterized by higher and lower maternal chronic stress, and overall findings indicated HCC as a useful biomarker of cortisol in response to chronic stress.^[60]

Moreover, chronic stress is associated with low socioeconomic status (SES) in children of young age and adolescent population by activating HPA-axis and releasing a high level of the stress hormone cortisol. According to this study, chronically high hair cortisol levels were found in children of young age and adolescents in low SES.^[61] However, older people with increasing age have more chances for a stress-related disease that correlates with chronically elevated cortisol secretion.^[62]

Table 1: Studies on hair cortisol

Author	Year	Sample Size	Technique	Outcome
Yamada <i>et al.</i> ^[52]	2007	Infants ($n=60$) of more than 25 weeks at the time of birth	ELISA	Possible association of hair cortisol and chronic stress in infants
Liu <i>et al.</i> ^[53]	2016	Infants ($n=47$) and mothers ($n=41$)	Enzyme immunoassay	It is suggested that hair cortisol can be used as a potential biomarker for chronic stress detection within the 1 st year post-birth, and in postpartum state.
Kalra <i>et al.</i> ^[54]	2007	Pregnant females ($n=25$) having age between 18 and 45 years.	ELISA	Hair cortisol is suggested to be a possible biomarker of chronic stress in gestation.
Janssen <i>et al.</i> ^[56]	2017	Males ($n=61$) and females ($n=41$) having a mean age of 43.4 years.	Liquid chromatography-tandem mass spectrometry	The applicability of Hair Cortisol Concentration (HCC) is suggested to be limited regarding it being considered as a job stress biomarker; however, HCC can be used for detection of early symptoms of stress-associated illnesses, such as depression.
Chen <i>et al.</i> ^[59]	2015	Children ($n=87$) and caregivers ($n=86$)	Liquid chromatography-tandem mass spectrometry	The chronic stress of caregivers was assessed by elevated levels of hair cortisol concentration, which was found positively related to obesity in children having disabilities.
Ouellette <i>et al.</i> ^[60]	2015	Mothers ($n=60$) and daughters ($n=60$)	ELISA	The relationship between mother and daughter hair cortisol concentration was found strongly associated with a negative parenting state.
Vliegenthart <i>et al.</i> ^[61]	2016	Children and adolescents ($n=270$) having age between 4 and 18 years.	Liquid Chromatography-Tandem Mass Spectrometry	Increased cortisol levels are identified in children and adolescents belonging to families of the low socioeconomic state. Furthermore, the scalp hair cortisol estimation can be used for chronic stress analyses.
Feller <i>et al.</i> ^[62]	2014	Participants ($n=654$) having a mean age of 65.8 years, range: 47–82 years)	Chemiluminescence immunoassay	The findings confirmed previously reported association of hair cortisol concentration with age, gender, usage of alcohol, and type 2 diabetes mellitus, and also with smoking (a new factor presented by this study), which may lead to increased levels of cortisol in older people.
Henley <i>et al.</i> ^[63]	2013	Participants ($n=55$)	ELISA	Elevated concentration of hair cortisol was found in Walpole Island First Nation compared to non-First Nation volunteers, which represents increased chronic stress state, maybe due to factors including the socioeconomic and poor state of health.

