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ORIGINAL ARTICLE

Salivary Alpha Amylase Activity in Human Beings of Different Age Groups Subjected to Psychological Stress

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Abstract Salivary alpha-amylase (sAA) has been proposed as a sensitive non-invasive biomarker for stress-induced changes in the body that reflect the activity of the sympathetic nervous system. Though several experiments have been conducted to determine the validity of this salivary component as a reliable stress marker in human subjects, the effect of stress induced changes on sAA level in different age groups is least studied. This article reports the activity of sAA in human subjects of different age groups subjected to psychological stress induced through stressful video clip. Differences in sAA level based on sex of different age groups under stress have also been studied. A total of 112 subjects consisting of both the male and female subjects, divided into two groups on basis of age were viewed a video clip of corneal transplant surgery as stressor. Activity of sAA from saliva samples of the stressed subjects were measured and compared with the activity of the samples collected from the subjects before viewing the clip. The age ranges of subjects were 18–25 and 40–60 years. The sAA level increased significantly in both the groups after viewing the stressful video. The increase was more pronounced in the younger subjects. The level of sAA was comparatively more in males than females in the respective groups. No significant change in sAA activity was observed after viewing the soothed video clip. Significant increase of sAA level in response to psychological stress suggests that it might act as a reliable sympathetic activity biochemical marker in different stages of human beings.

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Introduction

Salivary alpha-amylase [(sAA); EC 3.2.1.1] is a calcium-containing metalloenzyme that hydrolyzes the α-1, 4 linkages of starch to glucose and maltose in oral cavity. The enzyme accounts for 40–50 % of the total salivary gland-produced protein, most of the enzyme being synthesized and secreted by acinar cells of parotid gland [1, 2]. In addition to starch digestion, sAA has also been shown to have an important bacterial interactive function [3]. Various findings have demonstrated that autonomic nervous system (ANS) and the sympathetic nervous system (SNS) in particular, is involved in the release of sAA.

In recent past, sAA has drawn the attention of researchers for its role as sensitive biomarker for stress-related changes in the body that reflect the activity of SNS [4–7]. A number of studies applying psychological stress protocols have shown that sAA is highly sensitive to stress-related changes. An increase in concentration of sAA has been observed in subjects under academic examination stress conditions [8]. Significant increase in sAA has also been reported in conditions such as during preparation for skydiving [9], using stressful video game [10], during mental arithmetic task [11], driving stimulation [12] etc. Also, the diurnal profiles of sAA and salivary cortisol in ballroom dancers of different age and sex have been investigated [13]. In addition to psychological stress, increase in sAA has also been observed in several studies during and after physical exercise [4, 14, 15].

While sAA has been suggested as non-invasive biomarker for SNS activity, most of the studies pertaining to psychosocial stress-induced sAA responses have been carried out in children and young subjects. To our knowledge, there is scanty of information pertaining to effect of psychological stress on sAA level in older population. Strahler et al. [16] for the first time compared the sAA level of old adults in response to stress with that of children and young adults. Since the health conditions and diseases prevalence pattern changes with the progression of age, the stress reactivity of people belonging to various age groups needs to be addressed. This would help to find out the health conditions through a variety of stress-induced markers.

Therefore in the present study attempt has been made to examine the activity of sAA in human subjects of different age groups subjected to psychological stress induced through stressful video clip. Level of sAA in male and female subjects of these age groups under stress has also been compared.

Materials and Methods

Place of Study and Characteristics of Subjects

The experiment was carried out in Biochemistry Laboratory of Faculty of Life Sciences of MATS University and was being approved by the ethical committee of the University. A total of 112 human subjects, consisting of equal number of males and females participated in the present study. The subjects were divided into two groups based on their age. The age of the first group ranged from 18 to 25 years and that of the second group from 40 to 60 years. The details of the participants were obtained through predesigned questionnaires. The study included the participants that were without physical and mental illness, pregnancy and any history of oral disease. Before the study, the health status of the subjects was examined by a medical doctor to find out current or past health related problems. Participants with habits like smoking, tobacco chewing, alcoholic, and using substances such as sleeping pills, oral contraceptives etc. were strictly excluded from this study. The subjects were instructed not to take any food or drink except water for at least 2 h prior to the experiment. The aim, objective and procedure of the study were explained to the subjects and written consent was obtained before conducting the experiment.

