Stress Biomarkers in Saliva – An Overview

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ABSTRACT

Stress has become an important concern in today's lifestyle. Stress is defined as a state in which homeostasis is altered, which is a dynamic balance of internal conditions necessary for the proper functioning of cells or the living organism as a whole. Evaluation of stress can be assessed using biomarkers. Biomarkers are biologically active substances in body fluids which is analysed qualitatively and quantitatively. Studies to evaluate stress biomarkers and information regarding reactions to stressors have attracted great attention in medical research field today. These biomarkers can be assessed via monitoring corporal fluids (serum, plasma, urine and salivary fluid). Interest on saliva as a diagnostic fluid has grown exceptionally in recent years. The compounds most used as salivary stress biomarkers are cortisol, Immunoglobulins, Lysozyme, Chromogranin A and α-amylase. Saliva has an advantage of collection being easy, non-invasive and permits multiple samples. This paper highlights an overview of the salivary biomarkers in stress.

Keywords: Stress; Salivary Biomarkers; Cortisol; Immunoglobulins,; Lysozyme; Chromogranin A; α-amylase

INTRODUCTION

In modern society, stress has become an inevitable part plaguing the daily lives of people. Stress is defined as a condition in which homeostasis is altered, which is a dynamic equilibrium of internal conditions necessary for the appropriate functioning of cells or the living organism as a whole. It is influenced by the action of various factors, known as stressors.[1,2,3] There are two categorization of stressors: physical (systemic or reactive) and psychological (emotional or processing).[4,5]

Stress reaction occurs as a sequel of stress system activities, occurring due to the actions of central and peripheral nervous system.[1,2] The central nervous system (CNS) plays an imporatant role in physical and psychological processes of the body and also in response to stressors. During stress (physical or psychological), there is an activation of the hypothalamic-pituitary-adrenal (HPA) axis (which results in the secretion of glucocorticoids (cortisol) into the blood circulation), activation of Autonomic Nervous System (ANS) and the release of catecholamines into the blood circulation. The sympathetic component of the ANS is responsible for effects on body like heart rate, respiratory rate, blood flow to muscles etc.[4,6,7]

Stress can be categorized into acute and chronic stress

Acute stress activates the sympathetic nervous system and HPA axis which synchronize a set of physiological responses, that prepares the body to deal with an immediate threat.[4,8,9]

Chronic stress can either increase or decrease the HPA activity, and the responses to the stress is governed by the stressor and the individual. Chronic stress is likely to cause various health hazards. Prolonged periods of activation of the HPA axis can increase the risk of fatigue, depression, cardiovascular diseases, oral diseases, neurodegenerative diseases, psychological disease, impair immune function, and diminish the cognitive functions of the individual.[4,10]

EFFECT OF STRESS AND HPA AXIS

While sympathetic neurotransmitters regulate the initial stage of the acute stress response, the neuroendocrine response follows in a delayed but prolonged and gene-mediated fashion. Upon precipitation of stress, the amygdala activates the HPA axis by signalling the hypothalamus to release corticotrophin releasing hormone (CRH). This hormone then triggers the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary and ACTH stimulates the release of cortisol from the adrenal cortex. Approximately, 15 minutes after the onset of stress, cortisol levels rise systemically and remain elevated for several hours. Increased levels of cortisol mobilize glucose and, promote fat and protein breakdown. Cortisol modulates and maintains a variety of important cardiovascular, metabolic, immunologic, and homeostatic functions, reduces inflammation and allow for the effective management of stress.[11]

BEHAVIOURAL ADAPTATION TO STRESS

Behavioural adaptation to stress includes heightened excitement, vigilance, caution, focus as well as euphoria or dysphoria. Also, increased temperature, with the inhibition of vegetative functions, such as appetite, feeding and reproductive functions is present. At the same time, for the motive of redirecting energy, physiological alterations occurs, so that oxygen and nutrients are indended towards the CNS and other bodily systems where they are in vital requirement at the moment.[1,3]

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The cardiovascular system shows an increase of arterial pressure, minute heart volume, and increased pulse. In the respiratory system there is bronchodilation and alveolar hyperventilation, and rapid breathing. Also, catabolic processes are activated which results in lipolysis, glycogenolysis, glyconeogenesis, increasing the plasma concentration of glucose, fatty acids and aminoacids, basic fuels for our body. Detoxification is increased, for the removal of mutilating metabolic products formed as an outcome of stress reactions, while, at the same time, the activity of digestive, reproductive and immune system has been impeded.[2,3]

Stress reaction becomes oversized, prolonged, going beyond the framework of adaptation, which may result in the development of pathological conditions. There is a sustained dynamic equlilibrium between the process that stimulate and the process that hinder the development of a stress reaction.[1]

METHODS OF EVALUATION OF STRESS

1. Psychological test: Physiological test is done by using various stress evaluation scales.

Eg: DAS scale, Adaptive behaviour scale, Psychological screening inventory.