Table 2: Studies on salivary cortisol

Author	Year	Sample Size	Techniques	Outcome
De <i>et al.</i> ^[64]	2003	Burnout patients (<i>n</i> =22) and healthy participants (<i>n</i> =23)	Enzyme immunoassay	Burnout patients were found to have increased cortisol levels in the first-hour post-awakening compared to healthy subjects.
Schulz <i>et al.</i> ^[65]	1998	Females (<i>n</i> =51) and males (<i>n</i> =49) having a mean age of 25.7 years (range 19-55 years)	Time-resolved immunoassay with fluorescence detection	Subjects who were chronically stressed showed an elevated level of cortisol post-awakening in comparison with non-stressed participants, and this increase in cortisol level was identified to be more in women who were chronically stressed versus men.
Soderstrom <i>et al.</i> ^[66]	2006	Participants with high (<i>n</i> = 9) and low (<i>n</i> = 11) burnout scores assessed by modified Shirom-melamed Burnout Questionnaire	Radioimmunoassay	Increased awakening cortisol in workday compared to the weekend and enhanced state of activation as well as mental fatigue on weakened which may exhibit an impaired state of recovery was identified for high burnout group compared to low burnout group. An increased stress-mediated arousal rate before sleep was linked to elevated cortisol's diurnal amplitude, and also to cortisol's earlier diurnal peak in the workday.
Grossi <i>et al.</i> ^[67]	2005	Patients have taken sick leaves because of burnout (<i>n</i> =22), and working participants with low (<i>n</i> =22) and intermediate (<i>n</i> =20) scores obtained on the Shirom-Melamed Burnout Questionnaire	Radioimmunoassay	Increased levels of cortisol (morning) were found in female burnout patients, however in males elevated cortisol levels were identified with moderate burnout state, and not in male patients or healthy male subjects.
Melamed <i>et al.</i> ^[68]	1999	Non shift blue-collar workers (<i>n</i> =111)	Radioimmunoassay	Chronic burnout state is found related to enhanced somatic arousal and also to increased concentrations of salivary cortisol.
Powell <i>et al.</i> ^[69]	2002	Chronically stressed women due to experiencing divorce or separation (<i>n</i> =20), and non-stressed women due to the stable nature of marriages (<i>n</i> =20)	Radioimmunoassay with time-resolved fluorometric detection	Stressed women were found to have increased levels of evening salivary cortisol compared to non-stressed women.
Siddiqui <i>et al.</i> ^[70]	2019	Newly detected diabetic Mellitus (NDDM=125) and normal glucose tolerance (NGT=125) Subjects	Radioimmunoassay method	Higher levels of oxidative stress and inflammation in association with significantly higher chronic psychological stress in patients with newly detected diabetes mellitus

Table 3: Study on urinary cortisol

Author	Year	Sample Size	Techniques	Outcome
Moch <i>et al.</i> ^[71]	2003	Women (South African) with and without burnout having ages between 25 and 59 years (<i>n</i> =16) controls (<i>n</i> =16)	Radioimmunoassay	Reduced urinary free cortisol was determined in burnout patients compared to control subjects and stress management intervention did not lead to the restoration of this hypocortisolism state in burnout patients who appeared to exhibit improvement in clinical and psychological condition. Furthermore, other hormonal and biochemical parameters studied remained insignificantly different.

Table 4: Studies on adrenocorticotrophic hormone

Author	Year	Sample size	Techniques	Outcome
Moch <i>et al.</i> ^[71]	2003	Women with and without burnout having ages between 25 and 59 years Tests (<i>n</i> =16) controls (<i>n</i> =16)	Radioimmunoassay	Reduced urinary free cortisol was determined in burnout patients compared to control subjects and stress management intervention did not lead to the restoration of this hypocortisolism state in burnout patients who appeared to exhibit improvement in clinical and psychological condition. Furthermore, other hormonal and biochemical parameters studied remained insignificantly different.
Borders <i>et al.</i> ^[72]	2015	Non-Hispanic black (NHB) women (<i>n</i> = 55) and non-Hispanic white (NHW) women (<i>n</i> = 57)	Enzyme immunoassay	NHB women were found to have elevated levels of CRP and ACTH in the second and third trimester of gestation, compared to NHW women.

HCC showed a positive relation with age, female sex, alcohol consumption, and other factors such as smoking, contributing to significantly elevated cortisol levels in older people.^[62] Moreover, increased hair cortisol suggested a high level of chronic stress among the Walpole island first nation volunteers compared to volunteers of the non-first nation.^[63] Thus, the most reliable biomarker of chronic stress and the most readily used validation exhibited hair cortisol measurements.

Salivary cortisol

There were a total of seven studies for salivary cortisol as a biomarker of chronic stress (Table 2). The use of salivary cortisol as a stress biomarker increases and provides useful results when estimating cortisol through saliva samples.^[64-70] However, when investigated the role of the physiological stress response and basal physiological values in health and stress (burnout), individual study suggested that stress (burnout) patients showed higher heart rate and high cortisol level during the 1st h after awakening from the sleep when compared with healthy subjects.^[64] Furthermore, this study also suggested the association between chronic stress and cortisol changes during the 1st h of post-sleep in the morning. The salivary cortisol was found to be elevated in chronically stressed subjects compared to unstressed subjects.^[65] According to Soderstrom *et al.*, 2006, burnout (stress), patients showed a high awakening cortisol response during the workday than during the weakened.^[66] However, when examined the role of free cortisol response after awakening among both genders, the study suggested high cortisol response in females with high (stress) burnout than to females with low burnout (stress) at awakening and +15 min, +30 min, and +60 min after awakening whereas, male participants with moderate burnout (stress) showed high cortisol level at +60 min, after awakening than to low burnout (stress) male participants.^[67] Therefore, the measurement of free cortisol in response to awakening should be considered a possible biomarker for chronic stress.^[64] Moreover, this study examined whether chronic stress (burnout) is associated with somatic and physiological arousal, intensified somatic arousals and elevated salivary cortisol levels.^[68] In addition, the study for determining the physiological biomarker of chronic stress through a salivary sample on middle-aged women revealed that stressed women have high evening (9 pm) salivary cortisol level and they had high urinary testosterone from the first-morning void whereas no association found between overnight urinary cortisol and catecholamines Table 6.^[69]

