

Effect of Psychosocial Stress on the Salivary Alpha Amylase Levels in Children going and not going to School

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ABSTRACT:

BACKGROUND: Salivary alpha-Amylase is a starch digesting enzyme. In addition to its function in the context of nutrition, it has also turned out to be useful for monitoring sympathetic nervous system activity.

AIMS: The aim of the present study was to evaluate the association of psychosocial stress on salivary alpha amylase responses in children going and not going to school aged 9-14 years.

METHODS: The study includes 60 subjects of which 30 children went to and 30 children did not go to school. These children were between the age of 9-14 years. Each group was further sub-divided into 15 each of boys and girls. STRESS in CHILDREN (SiC) Questionnaires were given to all the subjects to evaluate stress. One ml of fresh un-stimulated whole saliva was collected from all the participants.

RESULTS: The SiC scores between boys and girls showed a statistically significant difference ($p < 0.05$) within the group. The correlation between global mean score of stress in children questionnaire with salivary alpha amylase levels showed a statistically significant difference among boys not going to school (Group 2A) ($p = 0.03$) when compared to all the groups.

CONCLUSION: The results showed a possible influence of stress on salivary alpha amylase response in boys not going to school when compared to all the groups.

Key words: Psychosocial stress; Salivary Amylase; Sympathetic nervous system

INTRODUCTION

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.^{1&2}

Alpha-amylase is one of the major salivary enzymes in humans, and is secreted from the salivary glands in response to sympathetic stimuli.³ Chatterton⁴ reported that there was a good association between the concentration of salivary amylase and blood levels of catecholamines. Currently, it is considered that measurement of this salivary enzyme is a useful tool for evaluating the SAM system.^{5&6}

Salivary α -amylase is an enzyme important for carbohydrate digestion and its secretion is under strong neurohormonal control (i.e., released upon sympathetic stimulation). Saliva products are released by acinar cells after neurotransmitter stimulation.⁷ Acinar cells are innervated by sympathetic and parasympathetic branches of the autonomic nervous system.⁸ While functioning as a digestive enzyme, sAA has turned out to be useful for detecting autonomic activity.⁹ Recent studies in humans have found a relationship between intra-individual changes in sAA and physical^{4, 10, 11} and psychological stressors.^{4, 11-16}

MATERIAL AND METHODS

Subjects

The study included 60 subjects of which 30 were children going to school (Group 1) and 30 children not going to school (Group 2), aged between 9-14 years. Each group was further sub-divided into 15 each of boys and girls. The study conducted was approved by the ethical committee of the college and undertaken with the understanding and written consent from each subject. Questionnaires were distributed prior to the experiment to evaluate psychosocial stress. The subjects involved in the study had no history of any medical problem and were not engaged in any physically or mentally stressful activity for past one hour before collection of saliva sample. STRESS in CHILDREN (SiC) Questionnaire was given to all the subjects to evaluate stress.¹⁷ It includes the degree of distress and the presence of symptoms or impaired well-being, as well as some important protective aspects such as coping strategies, expectancy, and social support. Such inclusive measurement of various aspects relevant to stress could also be useful when delineating relationships with biological markers.

Each question was rated on a fourth-point Likert scale ranging from never (0), sometimes (1), often (2) to always (3). The resulting scores were calculated for each inventory by totalling their 20 response scores, which can range from 0 to 60. SiC Global Mean Score (GMS) for each participant was calculated by dividing the total score by the numbers of questions answered.

Saliva collection

Saliva was collected from all children between 10.00 am to 12.00 pm to avoid circadian variation.¹⁸ First oral cavity of these children was thoroughly washed with normal saline to wash away previously collected saliva in oral cavity. Fresh unstimulated whole saliva was collected by method described by Harmon^{19&20} in which a cotton pledget is placed below the tongue. When cotton pledget got fully saturated with saliva, it was removed and placed in an empty syringe with piston removed. By pressing piston, approximately 1 ml of saliva was expressed from cotton and collected in sterilized container. It was stored at -20 °C till it was sent to the lab for analysis of alpha amylase.

