Brief Note

Relationship Between Salivary Alpha-Amylase Activity and Self-Rated Anxiety Among School Non-Attendance Junior High School Students with Social Anxiety Disorder or Autism Spectrum Disorder

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Students in special needs education schools with a record of non-attendance may suffer significant mental problems. The salivary alpha-amylase (sAA) levels of such students were measured to evaluate their autonomic nervous system (ANS) function because sAA levels are known to rise as psychological stress is exacerbated. Furthermore, the relationship between psychological anxiety and ANS function (sAA levels) among school students with social anxiety disorder (SAD) or autism spectrum disorder (ASD) with a record of school non-attendance was examined. The subjects included junior high school students with SAD (n=33) or ASD (n=17) and age-matched healthy controls (n=69). We assessed the subjects’ sAA levels and State-Trait Anxiety Index (STAI) scores to evaluate their psychiatric states. The sAA levels of the SAD and ASD groups were significantly higher than those of the control group. Both the SAD and ASD groups exhibited significantly higher STAI scores than the control group. The results revealed that junior high school students with SAD or ASD exhibit high levels of sAA and anxiety symptoms. Because sAA measurements can be obtained easily and quickly, they may be beneficial for evaluating the psychological stress of school students in special needs education with a record of school non-attendance.

Key Words: salivary alpha-amylase, autism spectrum disorder, school non-attendance, social anxiety disorder, special needs education

Introduction

In Japan, school non-attendance is an important educational and psychiatric problem, with approximately 45,000 elementary school students (0.70%) and 120,000 junior high school students (3.65%) with such a record (Ministry of Education, Culture, Sports, Science, and Technology, Japan, 2018). School non-attendance has been defined as being absent for 30 or more days in a year (Ministry of Education, Culture, Sports, Science, and Technology, Japan, 2018). Social anxiety disorder (SAD) and autism spectrum disorder (ASD) are considered to be two of the main causes of school non-attendance.

Totsika, Hastings, Dutton, Worsley, Melvin, Gray, Tonge, and Heyne (2020) revealed that the most frequent reason among students with ASD for persistent school non-attendance was school refusal. González, Díaz-Herrero, Sanmartín, Vicent, Pérez-Sánchez, and García-Fernández (2019) found that adolescents with high school refusal behavior suffer higher rates of social anxiety. SAD, which is a common disorder, is characterized by an excessive fear of scrutiny and embarrassment in social situations. The incidence rates of the disorder are highest during childhood and adolescence, that is, among 10- to 20-year-olds (Beesdo, Bittner, Pine, Stein, Höfler, Lieb, & Wittchen, 2007). According to the Diagnostic and Statistical Manual of Mental Disorders (fifth edition), in the United States, 13.75% of patients with SAD develop the condition between the ages of 8 and 15 years. Furthermore, the prevalence rate of the disorder is 7% among both children and adults in the United States (American Psychiatric Association, 2013). Patients with SAD experience more frequent
anxiety and higher levels of anxiety in relation to any social evaluative threat (Wong & Rapee, 2016). Some students with SAD stop attending school because they suffer a social phobia of entering classrooms and attending lessons. On the contrary, ASD is a serious neurodevelopmental disorder, which impairs individuals’ ability to communicate and interact with others (Bujnakova, Ondrejka, Mestanik, Visnovcova, Mestanikova, Hrtanek, Fleskova, Calcovska, & Tonhajzerova, 2016). Students with ASD exhibit avoidance and crying in response to specific stimuli and/or situations, freezing behavior, fearful affect, clingingness, and an increased frequency of repetitive behaviors. In addition, irritability, tantrums, disruptive behavior, aggression, exacerbating sleep problems, and self-harm may indicate the presence of anxiety (Vasa, Mazurek, Mahajan, Bennett, Bernal, Nozzolillo, Arnold, & Coury, 2016). Anxiety in students with ASD may compound the social deficits and impairments they encounter in their daily living abilities and social relationships (Storch, Zavrou, Collier, Ung, Arnold, Mutch, Lewin, & Murphy, 2015). Therefore, students with ASD may stop attending school. Accordingly, it is imperative to understand the mental states and related physiological functions of students who suffer these conditions in order to support and assist them in their school lives in special needs education.

