Scientific Research Publishing

Journal of Behavioral and Brain Science, 2017, 7, 360-369
http://www.scirp.org/journal/jbbs
ISSN Online: 2160-5874
ISSN Print: 2160-5866

Increase in Salivary Alpha-Amylase Levels among Non-Attending Junior High School Students Diagnosed with Social Anxiety Disorder

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How to cite this paper: Inagaki, T., Nagahama, M., Kawano, K., Wake, R., Miyaoka, T. and Horiguchi, J. (2017) Increase in Salivary Alpha-Amylase Levels among Non-Attending Junior High School Students Diagnosed with Social Anxiety Disorder. *Journal of Behavioral and Brain Science*, **7**, 360-369.
https://doi.org/10.4236/jbbs.2017.78027

Received: June 25, 2017
Accepted: August 15, 2017
Published: August 18, 2017

Abstract

Purpose: Several studies have demonstrated that the measurement of salivary alpha-amylase (sAA) levels is a useful tool for evaluating the autonomic nervous system. Psychosocial stress increases the release of sAA as a useful marker for autonomic nervous system (ANS). To our knowledge, although some studies have evaluated sAA levels under psychosocial stress, no studies have investigated the changes in sAA activity that occur in junior high school students who are not attending school due to social anxiety disorder (SAD). We aimed to investigate the relationship between the sAA levels and psychiatric states of such patients. Methods: The study subjects consisted of SAD patients (n = 39) and healthy controls (n = 57). We used a portable hand-held monitor to measure the level of sAA and State-Trait Anxiety Index (STAI) to evaluate the psychiatric state. Results: The patients' sAA activity was significantly higher than that of the controls (n = 57) ($p < 0.001$). Significant differences in heart rate (HR) (76.10 ± 11.96 vs. 68.69 ± 10.61, respectively, $p < 0.01$) and STAI scores (both the STAI-State and STAI-Trait scores) (49.35 ± 10.57 vs. 41.24 ± 8.59, respectively, $p < 0.01$; 55.69 ± 10.44 vs. 45.61 ± 9.36, respectively, $p < 0.001$) were detected between the patients and healthy controls. Conclusions: These results indicated that junior high school students with SAD exhibit a higher state of anxiety and high autonomic activity, probably due to changes in the sympathetic nervous system. As a result, junior high school students with SAD are expected to exhibit high levels of sAA accompanied by anxiety symptoms.

Keywords

Autonomic Function, Junior High School, Salivary Alpha-Amylase, School Non-Attendance, Social Anxiety Disorder

1. Introduction

Social anxiety disorder (SAD) is characterized by a recurrent and intensely anxious response to social situations in which the patient expects to be evaluated by others [1]. Patients with SAD experience more frequent anxiety and higher levels of anxiety in relation to social evaluative threat [2]. SAD is a common disorder characterized by excessive fear of scrutiny, embarrassment, and humiliation in social or performance situations. The incidence rates of the disorder are higher during childhood and adolescence (e.g., between 10 and 20 years of age) [3] and according to the DSM-5, the median age of onset of social anxiety disorder in the US is 13 years old, with 75% of those with social anxiety disorder experiencing the onset at a range of ages 8 - 15 (American Psychiatric Association, 2013). Once the disorder occurs it usually follows a chronic course and occasionally results in the development of major depressive disorder [4]. SAD often occurs in adolescent age. The DSM-5 cites the annual prevalence of social anxiety disorder as 7%, in both children and adults in the United States (American Psychiatric Association, 2013).

Junior or High school students with SAD frequently exhibit school non-attendance or school avoidance because they feel that it is impossible for them to enter the classroom and take lessons because of their social anxiety. Even when they go to school, they often stay in a separate room and only remain at school for a few hours. In Japan, school non-attendance is an important educational and psychiatric problem. There are about 24,000 non-attending elementary school students (0.36%) and 95,000 non-attending junior high school students (2.69%) (Ministry of Education, Culture, Sports, Science, and Technology, Japan, 2013). School non-attendance is defined as absence on more than 30 days one month. Such students' psychological stress is expected to be severe and to affect their physiological state. Therefore, it is important to understand students' mental state in order to aid their school life.

