Salivary biomarkers in stress research

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ABSTRACT:
Stress researchers need biomarkers in order to study physiological mechanisms of stress response. Monitoring biomarkers in saliva is a fast and simple procedure, both in healthy subjects and patients. Saliva contains a wide range of potential parameters which reflect physiologic endocrine and immune responses, like the biologically active form of certain hormones (e.g. cortisol) and cytokines. Here we describe briefly the markers of endocrine and inflammation response to stress.

Key words: Stress, salivary biomarkers

REZUMAT:
Cercetãtorii stressului au nevoie de biomarkeri pentru a studia mecanismele fiziologice ale rãspunsului la stres. Monitorizarea biomarkerilor în salvã este o metodã simplã și rapicã de monitorizare a reacþiei la stres atât la subiectii sãnãtoºi cât ºi la pacienþi. Saliva conþine o gamã variatã de potentiali parametrii care reflectã rãspunsul fiziologic endocrin ºi imun, ca ºi forma activã biologic a unor hormoni (ex. cortizolul) ºi a citokinelor. În acest articol vom descrie pe scurt markerii endocrini ºi inflamatori ai rãspunsului la stres.

Cuvinte cheie: Stres, biomarkeri salivari

BIOLOGICAL CORRELATES IN STRESS RESEARCH

Stress is a major area of research, mostly for researchers interested in environmental and psychosocial influences on health. Since the definition given by Selye (1936), which implied that stress could be a mechanism in disease formation, definitions of stress included incapacity of the individual resource to reduce, master or tolerate external or internal solicitations (Folkman and Lazarus, 1985), biological, psychological and behavioral imbalance in reaction to external factors (Derevenco, 1995). Stress factors include physical, chemical and psychological ones. Iamandescu (2002) remarks the importance of two kinds of stress: eustress and distress, as the effect of positive stressors is both biological as well as behavioral. Iamandescu establishes the definition of secondary psychological stress, which is a psychological stress reaction continuing after a primary non-psychological stress. Secondary psychological stress includes stress represented by the disease itself.

There are three ways summarized by Cohen et al (1995) to assess the role of stress in disease risk. The environmental hypothesis treats stress as the result of environmental events or experiences that are usually associated with adaptive responses. The psychological hypothesis tests the subjective assessment of coping strategies and of the individual affective responses in the face of events or experiences. The biological hypothesis tests the way specific physiological systems are being activated by psychologically and physically demanding conditions.

Stress response is regulated by two primary neuroendocrine systems, the hypothalamus-pituitary-adrenocortical (HPA) and sympathetic adrenomedullary (SAM) systems. These are described in detail in Levi (1972), Baum et al (1981) and a more recent discussion upon biomarkers related to these neuroendocrine systems is made by Takai et al (2007).

The HPA (hypothalamus-pituitary-adrenocortical) axis is one of the main physiological systems involved in the neuroendocrine response to stress. Psychological stress is increasing HPA activity and subsequently there is a rise in cortisol hormone level, as a result of ACTH release at the command of CRF/AVP neurons in the paraventricular nucleus (PVN) of the hypothalamus. Glucocorticoid receptors (GR) mediate the actions of glucocorticoids. Cortisol increase in body fluids is the psychobiologic response of HPA axis to environmental and psychological stressors.

Cortisol contributes fundamentally to the maintenance of basal and stress-related homeostasis. Glucocorticoids influence the human genome and their effects are spread all over the human tissue and organs. Thus, psychiatric and somatic disorders, such as anxiety, depression, insomnia, chronic pain and fatigue syndromes, obesity, the metabolic syndrome, essential hypertension,
diabetes type 2, atherosclerosis and osteoporosis, as well as autoimmune inflammatory and allergic disorders, appear to have a glucocorticoid component (Chrousos and Kino, 2007).

As a result abnormalities and the reactivity of the HPA axis have been the focus of many investigations on psychiatric disorders. Furthermore, cortisol is considered to be the endocrine hormone modulating mental and physical states that are associated with psychosocial stressors (Shirotsuki et al, 2007).

The primary mechanism of defense against stressful stimuli is activation of the sympathoadrenal system (SAS), comprising the sympathetic nervous system and the adrenal medulla.