Recently, a number of stress markers, including cortisol and catecholamine levels, have been reported to be useful indicators for assessing physiological stress systems, for example, the hypothalamic-pituitary-adrenal (HPA) and sympathetic-adrenal-medullary (SAM) systems. The salivary alpha-amylase (sAA) level is a candidate marker of autonomic activity because secretion from the salivary glands occurs in response to neurotransmitter stimulation and the salivary glands are innervated by both sympathetic and parasympathetic nerves. Both the autonomic and somatic nervous systems work harmoniously in conjunction with one another to evoke salivary secretion (Nater & Rohlender, 2009). Salivary cortisol, sAA, and chromogranin-A levels, which can be measured non-invasively, have been evaluated as stress biomarkers (Noto, Sato, Kudo, Kurata, & Hirota, 2005).

An individual's sAA level has been proposed to be an indicator of stress-induced physical changes. Although to a lesser extent than the sAA level, the salivary cortisol level also increases in response to such changes (Takai, Yamaguchi, Aragaki, Eto, Uchihashi, & Nishikawa, 2004). Studies have shown that salivary cortisol and sAA levels displayed different reaction profiles in response to psychosocial stressors, specifically, there was a more significant and rapid increase in levels of sAA than salivary cortisol levels in response to psychological stressors (Gordis, Granger, Susman, & Trickett, 2006; Nater & Rohlender, 2009). Furthermore, the HPA axis responds more slowly to stressors than the SAM axis (Bitsika, Sharpley, Sweeney, & McFarlane, 2014). Studies on the reactivity of sAA levels to psychological stimuli have indicated that sAA levels may be a useful direct marker of SAM activity, namely, autonomic activation (Anderson, Colombo, & Unruh, 2013). Salivary sampling is advocated in that it is non-invasive, thus indicating that multiple samples can be obtained in an easy and stress-free manner (Takai et al., 2004). sAA levels vary directly with regard to norepinephrine levels, cardiac activity, and electrodermal activity, all of which are established indicators of anxiety (Bitsika et al., 2014). Therefore, in this study, we decided to employ sAA levels to evaluate the subjects' psychiatric states.

Elevated sAA levels are present in several psychiatric disorders and situations that involve psychological stress (Nater & Rohlender, 2009). Only a few studies have evaluated the sAA levels of child and adolescent patients with SAD or ASD. We have experienced clinical cases of school non-attendance that have been caused by various types of social psychiatric stress in students with SAD or ASD. It has been assumed that these students experience severe psychological stress, which affects their physiological state. Although many students with ASD suffer from comorbid anxiety disorders and it is difficult to distinguish the psychological states in both these disorders, there may be physiological differences.

The purpose of the present study was two-fold: first, to compare the sAA levels of junior high school students with SAD or ASD who exhibited school non-attendance to those of control students without SAD or ASD, and second, to investigate the psychological states and physical conditions (autonomic nervous system [ANS] function) of these students so as to clarify the relationships between these factors.
Method

Subjects

The study subjects included SAD and ASD outpatients (students) who were treated at the Department of Psychiatry of Shimane University School of Medicine and healthy controls. The patients all had a record of school non-attendance between April 2010 and March 2018 for which they provided several reasons. Attempts were made to ensure that the age range of the healthy control students who were recruited from a junior high school near our clinic was matched as closely as possible to those of the SAD and ASD patients. All the students attended regular classes, not special needs classes. In addition, 50 outpatients (mean age: 13.80±0.94 years; 16 males and 34 females), who were diagnosed with SAD or ASD at our hospital in accordance with the Diagnostic and Statistical Manual of Mental Disorders IV Text Revision (DSM-IV-TR) criteria (American Psychiatric Association, 2000), were divided into two groups: the SAD group (33 students; 8 males and 25 females) and ASD group (17 students; 8 males and 9 females). Patients with other psychiatric conditions involving attention deficit hyperactivity disorder (ADHD) were excluded in order to simplify the comparisons between the patient groups and avoid the effects of the co-occurrence of ASD and ADHD. We also excluded any patients who were considered to have serious psychosocial conditions, including schizophrenia, mood disorders, mental retardation, and personality disorders. We were unable to assess the subjects’ IQ levels. Furthermore, none of the outpatients had been administered medication. The patients’ data were compared with those obtained for the 69 healthy controls (mean age: 13.40±0.49 years; 43 males and 26 females). The contents of the survey were explained fully to the subjects. Furthermore, consent was obtained for the saliva sample collection and sAA measurements. The participants provided written informed consent before participating in the study. Furthermore, they did not suffer from neurological and cardiovascular diseases that are known to cause autonomic dysfunction as well as chronic diseases such as cancer and diabetes mellitus. None of the healthy control subjects had a history of psychiatric illness and neurodevelopmental disorders and none were taking any medication. The subjects’ personal information and that of their schools were kept confidential.