Measurement of Physical Parameters

The height and weight of each participant were measured. The body mass index (BMI) was calculated using the formula weight in kg/height in m^2. Blood pressure of the participants was also measured during the health check up before the commencement of the experiment. These measurements were all carried out by the medical doctor.

Collection of Saliva Sample

The collection of saliva sample from the subjects was carried out according to the method as followed by Takai et al. [17]. All the participants were shown a video clip of corneal transplant surgery for 20 min. The video clip was used as a stressor. The TV monitor was placed at 120 cm away from the subjects. The participants were asked to wash their mouth cavity thoroughly before viewing the clip. On a different day the same subjects were shown a scenic beauty video clip as a soother. The subjects had never viewed these videos earlier. In both the cases the subjects were instructed to tilt their heads slightly forward without taking their eyes off the TV monitor, and to accumulate the saliva in mouth cavity for 10 min. The saliva from the mouth cavity was collected in pre-weighed plastic vials to measure the weight. Then, the saliva samples were centrifuged in a cooling centrifuge at $10{,}000 \times g$ and the supernatant was stored at -20 °C until biochemical analysis. The above experimental sessions were limited to morning hours between 8.00 to 10.00. The saliva samples collected from each of these subjects 1 h before viewing the video clips were considered as the baseline samples. The weight of the saliva collected per minute is the saliva flow rate and was expressed as mg/min.

sAA Activity

The activity of sAA was determined according to Gunatillaka et al. [18]. The saliva sample was thawed and diluted to 1/250 with normal saline. Buffered starch prepared by dissolving the paste of 0.4 g of soluble starch with the boiling solution containing 200 mM disodium hydrogen phosphate, 30 mM sodium chloride and 70 mM of sodium benzoate. The mixture was cooled and the pH was adjusted to 7.0. 1 ml of the buffered starch solution was taken in test tubes and were placed in water bath at 37 °C for 5 min. 20 µl of the diluted saliva sample was then added to the tube. The contents of the tubes were mixed thoroughly in a vortex mixer and incubated in a water bath at 37 °C for exactly 7 min 30 s. After incubation, immediately 1 ml of 5 mM iodine solution and 8 ml of distilled water was added to the tubes. The contents were mixed thoroughly and the absorbance was measured at 660 nm using an UV–visible spectrophotometer. The tube containing all the above contents except the diluted saliva sample was used as blank. Amylase activity was calculated using the formula (B–T)/B × 1,470 where B and T are absorbances of the reagent blank and test samples respectively and 1,470 is the factor to express

Table 1 Sample characteristics of participants

Variable	Young adults (n = 56)		Older adults (n = 56)	
	Mean (SD)	Range	Mean (SD)	Range
Age (years)	21.3 (2.2)	18–25	52.4 (6.3)	40–60
Height (cm)	164.3 (4.6)	157–180	168.8e (6.2)	152–175
Weight (kg)	51.21 (7.8)	48–68	72.5 (9.7)	52–89
Blood pressure (mm Hg)	116.32 (3.5)/ 83.21 (2.1)	110/78–130/90	134.61 (4.1)/ 89.13 (2.4)	110/82–140/90
BMI (kg/m^2)	21.3 (3.1)	17–24	25.6 (4.2)	23–27

values in U/l. The enzyme activity of sAA was expressed in U/ml. One unit of the enzyme is defined as that liberates 1.0 mg of maltose from starch in 1 min at pH 7.0 at 37 °C.

