Salivary α-amylase Activity - a Novel Non-Invasive Biomarker in Clinical Pathology

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Salivary biomarkers have developed into biological clinical research because they are accessible and easily obtained. Salivary α-amylase was proposed as an associat marker of salivary cortisol in assessing stress and at the same time as a possible non-invasive indicator of autonomic nervous system activation. Studies conducted so far have tested α-amylase activity in psychiatric disorders, diabetic neuropathy, lung disease, and autonomic nervous system activation in hypertension or heart failure.

Keywords: salivary α-amylase, salivary cortisol, stress, autonomic nervous system

In recent years there has been increasing interest in salivary biomarkers and their potential diagnosis. In the early 1970s, Brown has been suggested that changes in salivary hormones might be indicative of some psychopathological troubles [1]. Since then, salivary determinations have progressed rapidly. Along with salivary cortisol and dehydroepiandrosterone, salivary α-amylase could be a non-invasive marker for several pathological disorders. Most arguments are gathered around salivary α-amylase as a possible indicator of dysfunction of the autonomic nervous system [2, 3]. In cardiology, hypertension and heart failure are the main candidates for activation and dysfunction of the autonomic nervous system (ANS) from the earliest stages [4, 5]. Moreover, was demonstrated that salivary á-amylase is influenced by physical and psychological stress [6, 7]. In clinical research, subjects could be studied in their own environment or in laboratory stress conditions.

Salivary α-amylase is a calcium-containing metallo-enzyme released into the saliva to decompose insoluble starch in soluble maltose and dextrin. Salivary sampling is non-invasive and is followed by the assessment of enzyme activity using specific kits. By concurrent dosage of salivary α-amylase and cortisol, clinical studies could simultaneously evaluate the activation of the hypo-thalamus-pituitary adrenal (HPA) axis and ANS in different pathological conditions.

Even it is considered a surrogate marker of ANS activity, in some studies α-amylase reflected stress-dependent changes more closely than the response of the heart rate or blood pressure. The concomitant increase in plasma catecholamines and the release of salivary α-amylase during physical and psychological stressors stated the remark that salivary α-amylase could be measured as a non-invasive substitute for catecholamines [8]. The aim of this review is to present the characteristics of the salivary á-amylase and the main arguments on it use as a subclinical marker of ANS activation in various pathological conditions.

Biological characteristics of salivary α-amylase in human

Sympathetic and parasympathetic nervous system innervate internal organs, being in a continuous, dynamic and complex interaction that contributes to physiological functions under basic conditions or after various demands. Acetylcholine is the predominant neurotransmitter in preganglionic sympathetic neurons. The internal organs namely the heart, kidney or vessels are innervated by postganglionic sympathetic neurons, the main neurotransmitter being noradrenaline, but other substances such as the neuropeptide Y may be involved [9, 10]. Acetylcholine is the main neurotransmitter for the vagus nerve effects at the cardiac level, counterbalancing adrenergic-mediated tachycardia and hypercontractility. Both systems are controlled by the hypothalamus and interact with endocrine axis, especially with the HPA axis and immune defense mechanisms [11]. In previous studies, salivary enzyme α-amylase synthesis was associated with the activation of both sympathetic and parasympathetic nervous system, but the releasing was attributed mainly to the sympathetic influence [8].

Salivary α-amylase belongs to the family of glycosyl hydrolases and participates in carbohydrate digestion. It was first described by Leuchs in the 19th century, is mainly generated in parotid glands and less in submandibular or sublingual glands. ANS plays an important role in the synthesis and release of α-amylase in response to alpha and beta-adrenergic stimuli [12-14]. In basal, unstimulated condition, 20% of saliva is secreted by parotids and increase at more that 50% after stimulation [3].

An important aspect that has been demonstrated by researchers refers to augmented secretion of α-amylase. Both heart rate, an important marker of ANS activation, and salivary α-amylase reflect the balance between sympathetic and parasympathetic nervous system. Thus, sympathetic effects on salivary α-amylase are amplified by concomitant parasympathetic stimulation [15, 16]. Bosch and coworkers describe several pathways through

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parasympathetic drive could influence the salivary α-amylase concentrations [3]:

-synthesis in the palate and sublingual glands which are mainly parasympathetically innervated;

-the increase in sympathetic effects by parasympathetic counterbalance feed-back;

-the effect of parasympathetic on salivary flow rate [3].

In this regard, α-amylase activity could be used as a potential autonomic biomarker that should be interpreted in comparison with other parameters like heart rate, blood pressure or catecholamines release [17].

