

Immune function in Japanese schoolchildren with sleep-disordered breathing: a preliminary study with analyses of salivary markers

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ABSTRACT

The aim of this study was to assess the prevalence of sleep-disordered breathing (SDB) symptoms among primary schoolchildren, and to objectively determine the influence of SDB on the intra-oral environment through the analysis of saliva. A questionnaire survey was conducted among approximately 400 children from a primary school in Hiroshima. Parents were asked to complete the questionnaire and provide their contact information if they allowed the collection of saliva samples from their children. Thirty-eight children agreed to participate in the saliva study. Habitual snoring and cessation of breathing during sleep were found in approximately 8% and 1% of children, respectively. The present results showed significant correlations between snoring and mouth breathing. A significant association between excessive daytime sleepiness (EDS) and learning problems was found. Furthermore, among children between the ages of 7 and 12 years, those with EDS and learning problems tended to be older. SDB symptom scores were statistically significant only in relation to EDS. The present study also demonstrated significantly higher levels of salivary IgA and cortisol in children with sleep-related disorders. The present study determined the prevalence and characteristics of SDB among Japanese primary schoolchildren and their effects on the oral environment. Approximately 8% of primary schoolchildren with habitual snoring might need to be carefully monitored for SDB symptoms and immune status to ensure proper psychological and physical development.

Keywords: Schoolchildren; Sleep-Disordered Breathing Symptoms; Immune Function; Salivary Markers

1. INTRODUCTION

Sleep-disordered breathing (SDB), including sleep apnea syndrome (SAS), is an important cause of morbidity for both adults and children [1-5]. In children in particular, SDB is of great concern because it can cause neurobehavioral and growth-related problems, and affect academic performance [6-9]. The estimated prevalence of SAS in children ranges from 1% to 3% and the incidence of habitual snoring has been reported to be 7% - 12% in previous studies [10-13]. Therefore, the prevalence of SDB and its influence on daily life are significant and cannot be neglected in growing children.

In the fields of dentistry and orthodontics, mouth breathing patients are known to have narrow upper dentition and long facial height resulting from the backward and downward displacement of the mandible. This association has been explained by the fact that mouth breathing causes reduced activity of the facial and masticatory muscles, leading to the appearance of skeletal deformities represented by an open bite with a small and distally located mandible. In addition, the descriptive term "adenoid face", consisting of narrow dentition, protruding teeth, and lip incompetence at rest has been documented in the literature for at least a century [14-16].

Over the last decades, the effect of SDB on the immune system has been reported frequently [17]. The measurement of secretory immunoglobulin A (IgA) levels in the saliva is a convenient and frequently used indicator of immune status. SDB during childhood differs significantly from that in adults in terms of various parameters such as symptoms and pathogenesis. Therefore, an appropriate differential study to discriminate SDB symptoms in children is necessary.

The aim of this study was to assess the prevalence of SDB symptoms in primary schoolchildren and to objectively determine its influence on the intra-oral environment by means of saliva analysis.

2. MATERIALS AND METHODS

2.1. Participants

A questionnaire survey was conducted among 412 children (197 boys and 215 girls) from a primary school in Hiroshima. All children were within normal range in both physical and psychological development and had no significant systemic diseases. Each subject gave written informed consent and the study protocol was approved by the Ethical Review Board of Hiroshima University Hospital.

2.2. Questionnaire

A questionnaire on self-reported symptoms for the evaluation of SDB was used according to the Tucson Children's Assessment of Sleep Apnea (TuCASA) study reported previously by Goodwin *et al.* [18]. This screening questionnaire was designed to assess the severity of sleep-related symptoms irrespective of age and gender. The questionnaire was translated into Japanese. Parents were asked to answer 13 questions pertaining to their child's sleep habits (APPENDIX). These questions were scored by the parent on a scale of "never", "rarely", "occasionally", "frequently", "almost always", or "don't know". Each questionnaire informed the parent that we would complete sleep studies on approximately 400 children. The children who had all 3 symptoms (excessive daytime sleepiness (EDS), witnessed apnea (WITAP), and snoring) or more were selected and classified into the SDB group and the children who had no symptoms at all served as the control group according to the TuCASA study.

The complete list of survey questions is shown in the "APPENDIX".

2.3. Saliva Sample Collection

Parents were asked to complete the questions and to provide their contact information if they agreed to allow study personnel to call and schedule a saliva test for their children. Of 412 children, 38 children (19 boys and 19 girls, ranging in age from 6.7 to 11.7 years) agreed to participate in the saliva study. Before the start of the saliva collection and analysis, informed consent in paper form was obtained from the parents again. The same number of children (19 boys and 19 girls) was selected for the control group with similar distribution of sex and age.

