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A case study on salivary amylase: A non-invasive biomarker of stress in humans under pathological and strenuous situations

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Abstract

Elucidation and identification of mechanisms underlying a dysregulation of major components of the stress response system particularly in humans, is a very challenging task. Saliva sampling has the advantage that it is non-invasive, making multiple sampling easy and stress free. We examined the effects of pathological conditions, psychological stressor and soother on the salivary amylase levels in different aged subjects preferably young adults, and compared the characteristics of parameters like difference in enzyme activity between different aged subjects under normal condition, difference in the enzyme activity between patients under various pathological conditions, students under demanding conditions and healthy subjects under psychological stress. Compared to base line data generated statistically the subjects under various stressor situation found to have higher enzyme activity with no significant difference in flow rate. Salivary amylase level was more significantly increased and reacted more rapidly by psychological stressor, suggesting that it is a better index of stress. Furthermore, it is suggested that the enzyme is a soothing or relaxation index.

Keywords: Non-invasive, Pathological, Psychological, Subjects, Enzyme activity.

1. Introduction

Stress and stress-related health impairments have become major problems in human life, investigations into the biological pathways linking stress and disease are of major importance [1]. Humans usually suffer from either of psychological and physical stress in their day to day life. It is widely accepted that psychological stress could produce physiological effects that are similar to those produced by physical challenges in a variety of physiological systems. Two primary systems are particularly involved in setting on the stress response, hypothalamus—pituitary—adrenocortical axis (HPA) and sympatho-adrenomedullary (SAM) system. The activation of HPA causes an increase in cortisol secretion in adrenal cortex [2, 3]. Salivary cortisol concentrations are closely correlated to serum cortisol concentration [4]. Thus, salivary cortisol reliably reflects the HPA activity, and is a more practical assessment tool than blood collection in stress research due to its potential to elicit spurious increases in cortisol secretion reflecting a hyperstress component [5]. α -amylase and cortisol [6] are frequently used to assess stress. Salivary α -amylase activity is linked with the SAM system [7] and is significantly correlated with noradrenaline levels in saliva. It has been reported that salivary amylase levels rise in response to physiological and psychological stress [8]. Extreme environments allow us to examine various aspects of the hormone physiological relationship that is essential to fully understand the concept of adaptation of humans to the stresses of these environments [9]. It is known that the social environment can exert modulating effects on salivary cortisol stress responses. In men, brief social support resulted in significantly decreased salivary cortisol responses depending on the quality of support whereas women showed even marginally higher salivary cortisol responses when supported by their own partner in life. Kirschbaum *et al.*, (1995a) and Heinrichs *et al.* (2003) observed that the neuropeptide oxytocin enhances the buffering effect of social support on salivary cortisol stress responsiveness in men pointing to a potential underlying biological mechanism for stress-protective effects of positive social support [10, 11]. Recent observations indicate a relation between salivary amylase secretion and experience stressful condition. The enzyme concentration increases under both physical stress, such as treadmill exercise, running, bicycle exercise and cold exposure and psychological stress as well such as

watching highly negative emotional pictures of mutilation or accidents, participating in collegiate level individually oriented athletic competition, written examination, and Trier Social Stress Test (TSST). When the concentration of catecholamines (epinephrine and nor-epinephrine) increases in the blood due to stress, the salivary amylase concentration also increases [12].

An extensive phenotyping including salivary cortisol responsivity is essential in order to be able to uncover mechanisms mediating stress-related disorders and to potentially develop new therapeutic strategies in the future. Such a research agenda depends on substantial knowledge of moderating and intervening variables that affect free cortisol responses to different kinds of stressors and stimuli [1].

Thus, the measurement of salivary amylase may be utilized for evaluation of the stress level of people who were in extreme and isolated environments, our study aimed to know whether enzyme activity of salivary amylase is useful as a biomarker for stress in humans under pathological and most demanding conditions.