Advantages and limits in available quantification method

The use of salivary dosages is advantageous due to affordable, non-invasive and easy to reach at home characteristics. Therefore, it can be adapted to studies where participants are working at home or at profession place. Sampling of blood by venipuncture could be a stressor and therefore a confounding factor with the dosage of stress markers such as catecholamines. The method can be applied to all age groups after explaining the basics. The authors report that while cardiovascular parameters are more influenced by posture, salivary markers are largely dependent on psychical changes [12, 18]. Reassessment of the method used to determine salivary α-amylase has shown that a confounding factor is ignoring the salivary secretion rate for expressing the values that were measured. For this, the passive technique recommends noting the amount of time required to collect aα-amylase is the expression of salivary flow resulting from parasympathetic stimulation and protein secretion that is determined by sympathetic activation. For example, Bosch and coworkers reported changes in salivary α-amylase activity with 25-40% due to changes in salivary fluid secretion comparing the effects of different laboratory stress tasks on saliva flow and salivary α-amylase secretion and found that changes in salivary α-amylase activity were for 25-40% due to changes in salivary fluid secretion [2, 19, 20].

For standard collection subjects are prepared for absorption of saliva in cotton or for passive drool. Coton Salivette (Sarstedt, Germany) are saturated with saliva in the mouth and after saliva is extracted through centrifugation. Despite the ease of self-collection, Salivettes introduce error variance in the measurement of some hormone, particularly at low sample volumes. Passive saliva collection is followed by full sample recovery; the inconvenient could be in this case the azide content of the collection vessels, which is a strong α-amylase activator [17, 21, 22]. In fact, the majority of the salivary α-amylase studies use the method of collection based on the mechanical stimulation in which the activation of the local innervation could be a confounding factor with the central nervous system activity [2, 23, 24]. This confounding factor is similar with the influence of posture changes on the heart rate modulation, when we choose heart rate as a marker of ANS activity. The mechanical stimulation of the saliva secretion will change the protein content of the saliva due to different content of the parotid and submandibular glands. In passive sampling only 20% amount of saliva will come from parotid glands, which have the maximum content in salivary α-amylase [2, 25, 26]. After sampling, it is recommended to store saliva in a refrigerator until dosing is done. In healthy persons salivary α-amylase has a particular diurnal profile with a decrease immediately after awakening and a constant increase during the morning and afternoon [27, 28].

Salivary α-amylase as a non-invasive biomarker in stress response and ANS activation

Based on previous studies salivary α-amylase was proposed as a potential biomarker in acute and chronic stress, still under debate if the marker represent the activity of sympathetic nervous system or show, in the same time, the influences of the parasympathetic nervous system.

Several studies have been conducted in healthy subjects where psychosocial stress has led to significant increases in salivary α-amylase. For stress simulation under experimental conditions, Trier Social stress test, mental arithmetic load, driving simulation, competitive ballroom, were used. Chronic stress was less used but some correlations were found with salivary α-amylase [12, 29-32]. In a recent review, we found that stress impact highlighted by salivary cortisol and α-amylase was correlated with cardiovascular and cardiometabolic risk factors [33].

Salivary α-amylase is a promising marker of ANS dysregulations in psychopathology, cardiology and diabetology:

-while hypercortisolism was well established in depression, the interaction between the 2 systems and salivary α-amylase fluctuations were supported by the research concerning anxiety-related disorders, borderline personality disorder, depressive disorder, schizophrenia, anorexia nervosa [12, 34, 35];

-in combination with the HPA axis, the ANS was involved in many pathological disorder; in the context of somatic disorder, salivary biomarkers were measured in patients with HIV, in atopic disease, chronic dermatitis, oral lichen planus, obstructive sleep apnea or asthma disease [3, 36-41];

-recently, salivary α-amylase was evaluated in patients with hypertension, heart failure and with diabetic neuropathy; in the regression analysis of the patients with diabetic neuropathy were found significantly correlations between AUC of salivary α-amylase with heart rate variability parameters obtained on Holter recording [4, 42, 43].

Conclusions

In summary, present data sustain salivary α-amylase as new non-invasive, complementary tool for the detection and following of the ANS activity and in stress research. In hypertension or heart failure the small number of patients evaluated did not allow to the authors to state the salivary α-amylase as a new biomarker for diagnostic and management. Further research will open many possibilities in different field of ANS activation and salivary α-amylase expression.

References

1.BROWN, C.C. The parotid puzzle: a review of the literature on human salivation and its applications to psychophysiology. Psychophysiology., 7, 1970, 65-85.

2.BOSCH, J.A., VEERMAN, E.C.I., DE GEUS, E.J., et al. Alpha-amylase as a reliable and convenient measure of sympathetic activity: don't start salivating just yet! Psychoneuroendocrinology., 36, 2011, 449-453.

3.NATER, U.M., ROHLEDER, N. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. Psychoneuroendocrinology., 34, 2009, 486-496.

