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Correlation of Salivary Alpha Amylase Level and Adenotonsillar Hypertrophy with Sleep Disordered Breathing in Pediatric Subjects

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SCIENTIFIC INVESTIGATIONS

Study Objectives: Obstructive sleep apnea syndrome (OSAS) and sleep disordered breathing (SDB) can affect the sympathetic adrenomedullary system (SAM). As a biomarker of SAM activity, salivary α-amylase (sAA) in pediatric subjects was evaluated whether it has any correlation with polysomnographic (PSG) parameters related to SDB.

Methods: Sixty-seven children who attended our clinic during 1 year were enrolled prospectively and underwent clinical examinations and in-lab polysomnography. The sAA was measured at 2 points—at night before PSG and in the early morning after PSG

Results: Subjects were divided into control (n = 26, apnea-hypopnea index [AHI] < 1) and OSAS (n = 41, AHI ≥ 1) groups. The OSAS group was subdivided according to AHI (mild-moderate, 1 ≤ AHI < 10; severe, AHI ≥ 10). The sAA subtraction and ratio (p = 0.014 and p < 0.001, respectively) were significantly higher in severe OSAS than in the mild-moderate and control groups. Although oxygen desaturation index (ODI) and AHI were significantly associated with sAA, sAA in the OSAS group was not related to lowest oxygen saturation or adenotonsillar hypertrophy.

Conclusion: sAA was well related to polysomnographic (PSG) parameters related to SDB, such as AHI and ODI. Therefore, screening test for sAA in children suspected to have SBD may help to identify OSAS patients from control.

Keywords: amylase, child, saliva, polysomnography, sleep disordered breathing

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Obstructive sleep apnea (OSA) is a relatively common disorder, with an estimated frequency of 2% to 3% in children. Sleep disordered breathing (SDB) has a wide spectrum of respiratory abnormalities from primary snoring to OSA, and its prevalence is estimated to be from 0.9% to 13% in children.[1] Reduced airflow (flow limitation, hypopnea), increased respiratory effort, and repetitive cessation of airflow (apnea) are usually observed in subjects with SDB. As a result, SDB can cause intermittent hypoxia and hypercapnia followed by successive restoration of normo-oxygenation after arousals, which can activate stress responses mediated by the hypothalamic-pituitary-adrenocortical (HPA) axis and the autonomic nervous system (ANS).[2-5]

It is well known that ANS parasympathetic tone is elevated by apnea and hypopnea, and sympathetic tone is elevated following respiratory events.[6] As a result of activation of ANS, the ANS during sleep may be unstable in children with SDB, and the balance of sympathetic and parasympathetic nervous system during sleep may be unstable. Most previous research has focused on investigations of the relationship between SDB and the ANS, the role of repetitive hypoxia in causing the change of ANS balance,[3,7] and the correlation with cardiovascular, neurocognitive, immune, endocrine, and metabolic comorbidity with autonomic imbalance in OSA.[8,9]

BRIEF SUMMARY

Current Knowledge/Study Rationale: Salivary α-amylase (sAA) is a biomarker of SAM activity. To evaluate the effect of obstructive sleep apnea on the sAA, we measured the correlation between sAA and polysomnographic parameters in pediatric subjects.

Study Impact: Sampling for sAA was easy and pain-free to collect and optimal for multiple sampling in children. sAA in OSA children was related to the presence of OSA (AHI, ODI) and may be used as a biomarker of OSA.

Recently, salivary proteins have gained become of interest in diagnosing a variety of diseases, and several salivary proteins have been suggested as biomarkers to predict a variety of diseases. Among them, salivary cortisol may indicate a chronically stressed HPA axis and be a useful biomarker for OSAS.[10] Salivary protein α-amylase (sAA) regulated by the sympathetic nervous system has been suggested as a biomarker for sympathetic activity because sAA activity is a readily accessible, noninvasive parameter that reflects human responses to physiological and psychological stress.[11-13] For example, sAA activity can be used with moderate sensitivity and specificity to detect acute myocardial infarction in patients with acute chest pain.[14] A few studies have been published on α-amylase (AA) related to

acute and chronic hypoxia, but these studies have tried to evaluate hypoxia-related changes of AA in a certain circumstance (high altitude) or with rat plasma (not with saliva).[15,16] However, to our knowledge, there have been no studies on the changes of sAA during sleep and its relationship with PSG parameters and adenotonsillar hypertrophy (ATH), the most common cause of pediatric SDB in pediatric subjects.

