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Urine cysteinyl leukotriene levels in children with sleep disordered breathing before and after adenotonsillectomy

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ABSTRACT

Objectives: Obstructive sleep apnea (OSA) is a common problem in children and is associated with increased cardiovascular, neurobehavioral and somatic growth consequences. Cysteinyl leukotrienes (CysLTs) play a major role with local and systemic relations to the pathophysiology of OSA. The level of CysLTs in urine, blood, exhaled breath and adenotonsillar tissue of OSA children are increased. However it remains unclear whether inflammatory marker levels are alleviated after adenotonsillectomy. Therefore, we compare the urine leukotriene E4 (uLTE4) levels in children before and after adenotonsillectomy and evaluate clinical outcomes on resolution of OSA.

Methods: Children under 15 years who suspected OSA with planned adenotonsillectomy were recruited. Sleep questionnaires, quality of life assessment by OSA-18, physical examination, lateral neck radiographs, overnight SpO_2 monitoring and uLTE4 levels were collected. 4 ± 2 weeks post-surgery, OSA-18 was reevaluated and urine was collected again.

Results: Thirty-three children with sleep disordered breathing (SDB) were included (mean age 8.1 ± 2.8 years). After adenotonsillectomy, the $uLTE_4$ levels decreased from 961.9 (684.8–1438.2) to 708.6 (538.2–1038.8) pg/mg Cr ($P = 0.009$). The post-surgery score from sleep questionnaire, OSA-18 questionnaire were significant improved ($P < 0.001$). Obese children demonstrated an improved quality of life post-surgery, but results were poorer than normal-weight children ($P = 0.01$). The uLTE4 no obvious improved in obese children.

Conclusions: Adenotonsillectomy remains an effective treatment for SDB children that not only alleviated the severity of SDB and improved quality of life; it also decreased levels of the systemic inflammatory marker, uLTE4. However, benefits were more obvious in non-obese children.

1. Introduction

Obstructive sleep apnea (OSA) is a common problem in school-age children with a prevalence range of 1–4% [1], and adenotonsillar hypertrophy is the major cause due to disproportionate growth of the pharyngeal lymphoid tissue in comparison to the cranial skeleton [2–5]. This can result in upper airway obstruction especially when sleeping. Other strong factor for OSA is obesity. Previous evidence suggests that the prevalence of obesity in OSA children has increased [6–8]. In the other hand, sleep disordered breathing (SDB) was found markedly increased in obese children [9–11]. Dayyat and colleagues found that for every increment in body mass index (BMI) of 1 kg/m^2 beyond the mean BMI for age and gender, the risk of OSA is increased by 12% [8,12]. This fact is similar trends that have been reported from worldwide [6–8]. Other contributors to pediatric OSA are allergic rhinitis, craniofacial abnormalities and neuromuscular disorders. If left untreated, OSA can

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increase the risk of cardiovascular, neurobehavioral and somatic growth consequences [13–15].

Evidence suggests that inflammation contributes to the pathogenesis of OSA [14,16,17]. It has been further reported that the increased inflammatory markers found in the upper airway tissue of OSA patients are systemically found [17–21]. Leukotrienes (LTs) are key inflammatory mediators in the respiratory system; they are lipid compounds and major arachidonic acid metabolites derived from the 5-lipoxygenase pathway. The LTs family includes LTB4 and cysteinyl leukotrienes (LTC4, LTD4, and LTE4). All of the compounds have a specific role in inflammatory responses [22], but cysteinyl leukotrienes (CysLTs) play a major one with not only local effects but with systemic relations to the pathophysiology of OSA. Many studies confirm an increased level of CysLTs in urine, blood, exhaled breath and adenotonsillar tissue of OSA children [23–32] with disease severity dependency [31,32].

Adenotonsillectomy is the treatment of choice [14,33–36], although sometimes its indication and effectiveness remains controversial. Meta-analysis suggests a high success rate of adenotonsillectomy, but in some cases residual symptoms present after surgery [14,37–39]. Obesity has been reported as a risk factor for unsatisfactory outcome after adenotonsillectomy [40–43]. Although inflammation plays a significant role in the pathophysiology of SDB, it cannot be determined whether the inflammatory mechanism is a cause, a consequence, or both in the disorder. Yet, it is not clear whether inflammatory marker levels return to control levels after adenotonsillectomy [44,45]. Therefore, in order to better understand this correlation, we compare the urine leukotriene E4 (uLTE4) levels in children before and after adenotonsillectomy and we also evaluate clinical outcomes of adenotonsillectomy on resolution of OSA.