4. SUSKA, A., ALEHAGEN, U., LUNDSTROM, I. et. al. Salivary alpha-amylase activity, a new biomarker in heart failure ? J. Clin. Exp. Cardiolog., S:2, 2012, 1-7.

5.STRAHLER, J., KIRSCHBAUM, C., ROHLEDER, N. Association of blood pressure and
antihypertensive drugs with diurnal alpha--amylase activity. Int. J. Psychophysiol., 81, 2011, 31-37.
6.PATACCHIOLI, F.R., GHICIUC, C.M., BERNARDI, M., et al. Salivary a-amylase and cortisol after exercise in menopause: influence of long-term HRT. Climacteric., 20, 2015, 1–20.
7.GRANGER, D.A., KIVLIGHAN, K.T., EL-SHEIKH, M., et al. Salivary alpha-amylase in biobehavioral research: recent developments and applications. Ann. N. Y. Acad. Sci., 1098, 2007, 122-144.
8.NATERA, U.M., LA MARCAA, R., FLORINA L., et al. Stress-induced changes in human salivary alpha-amylase activity - associations with adrenergic activity. Psychoneuroendocrinology., 31, 2006, 49-58.
9.DAVY, K.P., ORR, J.S. Sympathetic nervous system behavior in human obesity. Neurosci. Biobehav. Rev., 33, nr. 2, 2009, 116-124.
10.VINCENTZ, J.W., RUBART, M., FIRULLI, A.B. Ontogeny of cardiac sympathetic innervation and its implications for cardiac disease. Pediatr. Cardiol., 33, nr. 6, 2012, 923-928.
11.THAYER, J.F., STERNBERG, E.M. Neural aspects of immunomodulation: focus on the vagus nerve. Brain. Behav. Immun., 24, nr. 8, 2010, 1223-1228.
12.SCHUMACHER, S., KIRSCHBAUM, C., FYDRICH, T., et al. Is salivary alpha-amylase an indicator of autonomic nervous system dysregulations in mental disorders? A review of preliminary findings and the interactions with cortisol. Psychoneuroendocrinology., 38, 2013, 729-743.
13.CHATTERTON Jr., R.T., VOGELSONG, K.M., LU, Y.C., et. al. Salivary alpha-amylase as a measure of endogenous adrenergic activity. Clin. Physiol., 16, 1996, 433-448.
14.HUMPHREY, S.P., WILLIAMSON, R.T. A review of saliva: normal composition, flow, and function. J. Prosthet. Dent., 85, 2001, 162-169.
15.BERNTSON, G.G., NORMAN, G.J., HAWKLEY, L.C., et al. Cardiac autonomic balance versus cardiac regulatory capacity. Psychophysiology., 45 nr. 4, 2008, 643-652.
16.PROCTOR, G.B., CARPENTER, G.H. Regulation of salivary gland function by autonomic nerves. Auton. Neurosci., 133 nr. 1, 2007, 3-18.
17.DECARO, J.A. Methodological considerations in the use of salivary α-amylase as a stress marker in field research. Am. J. Hum. Biol., 20, 2008, 617-619.
18.VAN STEGEREN, A.H., WOLF, O.T., KINDT, M. Salivary alpha-amylase and cortisol responses to different stress tasks: impact of sex. Int. J. Psychophysiol., 69, 2008, 33-40.
19.ROHLEDER, N., WOLF, J.M., MALDANADO, E.F., et al. The psychosocial stress-induced increase in salivary alpha-amylase is independent of saliva flow rate. Psychophysiology., 43 nr. 6, 2006, 645-652.
20.BOSCH, J.A., DE GEUS, E.J.C., VEERMAN, E.C.I., et al. Innate secretory immunity in response to laboratory stressors that evoke distinct patterns of cardiac autonomic activity. Psychosom. Med., 65 nr. 2, 2003, 245-258.
21.HARMON, A.G., HIBEL, L.C., RUMYANTSEVA, O., et al. 2007. Measuring salivary cortisol in studies of child development: watch out-what goes in may not come out of saliva collection devices. Dev. Psychobiol., 49, 2007, 495–500.
22.MAURUS, R., BEGUM, A., WILLIAMS, L.K., et al. Alternative catalytic anions differentially modulate human alpha-amylase activity and specificity. Biochemistry., 47, 2008, 3332-3344.
23.BOSCH, J.A., DE GEUS, E.J., CARROLL, D., et. al. A general enhancement of autonomic and cortisol responses during social evaluative threat. Psychosom. Med., 71, nr. 8, 2009, 877-885.
24.GARRETT, J.R. The proper role of nerves in salivary secretion: a review. J. Dent. Res., 66, nr. 2, 1987, 387-397.
25.PROCTOR, G.B., CARPENTER, G.H. Chewing stimulates secretion of human salivary secretory immunoglobulin A. J. Dent. Res., 80, nr. 3, 2001, 909-913.