Statistical Analysis

The activity of sAA for each subject was measured minimum for three times from the collected sample and presented as mean ± SD (standard deviation). Student's *t* test was used to evaluate the salivary parameters between male and females as well as between age groups.

Results

Sample Characteristics

The sample characteristics of the participants for this study including height, weight, BMI and blood pressure have been presented in Table 1.

Effect on Flow Rate of Saliva

A wide range of saliva flow rate in individual subjects was observed. The flow rate increased in subjects of 18–25 age group as compared to subjects of 40–60 age group. However, much variation was not found in saliva flow rate of male and female subjects (data not shown).

Effect of Stressful Video Clip on Salivary Amylase Activity

Effect on 18–25 Age Group

Biochemical analysis of the saliva sample showed a noticeable difference in baseline amylase activity and stressed amylase activity in subjects of 18–25 age group of both the genders. The baseline amylase activity of the male subjects of 18–25 age group varied between 91.86 and 249.61 U/ml where as the activity in the same subjects in response to stressed video clip ranged from 152.13 to 297.64 U/ml. When the mean baseline activity of the male subjects was compared with that of the activity of stressful subjects, a significant increase in amylase activity ($P \leq 0.05$) was observed in stressful subjects. The amylase activity of the female subjects of 18–25 age groups also varied to a greater extent. The minimum baseline amylase activity observed for the female subjects was 85.75 U/ml and the maximum activity was 189.25 U/ml. After being subjected to stress through video clipping the activity of the enzyme of each of these female subjects increased in all the stressed female subjects. The activity in these stressed females varied between 132.23 and 220.81 U/ml. As in the stressful male subjects, a significant increase in amylase activity ($P \leq 0.05$) was also observed in stressful female subjects. The activity of amylase in baseline sample and that of stressful subjects of 18–25 age group of both the sexes is presented in Fig. 1.

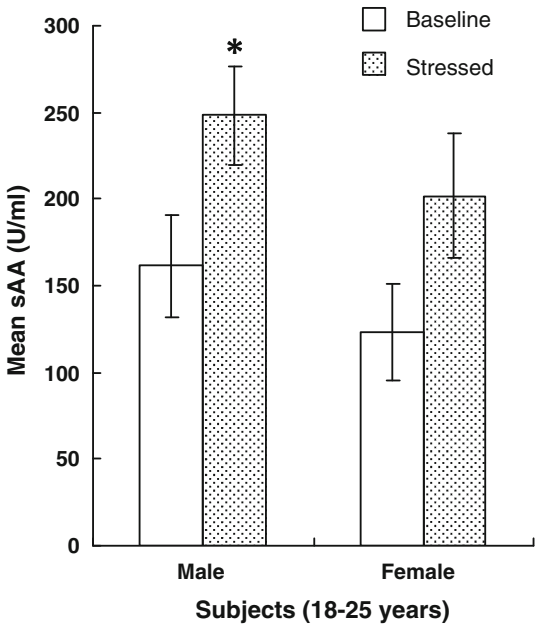

Fig. 1 Effect of stressful video viewing on sAA levels of subjects of both the sexes belonging to 18–25 age group. *Bars* represent ±SD. Statistically significant difference at $^*P \leq 0.05$

Effect on 40–60 Age Group

The baseline activity of sAA of the male subjects of 40–60 age group ranged from 76.23 to 159.14 U/ml and that of female subjects ranged between 75.75 and 178.62 U/ml. The activity of the enzyme increased in all the male and female stressed subjects of 40–60 age groups. The activity in the stressed male subjects varied from 102.68 to 221.0 U/ml where as in stressed females varied from 112.13 to 209.81. The mean amylase activity of both stressed male and female subjects increased significantly