2. Methods

2.1. Participants

The study was approved by the Institutional Ethics Committee of Thammasat Hospital, and informed consent was obtained from all parents or legal caretaker. Assent was also obtained from children >7 years old. Children <15 years old with symptoms of OSA who underwent adenotonsillectomy from March 2014 to February 2015 were eligible for recruitment. Indications for surgery were the following: (1) adenotonsillar hypertrophy (adenoid >50%, tonsils size > 2+) [35]; (2) symptoms of SDB > 3 nights per week; and/or (3) detected significant desaturation via overnight SpO_2 monitoring. Exclusion criteria were the following: (1) cardiovascular, neuromuscular, craniofacial, or genetic disorders; (2) acute symptoms or signs of respiratory tract infection.

2.2. Anthropometry and clinical evaluation

Weight and height were measured and analyzed based on Thai growth curves, and body mass index (BMI) z score was calculated using WHO AnthroPlus software; Overweight were classified as those with BMI z score > +1SD, obese were classified as those with BMI z score > +2 SD [46,47]. The questions in the case record form collected referred to symptoms and duration of SDB, past medical history and medication used (especially for montelukast), history of recent respiratory tract infections within the past month, and family history. Sleep symptoms were recorded by using a sleep questionnaire modified from Tucson Children's Assessment of Sleep Apnea (TuCASA) screening questionnaire [48]. This questionnaire represents symptoms during sleep in the past 4 weeks. The score was graded from 0 to 5; 0 = no symptom, 1 = 1–2 night/month, 2 = 1–2 night/week, 3 = 3–5 night/week, 4 = > 5 night/week. The quality of life was measured by Thai version Quality of Life Questionnaire (OSA-18) to describe frequency of symptoms has occurred during the past 4 weeks. It consists of 18 items grouped in 5 domains of sleep disturbance. Each item was graded from 1 to 7; 1 = none of the time, 2 = hardly any of the time, 3 = a little of the time, 4 = some of the time, 5 = a good bit of the time, 6 = most of the time, 7 = all of the time [49]. The OSA-18 total score is the sum of the 18 items, and were classified into 3 groups: minor (score < 60), moderate (score 60–80) and major (score > 80) [50]. Tonsil size was graded from 1 to 4 by direct inspection of the oropharynx [51,52]. For assessment of nasopharyngeal airway patency, lateral neck radiographs were performed pre-surgery. The adenoidal/nasopharyngeal ratio was measured according to the method described by Fujioka and colleagues [2]. The severity of desaturation by overnight pulse oximetry were classified into 4 groups by The McGill Oximetry Scoring System: normal (<3 clusters desaturation < 90%), mild (≥3 clusters desaturation < 90%), moderate (>3 clusters desaturation < 85%), severe (>3 clusters desaturation < 80%) [53]. Before surgery, a urine sample was taken, and during the surgery, adenotonsillar tissue was collected and stored at −80 °C until later analysis. 4 ± 2 weeks after surgery, the questionnaire previously given was distributed to the patients, and urine samples were recollected.

2.3. Measurement of uLTE4

Urine samples were centrifuged at 4000 rpm (Hettich; universal 320 R) for 15 min at 4 °C; the supernatant was then transferred to clean test tubes and frozen at −80 °C until assayed. The concentration of uLTE4 was measured by commercially available enzyme linked immunoassay kits (The Invitrogen Human Leukotriene E4 ELISA Kit, Invitrogen, USA) according to the manufacturer's instructions. Samples (100 μl) or standards were added to wells in duplicate. LTE4 alkaline phosphatase tracer (50 μl) was added to each well except the blank wells and total activity (TA) wells. LTE4 antiserum (50 μl) was added to each well except the blank wells, TA wells and non-specific binding (NSB) wells. Each plate was covered for 2 h to incubate at room temperature on an orbital shaker. Wells were then washed five times with wash buffer and para-Nitrophenyl phosphate (pNPP) solution (200 μl) added to each well including blank and TA wells. LTE4 alkaline phosphatase tracer (5 μl) was added to TA wells. The plates were covered and allowed to develop in the dark on an orbital shaker. A stable yellow-colored product was produced that was proportional to the amount of enzyme present. The absorbency (412 nm) was read after 90 min and compared with standards by a computer program using a 4-parameter logistic or a log-logit curve fit for the calculation of sample LTE4. The uLTE4 concentrations were reported as pg/ml. Urine creatinine concentration was measured by adjusting the uLTE4 levels.

2.4. Statistical analysis

Statistical analysis was performed by using SPSS. Continuous data were presented as mean ± SD. uLTE4 were presented as median (interquartile range) due to skew distribution. A comparison between the pre- and post-surgical value of the biological parameters was done using Wilcoxon Signed Rank Test. A comparison between the pre- and post-surgical value of the quality of life was done using paired *t*-test. Comparisons according to group assignment were made with student t-test for continuous variables and X^2 test for categorical data. A 2-sided p value of <0.05 was used to define statistically significant.