Saliva was collected from each participant and analyzed for IgA and cortisol levels according to the guidelines of Hanrahan *et al.* [19]. All subjects were requested to collect the saliva sample within the first 20 min after waking up. The samples were then taken to school on the same day, collected by the study personnel and immediately frozen at -20°C until their subsequent analysis.

2.4. SIgA and Cortisol Assay

Samples were recovered after thawing by centrifugation at 1500 g for 15 min. Salivary IgA ($\mu\text{g/mL}$) and cortisol ($\mu\text{g/dL}$) were quantified by means of an enzyme-linked immunosorbent assay (ELISA) using the Salivary Secretory IgA Indirect Enzyme Immunoassay Kit and High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit, respectively (Salimetrics, LLC, State College, PA, USA).

A ready-to-use 96-well microtiter plate coated with highly purified human SIgA was used to assess IgA levels and for generating standard immunoglobulin level curves. As a detecting antibody, goat anti-human SIgA conjugated to horseradish peroxidase was used. The first 2 columns of each plate contained blank controls and 6 dilutions of purified human SIgA (600, 200, 66.7, 22.2, 7.4, and 2.5 $\mu\text{g/mL}$) in duplicates. The rest of the plate was filled with a high and low control, and diluted (1:120) saliva samples in duplicates. After incubation for 90 min at room temperature with continuous mixing at 400 rpm, the wells were washed 6 times with wash buffer (phosphate-buffered solution) and filled with TMB solution (tetramethylbenzidine). After 5 min of mixing at 500 rpm and additional 40-min incubation in the dark at room temperature, the wells were filled with an acidic formulation stop solution. The plate was mixed at 500 rpm for 3 min and the developed color was measured at an optical density of 450 nm on a microplate reader (Model 550, Bio-Rad Laboratories, Hercules, CA, USA).

For the assessment of the salivary cortisol concentration, all samples were assayed in duplicate. Absorbance was measured at an optical density of 450 nm on a microplate reader (Model 550, Bio-Rad Laboratories) and the quantity was derived from the standard curve.

2.5. Data Analysis

Thirteen questionnaires for the evaluation of SDB symptoms and school activities were initially scored according to frequency of occurrence as never (0), rarely (1), sometimes (2), frequent (3), or always (4). Blanks were regarded as missing data and "don't know" was neglected. Five principal subjective parameters regarding respiration and sleep were analyzed and the findings were compared with previous results obtained from the same school in 2003.

To examine saliva data, the subjects were judged as experiencing EDS if the parent reported that their child was sleepy in the daytime "frequently" or more. WITAP was present if the parent reported that their child stopped or struggled to breath during sleep "frequently" or more. Snoring was present if parents reported loud snoring from their child "frequently" or more.

Data are presented as percentages. A similar study with the same questionnaire was conducted in 2003, and

the statistical significance of the symptom scores in the previous and current surveys was examined with the Mann-Whitney U test. A Spearman rank correlation analysis was used for non-parametric variables. To compare the control and SDB symptom groups, Student's *t*-test was conducted. A *p* value < 0.05 was considered significant.

3. RESULTS

3.1. Demographics of the Participants

Tables 1 and 2 show the demographic data of the previous survey in 2003 and of the present study. The number of participants in the present study was 69 (34 boys, 35 girls) in the first grade, 63 (29 boys, 34 girls) in the second grade, 65 (30 boys, 35 girls) in the third grade, 72 (34 boys, 38 girls) in the fourth grade, 76 (37 boys, 39 girls) in the fifth grade, and 67 (33 boys, 34 girls) in the sixth grade. The children ranged in age from 6 (first grade) to 12 years (sixth grade), and the mean age was 9.6 (0.4) years. In addition, no significant differences in the distribution of boys and girls were found between the previous and current demographic data shown in Tables 1 and 2.

Table 1. The number of participants in the previous survey in 2003.

			Boys		Girls	
Total 418	Male	206	Grade 1	46	21	25
			Grade 2	80	41	39
			Grade 3	74	32	42
	Female	212	Grade 4	78	40	38
			Grade 5	82	43	39
			Grade 6	58	29	29

Table 2. The number of participants in the current survey.