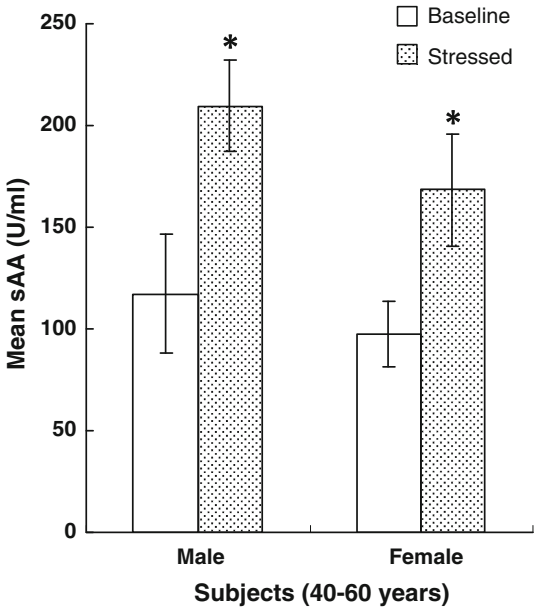

Fig. 2 Effect of stressful video viewing on sAA levels of subjects of both the sexes belonging to 40–60 age group. *Bars* represent ±SD. Statistically significant difference at $*P \leq 0.05$

($P \leq 0.05$) as compared to the baseline activity. Amylase activity of the baseline sample and that of the stressful subjects of 40–60 age groups of both the sexes is presented in Fig. 2.

Effect Based on Sex Differences

The activity of sAA of the male subjects in both the age groups was 15–28 % higher than the corresponding female subjects before and after viewing the video clips. The difference in sAA activity of male subjects of both the age groups in response to stress was almost same with that of female subjects.

Effect of Soothing Video Clip on Salivary Amylase Activity

The amylase activity of the subjects of 18–25 age groups after viewing the soothing video clip increased in none of the subjects in both the sexes. However, the activity either decreased slightly or remained same in these subjects. No significant ($P \leq 0.05$) difference in amylase activity of the baseline samples and stressed samples was observed in the subjects of 18–25 age group of both the sexes.

In case of subjects belonging to 40–60 age group the activity of amylase slightly increased in 40 % subjects, decreased in 20 % subjects and remained same in 40 % subjects. The decrease and increase were not found to be significant ($P \leq 0.05$). The effect of soothing video on salivary amylase activity of both the age groups is presented in Fig. 3.