2. Materials and Methods

2.1 Sample collection from different age group subjects

The subjects were 12-16 years (healthy young subjects), 20-30 years (healthy adults) and 50-60 years (healthy old subjects without Diabetes) who were eligible for the study and free of acute and chronic medical conditions. The saliva was collected from the same subjects under normal conditions according the method described previously by Ran-Hi Hong *et al.*, (2009) wherein sample collection was done for six times a day at 7:00 (awakening), 8:00 (1st hour after awakening), 10:30, 12:00, 17:30, and 22:30 (before sleep). Subjects were requested in advance to rinse their mouth with water and not to eat or drink 1 hour before saliva collection. After the collections, all samples were kept in an icebox and immediately transferred to the laboratory, where they were stored at -20°C until analysis. The collected saliva sample was centrifuged at 5000 rpm for about 10 min and the supernatant was taken for further analysis [13].

2.2 Sample collection from patients: The subjects were 20-39-year-old both men and women who were eligible for the study and free of acute and chronic medical conditions. Saliva samples were collected from patients who were under pathological conditions from the Logic and Clue Diagnostics centre before medication. Subjects were requested in advance to rinse their mouth with water and not to eat or drink 1 hour before saliva collection. After the collections, all samples were kept in an icebox and immediately transferred to the laboratory, where they were stored at -20°C until analysis. The collected saliva sample was centrifuged at 5000 rpm for about 10 min and the supernatant was taken for further analysis.

2.3 Sample collection from students under demanding condition:

The subjects (20 volunteer's) were aged between 20-23 years both boys and girls, who were eligible for the study and free of acute and chronic medical conditions. The saliva was collected from the college students who were instructed to deliver seminar on life science topics provided the two days prior intimation. The sample was collected 5 min before and after the seminar. Subjects were requested in advance to rinse their mouth with water and not to eat or

drink 1 hour before saliva collection. After the collections, all samples were kept in an icebox and immediately transferred to the laboratory, where they were stored at -20°C until analysis. The collected saliva sample was centrifuged at 5000 rpm for about 10 min and the supernatant was taken for further analysis.

2.4 Sample collection from adults subjected to psychological stress:

This experiment was followed according to the method previously described by Noriyasu Takaia *et al.*, (2004) with slight modifications as follows; Experimental sessions were limited to the hours between 15:00 and 17:00 to minimise time of day effects. Subjects (volunteer's) sat unrestrained in a comfortable chair with lumbar support opposite a 19-in. TV monitor placed 100 cm away at eye level after thoroughly rinsing their mouths. A video recording of post-mortem surgery, which included scenes of dissecting out of body parts with scissors, served as the psychological stressor for 20 min. A scenic beauty video viewing was also used as the soother. Thirty subjects viewed only the stressful video or the soothing video each. Twenty subjects viewed both the videos, but both the experimental sessions were not carried out on the same day. All subjects viewed each video for the first time. After the video ended, they remained quiet for 15 min. Subjects were instructed to tilt their heads slightly forward without taking their eyes off the TV monitor, and to accumulate the saliva in the floor of the mouth before spitting the saliva into a pre-weighed plastic vial to measure the weight. All the accumulated saliva was collected every 3 min throughout the session. Salivary flow was expressed in mg of saliva per min (mg/min). Then, the saliva samples were centrifuged and the supernatant was stored at -20°C until analysed [14].

2.5 Determination of specific enzyme activity: Salivary α -amylase collected from the healthy subjects and patients who were under different kind of stress either by situation or by disease was incubated with the starch and evaluated for its activity by DNS reduction assay wherein activity was calculated by taking maltose standard curve followed by determination of specific activity calculated based on its protein concentration estimated by Lowry's method by taking Bovine serum albumin as standard with concentration ranges between 0-75 $\mu\text{g/ml}$ [15].

3. Results

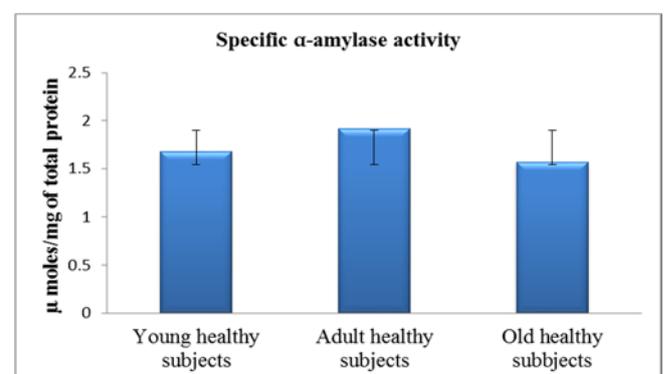


Fig 1: Specific α -amylase activity of different age group healthy subjects

The salivary α -amylase which was collected from different aged healthy subjects at different time intervals was subjected to interact with its substrate i.e., starch under

previously mentioned standard conditions and activity followed by specific activity was calculated for all the samples collected throughout the day wherein the values obtained represents Standard deviation \pm mean. The result indicates specific activity was found maximum in case of adult healthy subjects aged between 20-30 years under normal non pathological conditions. The values provide the base data for the specific amylase activity among the tested healthy subjects.