WHY DO WE NEED BIOMARKERS IN STRESS RESEARCH

According to National Institute of Health (USA), the definition of a biomarker is the following: “a istic that is objectively measured and evaluated as an of normal bio-
logic processes, processes, or pharmacologic res to a therapeutic intervention” (Biomarkers definitions working group, 2001).

Physiological stress responses can be normal biological processes, or pathogenic processes and thus are important both in health and in disease, including psychiatric ones (McEwen, 2004).

In stress research we study physiological stress responses because they are important either for health or disease progression, thus researchers utilize biomarkers in order to establish whether a subject has a reaction to stress or not.

The integration of salivary biomarkers into stress research has increased exponentially (see reviews by Kirschbaum et al., 1992; Malamud, 1993). Monitoring biomarkers in saliva has several distinct advantages over doing so in other biological fluids (i.e., urine, serum or plasma, as it is an accessible fluid for investigation of stress. We can investigate whole saliva as a source of biomarkers to distinguish individuals who have and who have not been chronically exposed to stress, including life difficulties (threatening or not), experimental conditions, noise exposure, academic stress, etc.

SALIVARY BIOMARKERS

Saliva contains primarily water (pH: 6.4–7.4) and a large pool of biochemical constituents: heavy metals, hormones – like cortisol (diurnal peak: 13.8–48.9 nmol/l, compared to blood: 190–690 nmol/l), dehydroepiandrosteron (DHEA), testosterone, estradiol – toxins, metabolites – cotinine, enzymes – lyzosyme, alpha-amylosis, immunoglobulins – IgA, other proteins and DNA (Vitorino et al, 2004; Xie et al, 2005; Chiappelli et al, 2006). Saliva is assessed as resting (unstimulated) or stimulated. Under unstimulated conditions, 20% of saliva is derived from the parotid, 65% from the submandibular, and 7–8% from the sublingual glands (Nater and Rohleder, 2009).

The content of saliva makes it a valuable biological fluid for diverse studies, including detection of diagnostic and prognostic biomarkers in various diseases and in physiological responses, like stress. We use saliva as a clinical instrument because it contains unbound biologically active form of certain hormones (e.g. cortisol) and cytokines which reflect physiologic endocrine and immune responses.

Different collection techniques and devices for standardized collection of saliva as described in studies (Granger et al, 2007) include passive drool, filter paper, and micro sponges. All of these have both advantages and disadvantages. Passive drooling makes the measurement of multiple salivary biomarkers safe without concern of interference caused by material employed to stimulate or absorb the sample (Schwartz et al, 1998). The technique, however, requires a compliant research subject. Filter paper is better to use when small volumes of saliva are available (e.g., pre-term infants), especially in the case of salivary cortisol (Neu et al, 2007).

ENDOCRINE MARKERS IN STRESS RESEARCH

Cortisol and ß-amylase can outline the neuroendocrine status (Chiappelli et al, 2006), as the two neuroendocrine systems involved in stress response are sympathoadrenal system and HPA axis.

CORTISOL

The use of salivary sampling as a noninvasive tool for the assessment of free cortisol and therewith as a marker for activity of the hypothalamic pituitary adrenal (HPA) axis is well established in human stress research (Kirschbaum, 1994) and used both in normal subjects and patients. Various values of normal salivary cortisol levels have been evidenced 0,67+/-0,12mcg/dl (Restituto et al, 2008), 8,7+/− 4,8 nmol/l (Lo et al, 1992) and 15.5 ± 0.8 nmol/L (range, 10.2–27.3) at 0800 h (Laudat et al, 1988).

One of the most used and evidence-based protocols is the one described by Pariante et al (2002, 2004) and it uses as
outcome measure the total salivary cortisol output, calculated as the area under the curve (AUC) during the day (0 min, noon and 8 pm) and the AUC of the increase (AUCi) of cortisol levels after awakening (from 0 min to 15, 30, and 60 min after awakening) (Pariante et al 2002, Pariante et al, 2004).

