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Reduction in oxidative stress biomarkers after adenotonsillectomy

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ABSTRACT

Objectives: A number of otolaryngic conditions such as chronic tonsillitis, adenoid hypertrophy, and obstructive sleep apnea are associated with oxidative stress and elevated levels of serum oxidants. The objective of this study is to measure changes in urine biomarkers of oxidative stress in children after adenotonsillectomy.

Methods: Twenty-two children with sleep disordered breathing (SDB) with tonsil and adenoid hypertrophy were enrolled prior to adenotonsillectomy. Controls consisted of 20 healthy children. Urine samples were collected from all patients. Levels of three urinary biomarkers for oxidative status, 8-hydroxy-2-deoxyguanosine (8-OxodG), F^2-isoprostane, and malondialdehyde (MDA) were measured using high performance liquid chromatography. For the study group, urine samples were repeated 3 weeks after surgery.

Results: In the study group, preoperative urinary levels of 8-OxodG were higher than in controls ($p = 0.015$). Levels decreased after surgery compared to preoperative levels ($p = 0.002$), and reached control levels ($p = 0.167$) at 3 weeks. Levels of urinary F^2-isoprostane were similar in both groups ($p = 0.252$), but decreased significantly after surgery ($p = 0.020$).

Conclusions: Children with SDB have elevated levels of urinary 8-OxodG, a marker of oxidative stress. Adenotonsillectomy results in decreased 8-OxodG and F^2-isoprostane. These findings suggest that urine analysis may represent a valuable tool for the measurement of oxidative stress.

1. Introduction

Oxidative stress reflects an imbalance between formation of reactive oxygen species (ROS) and antioxidative defense mechanisms [1]. ROS are produced continuously from normal cellular metabolism, however elevated levels are associated with a variety of neurodegenerative and cardiovascular diseases, aging, and cancer, etc [1,2]. Oxidative stress has harmful effects on proteins, lipids, and DNA when antioxidant defenses are overwhelmed [2]. Studies have implicated oxidative stress with common conditions in the head and neck including chronic tonsillitis and adenoid hypertrophy [3,4].

Studies have provided evidence that supports an increase of oxidative stress in adults with obstructive sleep apnea (OSA). Doğruer et al. [5] and Dyugovskaya et al. [6] detected increase production of ROS in adults with OSA, while Christou et al. [7] found that patients with severe OSA have a reduced antioxidant capacity. These studies indicate a significant relationship between OSA and oxidative stress.

Several investigators have demonstrated similar findings of oxidative stress in children with adenotonsillar hypertrophy and sleep-disordered breathing (SDB) [3–5]. However, these studies have relied on serum blood tests to measure ROS. Obtaining blood is difficult in children, and can be an obstacle in many cases. The objective of this study is to measure urinary ROS before and after tonsillectomy in children, and to compare the results with healthy controls. Utilization of urine to measure ROS would represent a simple and non-invasive test for oxidative stress.

2. Methods

2.1. Subjects

This study was approved by Konkuk University Hospital Institutional Review Board (KUH1110034). For the study group,

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22 consecutive children were enrolled who were referred to the department of otorhinolaryngology, Konkuk University Hospital (Seoul, Korea) for adenotonsillectomy with sleep disordered breathing. For comparison, 20 healthy children were enrolled who never or rarely snored as reported by their parents. Exclusion criteria were as follows: acute or chronic cardiorespiratory or neuromuscular disease, dysmorphism, chronic inflammatory diseases, major craniofacial abnormalities, chromosomal syndromes, previous adenotonsillecotmy or adenoidectomy, taking any kind of medication within recent 2 weeks, and age under 4 or over 9. Informed consent was obtained from the parents of each child before enrollment in the study.

2.2. Methods

The children's height and weight were measured to calculate their body mass index (BMI) and the tonsil size was classified into four grade with a physical examination: [8] Grade 1 was defined when tonsil occupied <25% of oropharyngeal width, and grade 2, 3, and 4 were 25% <and <50%, 50% < and <75%, and >75%, respectively. The presence or absence of allergies was measured by skin prick test (SPT). SPT was done for six most common allergens for Korean children, namely *Dermatophagoides pteronyssinus, Dermatophagoides farinae*, cat, dog, mugwort, and pollen mixture in a method previously reported [9]. If the wheal was larger than 3 mm or the histamine control for at least one allergen, it was defined to be a positive SPT. We asked the parents about degree of SDB symptoms including loud snoring, apnea, choking or gasping, and frequent awakening or restlessness by using visual analogue scale (1 for never to 7 for always, Fig. 1).