In this study, we have researched the relationship between the psychiatric state and physiological condition of junior high school students with SAD based on salivary alpha-amylase (sAA) measurements. Recently, numerous studies have shown that changes in sAA levels are dependent on stress stimuli (which can be physiological or psychological in nature) [5]. Yorbic *et al.* [6] reported that the sAA levels significantly increased in anxiety group compared to control group in children and adolescent aged 8 - 16 years. Several reports have suggested that psychosocial stress increases the release of sAA, and marked increases in sAA have been detected following psychosocial stress, which is indicative of the stress-dependent activation of sAA. Therefore, it is supposed that the mea-

surement of sAA levels is a useful tool for evaluating the sympathetic-adrenal-medullary (SAM) system [5] [7]-[14]. sAA has been mostly considered as an index of sympathetic nervous system (SNS) activity [6]. To the best of our knowledge, no studies have evaluated sAA levels under psychosocial stress or examined the changes in sAA activity that occur in non-attending junior high school children with SAD. The aim of this study was to investigate the relationship between the sAA levels and psychiatric states of junior high school students with SAD and to discuss the correlation between their psychiatric states and physiological changes. To do this, we used a portable hand-held monitor to obtain sAA measurements [15] [16].

2. Methods

2.1. Participants

The study subjects consisted of SAD outpatients that were being treated at the Department of Psychiatry of Shimane University School of Medicine and healthy control controls who were recruited between April 2010 and December 2016. Junior school students were recruited to make the range of age as identical as possible. Thirty-nine outpatients (mean age: 13.80 ± 0.98 years; 14 males, 25 females) who were diagnosed in our hospital with SAD according to the Diagnostic and Statistical Manual of Mental Disorders IV Text Revision (DSM-IV-TR) criteria (American Psychiatric Association, 2000) were enrolled in this study. The mean duration of the subjects' illness was 10.41 ± 8.24 months. None of the outpatients had been administered medication. We excluded any patients who were considered to be in a serious psychosocial condition (*i.e.* schizophrenia, mood disorder and personality disorders). The patients' data were compared with those obtained for the 57 healthy controls (mean age: 13.60 ± 0.50 years; 32 males, 25 females). We excluded the students with chronic disease and neurodevelopmental disorders. They were recruited from a local junior high school as a sample of convenience. The 39 outpatients with SAD and 57 healthy controls gave their written informed consent before participating in this study after the study's purpose and procedure had been fully explained to them, in compliance with the Declaration of Helsinki. The study protocol was performed in accordance with the Institutional Review Board of Shimane University School of Medicine. Both sets of participants were free of neurological and cardiovascular diseases that are known to cause autonomic dysfunction and chronic diseases, such as cancer or diabetes mellitus. None of the control subjects had any history of psychiatric illness or neurodevelopmental disorders, and none of them were taking any medication.

2.2. Measurement of Salivary Alpha-Amylase

sAA measurements, which can be obtained non-invasively, have been evaluated as a stress biomarker [9] [17]. Salivary sampling has the advantage that it is non-invasive, making obtaining multiple samples easy and stress-free [8]. sAA has

been proposed to be a marker of stress-induced physical changes. We used a hand-held monitor (Nipro Co., Japan) to measure enzymatic sAA activity automatically using reagent paper. The hand-held monitor consisted of a disposable test strip and a monitor designed by Yamaguchi *et al.* [15] [16]. The monitor was equipped with a saliva transfer device and an optical device. Approximately 20 - 30 μl of whole saliva were collected from under the tongue within about 30 seconds. Using this hand-held monitor, the saliva sampling took 30 seconds, and the saliva transfer and measurement took a further 30 seconds. Thus, a total of one minute was required for measuring sAA activity. We previously used this methodology to analyze sAA in other studies [14] [18] [19]. To control for circadian variations in sAA, one-time saliva was conducted in the morning (10:00 - 12:00 AM). All subjects were requested to rest in a chair for at least 10 minutes before the measurement were obtained [17]. Saliva samples were collected and immediately measured using the device.