Urinary cortisol

Free cortisol was found in chronically stressed (burnout) patients when compared to control healthy subjects. Hormonal and biochemical changes remained indifferent in these patients versus control subjects. These results indicated hypocortisolism in the stressed patient Tables 3 and 6.^[71]

Adrenocorticotrophic hormone (ACTH)

Corticotrophin releasing hormone (CRH) is a crucial mediator of endocrine, autonomic, and immune responses to stress.

There are two studies in which ACTH was used as a measure to evaluate chronic stress (Table 4).^[71,72]

Brain-derived neurotropic factor (BDNF)

BDNF is considered necessary in the regulation, development, and survival of neurons and the maintenance of their physiological activity.^[35] However, the studies suggested that the stress (burnout) subjects have significantly lowered the level of BDNF and symptoms such as altered mood and cognition factor when compared with healthy control subjects (Table 5).^[36]

Biomarkers of Autonomic Nervous System (ANS)

Two physiological systems commonly; HPA-axis and sympathetic adrenergic medullary-axis (SAM) are involved in biomarkers of the autonomic nervous system. HPA-axis has a slow response toward a stressor whereas the SAM axis activates instantaneously and shows a very adaptive response to a stressor.^[9,10] Moreover, SAM deals with immediate sympathetic activation. Hence, it stimulates the ability of an individual to cope with the conditions such as increased heart rate, blood pressure, and the release of catecholamines which include epinephrine and norepinephrine.^[65]

Catecholamines

Catecholamine is one of the major biomarkers for the ANS activity. Epinephrine and norepinephrine are mainly synthesized by the central noradrenergic neurons and are released mainly from the adrenal medulla in the bloodstream.^[20]

Biomarkers of Metabolic Processes

Like HPA-axis and SAM-axis, several metabolic biomarkers play a major role in the physiology of stress, entailing a group of biomarkers such as glucose, glycosylated hemoglobin (HbA1c), cholesterol, and insulin. Thus, categorizing this group as metabolic biomarkers can be an effective tool for a biological measure of chronic stress level in the body.

Glucose, HbA1c, triglycerides, and cholesterol

The first study in Table 7 shows that the association of chronic stress with fasting glucose, glucose tolerance, HbA1c also involves the indirect effect of stress through inflammatory marker CRP.^[73,74] However, it was found that high chronic stress leads to elevated levels of fasting glucose, post loaded glucose, and HbA1c level that there was no indirect effect of stress with CRP, this result suggested that high chronic stress is associated with poor glucose regulation in Hispanics subjects before onset of

clinical diabetes diagnosis.^[42]**Endocrine Hormones****Table 5:** Study on brain-derived neurotropic factor

Author	Year	Sample Size	Techniques	Outcome
Sertoz <i>et al.</i> ^[35]	2008	Burnout (<i>n</i> =37) and healthy subjects (<i>n</i> =35)	ELISA	Lowered levels of BDNF were found in burnout participants compared to healthy controls, which is suggested to possibly participate in burnout syndrome neurobiology and the processing of burnout symptoms.

Table 6: Studies on catecholamine

Author	Year	Source	Sample size	Techniques	Outcome
Powell <i>et al.</i> ^[69]	2002	Urine	Chronically stressed women due to experiencing divorce or separation (<i>n</i> =20), and non-stressed women due to the stable nature of marriages (<i>n</i> =20)	High-Performance Liquid Chromatography with Electrochemical Detection	Stressed women had a non-significant trend toward elevated platelet catecholamine.
Moch <i>et al.</i> ^[71]	2003	Blood	Women with and without burnout having ages between 25 and 59 years Tests (<i>n</i> =16) controls (<i>n</i> =16)	High-pressure liquid chromatography	Reduced urinary free cortisol was determined in burnout patients compared to control subjects and stress management intervention did not lead to the restoration of this hypocortisolism state in burnout patients who appeared to exhibit improvement in clinical and psychological condition. Furthermore, other hormonal and biochemical parameters studied remained insignificantly different.