We hypothesized that if autonomic imbalance by SDB could be represented by sAA and sAA parameters might be correlated with the PSG parameters such as AHI and ODI, the estimation of sAA might allow us to predict the presence and the severity of SDB in children with SDB. Therefore, the aim of the present study was to evaluate the change in sAA during sleep, the correlation between sAA levels and PSG parameters, and the relationship between ATH and sAA in pediatric subjects with and without SDB.

PATIENTS AND METHODS

Subjects

Sixty-seven children who attended the department of Otolaryngology-Head and Neck Surgery at St. Vincent's Hospital with suspected OSAS or enlarged tonsils/adenoid between July 2011 and June 2012 were prospectively enrolled in the present study. The obesity was defined as BMI > 25 or > 95th percentiles for age and gender.[17] Exclusion criteria were antrochoanal polyp, nasal polyposis, congenital anomaly with craniofacial abnormalities, neuromuscular dystrophies, history of systemic medical conditions such as diabetes, and hypertension, prior history of cardiac or airway surgery, and history of cardiopathy or pneumopathy.

All children underwent a complete physical examination, and the parents of each child completed 2 sleep questionnaires: the Korean version of the OSA-18 (KOSA18) for quality of life and the modified Epworth Sleepiness Scale (KMESS) for sleepiness, from which the mention of alcohol had been deleted and the question when driving a car had been changed as the question when sitting as a passenger in the car.

We performed full attended overnight PSG and collected saliva from each child using specialized tubes around 22:00 prior to PSG and again the next morning after PSG (within 30 min after waking up). The study and its protocol were explained to all participants and their parents, and obtained informed consent was obtained from the participant's mother or father before enrollment. The study protocol was reviewed and approved by the Ethics Committee for Clinical Studies at St. Vincent's Hospital, The Catholic University of Korea.

Tonsil Size and Adenoid Size

Tonsil size was graded from 0 to 4,[18] and measured as a sum of each subjective tonsil size scales by one doctor because some subjects had asymmetric tonsillar sizes. To evaluate adenoid size, the adenoidal-nasopharyngeal ratio (AN ratio) was measured in the lateral neck radiograph or cephalometry, as described by Fujioka et al.[19]

Polysomnography

Each child underwent PSG in a quiet, darkened room with 20 ~25°C temperature. PSG include 4 electroencephalographic channels, electrocardiographic channels, 2 electro-oculographic channels (right and left outer canthi), chin and bilateral anterior tibialis electromyographic channels, fingertip pulse oximetry (with viewable pulse-wave form), snore sensor, nasal and oral airflow (with a nasal pressure transducer and thermistor), and chest and abdominal movement (with piezoelectric strain gauges). All measures were digitalized using a commercially available polysomnography system (Somnologica software and Embla S700/A10 hardware, Broomfield, CO, USA), scored initially by a certified technician, and then reviewed by a doctor.

The AHI was defined as the number of obstructive apnea and hypopnea events per hour. The diagnostic criterion for OSA in pediatric subjects was an AHI ≥ 1 event/hour. Obstructive apnea was defined as the absence of airflow with continued chest wall and abdominal movement lasting ≥ 2 regular breaths. Obstructive hypopnea was defined as ≥ 50% air flow reduction for > 90% of the entire event, which lasted ≥ 2 regular breaths with a desaturation ≥ 3%, awakening, or EEG arousal (compared with the previous baseline amplitude).

Measurement of Salivary α-Amylase

According to manufacturer's protocol, salivary α-amylase was measured in the same method described in our previous study.[10] In brief, we prevented enrolled children from eating a major meal for 60 min before collecting the saliva and educated parents for children not to eat or drink dairy products and sugar foods on day of PSG test. Child' mouth was rinsed with water to minimize errors 10 min before saliva sampling. Parents were also educated on their child's exercise, timing, and composition of the meals, and even teeth brushing before sampling on day of PSG test.