Sampling this endocrine marker of hypothalamus–pituitary–adrenal axis activity by assessment of the amount of bioavailable cortisol in saliva with immunoassays is of major interest in both research and clinical practice (Van Stegeren et al, 2006). Sampling in special population like children and old people must be in accordance with literature reviews (Tlili et al, 2011).

Besides immunoassay, in order to facilitate point-of-use measurement of salivary cortisol levels Tlili et al (2011) have described the development of an ultrasensitive, nano-tube immunosensor which is capable of rapid, label-free measurement of salivary cortisol (Tlili et al, 2011).

**SALIVARY ALPHA-AMYLASE (SAA)**

Many studies have provided evidences on hypothalamic-pituitary-adrenal (HPA) axis response in stress and salivary cortisol, while there are fewer studies regarding sympathetic nervous system (SNS) biomarkers. One possible reason is that SNS response has been more difficult to assess than HPA axis markers. Nater et al (2009) have recently reviewed salivary á-amylase (sAA) as a salivary biomarker for SNS activity. The potential of alpha amylase as a salivary marker of adrenergic activity could be of substantial interest for human stress research since it would allow the parallel investigation of the two major neuro-endocrine stress systems with salivary sample (Chatterton, 1996). In humans, sAA levels have been reported to rise in response to physical stress as well as to psychological stressors (Chatterton, 1996) and (Nater et al, 2005). Moreover, one study reported that sAA levels were significantly associated with noradrenaline levels measured out of plasma samples (Chatterton, 1996). The authors concluded that salivary alpha-amylase concentrations are predictive of plasma catecholamine levels under a variety of stressful conditions.

In summary, assessment of sAA as a non-invasive biomarker for the sympathetic nervous system offers a multitude of applications in different research areas (studies on dysregulation of autonomic nervous system, treatment studies. More studies are needed to assess physiological response to stress and sAA is a potential and easy to assess parameter.

**DHEA**

DHEA (dehidroepiandrosterone) is a steroid hormone produced by the zona reticularis of the adrenal cortex and can be measured in saliva. DHEA has been shown in studies to be in inverse correlation with cortisol, supposedly having a protective effect for the individual against depression, stress. Shirotushki et al (2009) have shown the complete HPA axis reactivity to acute psychosocial stressors in individuals with high social anxiety might be blunted, e.g. cortisol to DHEA ratio in response to psychosocial stress was found lower in the highly socially anxious group (Shirotushki et al, 2009).

**SALIVARY CHROMOGRANINA (SCGA)**

The stress biomarkers sAA and sCgA showed a significant increase in concentrations in relation to naturalistic traffic noise exposure and they appear to be feasible biomarkers to measure direct effects of naturalistic traffic noise on the Sympatho-Adrenal-Medullary/HPA axes in a laboratory setup (Wagner et al, 2009).

**GLUCORTICOID RECEPTOR GENES**

The last but not the least in importance is the genetic material salivary biomarker. Salivary DNA and RNA can be studied for the glucocorticoid receptor genes. Bergen et al. observed reduced expression of glucocorticoid receptor-regulated genes) in whole saliva RNA from individuals exposed to chronic stressors, as compared to those with no exposure.

**INFLAMMATION MARKERS IN STRESS RESEARCH**

Biomarkers used in the assessment of inflammation not only document clinically significant infection status but also offer an important tool to explain the role of inflammation in physiology of stress. The response on stress of the hypothalamic–pituitary–adrenal (HPA) axis and the autonomic nervous system manifests in lowering the immune body response, as it was shown by various research studies on patients with viral infections, which have a worse evolution if stress is higher (Kemeny and Schedlowski, 2007). Also chronic psychosocial stressors are related to dysfunctionalities of the inflammatory response. Job stress, low socioeconomic status, childhood adversities as well as caregiver stress, and loneliness were
all shown to exert effects on immunologic activity (Hansel et al., 2010).

There are numerous studies that have linked the increase in cytokine level with symptoms of depression, like it is seen in treatment-induced depression in the case of therapy of hepatitis C with alpha-interpheron.

Salivary immunoglobulin A (IgA) and lysozyme were found to be inversely correlated with self-reported levels of stress among female nurses (Ng et al., 1999; Yang et al., 2002), stating further that IgA and lysozyme could be potential biomarkers in stress research.

REFERENCES