Table 7: Studies on glucose, HbA1c, cholesterol, and triglycerides

Author	Year	Biomarker	Source	Sample Size	Techniques	Outcome
McCurley <i>et al.</i> ^[41]	2015	Glucose, HbA1c	Blood	923 non-diabetic men and women aged between 18 and 74	Hexokinase enzymatic method (Fasting glucose and post-load glucose were measured in plasma) OGGT (Glucose tolerance) Tosoh G7 Automated HPLC Analyzer (HbA1c)	There was no indirect effect of stress through Inflammation. Hence suggest that higher chronic stress is associated with poorer glucose regulation.
Aguiló <i>et al.</i> ^[40]	2017	Glucose, HbA1c	Blood	Non-caregivers (<i>n</i> =19), geriatric caregivers (<i>n</i> =17) & oncologic caregivers (<i>n</i> =20)	Plasma samples biochemical and immunological analysis HbA1c: ethylenediaminetetraacetic acid (EDTA).	Caregivers of oncologic patients perceive higher stress and have higher levels of glucose in plasma than caregivers of geriatric patients with chronic diseases
Grossi <i>et al.</i> ^[73]	2003	HbA1c	Blood	High Burnout subjects (<i>n</i> =43) and Low Burnout Subjects (<i>n</i> =20)	Radioimmunoassay	TNF- α and HbA1c are physiological correlates of burnout among women. It enhances the inflammatory responses and results in oxidative stress.
Melamed <i>et al.</i> ^[74]	1992	Glucose, Cholesterol, Triglyceride	Fasting blood glucose	104 healthy male individuals in age ranged between 24 and 68 years. Tense burnout (<i>n</i> =68) Listless burnout (<i>n</i> =36)	Enzymatic method for glucose and cholesterol	Tense-burnout was related to reduced well-being (increased somatic complaints) as well as to elevated levels of cholesterol, uric acid, and glucose, and marginally higher levels of triglycerides
Siddiqui <i>et al.</i> ^[70]	2019	Glucose, HbA1c	Blood	Newly detected Diabetic mellitus (NDDM=125) and normal glucose tolerance (NGT=125) Subjects	Glucose-Glucose Oxidase Hb1Ac-HPLC	Higher levels of oxidative stress and inflammation in association with significantly higher chronic psychological stress in patients with newly detected diabetes mellitus

Table 8: Studies on prolactin and oxytocin

Author	Year	Biomarker	Source	Sample	Techniques	Outcome
Tops <i>et al.</i> ^[75]	2007	Prolactin, Oxytocin	Blood	Nine healthy volunteers (mean age 34 years, SD = 8) and 9 subjects fulfilling criteria for burnout (mean age 35 years, SD=9), all females	Chemiluminescence micro partial immunoassay (prolactin) Radioimmunoassay (oxytocin)	The burnout subjects displayed a significantly bimodal extreme distribution of basal prolactin levels, either displaying higher or lower levels compared to the controls.
Lennartsson <i>et al.</i> ^[76]	2014	Prolactin	Blood samples	Burnout n=24 men n=25 women No burnout n=25 men n=13 women	Immunochemiluminometric assay	This study indicates that prolactin levels are higher in men with burnout than men without burnout but not affected in women with burnout.