Saliva samples were collected in special tubes around 22:00 prior to PSG and at 07:00 the next morning after PSG (within 60 min of waking up). The samples were refrigerated within 30 min and frozen at or below −20°C within 4 h after collection. On the day of the assay, samples were thawed completely, vortexed, and centrifuged at 1500 × *g* (3000 rpm) for 15 min. Samples were at kept room temperature before transfer to the assay plate. The sAA activity was measured by use of a commercially available kinetic reaction assay (Salimetrics, State College, PA, USA). The m-sAA was the sAA level in the morning, and n-sAA was the sAA at night. The sAA ratio (r-sAA) was defined as the ratio of the measurements after PSG to those before PSG, and the sAA subtraction (sub-sAA) was calculated by subtracting the measurements before PSG from those after PSG.

Statistics

All statistical tests were conducted using the Statistical Package for the Social Sciences version 10.1(SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was performed to assess the normality of the data before further analysis. To correct substantial skews (out of normal distribution), sAA data were transformed by logarithmic transformation.[20] However, logarithmic transformation could not be used easily for all data, because one of the data points (sub-sAA) had a negative data value. Therefore, nonparametric statistics (Kruskal-Wallis Test, Wilcoxon rank sum test, multinomial logistic regression, and

Table 1—Basic demographic, anthropometric, polysomnographic questionnaire data of studied patients

	Total (n = 67)	Control (n = 26)	OSAS (n = 41)	p-value
Gender				
male	39 (58.2)	9 (34.6)	30 (73.2)	0.002 *, a
female	28 (41.8)	17 (65.4)	11 (26.8)	
Age	6 (3-16)	7 (4-12)	6 (3-16)	0.179
BMI	17.1 (10.4-27.6)	17.4 (13-25.9)	16.5 (10.4-27.6)	0.459
Obesity	4	1	3	0.566
Tonsil size	6 (2-8)	6 (2-8)	6 (4-8)	0.008 *
AN ratio	0.6 (0.3-0.9)	0.6 (0.3-0.8)	0.6 (0.5-0.9)	0.006 *
AHI	1.8 (0-39.8)	0 (0-1)	5.3 (1.1-39.8)	< 0.0001 *
ODI	0.4 (0-139.1)	0.1 (0.0-0.5)	1.5 (0.0-139.1)	< 0.0001 *
Lowest SpO_2	92 (0.4-98)	94 (0.4-98)	90 (66-97)	0.001 *

[a] N (%) tested by χ^2 test and Fisher exact test. Median (interquartile range) tested by Wilcoxon rank sum test. * p < 0.05. Obesity defined as BMI > 25 or > 95 percentiles for age and gender. BMI, body mass index; AN ratio, the ratio of the maximal adenoid thickness to the distance along a line from the posterior-superior edge of the hard palate to the spheno-occipital synchondrosis on the base of the skull; AHI, apnea hypopnea index; ODI, oxygen desaturation index.

Table 2—Salivary α-amylase levels in control and OSAS groups

	Total (n = 67)	Control (n = 26)	OSAS (n = 41)	p-value
Amylase				
n-sAA	131.55 ± 98.58	140.43 ± 108.76	125.92 ± 92.51	0.733
m-sAA	63.58 ± 50.52	60.11 ± 51.78	65.78 ± 50.23	0.653
sub-sAA	-67.97 ± 75.26	-80.32 ± 79.38	-60.13 ± 72.43	0.175
r-sAA	0.54 ± 0.29	0.48 ± 0.20	0.58 ± 0.34	0.306

* p < 0.05. p value of difference between control versus OSAS by Wilcoxon rank sum test. n-sAA, the level of salivary α-amylase before PSG; m-sAA, the level of salivary α-amylase after PSG; sub-sAA, the subtraction of the level of salivary α-amylase before PSG from the level of salivary α- amylase after PSG; r-sAA, the ratio of the level of salivary α-amylase after PSG to the level of salivary α-amylase before PSG.

