Salivary Cortisol Levels as a Biological Marker of Stress Reaction

Djordje Bozovic1, Maja Racic2, Nedeljka Ivkovic1
Department of Stomatology, Faculty of Medicine Foca, University of East Sarajevo, Bosnia and Herzegovina1
Family Medicine Department, Faculty of Medicine Foca, University of East Sarajevo, Bosnia and Herzegovina2

Aim: To determine the validity and psychobiological significance of salivary cortisol as a biomarker of stress in the experiments. Results: Stress is defined as a state in which homeostasis is jeopardized by the action of various external and internal stressors. The effect of cortisol is made through specific receptors located in the cytoplasm of the target cells. Determining blood cortisol levels, which has been the most widely used method, is characterized by certain shortcomings. The process of taking blood samples from the vein is accompanied by additional stress, which results in falsely positive results. Another flaw is found in the fact that cortisol taken and measured from serum or plasma represents total cortisol, not the free, biologically active one. Cortisol response lags behind ACTH by 5-20 minutes, with peak blood levels achieved in 10-30 min. Conclusion: Although, the studies on correlation between saliva cortisol concentrations and free levels of this hormone in blood samples are lacking, salivary cortisol offer a novel approach in research of stress biomarkers with its ease of collection and potentially wide scope for application.

Key words: salivary cortisol, stress, biological marker.

Corresponding author: Djordje Bozovic, DMD, MsD. Address: Studentska 4, 73300 Foca. Phone: +387 65585400. E-mail: djordje.bozovic@hotmail.com

1. INTRODUCTION

Stress

The notion of stress was first introduced by the Canadian physician Hans Selye in the 1930’s. He defined stress as a “non-specific response of the body to any demand for change” (1). Selye discovered and documented that there exists “good stress” (eustress) as well as “bad stress” (distress), depending on whether one reacts to good news or bad news, or whether the impulse is positive or negative. Today, stress is defined as a state in which homeostasis is jeopardized by the action of various external and internal stressors (2, 3). Homeostasis seeks to re-establish itself, through complex mechanisms of physiological and behavioral adaptation. Body adaptation is manifested in the form of a synchronized interaction of almost all systems in the body, especially the nervous, endocrine and immune systems (Fig.1). The quality and quantity of adaptation (reaction) depends on numerous factors: type of stress, the intensity and duration of exposure to the stressor, as well as on individual characteristics of the body.

Stress reaction is a result of activities of the so-called stress system, situated in the central and peripheral nervous system (CNS). The central, main components of the stress system are localized in the hypothalamus and the brain stem. The paraventricular nuclei (PVN) of the hypothalamus contain neurons in charge of the corticotropin-releasing hormone (CRH) synthesis and secretion as well as arginine vasopressin (AVP), while certain nuclei of the brain stem (paragigantocellular and parabrachial) are in charge of CRH secretion only (4). The extended marrows and pons host the locus ceruleus and other, mostly noradrenergic, nuclei related to the activity of the vegetative sympathetic nervous system.

The peripheral part of the stress system consists of the hypothalamic-pituitary-adrenal axis (the HPA axis), the adrenal sympathetic efferent system, as well as certain components of
the parasympathetic system (2, 3, 5, 6).

The hypothalamic-pituitary-adrenal axis

During a stress reaction, the HPA axis activity is increased. At the hypothalamic level, the stressor activates CRH and AVP secretion (2,3,5). These hormones stimulate the frontal lobe of the pituitary gland, which releases the ACTH (adenocorticotropic hormone), which in turn stimulates a glucocorticoid (cortisol) synthesis and secretion in adrenal cells. The effect of cortisol is made through specific receptors located in the cytoplasm of the target cells. The hormone-receptor complex enters the nucleus where it activates a transcription of specific genes for mRNA creation. The mRNA created this way is diffused into the cytoplasm and stimulates in ribosomes the process of translation for the creation of new proteins that operate as enzymes or transport proteins (3,7).