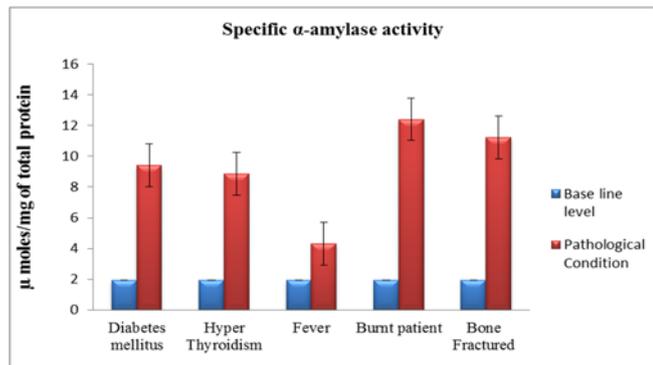


Fig 2: Specific α -amylase activity of patients suffering from various pathological conditions

The salivary α -amylase which was collected from patients aged between 20-30 years, specific activity was calculated for all the samples collected prior to medication wherein the values obtained represents Standard deviation \pm mean and the error bars indicate the S.E (N=20 for each condition). The result indicates specific activity was found maximum in all the cases of adult subjects under pathological conditions compared to the base line data for the specific amylase activity among the tested adult subjects.

Table 1: difference in percentage of increase in specific amylase activity.

Sl. No.	Pathological condition	Difference in Percentage activity of increasing activity compared to base line data
1.	Diabetes mellitus	79.57%
2.	Hyperthyroidism	78.32%
3.	Fever	55.55%
4.	Burnt patient	84.51%
5.	Bone fractured	82.90%

Percentage difference in enzyme activity indicates increase in the activity of amylase as a consequence of pathological stress wherein under fever conditions percentage increase in activity is comparatively less over the other conditions.

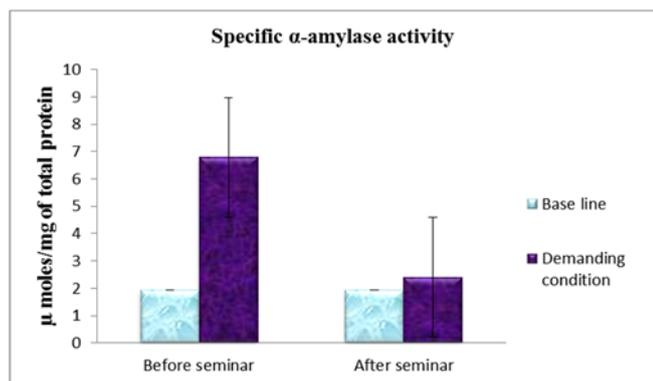


Fig 3: Specific α -amylase activity of students under demanding conditions

The salivary α -amylase which was collected from healthy students aged between 20-23 years, specific activity was calculated for all the samples collected 5 min prior to the seminar and 5 min after the seminar wherein the values obtained represents Standard deviation \pm mean and the error bars indicate the S.E (N=20). The result indicates specific activity was found maximum before delivering the seminar in all the subjects and the activity could come to the normal level after delivering the seminar from the same subjects which indicates possible stress free condition.