Spearman correlation) were used for the analysis of data. Quantitative variables of the case (AHI ≥ 1) and control (AHI < 1) groups adjusted for age and gender were compared using the multinomial logistic regression. Spearman correlation coefficients were used to assess the relationships between the sAA and ATH measurements and the PSG parameters. Receiver operating characteristic (ROC) curves were constructed to determine the sensitivity and specificity associated with a given cutoff of the ATH and sAA in terms of predicting a positive PSG (AHI ≥ 1 and AHI ≥ 10, respectively).[21,22] A p-value < 0.05 was considered to indicate statistical significance.

RESULTS

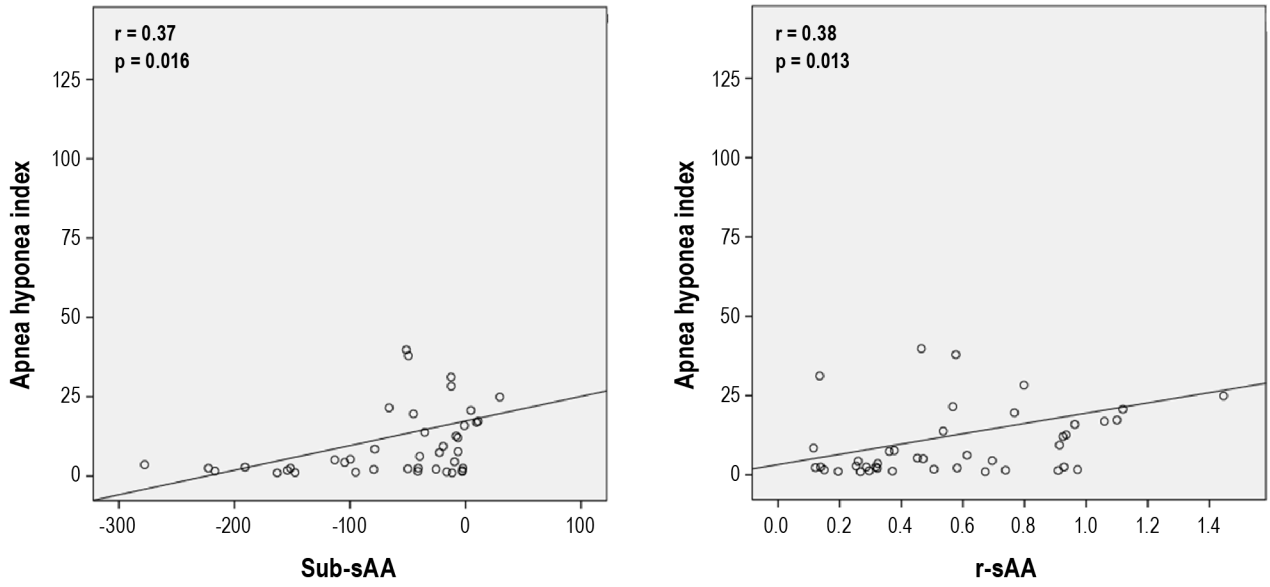
The subjects (n = 67) were divided into control (n = 26, AHI < 1) and OSAS (n = 41, AHI ≥ 1) groups. The OSAS group was then subdivided according to AHI score into mild-moderate (n = 26, 63.4%, AHI 1 to 10) and severe (n = 15, 36.6%; AHI ≥ 10) groups. In demographic data, the OSAS group included 30 males (73.2%) and 11 females (26.8%); the male-to-female ratio differed from that of the control group (9 males [34.6%], 17 females [65.4%]). Age was not significantly different between the groups (p = 0.178). In PSG findings, the AHI, oxygen desaturation index (ODI), tonsil size, and AN ratio were significantly higher in the OSAS group than in the control group; the lowest oxygen saturation in the OSAS group was significantly lower than that of the controls (**Table 1**). There were no significant differences between the OSAS and control groups in sAA measurements (**Table 2**).

Comparisons between the control group and each OSAS subgroup (mild-moderate and severe) revealed a significant difference in sub-sAA and r-sAA (p = 0.014; p < 0.001), such that the sub-sAA and r-sAA were significantly higher in the severe subgroup than in the control group and the mild-moderate subgroup.

Regarding ATH, we found that there were significant differences between the control group and each OSAS subgroup in tonsil size and AN ratio. Tonsil size was significantly larger in the severe subgroup than in the control group. AN ratio was significantly higher in the mild-moderate and severe subgroups than in the control group. However, no significant difference was found between the 2 subgroups in tonsil size and AN ratio (**Table 3**).