			Boys		Girls	
Total 412	Male	197	Grade 1	69	34	35
			Grade 2	63	29	34
			Grade 3	65	30	35
	Female	215	Grade 4	72	34	38
			Grade 5	76	37	39
			Grade 6	67	33	34

3.2. SDB-Related Symptoms

The percentage of children reported to snore loudly “almost always”, “frequently”, and “occasionally” was 6.8%; those reported to stop breathing during sleep “almost always”, “frequently”, and “occasionally” were 1.3%, and those reported to be daytime mouth breathers “almost always” and “frequently” were 14.3%. Furthermore, in association with school activities, the percentage of children who answered that they were sleepy during the daytime “almost always” and “frequently” were 3.9%, and the percentage in those who had learning problems “almost always”, “frequently”, and “occasionally” were 2.8%. In the 2003 data, the percentage of children who answered these questions similarly was almost the same (Table 3).

Table 4 shows the comparison of the distribution of SDB problems after the children were divided into 2 groups based on their school grades. In the older group comprising grade 4 - 6 children, the percentage of children who reported daytime sleepiness and learning problems was higher than in the younger group, whereas the percentage of children who reported snoring loudly was lower in the older group than in the younger group.

As shown in Table 5, statistically significant correlations were found among the 5 main questions. All significant correlations were positive (*r* = 0.11 - 0.27). The snoring scores were significantly correlated with most of the other variables, excluding learning problems. On the other hand, the learning problems score was significantly correlated with that of EDS in both the previous and the present surveys (*p* < 0.01).

With respect to the SDB symptom scores, the only

Table 3. Distribution of common SDB problems in the sleep survey.

Question	Responses	Previous survey in 2003 (N = 418)	Current survey (N = 393)
Q1. How often does your child snore loudly?	Almost always/Frequently/Occasionally	8.8	6.8
Q2. How does your child stop breathing during sleep?	Almost always/Frequently/Occasionally	1.4	1.3
Q3. Is your child a daytime mouth breather?	Almost always/Frequently	14.3	14.3
Q4. Is your child sleepy during the daytime?	Almost always/Frequently	4	3.9
Q5. Does your child ever have learning problems?	Almost always/Frequently/Occasionally	2.4	2.8

%

Table 4. Distribution of common SDB problems in sleep survey.

Question	Responses	Grade 1, 2, 3		Grade 4, 5, 6	
		Previous survey (N = 200)	Current survey (N = 197)	Previous survey (N = 218)	Current survey (N = 215)
Q1. How often does your child snore loudly?	Almost always/Frequently/ Occasionally	9.0	5.1	8.7	7.0
Q2. How does your child stop breathing during sleep?	Almost always/Frequently/ Occasionally	2.0	1.5	0.9	0.9
Q3. Is your child a daytime mouth breather?	Almost always/ Frequently	14.0	12.7	20.6	14.4
Q4. Is your child sleepy during the daytime?	Almost always/ Frequently	1.5	2.5	6.0	14.0
Q5. Does your child ever have learning problems?	Almost always/Frequently/ Occasionally	1.0	1.5	3.7	3.7

%

Table 5. Correlation coefficients between questions.

	Q1	Q2	Q3	Q4	Q5
Q1		0.23** 0.10	0.27** 0.25**	0.18** 0.13**	0.09 0.07
Q2			0.21** 0.08	0.11* -0.01	0.11* 0.03
Q3				0.08 0.25**	0.15** 0.07
Q4					0.13** 0.15**
Q5					

◻ : previous survey *significantly different (p < 0.05)
 ◼ : current survey **significantly different (p < 0.01)

EDS question that was used in the previous and current questionnaires showed a significantly large difference in the score in the older group (Tables 6 and 7).

3.3. Saliva Analysis

Because no significant differences in IgA and cortisol levels were found between boys and girls in both the control and symptomatic groups, the means obtained from 38 subjects in total were used for the following statistical comparison.

Measurement of IgA levels showed that the symptomatic group had significantly higher levels than the controls did (p < 0.05) (Figure 1). With respect to the cortisol levels, the symptomatic group also showed a considerably higher level than the control group, although a statistically significant difference was not found (Figure 2).

Table 6. Descriptive statistics and comparisons between SDB symptom scores in the 2 groups.

	2003						
	Grade 1, 2, 3			Grade 4, 5, 6			p
	Med.	25%	75%	Med.	25%	75%	
Q1	1	0	2	1	0	2	NS
Q2	0	0	0	0	0	0	NS
Q3	2	1	2	2	1	2	NS
Q4	0	0	1	1	0	1	*
Q5	1	1	2	1	1	2	NS

Med., median; 25%, 25th percentile; and 75%, 75th percentile. *Statistical significance was defined as p < 0.05.

Table 7. Descriptive statistics and comparisons between SDB symptom scores in the 2 groups.