2. Biomarkers

Biomarkers are defined as quantifiable biological indicators of a physiological process (normal, pathophysiologic, or risk of developing a pathology). These molecules have the capability to enhance the identification rate of individuals at the probability of progression to various diseases. These biomarkers can be evaluated by monitoring the corporal fluids of the body (serum, plasma, urine and salivary fluid).[4,12] Efficient biomarkers should be sensitive, stable, and easy to measure, non-invasive and without painful collection procedures for patients.[12] Therefore, marked heed has been given to identify the physiological biomarkers of stress in salivary fluid. Saliva has been progressively used as a better diagnostic or disease activity marker in various diseases (cancer, asthma, diabetes, cardiovascular diseases), and in stress research.[4,13]

Examples of salivary biomarkers for stress: Immunoglobulin, Chromagranin- A, Alpha amylase, Lysozyme, Cortisol

The mechanism of entry of the constituents of the blood into the saliva is by transcellular, passive intracellular diffusion and active transport, or paracellular routes by extracellular ultrafiltration within the salivary glands or through the gingival crevice.[14,16]

FUNCTIONALITY OF SALIVA

Saliva is a dynamic fluid that reflects the physiological and pathological state of the body and bearing functional equality of composition with serum. Saliva is an ideal medium to be analysed for health and disease surveillance. Interest on saliva as a diagnostic biofluid has grown exceptionally in recent years. Broad spectrum of biomarkers is quantafiable in saliva as it contains wide array of constituents. Salivary sample collection is easy, self-collection after proper guidance is possible and expertise not required. It does not carry the risk of needle stick injury and is also cost effective.[14,15]

SALIVARY STRESS BIOMARKERS

Cortisol

Cortisol has received considerable attention as a highly useful biomarker because of its sensitivity to psychological stress, utility as an indicator for neuroendocrine health and function and a predictor of general health and ailment.[17]

Cortisol is a steroid hormone which is synthesized from cholesterol and is the main glucocorticoid in the zona-fasciculata of human adrenal cortex. Cortisol is a primary stress hormone. Cortisol has a wide range of influences on metabolism, immunoregulation, vascular responsiveness, cognition and behaviour. Cortisol levels in blood increase during the early morning (highest at about 8 a.m.) and decrease slightly in the evening and during the early phase of sleep.[18] Under normal conditions, it shows an increase in the early morning hours, reaches a peak before awakening and decreases in the evening (diurnal variation).[19] Normal value of serum cortisol is 2-25 mg/dl and salivary cortisol is 3.5-27.0 mg/dl.

Upon precipitation of stress, the amygdala activates the HPA axis by signalling the hypothalamus to release corticotropin releasing hormone (CRH) which triggers the release of adrenocorticotropic hormone (ACTH) from anterior pituitary and ACTH stimulates the release of cortisol from a/drenal cortex. The eventual effect of the HPA axis activation is the domination of catabolic processes in the body, in order to provide the body, enough energy substrates, with the aim of meeting the increased need of the body at the moment important for its survival. The high plasma concentration of cortisol stimulates gluconeogenesis in the liver and produces insulin resistance of peripheral tissues, while the increased secretion of adrenalin and noradrenalin stimulates glycogenolysis and lipolysis. Salivary cortisol is elevated in response to stress.[1,3,20]

Cortisol - Serum to saliva: In serum, cortisol is mainly protein-bound and is usually measured as such. Cortisol is 90-95% bound to proteins, about 60% to transcortin and 30% to albumin.[21] The transfer from serum to saliva occurs by free diffusion of unbound cortisol through the acinar cells of the salivary glands and the equilibrium between serum and saliva is reached in less than 5 minutes.[1] The salivary cortisol concentration is independent of saliva flow rate and of the serous and mucous content. The mechanism of entry of cortisol from the blood in to the saliva is by transcellular, passive intracellular diffusion and active transport or paracellular routes by extra cellular ultrafiltration within the salivary glands or through the gingival crevices into the oral cavity.[14,22]

IMMUNOGLOBULINS

Secretory immunoglobulin A (sIgA) is the most frequent class of antibodies in mucous membrane which is a very important factor in protection against infectious agents, allergy external proteins and has a concentration that is affected by stress.

Daily secretion is 66 mg/kg/day, while the average half-life (elimination) of secretory IgA ranges from 3 to 6 days.[1] Secretory IgA in saliva peaks during the initial 30 minutes after waking up, and then declines gradually over the following 4 hours, after which it reaches plateau for the rest of the day.[1,23] Normal range of serum IgA is 80-300 mg/dl. Certain studies have showed normal salivary IgA range of 20-25 mg/l.[26,29] Secretory immunoglobulin A (sIgA) is found in various secretory fluids, including saliva, breast milk, and nasal, gastrointestinal, bronchial, and urogenital secretions at high levels of concentration.[24,25]