Although there were no significant correlations among tonsil size, AN ratio, and sAA measurements in the OSAS group (**Table 4**), the sub-sAA and r-sAA were positively associated with the AHI and ODI (the number of desaturations per sleep hour) (**Figure 1**). But No sAA measurements were associated with the lowest oxygen saturation. In contrast, the tonsil size in the OSAS group was not related to the AHI, ODI, and lowest oxygen saturation. The AN ratio in the OSAS group was negatively related to only lowest oxygen saturation (**Table 5**).

Figure 1—Scatter-plot of salivary amylase and AHI.



Sub-sAA and r-sAA significantly correlated with indexes of sleep apnea.

Table 3—Measurement of salivary α-amylase according to OSA severity

	OSA severity subgroup						
	Normal (0) 0 ≤ AHI < 1 (n = 26)	Mild-Moderate (1) 1 ≤ AHI < 10 (n = 26)	Severe (2) 10 ≤ AHI (n = 15)	p-value [a]	0 vs 1 p-value [b]	0 vs 2 p-value [c]	1 vs 2 p-value [d]
Amylase							
n-sAA	140.43 ± 108.76	143.88 ± 106.51	94.78 ± 50.54	0.940	0.915	0.104	0.133
m-sAA	60.11 ± 51.78	58.78 ± 51.80	77.91 ± 46.58	0.449	0.659	0.450	0.195
sub-sAA	-80.32 ± 79.38	-85.098 ± 78.78	-16.86 ± 27.23	0.010*	0.670	0.012*	0.018*
r-sAA	0.48 ± 0.20	0.449 ± 0.27	0.81 ± 0.32	0.003*	0.500	0.005*	0.005*
Tonsil size	5.27 ± 1.46	6.15 ± 1.22	6.47 ± 1.06	0.013*	0.093	0.036*	0.155
AN ratio	0.55 ± 0.13	0.63 ± 0.09	0.65 ± 0.12	0.002*	0.035*	0.010*	0.133

* p < 0.05. Tested by multinomial logistic regression, p values were adjusted by gender and age. [a] p value of difference between normal vs mild-moderate vs severe; [b] p value of difference between normal vs mild-moderate; [c] p value of difference between normal vs severe; [d] p value of difference between mild-moderate vs severe.

Table 4—Relationship of salivary α-amylase with degree of upper airway obstruction in the OSAS group.

	Tonsil size (n = 41)		AN ratio (n = 38)	
	r	p-value	r	p-value
Amylase				
n-sAA	0.12	0.453	0.03	0.840
m-sAA	0.10	0.553	0.08	0.639
sub-sAA	-0.09	0.586	0.02	0.928
r-sAA	-0.09	0.568	-0.03	0.853

* p < 0.05. Spearman correlation.

ROC curves were constructed; the area under the curves represented the discriminatory power of tonsil size, AN ratio, and sAA in predicting OSAS and the severe subgroup in the 67 children. Although tonsil size and AN ratio were significant predictors of OSAS ($p < 0.05$), the areas under the curve were no more than 0.7 (**Figure 2**; **Table 6**). However, the areas under the curve of sub-sAA and r-sAA for predicting severe OSAS were 0.80 and 0.81, respectively (**Figure 3**; **Table 7**).