	2006						
	Grade 1, 2, 3			Grade 4, 5, 6			p
	Med.	25%	75%	Med.	25%	75%	
Q1	0	0	1	0	0	1	NS
Q2	0	0	0	0	0	0	NS
Q3	0	0	1	0	0	2	NS
Q4	0	0	1	1	0	2	*
Q5	0	0	0	0	0	0	NS

Med., median; 25%, 25th percentile; and 75%, 75th percentile. *Statistical significance was defined as p < 0.05.

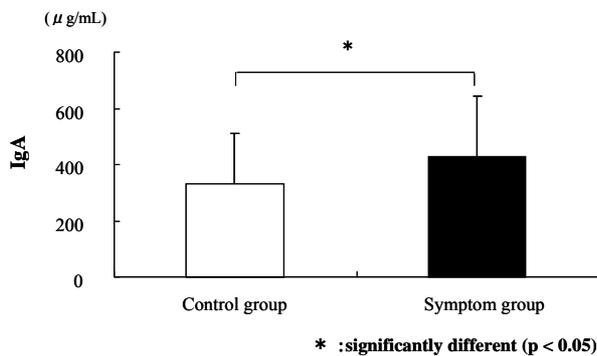


Figure 1. Comparison of the IgA levels between control and SDB symptom groups.

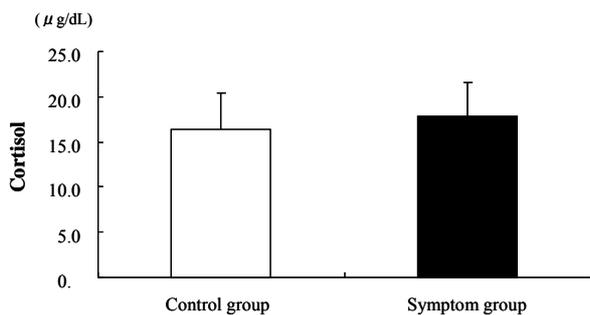


Figure 2. Comparison of the cortisol levels between control and SDB symptom groups.

4. DISCUSSION

In the present study, a consciousness survey was conducted for the assessment of SDB symptoms and problem behaviors among primary schoolchildren. The influence of these factors on salivary secretory immunoglobulin A and cortisol levels was also examined. Sleep apnea syndrome (SAS) has been of increasing concern among the middle-aged population in Japan in recent years, and the physical and psychological development of children is considered to be greatly influenced by sleep quality [2-4]. Mental retardation from chronic hypoxia, low stature from hyposecretion of growth hormones, and slow learning due to daytime sleepiness are listed as examples of the association between SDB and physical and psychological development; hence, children in primary school were defined as the target of the present study.

4.1. Prevalence of SDB Symptoms

In this study, habitual snoring and cessation of breathing during sleep were found in approximately 8% and 1% of children, respectively. Several studies from other countries have reported the prevalence of sleep problems in a population-based sample of children. Gozal and Pope reported that the mean prevalence of snoring among 1588 children was 9% in the United States [7]. A similar pre-

valence of snoring among children was reported in France (10%) [20] and in Italy (7.3%) [12]. The overall snoring prevalence in the previous and current surveys from our group was at the upper limit of the range reported by others.

With respect to cessation of breathing, the present results were similar to those of previous studies reporting that SAS affects approximately 1% - 2% children [2, 10, 13]. Cessation of breathing during sleep is not the same as diagnostic SAS. However, apneic symptoms such as cessation of breathing, difficulty in periodic breathing, and nocturnal choking might be the indicators of the presence of SAS.

The present results showed significant correlations between snoring and mouth breathing. Mouth breathing is one of the characteristic SAS symptoms [13]. Furthermore, nasal influences on mouth breathing, snoring, and sleep apnea have been reported [21]. Therefore, it is reasonable to assume that mouth breathing caused by nasal obstruction leads to loud snoring, apneic breathing, and disturbed sleep.

In this study, a strong association between EDS and learning problems was detected. This finding suggests that poor sleep quality such as sleep fragmentation and nocturnal hypoxemia might induce EDS, which ultimately influences academic performance and ability. In agreement with our results, EDS, as well as various behavioral problems such as inattention, hyperactivity, and aggression in school were reported in association with sleep-related disorders in previous studies [7-9]. Interestingly, Gozal demonstrated that improvements in school performance could be induced by remediation of SAS in first-grade children with substantial academic impairment in school-related activities [6].