Measurement of sAA Levels

sAA measurements, which can be obtained non-invasively, have been used as a biomarker of stress in studies (Nater, Rohlender, Gaab, Berger, Jud, Kirshbaum, & Ehlert, 2005; Yamaguchi, Kanemori, Kanemaru, Takai, Mizuno, & Yoshida, 2004; Yamaguchi, Deguchi, Wakasugi, Ono, Takai, Higashi, & Mizuno, 2006). A simple hand-held sAA monitor (Nipro, Co., Japan) was employed to obtain the sAA measurements. The saliva collection was conducted in the morning, specifically, between 10:00 and 12:00 to control for circadian variations in sAA levels less than two hours after the subject’s last meal. It was conducted after the subjects had rested for 10 minutes in a sitting position. We collected saliva samples from the patients who visited our clinic in the morning. The sAA level was measured immediately after each saliva sample was collected. This method has been utilized to analyze sAA levels in studies (Ieda, Miyaoka, Wake, Liaury, Tsuchie, Fukushima, Araki, Ezoe, Inagaki, & Horiguchi, 2013; Inagaki, Miyaoka, Okazaki, Yasuda, Kawamukai, Utani, Wake, Hayashida, Horiguchi, & Tsuji, 2010).

Evaluation of Anxiety State

The Japanese version of the State-Trait Anxiety Index (STAI; Form X) (Sankyoubou, Co., Kyoto, Japan) was employed to measure the subjects’ anxiety state (Mizuguchi, Shimonaka, & Nakazato, 1991; Spielberger, Gorsuch, & Lushene, 1970). The STAI measures two types of anxiety, namely, state anxiety and trait anxiety (STAI-state and STAI-trait). Furthermore, the measure has been employed widely to assess individual differences in anxiety (Spielberger et al., 1970). Both subscales comprise 20 items, which are rated on 4-point scale, ranging from 1 (not at all) to 4 (very much so). Higher STAI scores are positively correlated with greater anxiety. It is noteworthy that the subjects completed the STAI as a self-report measure of their anxiety levels. The validity and reliability of this version have been demonstrated (Shimizu & Imae, 1981). We evaluated the subjects’ anxiety levels in relation to their school lives by employing both their STAI-trait and STAI-state scores.

Data Analysis

The results are expressed as mean±standard devia-
tion (SD) values (Table 1). One-way ANOVA was performed to investigate the impact of individual variables on the three groups. Multiple comparisons of all of the variables that exhibited significant results were subsequently performed to estimate quantitative effects. The correlations between variables were evaluated using Pearson's correlation coefficient by employing Excel Statistics 2012 (ver.1.11) (Social Survey Research Information Co., Ltd., Japan). The level of significance was set at \( p < .05 \).

**Results**

The characteristics of the subjects with SAD and ASD as well as those of the healthy controls are displayed in Table 1. The mean duration of school non-attendance period was 12.64±13.57 months in the SAD group and 14.18±15.23 months in the ASD group. In this regard, there was no significant difference between the groups (\( p = .36 \)).

**Comparisons of sAA Levels Among the Outpatient Groups and Healthy Controls**

The mean sAA levels of the subjects with SAD and ASD and the controls were 55.94±28.91 (95% CI: 45.69–66.19) kU/l, 54.91±25.91 (95% CI: 41.62–68.26) kU/l, and 38.06±19.60 (95% CI: 33.35–42.77) kU/l, respectively. The results revealed significant differences among the sAA levels of the SAD, ASD, and healthy control groups (\( F_2, 116 = 8.14, p = .0005 \)). While the sAA levels of both the subjects with SAD and ASD were significantly higher than those of the controls (Bonferroni's test, \( p < .001 \) and \( p = .027 \), respectively) (Fig. 1), there was no significant difference between the sAA levels of the SAD and ASD groups (\( p = .66 \)). In addition, there were no sex-dependent differences in the sAA levels of the outpatient groups and healthy control group (SAD group: \( p = .49 \), ASD group: \( p = .45 \), control group: \( p = .34 \)).