DISCUSSION

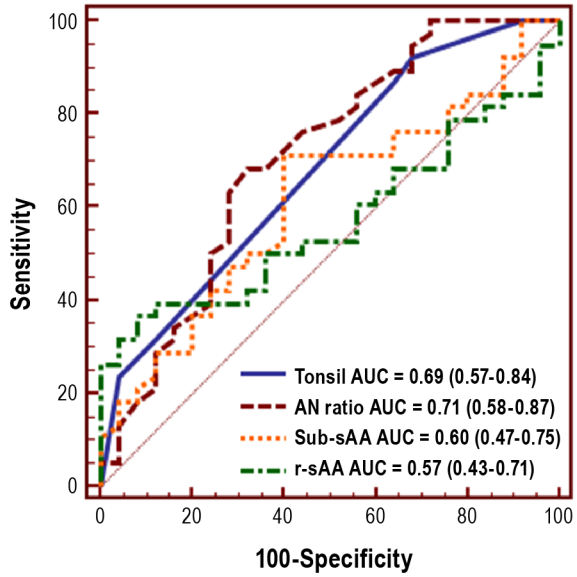
In order to evaluate the effect of SDB on the sAA activity, it is very important to consider its diurnal pattern, as well as the determinants that affect this pattern. This information will enable clinicians to understand the systemic effects of SDB related to the sympathetic adrenomedullary system (SAM) and to choose an appropriate methodology in investigating SDB-related conditions. For example, sAA activity showed a distinct diurnal pattern, with a decrease within 60 min after awakening and a steady increase in activity during the course of the day.[13] A previous study using microneurography demonstrated that SAM activity was markedly decreased during sleep.[21] Polysomnography (PSG) was recommended by an expert consensus panel and was designated by the American Academy of Pediatrics as the "gold standard" test for establishing the presence and

Table 5—Relationship of α-amylase to PSG parameters in OSAS patients

		AHI		ODI		Lowest O_2 saturation	
	n	r	p-value	r	p-value	r	p-value
Amylase							
n-sAA	41	-0.17	0.280	-0.08	0.605	-0.08	0.610
m-sAA	41	0.20	0.179	0.32	0.040*	-0.24	0.127
sub-sAA	41	0.37	0.016*	0.37	0.017*	-0.14	0.369
r-sAA	41	0.38	0.013*	0.45	0.003*	-0.16	0.314
Tonsil	41	0.09	0.575	0.18	0.263	-0.16	0.332
AN ratio	38	0.14	0.381	0.32	0.050	-0.45	0.005*

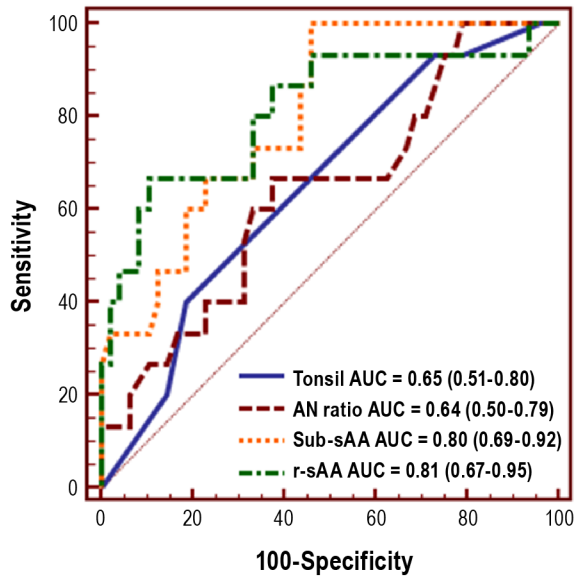
* p < 0.05. Spearman correlation.

Figure 2—Receiver operating characteristic curve for the tonsil size and AN ratio predicting the OSAS group.



Areas under the curve in the tonsil size and AN ratio were 0.69 and 0.72 (95% CI, 0.55 to 0.79; 0.58 to 0.82), respectively.

Figure 3—Receiver operating characteristic curve for salivary α-amylase predicting the severe OSAS subgroup.



Area under the curve in the sub-sAA and r-sAA were 0.80 and 0.81 (95% CI, 0.69 to 0.89; 0.70 to 0.80), respectively.

Table 6—Evaluation of effectiveness of screening measurements in identifying the OSAS group

			Asymptomatic 95% Confidence Interval	
	Area	p-value	Lower Bound	Upper Bound
Tonsil size	0.69	0.007*	0.57	0.84
AN ratio	0.72	0.003*	0.58	0.87
sub-sAA	0.61	0.160	0.47	0.75
r-sAA	0.57	0.392	0.43	0.71

* p < 0.05. Receiver operating characteristic curve.