26.HUMPHREY, S.P., WILLIAMSON, R.T. A review of saliva: normal composition, flow, and function. J. Prosthet. Dent., 85, nr. 2, 2001, 162-169.
27.GHICIUC, C.M., COZMA-DIMA, C.L., PASQUALI, V., et al. Awakening responses and diurnal fluctuations of salivary cortisol, DHEA-S and α-amylase in healthy male subjects. Neuro. Endocrinol. Lett., 32, nr. 4, 2011, 475-480.
28.ROHLEDER, N., NATER, U.M., WOLF, J.M., et al. Psychosocial stress-induced activation of salivary alpha-amylase: an indicator of sympathetic activity? Ann. N.Y. Acad. Sci., 1032, 2004, 258-263.
29.KIRSCHBAUM, C., PIRKE, K.M., HELLHAMMER, D.H., 1993. The 'Trier Social Stress Test' - a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology., 28, 1993, 76-81.
30. DIMA-COZMA, C., PATACCHIOLI, F.R., GHICIUC, C.,M., et al. Current perspectives in stress research and cardiometabolic risk. Rev. Cercet. Interv. So., 45, 2014, 175-188.
31.GORDIS, E.B., GRANGER, D.A., SUSMAN, E.J., et al. Asymmetry between salivary cortisol and alpha-amylase reactivity to stress: relation to aggressive behavior in adolescents. Psychoneuro-endocrinology., 31, 2006, 976-987.
32.STRAHLER, J., BERNDT, C., KIRSCHBAUM, C., et al. 2010a. Aging diurnal rhythms and chronic stress: distinct alteration of diurnal rhythmicity of salivary α-amylase and cortisol. Biol. Psychol., 84, 2010, 248-256.
33.COZMA, S., DIMA-COZMA, L.,C., GHICIUC, C.M., et al. Salivary cortisol and α-amylase: subclinical indicators of stress as cardiometabolic risk. Braz. J. Med. Biol. Res. 50, nr. 2, 2017, e5577.
34.MONTELEONE, P., SCOGNAMIGLIO, P., CANESTRELLI, B., et al. Asymmetry of salivary cortisol and αamylase responses to psychosocial stress in anorexia nervosa but not in bulimia nervosa. Psychol. Med., 41, 2011, 1963-1969.
35.FISHER, A.J., GRANGER, D.A., NEWMAN, M.G. Sympathetic arousal moderates self-reported physiological arousal symptoms at baseline and physiological flexibility in response to a stressor in generalized anxiety disorder. Biol. Psychol., 83, 2010, 191-200.
36.COLE, S.W., NALIBOFF, B.D., KEMENY, M.E., et al. Impaired response to HAART in HIV-infected individuals with high autonomic nervous system activity. Proc. Natl. Acad. Sci. U.S.A., 98, 2001, 12695-12700.
37.CRESPI, H., ARMANDO, I., TUMILASCI, O., et al. 1982. Catecholamines levels and parotid secretion in children with chronic atopic dermatitis. J. Invest. Dermatol., 78, 1982, 493-497.
38.PIPPI, R., PATINI, R., GHICIUC, C.,M., et al. Diurnal trajectories of salivary cortisol, salivary α-amylase and psychological profiles in oral lichen planus patients. J. Biol. Regul. Homeost. Agents. 28, nr. 1, 2014, 147-154.
39.WOLF, J.M., NICHOLLS, E., CHEN, E. Chronic stress, salivary cortisol, and alpha-amylase in children with asthma and healthy children. Biol. Psychol., 78, 2008, 20-28.
40.PATACCHIOLI, F.R., TABARRINI, A., GHICIUC CM, et al. Salivary biomarkers of obstructive sleep apnea syndrome in children. Pediatric. Pulmunology., 49, nr. 11, 2014, 1145-1152.
41.GHICIUC, C.,M., DIMA-COZMA, L.,C., BERCEA, R.,M., et al. Imbalance in the diurnal salivary testosterone/cortisol ratio in men with severe obstructive sleep apnea: an observational study. Braz. J. Otorhinolaryngol., 82, nr. 5, 2016, 529-535.
42.AL'ABSI, M., WITTMERS, L.E. Enhanced adrenocortical responses to stress in hypertension-prone men and women. Ann. Behav. Med., 25, 2003, 25-33.
43.DIMA–COZMA, L.C., GHICIUC, C.M., MITU, F., et al. Salivary alpha-amylase activity – a possible non-invasive biomarker of cardiovascular involvement in type 2 diabetes mellitus. Interdiab 2016: Diabetes mellitus as cardiovascular disease.Book Series: International Conference on Interdisciplinary Management of Diabetes Mellitus and its Complications, Editura Niculescu, Bucuresti, 2016, 211-220.

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