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A novel method for evaluation of oxidative stress in children with OSA

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Objectives: To evaluate the role of adenotonsillar hypertrophy and the outcomes of adenotonsillectomy (AT) on oxidative stress for obstructive sleep apnea (OSA) in children using a new method; thiol/disulfide homeostasis.

Methods: The study is consisted of 45 children with OSA and 38 healthy control subjects with similar age and sex. Children 3–12 years of age with OSA, defined as having an apnea/hypopnea index (AHI) of 5 or more in an overnight polysomnography, underwent adenotonsillectomy. OSA was classified as mild ($1 \leq$ AHI<10), moderate ($10 \leq$ AHI<20) or severe (AHI$\geq$20). Venous blood samples were taken preoperatively and one month after surgery. The blood levels of thiol/disulfide homeostasis were assessed and compared between patients and control group, before and after adenotonsillectomy.

Results: Body mass index (BMI), mean age and gender distribution were similar between the study and control groups. Statistically significant higher disulfide levels and ratios were found in the study group compared to the control group; in patients with moderate to severe OSA compared to mild OSA; in the preoperative period compared to postoperative period ($p < 0.001$, for all).

Conclusions: The current study provides preliminary evidence between oxidative stress and OSA in children with adenotonsillar. Adenotonsillectomy for OSA may result in a dramatic improvement in oxidative stress as measured by thiol/disulfide homeostasis.

1. Introduction

1.1. Background

Although pediatric obstructive sleep apnea (OSA), which affects 1%–4% of the pediatric population, has multiple etiologies, the essential feature of OSA in children is increased upper airway resistance during sleep [1]. Airway narrowing may be due to craniofacial abnormalities or soft tissue hypertrophy including adenotonsillar hypertrophy (ATH). ATH resulting in OSA is a common problem and can lead to significant cardiopulmonary complications, poor growth and problems with learning and behavior, which is mostly treated with adenotonsillectomy as a first line of treatment.

1.2. Literature review

Upper airway obstruction in children with ATH may result in sleep fragmentation, difficulty in breathing, alveolar hypoventilation and intermittent hypoxemia. Hypoxemia occurs in two ways: either cessation of air flow (apnea) or decreased air flow (hypopnea). Repetitive cycles of hypoxia and reoxygenation during periods of apnea and hypopnea, promote systemic oxidative stress and inflammation [2–4]. Impaired protein oxidative damage is previously observed in patients with OSA [5]. It is very difficult to measure amounts of reactive oxygen metabolites (ROS) directly in clinical setting because they are extremely unstable. Therefore, reactions between ROS and biological molecules including lipids, proteins and DNA are measured as biomarkers of ROS. In a previous study, proteins, amino acids and peptides were shown to be

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vulnerable to attacks by free radicals and oxidants [6]. Proteins, such as albumin, which have thiol groups, are oxidized by oxygen molecules and are reversibly converted to disulfide bonds. These formed disulfide bonds can be reduced to thiol groups in a condition of decreased oxidative stress. Thus, the thiol-disulfide balance is maintained. Cysteine modifications of proteins are considered a hallmark of oxidative stress associated with disulfide formation. Disulfide formation has been considered a reliable indicator of oxidative stress because it reflects the antioxidant status and pro-oxidant reactions and the protein thiol redox status [7]. Thiol-disulfide levels are measured one by one and cumulatively with a novel and automated method [8]. Thiol-disulfide homeostasis is a unique, easy and new method to prove oxidative stress when compared to others [8].

1.3. Study objectives

In this prospective study, we aimed to find out if ATH resulting in OSA plays a pivotal role in progression and development of oxidative stress in children and to assess the efficacy of surgery on reducing oxidative stress in pediatric population.