The first urine in the morning was collected to measure biomarkers for oxidative status. It was quickly stored at −80 °C until analysis. The concentration of 8-hydroxy-2-deoxyguanosine (8-OxodG) and F2-isoprostane were measured by using high performance liquid chromatography with triple tandem mass detector (HPLC-MS/MS Agilent 6410, Agilent Technologies, Santa Clara, US), and those of malondialdehyde (MDA) were measured by using rapid resolution liquid chromatography (RRLC Agilent 1200SL, Agilent Technologies). Tests were performed at the Seegen Medical Foundation in Seoul, Korea. All the measurement values were corrected for creatinine level.

For snoring children, we repeated urine sampling and parent's survey regarding SDB at 3 weeks after adenotonsillectomy. This study was approved by Konkuk University Hospital Institutional Review Borad (KUH1110034).

2.3. Statistics

For comparison of age and BMI between snoring children and control, a Student *t*-test was used. Pearson χ^2 was used for comparison of the sex ratio and prevalence of positive SPT, and Mann–Whitney test was for tonsil size and SDB symptoms.

Paired *t*-test and Wilcoxon sign ranked test were used for comparison of urinary biomarkers and SDB symptoms between pre- and post-operation state, respectively. Student *t*-test was used for those between pre-operation and control or between post-operation and control. All *p*-values reported were 2-sided and the statistical significance was set at <0.05. These calculations were completed with statistical software (IBM SPSS statistics 21, IBM, NY, US).

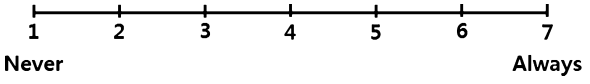

Fig. 1. Visual analogue scale for measuring the degree of sleep-disordered breathing.

3. Results

Twenty-one snoring children and 19 control children completed the study. Age, sex ratio, and incidence of allergies on SPT were not different between two groups ($p > 0.05$). However, BMI ($p = 0.028$) and tonsil size ($p < 0.001$) were greater among snoring children than control. SDB symptoms were much more severe among snoring children than controls. All the results were summarized in Table 1. After adenotonsillectomy, all the SDB symptoms among snoring children improved significantly (Fig. 2).

3.1. Urinary biomarkers of oxidative status

3.1.1. (1) 8-hydroxy-2-deoxyguanosine

In pre-operation state, 8-OxodG was significantly increased among snoring children compared to controls (4.77 ± 2.88 vs. 2.72 ± 2.18 μg/g creatinine, $p = 0.015$). After adenotonsillectomy, it decreased significantly compared to pre-operation state (4.77 ± 2.88 vs. 1.84 ± 1.80 μg/g creatinine, $p = 0.002$). There was no difference between post-operation state and control (1.84 ± 1.80 vs. 2.72 ± 2.18 μg/g creatinine, $p = 0.167$). The results are summarized in Fig. 3.

3.1.2. (2) F^2-isoprostane

In pre-operation state, F^2-isoprostane was not higher among snoring children compared to control (0.630 ± 0.647 vs. 0.449 ± 0.220 ng/g creatinine, $p = 0.252$). After adenotonsillectomy, it decreased significantly compared to pre-operation state (0.630 ± 0.647 vs. 0.297 ± 0.212 ng/g creatinine, $p = 0.020$) or even to control (0.449 ± 0.220 vs. 0.297 ± 0.212 ng/g creatinine, $p = 0.031$). The results are summarized in Fig. 4.

3.1.3. (3) Malondialdehyde

The levels of MDA were not different among control (2.66 ± 1.07 μM/g creatinine), pre- (2.27 ± 1.07 μM/g creatinine) and post-operation (2.03 ± 0.68 μM/g creatinine) state. The results are summarized in Fig. 5.

4. Discussion

It is thought that repeated episodes of hypoxia and reoxygenation which occurs during SDB can result in the production of reactive oxygen species (ROS) and a state of oxidative stress [10]. Examples of common ROS include hydrogen peroxide, hydroxyl radicals, superoxide anion, and nitrous oxide [1]. It is very difficult to measure amounts of ROS directly in clinical setting because they

Table 1
Comparison of demographic data between patients and control.

	Patients	Controls	*p*-value
Number	21	19	
Age (years)	7.1 ± 1.2	6.9 ± 1.8	0.682
Boys/Girls	17/4	13/6	0.473
BMI (kg/m^2)	17.3 ± 1.9	15.7 ± 2.3	0.028*
Tonsil size (1/2/3/4)	3/5/8/5	12/7/0/0	0.001<*
Positive SPT	15/21 (71.4%)	10/19 (52.6%)	0.328
SDB symptoms			
Loud snoring	4.6 ± 1.6	1.8 ± 0.7	0.001<*
Apnea	2.8 ± 1.3	1.2 ± 0.4	0.001<*
Choking or gasping	2.3 ± 1.4	1.2 ± 0.5	0.005*
Frequent awakening or restlessness	3.6 ± 0.9	2.3 ± 1.3	0.002*

* Means *p*-value <0.05.
BMI: body mass index.
SPT: skin prick test.
SDB: sleep-disordered breathing.