Salivary Analysis

Measurement of salivary alpha amylase was done in a biochemistry lab using an enzyme kinetic assay which is recommended by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) in 1998.^{21&22}

10 μ l of collected saliva was incubated with 4,6-ethyliden-G7-PNP and the auxiliary enzyme alpha-glucosidase. Alpha Amylase was broken down by the auxiliary enzyme into p-nitrophenol (PNP) and glucose. PNP absorbs light at a wavelength of 405 nm (yellow). The more Alpha amylase is present in the saliva sample, the more substrate is broken down into PNP in a specific period of time, and the higher the optical density that can be measured at 405 nm. The color change was quantified by measuring the microplate in a regular photometric plate reader twice, first after warming up to 37.8°C and a second time after a defined period of 2-5 min with a 405 nm filter. The resulting color change was directly proportional to amylase activity.

STATISTICAL ANALYSIS:

The salivary alpha amylase level in saliva was compared with SiC Global Mean Score (GMS) using various statistical tests like one way ANOVA test, Tukeys multiple post hoc procedure and Karl-Pearson's correlation coefficient method. Statistical significance was accepted at $p < 0.05$.

RESULTS:

The values of global mean score of SiC and salivary alpha amylase levels in school going children (Group 1A and 1B) was more when compared to non school going (Group 2A and 2B) but there was statistically no significant difference when assessed by Tukeys multiple posts hoc procedures (Fig. 1 & 2). When assessed by gender, the SiC scores between boys and girls showed a statistically significant difference ($p < 0.05$) within the group with higher levels in girls (Table 1). The amylase levels did not show any statistically significant difference in school going children and non school going children and in boys and girls (Table 2). The correlation between global mean score of stress in children questionnaire with salivary alpha amylase levels is statistically significant among boys not going to school (Group 2A) ($p = 0.03$) when compared to all the groups when assessed by Karl-Pearson's correlation coefficient (Table 3). No significant correlation was found in any of the other groups.

DISCUSSION:

The idea that sAA may serve as indicator of psychological stress emerged in the late 1970s. To our knowledge, the relationship between sAA and psychological stress was first examined in a study by Gilman et al. (1979a),²³ in which subjects were exposed to eight days of hyperbaric pressure. Findings showed increased concentrations of sAA. The authors concluded that these increases were not only due to the hyperbaric exposure and its effects on the ANS, but also due to the psychological stress caused by the procedure itself. In the 1980s, Donald Morse and his group undertook a series of experiments with the goal of examining the impact of stress on various salivary parameters. Their findings proved quite counterintuitive for

researchers interested in salivary alpha-amylase. Morse et al. found that relaxation, not stress, leads to increases in sAA.^{24,25} However, these studies were methodologically flawed in that there was no control condition or random allocation to treatment groups. Furthermore, the "relaxation condition" always followed immediately after the "stress condition," suggesting that the increases in sAA might be attributable to a stress response occurring after the stressor. Since Morse concluded that salivary parameters were valid and reliable indicators for psychological stress and relaxation, attempts were made to re-evaluate this statement by comparing the usefulness of salivary indicators with other known psychophysiological parameters.²⁶

Whereas other stress-related factors in saliva, such as salivary cortisol,^{27,28} received widespread scientific attention in the following decade, interest in sAA as a stress marker was not sparked again until Chatterton and colleagues published their findings of increases in sAA in a variety of stressful conditions.⁴

In the present study, we examined salivary alpha amylase responses to stress in children going and not going to school. So far not many studies have been reported where examination of stress in children going and not going to school and their correlation with amylase levels has been done. There was significant correlation found between the salivary alpha amylase responses and global mean score of SiC (stress in children) questionnaire only in non school going boys ($p = 0.03$). Granger et al., 2006, did a study in young children where in under acute stress conditions, some evidence for socio economic status dependent differences in salivary α -amylase were found.²⁹ However, a statistically non-significant correlation suggested that there was an inverse proportion between salivary alpha amylase response and SiC score. This result is in accordance with the previous study by Wolf et al., 2008, where no association found between chronic home life stress and sAA (salivary alpha amylase) output variables in healthy children, it might be that disease related autonomic nervous system dysfunction is necessary to detect stable differences in salivary alpha-amylase.³⁰

Interestingly, only a few studies have found no changes in sAA in response to stressful stimuli including noise,³¹ the heel prick test in neonates,³² or a strange situation paradigm (Hill-Soderlund et al., 2008).³³ The scarcity of non-significant changes suggests that sAA is indeed a highly sensitive parameter reflecting changes caused by psychological stressors.