2. Methods

2.1. Subject recruitment

Totally, 108 children that admitted to Ataturk Training and Research Hospital, Department of Otolaryngology between February 2015 and January 2016 were planned to undergo adenotonsillectomy. The children who had signs and symptoms of a sleep disturbance including snoring, mouth breathing, and witnessed breath holding for at least 3 months' duration were included in the study and an overnight polysomnography (PSG) was done. The objective criteria for the diagnosis of OSA based on PSG were an obstructive apnea/hypopnea index (AHI) equal to or greater than 5 or an obstructive apnea index equal to or greater than 1. Exclusion criteria were as follows; body mass index (BMI) at the 95th percentile or higher (obesity), craniofacial abnormalities, neuromuscular disease, previous adenoidectomy, taking any kind of medication, chronic tonsillitis or symptoms/signs of acute respiratory tract infection. Thus, 45 children who had airflow obstruction due to ATH without a history of recurrent tonsillitis and planned to undergo traditional adenoidectomy and total tonsillectomy were included in the study. In addition, 38 healthy children that attended to our outpatient clinic who never snored as reported by their parents were also included in this study and comprised the control group. Informed consents were obtained from children and their parents before enrollment, and the study was approved by the ethics committee of the hospital (2014-225).

2.2. Polysomnography

To assess the severity of OSA in a definitive manner, all patients underwent full-night PSG before surgery. No sedation or sleep deprivation was used to induce sleep. They were accompanied by at least one parent throughout the night. A sleep medicine physician interpreted the results of PSG. AHI, defined as the number of obstructive apneas and hypopneas per hour of sleep, was measured and grouped into three categories: mild ($1 \leq$ AHI<10), moderate ($10 \leq$ AHI<20) or severe (AHI$\geq$20) [9].

2.3. Grading system for adenotonsillar hypertrophy

Size of adenoids was subjectively graded (with flexible fiberoptic nasopharyngoscopy) and reported based upon a numerical scale. An adenoid size of 1 + denotes 0–25% obstruction of the choanae, 2 + denotes 25–50% obstruction, 3 + denotes 50–75% obstruction, and 4 + denotes 75–100% obstruction. This scale has been proposed as a standardized grading scale for adenoid size, which was adopted from the tonsillar hypertrophy grading scale [10]. Tonsillar hypertrophy was graded using a standardized grading system [11].

2.4. Biochemical analysis

Venous blood samples were collected from the subjects before the surgery and one month after the surgery to allow enough time for resolution of postoperative edema and to ensure that upper airway remodeling is complete. All samples centrifuged in the cold at 2300× g for 10 min. Serum samples were separated and stored at −80 °C. Serum thiol-disulfide homeostasis was determined with a recently developed novel and automatic measurement method by using an automated clinical chemistry analyser (Roche, cobas 501, Mannheim, Germany). Native thiol (-SH) and total thiol (-SH + -S-S-) were measured directly, and disulfide (-S-S-) level, disulfide/total thiol ratio (-S-S−/−SH + -S-S-), disulfide/native thiol ratio (-S-S−/−SH) were obtained with calculation [8].

2.5. Data analysis

The statistical analyses of our study were done using SPSS v22.0 for Macintosh. The descriptive data were given as mean ± standard deviation. The distribution of data was calculated by using the Kolmogorov–Smirnov test. Continuous variables with normal distribution were determined to have mean standard deviation. Categorical variables are shown as numbers and percentages. Thiol/disulfide homeostasis in preoperative and postoperative blood were statistically compared with each other and control group using Student's *t*-test. One-way ANOVA test and then post-hoc tukey analysis were used for multiple comparisons of OSA severity. The significance was taken as $p < 0.05$.

3. Results

3.1. Recruitment

The initial study was comprised of 108 children that attended to our clinic for adenotonsillectomy. Fifty-six children did not meet inclusion criteria. Of the remaining 52 children, 5 without postoperative blood test and 2 with incomplete data were excluded. The final study population included 45 children, 30 of whom were boys. The mean age of the children at the time of inclusion to the study was 7.1 (3.2–11.8) years. Based on preoperative age- and sex-corrected BMI percentiles, 8 (17.7%) children were underweight, 34 (75.5%) were of normal weight, and 3 (6.6%) were overweight. The demographic and clinical characteristics of the participants are shown in Table 1. Body mass indexes (BMI), mean age and gender distribution were similar between the study and control groups ($p > 0.05$).

3.2. Thiol-disulfide homeostasis between study and control groups

The mean native/total thiol ratio was significantly lower and the disulfide level and the disulfide/total thiol, the disulfide/native thiol ratios were significantly higher in the study group than the control group ($p < 0.001$) (Table 2). The other thiol-disulfide parameters were not significantly different between the groups ($p > 0.05$).