**Evaluation of Anxiety State**

In the evaluation of anxiety, significant differences in the STAI were detected among the three groups (\( F_2, 102 = 19.31, p < .001 \)). The mean STAI-state scores of the SAD group (48.57±10.20, Bonferroni's test, \( p < .001 \)) and ASD group (55.33±14.19, \( p < .001 \)) were significantly higher than those of the control group (38.96±9.25). However, there were no significant differences between the mean STAI-state scores of the SAD and ASD groups (\( p = .16 \)) (Fig. 2). Similarly, there were significant differences in the STAI-trait scores among the groups (\( F_2, 102 = 20.83, p < .001 \)).

![Fig. 1 Comparison of sAA Levels between the Patient and Control Groups](image)

**Table 1 Subjects’ Characteristics**

|                      | SAD patients | ASD patients | Controls |
|----------------------|--------------|--------------|----------|
| No. of subjects (n)  | 33           | 17           | 69       |
| Male/female (n)      | 8/25         | 8/9          | 43/26    |
| Age (years)          | 13.9±0.90    | 13.7±0.85    | 13.4±0.49|
| Salivary alpha-amylase level (kU/l) | 55.94±28.91** | 54.91±25.91* | 38.06±19.60 |
| STAI-state score     | 48.57±10.20** | 55.33±14.19** | 38.96±9.25 |
| STAI-trait score     | 57.81±9.24** | 54.93±15.77** | 43.36±8.70 |
| Duration of school non-attendance (months) | 12.64±13.57 | 14.18±15.23 |

Note. Comparisons were performed between the ASD group and controls, and between the SAD group and controls. The data are shown as mean ± SD values. Statistical analyses were conducted using the one-way ANOVA to investigate the impact of individual variables on the three groups. Multivariate analysis, including all the variables that exhibited significance, was subsequently performed to estimate quantitative effects. *: \( p < .05 \), **: \( p < .01 \).
The mean STAI-trait scores of the SAD group (57.81±9.24, Bonferroni’s test, p<.001) and ASD group (54.93±15.77, p<.001) were significantly higher than those of the control group (43.36±8.70). However, the mean STAI-trait scores of the SAD and ASD groups did not differ significantly (Bonferroni’s test, p=.81).

In addition, there was no correlation between the sAA level and severity of anxiety symptoms (STAI-state and STAI-trait scores) in any of the three groups (ASD: r=.093, p=.366, and r=.142, p=.307; SAD: r=.105, p=.290, and r=.096, p=.307; control: r=.180, p=.629, and r=.354, p=.294, respectively).

**Discussion**

We examined the relationships between psychological stress (anxiety) and ANS function (sAA levels) and subsequently compared the findings we obtained for the outpatients with SAD or ASD with those of the healthy controls. To the best of our knowledge, no previous studies have examined sAA levels under psychosocial stress and/or examined the changes in sAA levels in junior high school students with SAD or ASD that have a record of school non-attendance. High levels of sAA were detected in both the ASD and SAD patients who also obtained high STAI scores (STAI-state and STAI-trait scores).

**Comparison of Physiological Measures in the SAD and ASD Groups**

Several studies have indicated that psychosocial stress promotes the release of sAA, which is indicative of the stress-dependent activation of SAA secretion. Yorbic, Mutlu, Ozlurk, Alitnay, Tanju, and Kurt (2016), in a study involving 8- to 16-year-olds, found significantly higher sAA levels in the anxiety group than control group. Funke, Eichler, Distler, Golub, Kratz, and Moll (2016) also reported higher morning cortisol levels in plasma and saliva as well as increased sAA activity in pediatric patients with generalized anxiety disorder (n=26, mean age: 14.6 years).