Table 9: Studies on Dehydroepiandrosterone sulfate and Cytokines

Author	Year	Biomarker	Source	Sample size	Techniques	Outcome
Mommersteeg <i>et al.</i> ^[77]	2006	DHEA-S, Cytokines (TNF-a and IL-10)	Saliva, Blood	56 persons with burnout and 38 healthy control subjects	DHEA (Chemiluminescence assay) ELISA (Cytokine)	The burnout group showed higher DHEAS levels but no difference in cortisol levels after awakening or after dexamethasone intake in comparison to controls. Production of the anti-inflammatory cytokine IL-10 by monocytes was increased in individuals with burnout syndrome. T B NK CELLS shows no change
Grossi <i>et al.</i> ^[73]	2003	DHEA-S, Tumor necrosis factor-alpha (TNF-a)	Blood	High Burnout subjects (n=43) and Low Burnout Subjects (n=20)	Radioimmunoassay	TNF-a and HbA1c are physiological correlates of burnout among women.
Lennartsson <i>et al.</i> ^[27]	2015	DHEA-S	Blood Fasting glucose overnight samples	Burnout n=122 (62 men 60 women) healthy control n=47 (24 men 23 women)	Electrochemiluminescent immunoassay	The results show that, in the youngest group (25–35 years), DHEA-s levels were significantly lower in the patients than in the healthy controls, but among the other age groups (36–45 and 46–54 years) there were no differences in DHEA-s levels between patients and controls
Lennartsson <i>et al.</i> ^[32]	2016	DHEA	Blood	122 patients with clinical burnout. 62 men 60 women	Electrochemiluminescent immunoassay	About half of the patients exhibited increased DHEA-S levels during the year, while the other half exhibited decreased levels

Table 10: Studies on superoxide dismutase and catalase

Author	Year	Biomarker	Source	Sample	Sample Size	Techniques	Outcome
Moragón <i>et al.</i> ^[78]	2005	SOD, Catalase	Blood	Prehospitalary emergency service healthy workers	111 healthy workers of both sexes (69 men and 42 women), aged 20–69 years.	SOD activity (Minami and Yoshikawa method ^[80]) Catalase Activity (Aebi method ^[81])	Insignificant gender-associated changes were identified in activities of SOD and catalase and burnout conditions. Markedly evident changes in SOD activity were determined between groups of control subjects and pre-hospital emergency service workers. Furthermore, SOD activity was found to be higher in night shift workers versus evening shift workers, who also displayed higher but statistically insignificant burnout subscales scores.

Table 11: Study on Malondialdehyde

Author	Year	Source	Sample size	Techniques	Outcome
Casado <i>et al.</i> ^[28]	2006	Blood	Healthy workers of pre-hospital emergency service. Men ($n=69$) and women ($n=42$) between ages of 20 and 69 years	High-pressure liquid chromatography (Bull and Marnett method ^[83])	A positive relation was identified between MDA and occupational stress.

Table 12: Studies on anti-inflammatory and pro-inflammatory cytokines

Author	Year	Biomarker	Source	Sample Size	Techniques	Outcome
Von Känel <i>et al.</i> ^[31]	2008	TNF-a (pro-inflammatory), IL-6, IL-10 (anti-inflammatory)	Fasting blood samples	167 School teachers (median, 48 years; range, 23–63 years; 67% women)	ELISA	Burnout is associated with an imbalance between pro- and anti-inflammatory forces by increasing pro-inflammatory and decreasing the anti-inflammatory activity of the innate immune system.
Tian <i>et al.</i> ^[30]	2017	IL-6	Blood sample	Healthy volunteers (aged 18-30 years old), 10 males	Milliplex Map	Cytokine changes act as potential diagnostic biomarkers of stress-relevant diseases
Kiecolt-Glaser <i>et al.</i> ^[29]	2003	IL-6	Blood	225 adult participants (119 caregivers and 106 controls)	Immunoassay	Caregivers' average rate of increase in IL-6 was about four times as large as that of non-caregivers suggests that a chronic stressor is capable of, effectively prematurely aging the immune response.
Dutheil <i>et al.</i> ^[86]	2013	IL-8	Urine Sample	$n=17$ Emergency physicians	Enzyme-linked immunoassay	Urinary interleukin-8 is a strong biomarker of stress.

Table 13: Studies on Natural Killer cells and leukocytes

Author	Year	Biomarker	Source	Sample size	Techniques	Outcome
Bargellini <i>et al.</i> ^[87]	2000	NK cells	Blood	Physicians ($n=71$)	NK cell population was measured using indirect immunofluorescence and NK cell activity was determined by non-radioactive chromium (Cr) release assay from labeled K562 tumor cells.	No correlation was identified for NK cells and burnout.
Nakamura <i>et al.</i> ^[89]	1999	NK cells	Blood	Male workers between the ages of 36 and 51 years (SD: 44.9 ± 4.3) ($n=42$)	NK cells activity (chromium (Cr)-release assay) and NK cells subsets (monoclonal antibodies, and two-color flow cytometry)	NK cell activity was found correlated with depersonalization, and this association was determined to be independent of stress and health behaviors.