2.3. Cardiovascular Measures

To assess changes in autonomic function, the resting heart rate (HR) of the subjects was measured in the recumbent position during the same time period as the saliva sampling. Each participant's HR was recorder by manual pulse measurement. HR reactivity is an important indicator of cardiovascular reactivity and is considered to be an indicator of autonomic function [20].

2.4. Evaluation of Anxiety State

To evaluate the subjects' anxiety state, we used the Japanese version of the State-Trait Anxiety Inventory (STAI) (both the STAI-Trait and STAI-State) to assess their SAD symptoms [21]. Although the STAI usually evaluates the state of generalized anxiety but not that of social anxiety, we used STAI to evaluate students' anxiety state of school life in this study. The STAI is widely used to evaluate individual differences in anxiety [22]. Both subscales consist of 20 items. STAI items are rated on a 4-point scale from 1 "*not at all*" to 4 "*very much so*". The validity and reliability of this version have been demonstrated [23].

2.5. Statistical Analysis

The results are expressed as mean ± SD values. The Mann-Whitney U test or Chi-square test was used during comparisons of clinical variables between the patients and healthy controls. The correlations between variables were calculated using Spearman's correlation coefficients using the software Ekuseru-Toukei 2012 (ver. 1.11) (Social Survey Research Information Co., Ltd., Japan). The level of significance was set at $p < 0.05$.

3. Results

Table 1 shows the characteristics of both the SAD outpatients and healthy controls.

Table 1. Participants' characteristics.

	SAD patients	Controls	*P* value
No. of participants	39	57	
Gender (male/female)	14/25	32/25	0.051
Age (years)	13.80 ± 0.98	13.60 ± 0.50	0.097
Salivary alpha-amylase level (kIU/l)	61.28 ± 29.88	27.00 ± 13.50	<0.001
Herat rate	76.10 ± 11.96	68.69 ± 10.61	0.003
STAI-State score	49.35 ± 10.57	41.24 ± 8.59	0.0037
STAI-Trait score	55.69 ± 10.44	45.61 ± 9.36	0.0001
Duration of illness (months)	10.41 ± 8.24		

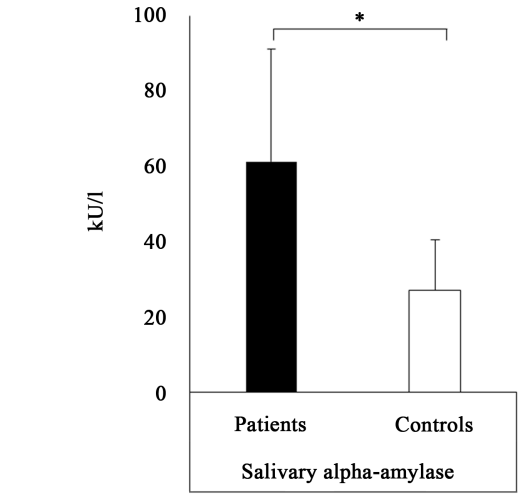
The data are shown as mean ± SD values. Statistical analyses were conducted using the Mann-Whitney U-test or χ^2 test.