Table 7—Effectiveness of screening for severe OSAS

			Asymptomatic 95% Confidence Interval	
	Area	p-value	Lower Bound	Upper Bound
Tonsil size	0.65	0.048*	0.51	0.80
AN ratio	0.64	0.107	0.50	0.79
sub-sAA	0.80	< 0.0001*	0.69	0.92
r-sAA	0.81	< 0.0001*	0.67	0.95

* p < 0.05. Receiver operating characteristic curve.

severity of OSA in children. However, it is not easy to routinely perform PSG for children suspected of having SDB because of the time and expense associated with PSG. Another major reason is the lack of availability of sleep labs with the ability and desire to test.[22,23] By contrast, it has been reported that clinical history and physical examination are not reliable for diagnosing OSA and that questionnaires alone do not provide a good diagnostic prediction for OSA in children.[23] Consequently, many researchers have tried to find a screening tool for the presence and severity of OSA in children.[23-25]

Calculation of the diurnal slope of the sAA was reported to be an alternative analysis strategy for altered ANS function.[13] Out et al. compared the distributions of each of the diurnal

slopes to the one based on four time points (waking, 12:00, 19:00, and 21:00) with regard to an optimal research design for assessing diurnal sAA. In their study, it was reported that the collection of a morning (waking) and an evening sample was sufficient to accurately characterize the diurnal slope.[26] Therefore, we measured the level of sAA at night before sleep and in the early morning after waking (two time points) for evaluating ANS diurnal pattern.

Contrary to our expectation, there was no significant difference in sAA variables between the OSAS and control groups. However, although not statistically significant due to the wide range of absolute values of n-sAA and m-sAA of each subject, n-sAA tended to decreased and m-sAA to increase in the severe OSAS subgroup. These trends may explain why both sub-sAA and r-sAA are higher in the severe OSAS subgroup than the mild-moderate and control groups. It could be suggested that the ratio of sAA might be a more consistent index than wide-ranging n-sAA and m-sAA in each subject.

Considering the relevance of SDB to chronic stress,[27] our finding for m-sAA as a wakening response may be similar to that of a previous report showing that sAA levels in the morning were elevated by chronic stress and stress reactivity, whereas perceived daily and momentary stress did not affect sAA levels.[13,28] In addition, the peak level of sAA after stress and the overall sAA level in response to stress across the entire day were reported to be higher in children with average-low sleep efficiency, compared with average-high.[29] Intermittent hypoxia and sleep fragmentation related to OSA may induce chronic ANS activation, which may elicit a negative baroreflex feedback loop for activated sympathetic tone.[30] A study by Prabhakar and Kumar suggested that persistent sympathetic activation related to sleep apnea might alter chemoreflexes and baroreflexes, which might result in sympathetic activation.[31] However, the suppression of n-s AA level in our results is hard to explain.

As distinctive indicators of sympathetic activity and HPA axis activity, respectively, salivary α-amylase and cortisol show characteristic diurnal patterns that are opposed to each other.[32] In our previous study,[10] the level of salivary cortisol at night before sleep and in the early morning after waking (two time points) were measured to evaluate the relationships with severity of OSAS, which exhibited that cortisol slope (the ratio of post- to pre-sleep cortisol) might reflect severity of OSAS because there were statistically significant changes (flattened slope) in severe OSAS subgroup, compared to control and mild-to-moderate groups. It would mean that severe OSAS could cause patients not to reach the expected peak level of cortisol.

In this study, like disturbed diurnal patterns of cortisol in previous study, the peak level of salivary amylase was lowered as severity of OSAS increased, which could explain why the amylase slope was flattened in severe OSAS subgroup. Considering the results of salivary cortisol and amylase, it might be deduced that the severity of pediatric OSAS might influence the diurnal pattern of stress system and decrease the gap between the highest and the lowest level of HPA axis and ANS systems. However, future studies will be needed to clarify this.

In this study, further attempts have been made to relate sAA to several factors related to SDB, such as PSG parameters of AHI and oxygen saturation, tonsil size, and AN ratio. The sAA measurements were positively associated with the AHI and ODI but have no relationship to the lowest oxygen saturation. In comparison with the control group and each OSAS subgroup, there were significant differences in sub-sAA and r-sAA according to AHI, except between the control group and the mild-moderate subgroup, which could match with the positive correlation of the sAA measurements with AHI.