On precipitation of stress, there is an immediate and delayed effect. There is increased SIgA secretion immediately after stress. Delayed stress effect is that; there is a decrease in the SIgA secretion several days after stress. The release of S-IgA is under robust neuroendocrine control (through the sympathetic autonomic nervous system by triggering the release of catecholamines) and acute stress studies have shown robust effects on S-IgA whereby its concentration typically increases. This probably occurs due to increased release of antibody from B-lymphocytes and/or increased transport of IgA across the epithelium into saliva.[26]

Lymphocytes which are a part of specific immunity have adrenergic receptors. During delayed stress, their responsiveness is reduced which causes a decreased production of cells responsible for immunity (T- helper cells, B cells-Antibodies (Ig A, IgE). This decreased production of sIgA leads to their decreased expression in saliva during stress, making it a biomarker of stress. [24,26]

Transport to saliva: Ig A produced locally by IgA producing plasma cells in the lamina propria of mucosal membranes or in the connective tissue of glands. Some of them diffuse through basement membranes to the basolateral surface of epithelial cells, where they are taken up by the epithelial cells with polymeric immunoglobulin receptors (poly-Ig receptor), then transcytosed to the apical surface of the epithelial cells, and released into secretory fluids in the form of sIgA.[24]

ALPHA-AMYLASE

Salivary alpha-amylase (sAA) is an enzyme made by the salivary glands and is regulated by the sympathetic nervous–adrenomedullary system. It is one of the considerable proteins in saliva and is produced by the epithelial acinar cells of the salivary glands. Alpha amylase release from glands that are solely para-sympathetically innervation mediated. Salivary glands secrete the enzyme α-amylase as a result of adrenergic stimulation.[4,27]

Considering the daily rhythm, the lowest level of α-amylase in saliva was reported in the morning hours, and the highest in the late afternoon, in contrast to cortisol and salivary IgA. Half-life (elimination) of α-amylase in plasma is about 12-24h, while values for saliva have yet to be determined with certainty.[1,28] Normal range of serum alpha-amylase is 0.05-1.25U/ml.

During stress, the sympathetic adrenal medullary system has increased activity, which activates the local parasympathetic system that regulates salivary secretion of Alpha amylase and causes its increased expression into saliva. α-amylase is sensitive to stress.

The two regulatory pathways for increasing salivary amylase activity are: hormonal regulation by noradrenaline and direct innervations (stimulates the secretion of salivary amylase and produces a rapid response than hormonal regulation). It has been reported that salivary amylase elicits a more sensitive response to psychological stress than cortisol produced in the hypothalamic – adrenocortical pathway.[4,29] Salivary alpha amylase is a, surrogate biologic marker of psychological stress not affected by corticosteroid use. Salivary alpha-amylase can serve as a non-invasive, quick and easy evaluation of adrenergic activity in humans.[4,30,31]

CHROMAGRANIN A

Chromagranin A (CgA) is an acidic glycoprotein, secreted by exocytosis from vesicles of adrenal medulla. Chromagranin A is co-stored and co-released with catecholamines with the stimulation of sympathetic nervous system.

The sympathetic neurotransmitters regulate the initial stages of stress response. During stress condition, there is rise in epinephrine, nor- epinephrine and dopamine secretion There is increase in expression of secretory granule proteins in adrenal medulla mainly Chromagranin A.[1,32]

A daily Chromagranin A rhythm is that, peak levels are reached during the night (23:00 h) and minimum levels in the morning hours (08:00 h). Average half-life of CgA in blood is 18.4 min, while correlation between the blood and saliva values has yet to be confirmed with certainty.[33] Normal value of serum Chromagranin A is 20-30mg/ml.

Presence in saliva: Chromagranin A is an acidic protein present in secretory granules of various endocrine and neuroendocrine cells. Chromagranin A production has been found in human submandibular salivary gland and is secreted into saliva by stimulation with nor- adrenaline and acetylcholine.[34]

LYSOZYME

Lysozyme is a low molecular mass cationic protein, extensively present in tissues and tissue secretions. Its synthesis takes place in monocytes and macrophage from where it is constantly released. Lysozyme is considered to belong to the defence system-innate immunity. Normal range of serum lysozyme is 9.6-16.8mg/dl and salivary lysozyme is 10-14 mg/l. Stress causes suppression of the effectiveness of immune system. This is hypothesised as a reason for reduced lysozyme expression during stress. Salivary lysozyme holds a negative correlation to the experienced level of stress.[1,35]

Limitations of saliva is that it does not express all the stress biomarkers in an accuracy level as present in serum and the situational anxiety and stress of hospital set up may alter the stress biomarkers.

CONCLUSION

The human body is intended to experience stress and to

react to it, but, stress becomes negative when a person faces constant encounteres to stress without any relief. In this context, the evaluation of stress biomarkers is vital, as it helps to prevent the progression and disorders related to stress and helps to improve quality of life in patients by various life style modifications. Saliva based diagnostic medium has unsurpassed opportunities for research and commercialization because of its intensified advantages and functional equality with serum.

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