The final effect of HPA axis or cortisol activation is a domination of catabolic processes, in order to provide the body with sufficient energy substrates to satisfy its increased needs during a stress reaction.

Concomitantly activated are inhibitory mechanisms, since a stress reaction outside the period of stressor operation is rather exhausting and damaging to the body. Cortisol has this effect too by affecting the central components of the stress system (perifrontal cortex, hippocampus and amygdala) by way of the negative feedback mechanism, which aims to limit and minimize, in terms of time, the catabolic, lipolytic, anti reproductive and immunosuppressive effect of the stress reaction (2, 3, 5, 8, 9).

Cortisol as a stress biomarker

Cortisol is a glucocorticoid synthesized from cholesterol, secreted by the adrenal cortex and released into blood. In blood plasma, most of the cortisol (65%) binds with high-level affinity and low capacity to corticosteroid-binding globulin (transcortin). A total of 30% of cortisol is bound to albumins, while 3-5% of cortisol remains in a non-bound (free) metabolically active form (10,11). Accordingly, the HPA axis activity during a stress reaction may be determined by determining the level of cortisol in extracellular fluids (blood, urine, saliva).

Determination of cortisol levels, which has been the most widely used method, is characterized by certain shortcomings. The process of taking blood samples from the vein is accompanied by additional stress, which results in falsely positive results (12). Another flaw is found in the fact that cortisol taken and measured from serum or plasma represents total cortisol, not the free, biologically active one. Further, certain diseases and the use of particular medicines affect the level of transcortin and albumin, which changes the level of total cortisol in relation to free cortisol (13, 14).

Cortisol is metabolized in the liver, 25% is secreted through bile, while the remaining 75% is secreted through kidneys in the free form. Renal secretion depends on glomerular and tubular functions. The rate of cortisol secreted daily via urine also depends on the proper procedure of collecting urine over 24 hours so that urinary cortisol is not always in correlation with the concentration of free cortisol in blood (15).

Nowadays, due to the mentioned shortcomings, attention is increasingly paid to determining salivary cortisol levels. The concentration of this hormone in the saliva accounts for 70% of the non-bound blood cortisol that enters saliva by diffusion through the basolateral membrane of salivary gland acini. Thanks to the low molecular weight and liposolubility, the non-bound cortisol passes through the cell membrane by simple diffusion (16). Salivary cortisol correlates highly (≥0.90, p<0.001) with free blood cortisol for it is independent from the transport mechanisms and the type, quantity and flow of saliva (17,18). The very method of taking salivary samples is simple, standardized, safe, non-invasive, less stressful, easy to repeat and does not require any special level of training or equipment. Cortisol is stable in the saliva, and at the room temperature samples may be kept for up to 4 weeks without any significant changes in cortisol levels. However, for the purpose of longer sample preservation, they must be frozen at -20 °C (11). Differences in temperature do not affect cortisol concentration in the saliva (19).

As of lately, enzyme immunoassay (EIA) with photometric analysis has been increasingly used for the biochemical analysis of salivary cortisol. The most widespread is the ELISA test (enzyme-linked immunosorbent assay), and represents an enzymatic variant of the sandwich testing (20).

Cortisol is subject to circadian (daily) rhythm of secretion with the half-life of < 1 hour, bearing in mind that several short episodes of increased secretion are repeated due to food intake (9). The highest levels of cortisol are reached in the morning hours (30-
Salivary cortisol levels may be shown in different measurement units: ng/ml; µg/dl and nmol/l. Conversion from one measuring unit to another is carried out using the following formulae (IBL international, 2009): Cortisol(ng/ml) × 2.76 = nmol/l TJ
Cortisol(µg/dl) × 27.6 = nmol/l.

Average salivary cortisol levels in healthy subjects are as follows:

- In the morning: 0.20 – 1.41 µg/dl (5.52 – 28.92 nmol/l)
- In the afternoon: 0.04 – 0.41 µg/dl (1.10 – 11.32 nmol/l).