Bosch et al, reported a two-fold increase in the salivary amylase level because of psychological stress.³⁴ In a study done by Takai et al, clearly showed that the salivary amylase level was more significantly increased and reacted more rapidly than cortisol by psychological stressor, suggesting that it is a better index of stress. Salivary amylase measurement will be a powerful tool for psychological research.³⁵ Salivary α -amylase has been found to respond to psychological stress,^{36,37,38} a finding that is also true in children.^{39,40} The present study showed statistically high significant difference in stress scores among school going and non school going children ($p=0.0002$).

Noto and colleagues,2005⁴¹ examined sAA levels during a mental arithmetic task observed significant increases in sAA. sAA increases have also been reported in response to other psychologically stressful conditions, such as experience of medical procedures (Yamaguchi et al., 2006a),⁴² the cold pressor test (West et al., 2006)⁴³ achievement and interpersonal stress (Stroud et al., 2006),⁴⁴ a driving simulation (Yamaguchi et al., 2006b),⁴⁵ execution of neck/face surgery by medical trainees (Yamakage et al., 2007),⁴⁶ a mental arithmetic task (Goi et al., 2007),⁴⁷ oral academic examination (Schoofs et al., 2007)⁴⁸ and in a peer rejection paradigm in adolescents (Stroud et al., 2009).⁴⁹

American Psychological Association (APA) has given that stress in children can come from a variety of sources including doing well in school, making and sustaining friendships, or managing perceived expectations from their parents, teachers, or coaches. Some stress can be positive in that it provides the energy to tackle a big test, presentation, or sports event. Too much stress, however, can create unnecessary hardships and challenges.⁵⁰

When the analysis was stratified by gender, the SiC scores between boys and girls showed a statistically significant difference within the group ($p<0.05$). There was a higher levels of stress scores in girls when compared to boys within group. Kessler, found gender differences in the response to stress, and especially in the reactivity to stress, may provide cues to explain the greater rates of depression in females.⁵¹ There were no significant differences between the sexes for the SiC in a study by Walter Osika,¹⁷ sex differences are shown to appear at an older age.⁵²

Rohleder et al.2008, recently showed that daily sAA secretion is associated with self-reported shame and depression in young women.⁵³

Thus, sAA can be regarded as an excellent indicator of stress related body changes. Only a handful of studies have reported associations of chronic stress and sAA. In one study, chronic stress was found to be associated with diurnal profiles of sAA.¹⁸ Subjects reporting relatively high chronic stress had higher momentary sAA activity than subjects reporting low chronic stress levels. So, this salivary marker can be used as an indicator in diseases with autonomic dysregulation. It can also be used in treatment of stress disorders and dementia as a monitoring tool. Taken together, sAA has already been used as a marker in a variety of conditions. More recent developments suggest that it may also be a useful marker in the context of pain⁵⁴ or sleep⁵⁵ research. Therefore, assessment of sAA through saliva as an indicator of autonomic functioning might present an easy, noninvasive, and efficient sampling method.

This study has several limitations: Firstly, because of the small sample size and young age of the children to evaluate questionnaire for stress. Even studies are needed which include other measures of autonomic nervous system (ANS) activity. Furthermore, associations between sAA and other markers of sympathetic and parasympathetic activity, for example heart rate variability, should be investigated before final conclusions about the usefulness of sAA in human stress research can be drawn.