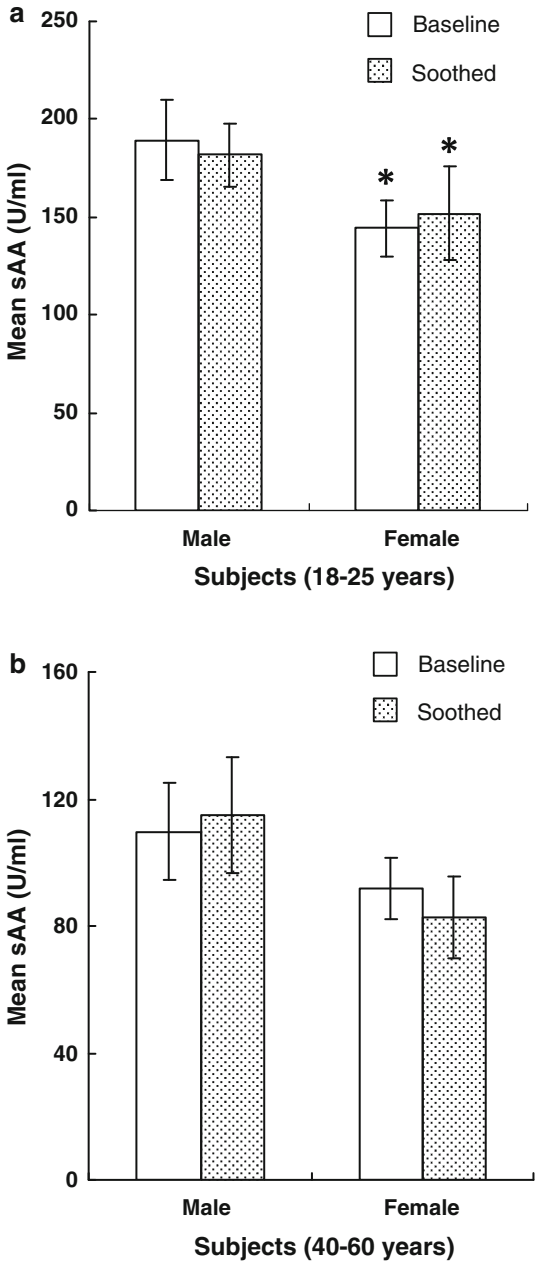

Fig. 3 Effect of soothing video viewing on sAA levels of subjects of both the sexes belonging to 18–25 age group (**a**) and 40–60 age group (**b**). *Bars* represent ±SD. Statistically significant difference at $*P \leq 0.05$

Discussion

The development of a new biochemical marker is an ongoing research in the field of psychophysiological and clinical practice. The use of salivary biomarkers has gained increased popularity over the past decade in psychological

and biomedical research. Increase in concentration of salivary components is known to be associated with psychological stress of various kinds such as public speaking, academic examination, viewing of suspense films etc. Though several studies have been conducted in human subjects to find the effect of psychological stress on sAA concentration, the effect of the stress on sAA concentration and activity on the basis of age and gender is least reported. In the present study, an increase in the activity of sAA was observed in both male and female human subjects of different age groups in response to stressful video.

Psychological stress factors employed in various manners have shown that sAA is highly sensitive to stress induced changes. Bosch et al. [19] have measured several salivary parameters in subjects exposed to academic examination and observed a two-fold increase in the level of sAA. An increased sAA was observed before jumping in subjects preparing for skydiving [9]. In another study, a significant increase in sAA has also been observed using a stressful video game [10]. The effect of psychological stress differs from persons to person on the basis of gender as well as age which could be estimated through the measurement of various biomarkers such as level of sAA and other salivary components. In our study, we observed a significant increase in sAA in young adults than the elder groups indicating that young subjects usually experience comparatively higher amount of acute stress than the adults. In a recent study, it has been reported that sAA concentration in children increases as compared to adults with response to psychological stress [16]. Increased sAA activity may not be due to increased saliva flow rate as previous studies have evidenced that stress induced activity of sAA is independent of salivary flow rate [20]. Studies have also shown that though the is age related changes in morphology of oral mucosa, the function of salivary gland and saliva composition remain almost unchanged [21].

The variation in sAA activity based on age differences in response to psychological stress could be due to the differences in response level of the nervous system in general and SNS in particular. Reduced reactivity of sAA in older adults corresponding to stress related SNS activity with aging is known in several cases [22, 23]. Also, as the activity of sAA that was measured before and after viewing the soothing video clip in subjects of both the age groups did not vary significantly, further substantiated that sAA secretion could be influenced by psychological stress of various natures.

In the present study, the sAA activity was comparatively more in male subjects as compared to the corresponding female subjects in all the experiments. sAA level based on sex differences is least studied. Kivlighan and Granger [24] have reported higher sAA levels in men as compared to that of women. Similarly, other previous studies have shown that male subjects possessed a significantly higher baseline sAA level than that of females [25]. Takai et al. [26] have observed no differences in amylase activity of men and women belonging to high and low anxiety groups. These data including the present regard to impact of sex on sAA level are in contrast with that as reported by Nater et al. [27] and depends on the time and other experimental conditions which perhaps affect the SNS and sAA levels more in men as compared to women.

Thus the present study along with the other previous relevant studies indicates that sAA changes in human subjects in response to psychological stressor could act as a sympathetic activity marker at all stages of human life. Further studies covering the subjects of both the sexes in groups of less difference of age or of same ages should be conducted to establish sAA and other salivary components as a reliable biomarker in response to stress. This also needs to be investigated whether these changes in salivary components including sAA are related with health conditions.

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