Furthermore, among children between 7 and 12 years of age, those with EDS and learning problems were more likely to be older than those without. Our data regarding SDB symptom scores were statistically significant only with respect to EDS. This may be due to the difference in mean sleep times between older and younger children, which were 9 h 18 min (32 min) (first grade) and 7 h 38 min (56 min) (sixth grade), respectively (data not shown). The current lifestyle of Japanese children, which includes longer study periods after school and the resulting delay in bedtime, might be a significant factor affecting children's sleep.

The Tucson Children's Assessment of Sleep Apnea (TuCASA) study is a prospective cohort study that was designed to determine the prevalence of objectively documented SDB in preadolescent children and to investigate its relationship to symptoms, performance on neurobehavioral measures, and physiologic and anatomic risk factors [18]. Although screening questionnaires for children can easily show bias, most TuCASA components

are regarded as standard questions that are found in sleep-habit questionnaires pertaining to SDB in children.

4.2. Association of SDB Symptoms with Salivary Immune Factors

The results of the present study showed significantly higher levels of salivary IgA and cortisol in children with sleep-related disorders. Secretory IgA, which is measured in the saliva, is the predominant antibody detected in bodily secretions. IgA levels are believed to indicate the functional status of the entire mucosal immune system [22]. As a source of biological fluid for physical examination, saliva has several advantages. Collection of saliva is less intrusive and easier than that of urine or blood, especially for children.

The most likely explanation for the significantly high concentration of salivary IgA in the symptom group is that sleep-related disorders negatively affect immune functions and increase IgA levels for protection against certain infections. The release of salivary IgA could be an indicator of intra-oral and upper airway inflammation. Therefore, salivary IgA provides a major defense against potential pathogens by preventing colonization and replication on the mucosal surfaces of the oral cavity and the upper respiratory tract. Previous studies demonstrated that recurrent upper respiratory infection was associated with increased prevalence of SDB symptoms in children [12,23,24].

The use of self-reported stress as a measurement method can be associated with bias and variation in study results [25]. The psychological status of patients can be reflected in their stress level. Cortisol is a stress hormone that indicates hypothalamic-pituitary-adrenal (HPA) axis activity and is a reliable biomarker of stress that is easily measured in saliva [26]. The cortisol awakening response, which is measured during the first 30 - 45 min after waking up in the morning, is an especially reliable marker of HPA axis activity [27]. Yehuda *et al.* demonstrated that a single salivary cortisol sample taken immediately after waking up can provide an accurate estimate of urinary cortisol and mean salivary cortisol throughout the day [28]. The present results indicated that SDB symptoms can constitute a stressor capable of elevating cortisol levels, and suggested that children in primary school with greater adrenocortical reactivity might have a considerable level of psychological stress.

In the field of dentistry, especially orthodontics, many studies have shown the influence of breathing disorders caused by respiratory obstruction with the large soft palate, tonsils, tongue, and nasal inflammation on craniofacial morphology. Animal studies demonstrated that specific skeletal and dental changes, including an increased lower facial height, anterior downward tipping of the

occlusal plane, and dual bites, crossbite, and open bites occur within 24 months after nasal obstruction in monkeys [29,30]. Bresolin *et al.* investigated the relationship between mouth breathing in allergic children and dento-facial development [29], and demonstrated that mouth breathers had longer faces with narrower maxilla and retrognathic mandibles [31]. These studies show that breathing disorders are strongly correlated with craniofacial morphology. Moreover, a small oral cavity with narrow dentition associated with retrognathism due to undergrowth of the mandible could induce SDB. Therefore, anatomical disorders carry a certain risk in terms of predisposition to the development of respiratory disorders such as SAS.

In conclusion, the present study determined the prevalence and characteristics of SDB among Japanese primary schoolchildren and its influence on the oral environment. Approximately 8% of primary schoolchildren with habitual snoring might need to be carefully monitored for SDB symptoms and immune status to ensure appropriate psychological and physical development.

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APPENDIX*Screening Questionnaire*

- 1) Does your child stop breathing during sleep?
- 2) Does your child struggle to breathe during sleep?
- 3) Do you ever shake your child during sleep to make him/her breathe again?
- 4) Do your child's lips ever turn blue or purple while he/she is sleeping?
- 5) Are you ever concerned about your child's breathing during sleep?
- 6) How often does your child snore loud?
- 7) How often does your child have a sore throat?
- 8) Does your child complain of morning headaches?
- 9) Is your child a daytime mouth breather?

- 10) Is your child sleepy during the daytime?
- 11) Does your child fall asleep at school?
- 12) Does your child fall asleep while watching television?
- 13) Does your child have learning problems?

Possible Responses

- 1) Almost always
- 2) Frequently
- 3) Occasionally
- 4) Rarely
- 5) Never
- 6) Don't know