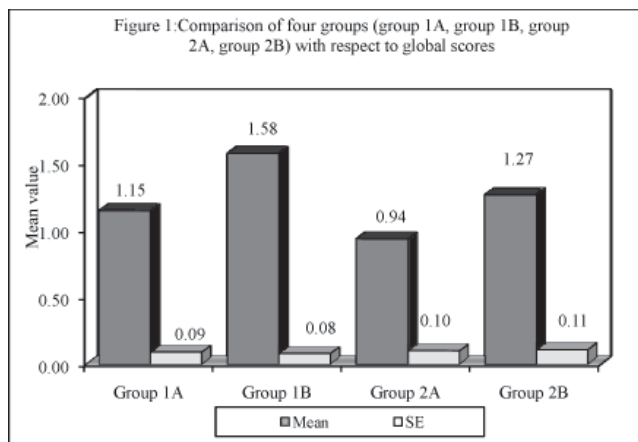


Fig. 1: Comparison of four groups (group 1A, group 1B, group 2A, group 2B) with respect to global scores

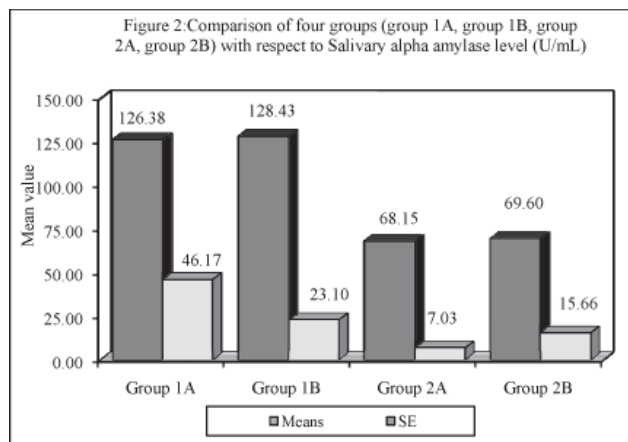


Fig. 2: Comparison of four groups (group 1A, group 1B, group 2A, group 2B) with respect to Salivary alpha amylase level (U/mL)

TABLE 1: Pair wise comparison of four groups (group 1A, group 1B, group 2A, group 2B) with respect to global score by Tukeys multiple posts hoc procedures

Group	Group 1A	Group 1B	Group 2A	Group 2B
Mean	1.1536	1.5800	0.9360	1.2736
Group 1A	P=1.0000			
Group 1B	P=0.0146*	P=1.0000		
Group 2A	P=0.3887	P=0.0002*	P=1.0000	
Group 2B	P=0.8222	P=0.1230	P=0.0449*	P=1.0000

*p<0.05

TABLE 2: Pair wise comparison of four groups (group 1A, group 1B, group 2A, group 2B) with respect to Salivary alpha amylase level (U/mL) by Tukeys multiple posts hoc procedures

Group	Group 1A	Group 1B	Group 2A	Group 2B
Mean	126.3800	128.4300	68.1470	69.6000
Group 1A	P=1.0000			
Group 1B	P=0.9999	P=1.0000		
Group 2A	P=0.4181	P=0.3716	P=1.0000	
Group 2B	P=0.4555	P=0.4089	P=1.0000	P=1.0000

TABLE 3: Correlation between global scores with Salivary alpha amylase level (U/mL) in total, group 1A, group 1B, group 2A, group 2B samples by Karl Pearson's correlation coefficient

Samples	Correlation coefficient r(X,Y)	t-value	P-value
Total	0.2984	1.0832	0.2999
Group 1A	0.2984	1.0832	0.2999
Group 1B	0.2958	1.0728	0.3044
Group 2A	0.5592	2.3368	0.0376*
Group 2B	-0.0251	-0.0869	0.9321

*p<0.05

CONCLUSION:

The study concludes that it is possible to use an easily administered self-completion questionnaire to screen for stress in children and quantitatively assess stress by using a non invasive stress biomarker such as salivary alpha amylase. Within the limitations of this study there was significant increase in salivary alpha amylase levels along with the stress (per the SiC - questionnaire) in school attending children. Amongst the group of school attending children girls were found to have higher stress level. In group of non school attending children, girls were found to have higher stress level. The association between the SiC - questionnaire score and the salivary amylase response was notable only in non school going boys but not among other groups and this finding need to be confirmed. So, we can conclude that sAA can be a characteristic biomarker for the measurement of stress.

CONFLICT OF INTEREST:

The authors have no conflicts of interest and declare no financial interests.

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