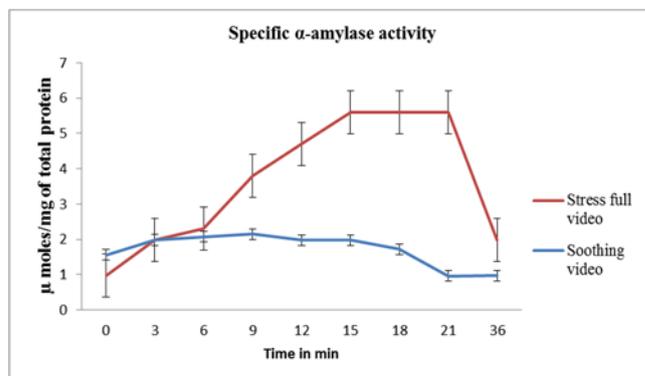


Fig 4: Specific α -amylase activity of healthy subjects under psychological stress.

The salivary α -amylase which was collected from the subjects aged between 20-30 years, specific activity was calculated for all the samples collected before, during and after the video viewing in both the cases of stress full video viewers and soothing video viewers for every three minutes wherein the values obtained represents Standard deviation \pm mean and the error bars indicate the S.E (N=20). The result indicates specific activity increases gradually in the subjects viewing stress full video till 15 min and remains same for 6 more min and decline after 15 min. No significant change in case of soothing video viewers and also in flow rate of saliva in either of the cases.

4. Discussion

The technique of diagnose the stress to be made simple and convenient. Origin of stress may vary but its effect is deleterious. It is a condition or circumstance which can disturb the normal physiological and psychological functioning of an individual. It is a well-known fact that stress of any nature produces a non-specific state in the organism i.e. the state of stress or “stress syndrome” which is characterized by adrenal hypertrophy, depletion of adrenal ascorbic acid and cortisol and a decrease in the size of lymphoid tissue [16]. During pathological conditions the salivary amylase could be used assess the level of stress that the patients are really suffering. In our studies it indicated that patients suffering from Diabetes mellitus have 79.57% higher activity (Fig 2) compared to base line data generated for the similar aged healthy subjects (Fig 1) accordingly other clinical conditions viz., hyperthyroidism (78.32%), Fever (55.55%), Mild Burnt patients (84.51%) and Bone fractured patients (82.90%) indicated increase in their salivary amylase activity compared to base line data (Table 1).

Previous studies indicated psychological stressors, such as facing the audience in public [17], academic examinations [18], dental procedures [19] and suspense films viewing [20], can induce significant increases in salivary cortisol levels.

Similar results using a stressful video viewing as a stressor were obtained in the present study, i.e., emphasizing the previous findings showing that the salivary amylase activity is a good stress index. Although there are numerous studies concerning the salivary cortisol response to HPA, only a few studies reported results according to the effect of the SAM system activities on salivary constituents such as amylase. A more recent study by Skosnik *et al.*, in which stressful video game playing served as the stressor, showed a 1.4-fold increase in the salivary amylase [21].

In our time-course experiment, the amylase level was increased just after the beginning of stressful video viewing which continues to increase till 15 minutes, remained constant till the end of the show, then it fell back to base line 15 min after the end of the video viewing (Fig 4). On the other hand, the subjects who viewed the soothing video did not show any significant change in enzyme activity during the show but the level was slightly decreased than its base line level 15 min after the end of the show. These results clearly showed that the psychological stressor increased in the amylase level due to increase in sensibility to the stressor. Bosch *et al.*, reported a two-fold increase in the salivary amylase level by psychological stress. They assayed unstimulated whole saliva from 28 dental students, and used a written examination as the psychological stressor [22]. We also created similar of situations for the students by asking them to deliver academic seminars their stress level increased as a consequence their lack of boldness to face the audience and fear of committing mistakes during the seminar which them to suffer from stress under most demanding conditions confirmed by our results (Fig 3). Herein we found 4 times the activity of amylase collected 5 min before the seminar and the level was almost equal to the base line 5 min after the seminar with the decrease in 35% activity. It is considered that the major stress response can be conceptualised as occurring in two stages: a short latency catecholamine component which represents the first system, and a slower acting glucocorticoid response representing the second system. The first response depends upon the SAM system. The second response depends upon HPA, resulting in an increase in cortisol secretion. This cortisol response in HPA is the final step in the normal stress response, and has a longer latency of secretion than that of catecholamine in SAM system [2, 3]. Presumably, the differences in the response time to stress reactions between stress full video viewers and soothing video viewers may result from the differences in the time latency between the stress response of SAM system and that of HPA which could be diagnosed by determining salivary amylase activity in humans.

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