Prolactin and oxytocin

Many other hormones such as prolactin, oxytocin, Dehydroepiandrosterone Sulfate (DHEA-S), and growth hormone are studied in various stress groups to determine the effects of these endocrine hormones as a physiological biomarker (Table 8).^[75,76]

Dehydroepiandrosterone sulfate (DHEA-S)

It is a steroid hormone with a capability of immunomodulatory function opposite to cortisol, and it is produced by the zona - reticularis in the adrenal cortex in response to ACTH Table 9.^[43,45,77] It has a significant role as a regeneration and protection function that's leads to maintenance of health status,^[46] whereas increased and decreased levels of DHEA-S are associated with the diagnosis of health issues and outcomes.

Antioxidants

Several enzymes such as SOD, catalase, and MOD are involved in the body's natural defense system against reactive oxygen species (ROS), contributing to aging, and disease-related problems.^[78]

SOD and catalase

The role of two antioxidant enzymes, SOD and catalase, in stress in healthy workers has been studied,^[78,79] it was shown that SOD was higher in workers of evening and night shift Table 10.

Malondialdehyde (MDA)

The stressful condition leads to free radicals along with MDA as an end product of lipid peroxidation. The relationship between MDA and burnout levels in workers, when identified at night and evening show increased level of MDA and high-

Table 14: Studies on C-reactive protein and fibrinogen

Author	Year	Biomarker	Source	Sample	Techniques	Outcome
Toker <i>et al.</i> ^[93]	2005	High-sensitivity C-reactive protein (hs-CRP) and fibrinogen	Blood sample (post-overnight fasting)	Healthy Women ($n=630$) and men ($n=933$) having a mean age of 44.8 ± 11.02 years and 45.9 ± 10.2 years, respectively.	hs-CRP (immunonephelometric assay centered on particle-enhanced immunonephelometry), and fibrinogen (Claus method ⁹⁰)	In women, burnout was related positively to the hs-CRP and fibrinogen levels, whereas anxiety was related negatively to their levels. In men, depression, but not burnout and anxiety, was related positively to hs-CRP and fibrinogen levels.
Gouin <i>et al.</i> ^[85]	2012	CRP and interleukin-6 (IL-6)	Blood sample	Caregivers ($n=53$) and non-caregiving controls ($n=77$)	CRP (chemiluminescence method) and IL-6 (immunoassay)	Caregivers were found to undergo different stressors (in the period of last 24 h) versus controls (non-caregiving), and the existence of many stressors (daily) was related to increased serum concentrations of IL-6 and CRP.
Borders <i>et al.</i> ^[72]	2015	CRP	Blood	Non-Hispanic black (NHB) women ($n=55$) and non-Hispanic white (NHW) women ($n=57$)	Enzyme immunoassay	NHB women were found to have elevated levels of CRP and ACTH in the second and third trimester of gestation, compared to NHW women.
Grossi <i>et al.</i> ^[73]	2003	CRP	Blood	High Burnout subjects ($n=43$) and Low Burnout Subjects ($n=20$)	Turbidimetry	The mean CRP levels were relatively low among low burnout women as compared to high.
McCurley <i>et al.</i> ^[41]	2015	CRP	Blood	923 non-diabetic men and women aged between 18 and 74	Immunoturbidimetric method (hs-CRP)	There was no indirect effect of stress through Inflammation. Hence suggest that higher chronic stress is associated with poorer glucose regulation.
Siddiqui <i>et al.</i> ^[70]	2019	CRP	Blood	Newly detected diabetic mellitus (NDDM=125) and normal glucose tolerance (NGT=125) Subjects	Serum hsCRP (EIA Method)	Higher levels of oxidative stress and inflammation in association with significantly higher chronic psychological stress in patients with newly detected diabetes mellitus

stress level. In contrast, MDA increases with age. No changes in MDA levels are found for sex; however, MDA is considered a biomarker of lipid peroxidation, occupational, and oxidative stress rather than chronic stress biomarker Table 11.^[29,82]

Biomarkers of Immune System

The immunological biomarkers are an effective tool in creating a link between pathways between the immune and neuroendocrine systems in chronic stress-related outcomes.^[84] However, few studies determine the role of immunological biomarkers and the association of different cytokines measures in plasma in the blood samples and it involves individual cytokine anti-inflammatory and pro-inflammatory or it showed the ratio of anti-inflammatory and pro-inflammatory in several studies to detect the role of cytokines as a biomarker of chronic stress.