Comparisons between the SAD Outpatients and Normal Controls

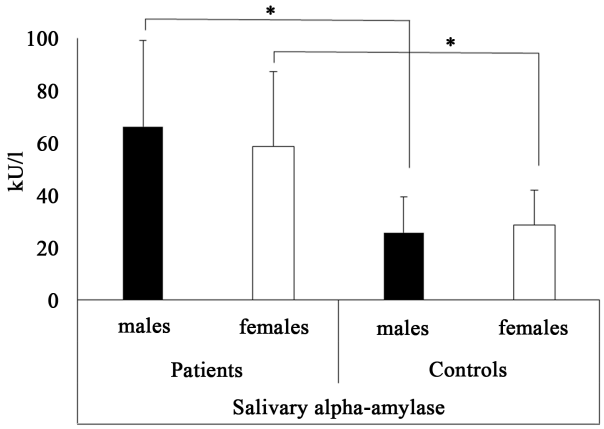
The patients and controls were individually matched for age and gender. The mean sAA levels of the SAD patients and controls were 61.28 ± 29.88 (kU/l) (range, 20 - 130) and 27.00 ± 13.50 (kU/l) (range, 4 - 59), respectively. The sAA activity of the SAD patients was significantly higher than that of the controls ($p < 0.001$) (Figure 1(a)). No significant gender differences in the sAA level were detected (patients: 14 males and 25 females, $p = 0.56$; controls: 32 males and 25 females, $p = 0.45$) (Figure 1(b)). In addition, a significant difference in HR was detected between the patients (76.10 ± 11.96 beats per minute [BPM] and healthy controls (68.69 ± 10.61 BPM) ($p = 0.003$) (Figure 2). The patients' HR was markedly higher than that of the controls. However, no significant gender differences in the HR were detected between males (76.1 ± 12.4) and females (75.5 ± 12.2) ($P = 0.91$) in patients. There was also no significant correlation between changes in sAA levels and HR in both groups. As for the relationship between the sAA level and each STAI score, both the STAI-State (49.35 ± 10.57 vs. 41.24 ± 8.59, $p = 0.0037$) and STAI-Trait (55.69 ± 10.44 vs. 45.61 ± 9.36, $p = 0.0001$) differed significantly between the patients and healthy controls (Figure 3). However, the correlations between the sAA level and the STAI scores (the STAI-State and STAI-Trait) were not significant ($p = 0.32$ and $p = 0.33$, respectively). Both HR and STAI scores were not directly proportional to the sAA activity.

4. Discussion

To the best of our knowledge, this is the first study to describe the association between changes in sAA levels and psychiatric state in junior high school students that are not attending school due to SAD. We found that the sAA levels of SAD outpatients were significantly higher than those of normal healthy controls. In the patient group, no significant gender differences in the sAA level were detected. Previous studies did not detect any gender differences in salivary biomarker responses to acute psychological stress [24] or in the sAA levels of schizophrenia patients, who also exhibit increased sAA levels [18].



(a)



(b)

Figure 1. Comparison of sAA levels between the patient and control groups. (a) The patient exhibited significantly higher sAA activity levels than the controls. *$p < 0.01$; (b) No gender difference of sAA levels were detected.

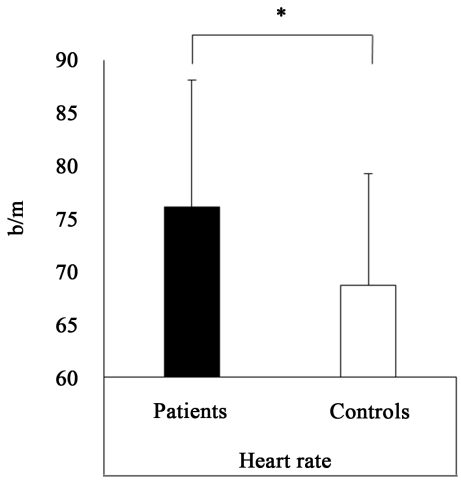


Figure 2. Comparison of HR between the patient and control groups. The patients HR were significantly higher than those of the controls. *$p < 0.01$.

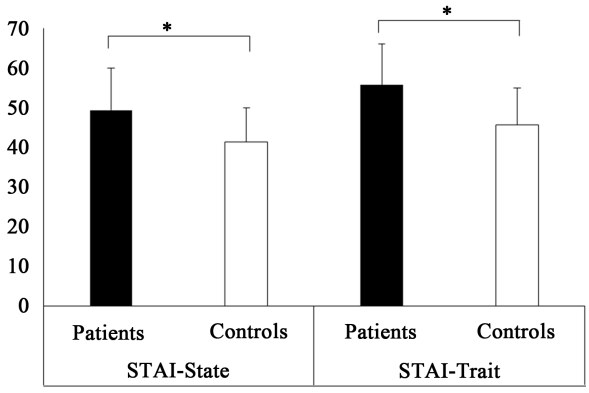


Figure 3. Comparison of the STAI scores of the patient and control groups. The patients' STAI-State and STAI-Trait scores were significantly higher than those of the controls. *$p < 0.01$.