As already known, cortisol secretion increases significantly in the state of acute stress (10, 11, 22, 23). The level of cortisol secreted in such situations stands in correlation with the intensity of the stress (24). Immediately upon exposure to stress, the adrenocorticotrophic hormone (ACTH) level begins to rise sharply within the first 5 minutes. Cortisol response lags behind ACTH by 5-20 minutes, with peak blood levels achieved in 10-30 min. (10) (Chart 2). The transfer of cortisol from blood to saliva takes place rather quickly, within no more than 2-3 min (22).

Unlike the acute stress, it is less known what effect the chronic stress has on cortisol (25). Results of numerous studies differ in that respect. On the one hand, results of several studies show that the extended exposure to stressors (work place stress, unemployment, detention, living in the vicinity of a nuclear plant, breast cancer) is related to increased levels of basal cortisol (26, 27, 28, 29,30,31). On the other hand, post-traumatic stress disorders, exposure to combat activities, death of a close family member and fibromyalgia are characterized by the chronically low secretion of basal cortisol (32, 33).

During a stressful experience, exactly which central components of the stress system will be activated in cortisol regulation depends on numerous factors. The first such factor is the type of stressor. Stressors may be physical (e.g. cold, heat, electrical shock, noise etc.); biological (e.g. bleeding, infection, lack of sleep etc.) and psychological (e.g. university exam, public appearance, graduation paper defense etc.). The physical stressor mostly engages amygdala (AG) while the psychological one affects hippocampus (HC) and perifrontal cortex (PFC) (34). Pruessner et al. have discovered a reduced HC activity during the subjects exposure to psychological stress, and concluded there existed an inverse connection between HC activation and cortisol response to stress. On the other side, various parts of PFC are related to cortisol secretion. Subjects who responded strongly to stress have been reported to have an increased ventrolateral cortex activity and a reduced medial, orbitofrontal and anterior cingulate cortex activity (34, 35, 36, 37) (Figure 2).

The measurement of cortisol in hair has the potential to serve as a biomarker of chronic stress in a variety of health conditions. Several studies found that hair cortisol analysis presents a complementary means of monitoring stress, capturing systemic cortisol exposure over longer periods of time. However, hair cortisol research is in an early stage and a clear picture of how to interpret and use the findings is only just emerging (38, 39).

2. DISCUSSION AND CONCLUSION

Several studies with momentary assessments of stress and salivary cortisol have been published supporting the view that even minor changes in negative affects can result in increased cortisol levels, whereas a positive affects has the opposite effect.

One of the major advantages of salivary cortisol is that samples can be obtained in the natural environment of the study participant as well as in special settings outside the laboratory. Also, the collection of saliva is a non-invasive sampling method and therefore does not induce additional stress in participants (7, 10).

In the course of stress and stress reaction research for the purpose of eliciting acute stress most of the studies applied the Trier Social Stress Test, one of the most prominent psychosocial laboratory stressors in humans (23, 40, 41). The results of the studies where oral examination was used as a stressor were homogenous and related to cortisol increase (42, 43). Dickerson and Kemeny have found that the peak cortisol response occurs between 21 and 30 minutes from the onset of the examination, and that it does not depend on the duration of the examination (23). The results related to written examinations (tests) were heterogeneous and not necessarily accompanied by cortisol increase (44, 45, 46, 47, 48).

Together, these results suggest the need to better define and consider the implications of both the specific measures of stress being used and individual differences in the subject samples in psychoneuroendocrine studies (49). Several studies suggest that stress-induced changes in cortisol level also occur in response to physical stressors, to competitive stressors or to more severe stressors only (50,51,52). It still remains unclear whether cortisol response to controlled experimental conditions (stressors) may fully apply to the response to actual life stressors (23, 48, 49).

Although, the studies on correlation between salivary cortisol concentrations and free levels of this hormone in blood samples are lacking, salivary cortisol offer a novel approach in research of acute stress biomarkers with its ease for application.