In this study, the mean sAA levels of both the SAD and ASD patients were significantly higher than those of the controls. It is difficult to distinguish between SAD and ASD based on sAA levels and thus, our results indicate that the high sAA levels present in the patient groups were associated with increased sympathetic activity. Sahu, Upadhyay, and Panna (2014) noted that in humans, sAA level changes that occur in response to psychological stressors could indicate sympathetic activity during all stages of life. Physiologically, anxiety affects ANS functions and induces high sAA levels. Other studies have also revealed that under psychosocial stress, the SNS plays a predominant role in the secretion of sAA in conjunction with parasympathetic nervous system withdrawal (Yamaguchi et al., 2006). Studies have revealed that pediatric and adolescent patients with ASD are affected by structural and functional central nervous system abnormalities, which may be associated with the dysregulation of the ANS (Anderson et al., 2013). By measuring their subjects’ heart rates, Fukasawa and Takeda (2012) found that sAA levels are a valid index of the SNS activity of children with autism. Other tools can also be employed to assess autonomic function. Martineau, Hernandez, Hiebel, Roché, Mertzger, and Bonnet-Brilhault (2011)
revealed that their ASD group's mean pupil size was significantly smaller than that of their control group. They explained that the patients with ASD may have had abnormally functioning nervous systems (ANS), which is consistent with the hypothesis that the balance between inhibitory and excitatory activity within the sympathetic and parasympathetic divisions of the ANS is altered in those with ASD. Furthermore, Kaartinen, Puura, Mäkelä, Rannisto, Lemponen, Helminen, Salmelin, Himanen, and Hietanen (2012) who investigated autonomic arousal during eye contact among children with ASD, which they measured by assessing skin conductance responses, revealed that enhanced arousal in response to a direct gaze was associated with impaired social skills in children with ASD. In addition, Kaartinen et al. (2012) also demonstrated that pupil response measurements were associated with a sensitive ANS. It has also been proposed that links may exist between ASD symptoms and ANS dysfunction-related sympathetic overarousal, parasympathetic underactivity, and/or atypical interactions between the two systems (Bujnakova et al., 2016).

Although social anxiety is one of the most common comorbidities in ASD, it is also one of the most difficult conditions to extricate from the social difficulties inherent in ASD (Vasa et al., 2016). Estimates have revealed that approximately 50% of children and adolescents with ASD meet the clinical diagnostic criteria for anxiety (Storch et al., 2015). Furthermore, Maddox and White (2015) found that half of adults with ASD suffered anxiety. Moreover, anxiety and ASD symptoms can overlap. It is difficult to distinguish clearly between the symptoms of social anxiety and ASD symptoms, particularly with regard to social difficulties and social avoidance, because of the cognitive and language impairments, compromised reporting of emotions, and unique behavioral expression of anxiety that patients with ASD exhibit (Burrows, Usher, Becker-Haimes, McMahon, Mundy, Jensen-Doss, & Henderson, 2018). Although there were no significant differences in sAA levels or anxiety levels between the SAD and ASD groups in this study, both patient groups exhibited higher anxiety states and physical stress levels than the control group.

**Practical Implications**

In this study, high sAA levels were detected in both the ASD and SAD groups. Furthermore, it was difficult to distinguish between social anxiety symptoms and ASD symptoms in both psychological and physiological measurements. Therefore, it is imperative that clinicians and school teachers ask questions to help determine whether overlapping symptoms are explained solely by ASD or are consistent with a co-occurring anxiety disorder (Vasa et al., 2016). The accurate assessment of social anxiety in such students may contribute to the identification of additional impairments. This would further assist them at school.

**Limitations of the Study**

This study has a few limitations. First, we did not compare students with SAD or ASD with a record of school non-attendance with those with such a record without SAD or ASD. Second, we did not compare students with SAD or ASD with a record of non-attendance at school with those without such a record. It is recommended that other biomarkers, including heart rate and heart rate variability (HRV) parameters, are measured to evaluate the relationship between sAA activity and ANS function. Nater, Marca, Florin, Moses, Langhans, Koller and Ehler (2006) reported a positive relationship between sAA levels and sympathetic tone, which they assessed with HRV parameters during stress. It is also recommended that a social anxiety scale involving a more comprehensive rating scale such as the Liebowitz Social Anxiety Scale (Liebowitz, 1987) be employed to evaluate psychiatric state in future research.

**Conclusion**

The results of this study revealed that junior high school students with SAD or ASD exhibit increased levels of anxiety and autonomic activity, probably due to changes in their SNS. The measurement of sAA levels and the STAI may be beneficial for assessing the psychological states and physical conditions (ANS functions) of junior high school students with SAD or ASD who have a record of school non-attendance.

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Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships.

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