Some studies have demonstrated marked increases in sAA levels following psychosocial stress, which was indicative of the stress-dependent activation of sAA. In addition, it suggested that high stress levels are associated with increased sAA levels [8] [9] [10] [14] [25]. sAA is a candidate marker of autonomic activity since salivary gland secretion occurs in response to neurotransmitter stimulation, and the salivary glands are innervated by both sympathetic and parasympathetic nerves [9] [26]. Previous studies suggested that the measurement of sAA reactivity to psychological stress was very useful as a direct marker of sympathetic-adrenal-medullary (SAM) activity [9] [13] [25] [27]. In addition, Nater *et al.* [10] reported a positive relationship between sAA and sympathetic tone, which was assessed using heart rate variability (HRV) parameters, during stress. Other reports indicated that the sympathetic nervous system (SNS) plays a predominant role in the secretion of sAA, together with parasympathetic nervous system (PNS) withdrawal, under psychosocial stress [5] [16]. HR reactivity is an important indicator of cardiovascular reactivity [20]. In the present study, we detected significantly increased HR in the outpatient group. Our results suggest that high levels of sAA are associated with a rise in HR probably due to increased sympathetic function. Sahu *et al.* [28] indicated that sAA changes in human subjects in response to psychological stressors could act as a sympathetic activity marker at all stages of human life. However, we did not examine autonomic function except by measuring HR and did not investigate the subjects' HRV. The physiological mechanism by which anxiety affects autonomic nervous function, and especially sAA levels, remains unclear. Ieda *et al.* [19] detected a significant increase in sAA levels in schizophrenia patients and speculated that the PNS was suppressed, whereas the SNS exhibited relatively high activity (according to HRV measurement). sAA has been suggested to be a surrogate for cardiovascular autonomic system balance correlating well with HRV parameters [27]. In addition, there were no gender differences in HR in this study. Gender difference in cardiovascular response to stressful tasks has not been consistent in the literature. Although many studies show women being more reactive than men, the

development of gender differences in cardiovascular and affective response to stress is not fully understand [20]. Further research is also needed to examine this topic in SAD patients.

Although we found significant differences between the STAI scores of the patients and healthy controls, no correlation was detected between the subjects' sAA levels and anxiety scores. Yorbic *et al.* [6] recently reported the sAA levels were significantly increased in anxiety group compared to control group in children and adolescent aged 8 - 16 years. However, there was no correlation between sAA and any anxiety scores of the scales. The STAI evaluates generalized anxiety, but does not necessarily measure fear due to interpersonal or social evaluative threat. This might explain why we did not find a significant correlation between the subjects' sAA levels and anxiety scores. A more comprehensive rating scale (e.g., the Liebowitz Social Anxiety Scale: LSAS) [29] should be used in a future study.

5. Limitations

We measured only HR to assess changes in autonomic function in this study. Further studies by HRV measurement are needed to acquire a better understanding of autonomic nervous system. Although we found that the sAA levels of SAD outpatients were significantly higher than those of normal healthy controls, it is not also clear that the higher sAA activity levels will continue or not with longer duration of illness.

6. Conclusion

We investigated the association between changes in sAA levels and SAD in junior high school students using a portable hand-held monitor. The students with SAD exhibited stress and anxiety, as well as high SNS activity. These results indicated that junior high school students with SAD exhibit a higher state of anxiety and high autonomic activity, probably due to changes in the sympathetic nervous system. As a result, schoolchildren who do not attend school due to SAD are expected to display high levels of sAA accompanied by severe anxiety symptoms. The measurement of sAA levels might contribute to evaluations of students' mental state and help to improve their school life and future.

Acknowledgements

The authors would like to thank Dr Motoi Itoga (Asahi Clinic, Izumo, Japan) and our colleagues at the Department of Psychiatry of Shimane University Faculty of Medicine for their support. We have no contribution and implications of this study. This research did not receive any specific grant from funding agencies in the public or commercial.

Conflict of Interest

All authors declare that they have no conflicts of interest.

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