In the OSAS group, n-sAA showed a tendency toward a negative correlation with ODI, and m-sAA showed a significantly positive correlation with ODI. These tendencies may have led to the significant positive correlation of sub-sAA and r-sAA with ODI in the OSAS group. Considering that intermittent hypoxemia related to SDB (ODI) might be related to reperfusion injury by oxidant and free radicals which might be related to SDB comorbidities,[27] the correlation of sub-sAA and r-sAA with ODI in the OSAS group would be meaningful.

ATH and airway dimensions have been thought to be useful predictors of the risk of upper airway obstruction in children.[33] Although several studies have reported that adenotonsillar size does not reliably predict SBD, many clinicians still use ATH as a clinical indicator for SDB in children.[34] So, in this study, we tried to investigate the association among sAA activity, OSA severity, and ATH (tonsil size and AN ratio) in children. In addition, we investigated the correlation between PSG parameters and sAA and between sAA and ATH with the aim of determining the predictive power of ATH and sAA for the severity of OSA.

Our results demonstrated that the ATH was not related to the AHI or ODI, and the AN ratio was negatively related to only lowest oxygen saturation. No significant difference was found between two subgroups of OSA in tonsil size and AN ratio (**Table 3**). As for the relevance of sAA to ATH, there was no significant correlation among tonsil size, AN ratio, and sAA measurements. Taking all these results (irrelevance to AHI, ODI, and sAA parameters) into consideration, it would be difficult to predict the severity of AHI on the basis of the adenotonsillar size alone.

As predictors of OSAS, the areas under the ROC of ATH were approximately 0.7, indicating these parameters were poor predictors of OSAS. A recent study reported that the correlation between tonsil size and OSAS severity was so inconsistent that clinicians must recognize the limitations of using tonsil size in clinical decision making.[35] Brooks et al.[36] reported that adenoid size was not related to the AHI in children, and that screening patients using AN ratio or visual inspection of the tonsils was associated with a marked risk of false-positive and false- negative. However, the sub-sAA and r-sAA were significantly higher in the severe OSAS than in the other groups, and the areas under the curve for severe OSAS were > 0.8. Furthermore, these measurements showed correlations with other PSG parameter like AHI and ODI. These results may suggest that these parameters may be better associated with OSAS than ATH.

Although PSG is the gold standard for diagnosis of pediatric SDB, many doctors still use ATH as an useful clinical indicator of SDB in children because of clinical and economic limitations of PSG.[37] Based on our results, the ATH can predict OSA roughly at both extremes, which might mean that the greater

ATH meant the more likely OSA, but our results showed that tonsil/adenoid size did not predict the severity of OSA according to AHI.

It has been reported that there is a high rate of residual OSAS after adenotonsillectomy in normal weight children with severe or moderate OSAS.[38] In addition, there is overwhelming evidence that children with more severe OSAS have a higher incidence of postoperative respiratory complications including postobstructive pulmonary edema, pneumonia, airway obstruction, and respiratory failure.[39] Therefore, when PSG assessment of OSAS is not available, the sub-sAA and r-sAA in combination with ATH or alone would be more helpful in predicting severe OSAS than clinical decision with ATH and/or history. This could help clinicians identify high-risk children. Although further studies need to be done, sAA would be clinically useful for the prediction and post-management plan of children with severe OSAS in combination with ATH. However, it should be noted that our results do not mean that sAA can replace the role of PSG in pediatric SDB.

Finally, it should be noted, though the present study was of a prospective design, our study did not control all factors that may affect pediatric SDB: (1) The control group was not age- and sex-matched and might be heterogeneous (including subjects with or without primary snoring and upper airway resistance syndrome). (2) The OSAS group was divided into two rather than three subgroups according to AHI (mild-moderate and severe) because the number of patients with each OSAS subgroups was not even. (3) No concurrent measurement of ANS function such as noninvasive heart rate variability measures, blood pressure, and urine epinephrine or norepinephrine was conducted, and the effects of treatment were not assessed for sAA. (4) sAA measurements were obtained at two time points.

In conclusion, sampling for sAA was easy, safe, and pain-free to collect and easy for multiple sampling in children. The sAA had a consistent relationship with AHI, which might be associated with instability of ANS by OSAS. Therefore, screening test of sAA in children suspected of having SDB may be a helpful tool in identifying those with OSAS when PSG is not available.

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