Cytokines

There are certain groups of cytokines such as growth factors, interleukins, and interferon. There are certain immune

biomarkers such as pro-inflammatory (IL-6, IL-1 β) cytokines, anti-inflammatory (IL-10), TNF-alpha, T, B cell, and NK cells and some of them are discussed below. All of these play a major role in immune responses by promoting the cell to cell communication and thus stimulates its role at the side of inflammation, infection, and trauma.^[30]

Anti-inflammatory and pro-inflammatory cytokines

As discussed earlier (Table 12), few studies demonstrated cytokines' role in stress physiology.^[32,85-88] A previous study has presented the association of stress (burnout) with circulating anti-inflammatory (IL-4, IL-10) and pro-inflammatory (TNF-alpha) cytokines, where burnout stress teachers possessed higher levels of TNF-alpha/IL-4 ratio,^[32] suggests burnout stress subjects are related to increased systemic inflammation.^[32]

Natural killer cells (NK) and leukocytes

Few studies have found the association between different types of white blood cells (leukocytes) and the levels of stress

(burnout) state (Table 13).^[28,89] The present study demonstrated the relationship between burnout (stress) state and immune variables indicating that high scores for stress lead toward an increased leukocyte level and several T cell counts above the normal range.^[90] Several studies do not involve the increased number of lymphocytes subsets due to burnout stress state.^[28,90] Hence, to summarize, burnout (stress) does not usually associate with a large number of leukocyte changes in the blood.^[28]

C-reactive protein (CRP) and fibrinogen

A study in support showed a significant relationship between chronic stress, inflammatory markers, and daily stressors (Table 14). It suggested that among family dementia caregivers, the increased daily stressors experience contributes to an elevation in the IL-6 and CRP levels. However, CRP showed more contrasting results with such variations, that either showing no relationship between burnout stress state and non-burnout state,^[42,74] while in some studies it showed increased levels of CRP for burnout (stress) state.^[91]

Discussion

All of the included 37 studies were original researches from the past 40 years and included any of the specific biomarkers that we have studied in this review. Among the biomarkers of HPA-Axis in relation to chronic stress, cortisol, ACTH, and BDNF were studied. A total of nine studies supported cortisol measurement as a chronic stress biomarker through hair samples. The useful cortisol parameters are urine, saliva, and blood; however, these parameters have some limitations.^[33] The best and recent innovation for cortisol estimation is from hair. According to Kimberly *et al.*, potential biomarker during maternal stress is cortisol; they proposed that hair cortisol as a valid biomarker rather than samples from urine or salivary samples. Hair cortisol concentration (HCC) is a useful tool in evaluating long-term cortisol levels. It provides a reliable measurement of HPA-axis activity throughout pregnancy and the reflecting total cortisol release.^[56] However, the working population depicted the association between chronic stress and hair cortisol concentration and suggested that hair cortisol concentration's limited applicability is an essential biomarker in work-related stress. Besides, it may be useful for the early detection of depression.^[57] Compared to salivary cortisol, hair cortisol appears to be a significant biomarker for evaluating stress and is a better indicator of stress system.^[58] Furthermore, the role of ACTH as a biological measure of chronic stress in the study of non-Hispanic black and white pregnant women suggested that black Hispanic pregnant women had elevated mean levels of ACTH and C-reactive protein (CRP) in the 2nd and 3rd trimester of pregnancy.^[72] However, another study did not show any ACTH association with biological measures of chronic stress in blood samples.^[72] Similarly, for BDNF studies suggest that the stress subjects have significantly low BDNF levels.^[35,36]

Among the ANS biomarkers, Catecholamines were studied while among those for the metabolic processes were glucose, HbA1c, triglycerides, and cholesterol. The included study suggested that there is no association found between ANS mediated hormones and chronic stress. The previous studies did not identify changes in stressed subjects versus healthy subjects,^[70,72] thus, it cannot be used as a valid biomarker for chronic stress detection. A study determined the role of the stressor on the individual, through metabolic endocrine, and immunological biomarker without any significant changes found through endocrine and immunological markers; however, statistical changes were found in glucose and HbA1c in two different caregivers group.^[41] However, another study determined metabolic, endocrine, and immune correlates of burnout (stress) state among women, and high HbA1c and TNF-alpha levels in women with burnout and suggested these women had enhanced inflammatory responses and oxidative stress.^[74] Moreover, the association of tensed burnout with cardiovascular disease (CVD) risk factors results showed reduced health state and elevated levels of glucose, cholesterols, triglycerides, and uric acid.^[75]

Prolactin, oxytocin, and Dehydroepiandrosterone Sulfate (DHEA-S) were the endocrine hormones studied. Three articles compared DHEA-S levels through blood samples and showed the association of DHEA-S levels between stress (burnout) subjects and controlled healthy subjects. The low dose dexamethasone and DHEA-S levels were identified; however, higher DHEA-S levels were found in burnout, and no difference was found in cortisol level. Furthermore, Pro-inflammatory cytokine IL-10 was found to be increased in stress subjects.^[78] When DHEA-S levels were compared between stress subjects and healthy subjects within each three 10 years interval age group, the youngest age group (25-34 years) showed lower DHEA-S levels more in females than in males of stress subjects. In comparison, no change in stress subjects for DHEA-S levels was found in the age group (35-44 years) and (45-54 years) when compared with control subjects.^[28] Moreover, changes in DHEA-S levels in stress patients during the 1st year of their treatment leads led to the development in health, hence changes in DHEA-S sulfate associated with the prognosis of outcome in stress patients.^[33] Furthermore, we studied SOD and catalase among the antioxidants, only one study analyzed the role of two antioxidant enzymes, SOD, MDA, and catalase, in stress in healthy workers, showed activity SOD was higher in workers of evening and night shift.^[80]

Biomarkers of immune system included cytokines, anti-inflammatory, pro-inflammatory cytokines, NK cells, leukocytes, CRP, and fibrinogen. A reviewed study showed high levels of IL-6 due to chronic stress that explains the changes due to chronic stress that produces adaptations in the immunological system reflected in the certain number of cytokines and immune cells, which do not return to normal baseline even after removal of stressors. That justify decreased

immune function in people with chronic stress provides a possibility to identify a personal vulnerability with specific biomarkers.^[31,86] Furthermore, the presented study that shows showed the association of IL-6 and chronic stress; hence, it was found that IL-6 was four times greater in caregivers than non-caregivers, and this provides an evidenced mechanism through which chronic stressors may be related to age-associated disease and ageing of the immune system.^[30] However, this present study indicates that chronic stress and its responses are widely associated with oxidative stress and inflammation, which may further lead to the risk of type 2 diabetes.^[71] The anti-inflammatory cytokines (IL-10) found to be increased in patients with burnout (stress) state.^[80] However, another study shows that interleukin-8 is a robust biomarker of chronic stress, and elevated levels of IL-8 are associated with cardiovascular disease and negative psychological consequences.^[87]

While, changes in NK cell activity and its number due to chronic stress were found in a few studies. One study shows that due to high burnout, depersonalization scores indicate decreased NK cell activity in male office workers and no changes in NK cell activity due to other burnout scores were found to in these male workers.^[89] The above studies summarized that the exact role of NK cell activity and variation in leukocyte number and activity due to chronic stress response is not clear. Hence, NK cells and leukocytes cannot be such valid biomarkers as compared to that of others. Furthermore, CRP is one of the significant biomarkers of inflammation,^[80] and studies have presented its enhanced levels in chronic stress conditions.^[92,93] A previous study has reported a positive relation of burnout with hs-CRP and fibrinogen levels in women, but not in men.^[94]

Summary of Main Findings and Study Limitations

Each biomarker plays an important role in stress, and each portray different outcomes. Moreover, this review helps to identify the relevance for biomarkers in chronic stress that can be helpful as the biological predecessor of disease, prognosticator of disease progression, and a potential target for behavioral interferences in chronic stress. Among the major limitation of this review was a few of the studies had a fragmented outcomes; however, conclusions are drawn based on this descriptive systematic analysis. However, we recommend a more extensive and yet specific literature search for each of these included biomarkers.

Conclusion

This review highlights the physiological biomarkers of HPA axis, ANS, immune, endocrine, metabolic, and defense system for how each of these play a major role in chronic stress. Hair and salivary cortisol, HPA axis biomarkers are considered as a major source of evaluating chronic stress levels in the targeted

individuals. As the salivary and urinary biomarkers provide convenient measurement methods.

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