Urinary Leukotriene E4 Levels in Children with Sleep-Disordered Breathing

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

Abstract

Objective. Due to limitations of polysomnography (PSG), novel ways to evaluate pediatric obstructive sleep apnea (OSA) are needed. Urinary leukotriene E4 (LTE4), an inflammatory marker, has been identified as a potential biomarker for pediatric OSA. The objective of the study was to assess whether urinary LTE4 levels correlate with OSA severity, as determined by obstructive apnea-hypopnea index (AHI) and nadir oxygen saturation.

Study Design. Prospective trial.


Subjects and Methods. Children (age, 3-16 years) with sleep-disordered breathing (SDB) who were referred for PSG were included. Urine samples were obtained the morning following PSG, and urinary LTE4 levels were quantified with enzyme-linked immunoassay kits.

Results. A total of 113 children were enrolled, and the mean age was 7.3 years. Thirty-nine percent (n = 44) were obese, and the majority were white (53%, n = 58). Seventy-eight percent (n = 88) were diagnosed with OSA (AHI >1), with 27% (n = 30) having severe disease (AHI >10). The mean urinary LTE4 level was 91.3 ng/mL. Urinary LTE4 levels did not correlate with AHI (P = .77) or nadir oxygen saturation (P = .64). There was a significant difference in urinary LTE4 levels between patients with mild SDB and those with moderate to severe OSA (P = .03).

Conclusion. Urinary LTE4 levels do not correlate with AHI in children with SDB. Compared with children with severe OSA, children with mild SDB have higher urinary LTE4 levels. Further research is needed to determine whether urinary LTE4 is a satisfactory biomarker for pediatric OSA.

Keywords

obstructive sleep apnea, urinary leukotriene E4, inflammatory biomarker

Received July 31, 2017; revised January 4, 2018; accepted January 30, 2018.
between urinary LTE4 and additional PSG parameters, such as nadir oxygen saturation.

**Methods**

**Subjects**

The study was approved by the Eastern Virginia Medical School Institutional Review Board. Subjects were recruited from outpatient pediatric otolaryngology clinics at the Children’s Hospital of The King’s Daughters, a tertiary care children’s hospital in Norfolk, Virginia, from March 2014 through October 2016. Children aged 3 to 16 years with sleep-disordered breathing (SDB) who were referred for PSG were included in the study. Exclusion criteria were as follows: (1) subjects with cardiovascular, neuromuscular, or craniofacial disorders; (2) subjects treated with oral corticosteroids and/or leukotriene receptor antagonists within 4 weeks of study enrollment; and (3) subjects who had previously undergone adenoidectomy and/or tonsillectomy. Children were excluded from the study if they failed to attend the PSG or if they were unable to provide a urine sample. Written informed consent was obtained from a parent or guardian. Those subjects aged ≥8 years also provided assent.

**Clinical Evaluation**

Patients presenting with SDB were evaluated and recruited during their initial clinic visits. Demographic data were recorded, including age, sex, and ethnicity. Weight and height were obtained, and body mass index (BMI) percentiles were calculated according to the guidelines of the US Centers for Disease Control and Prevention. A detailed history was obtained, including parental report of medication use, secondhand smoke exposure, and a history of asthma or allergic rhinitis. To assess quality of life, caregivers completed the validated OSA-18 survey at the time of enrollment. Tonsil size was graded from 1 to 4 by direct inspection of the oropharynx during the clinic visit according to the scale established by Brodsky.

**Polysomnography**

Full-night PSG was performed at our institution’s dedicated pediatric sleep laboratories. The average wait time for a PSG at our institution is 6 weeks. PSGs were scored in accordance with the guidelines of the American Academy of Sleep Medicine and interpreted by physicians with expertise in pediatric sleep medicine. PSG parameters were recorded, such as AHI, nadir oxygen saturation, and peak end-tidal carbon dioxide level. Children with an AHI >1 were diagnosed with OSA. The degree of OSA was further classified as mild (AHI, 1-5), moderate (AHI, 5.1-10), or severe (AHI, >10).

**Urinary LTE4 Levels**

On the morning following the PSG, a urine sample was collected from each patient. A portion of the sample was sent to our hospital laboratory to obtain a urinary creatinine level. The remainder was stored at −80°C until it was assayed. Urinary LTE4 levels were measured with commercially available LTE4 enzyme immunoassay kits (item 520411; Cayman Chemical Company, Ann Arbor, Michigan). The assay is based on the competition between LTE4 and LTE4-acetylcholinesterase. Urine samples were purified in accordance with manufacturer instructions, and each sample was loaded in triplicate and assayed at >2 dilutions. The plates were read at a wavelength of 420 nm. Absorbance results were analyzed with a 4-parameter logistic fit. Urinary LTE4 levels >2 SD from the mean were excluded. Urinary LTE4 levels were expressed as ng/mM of urinary creatinine to control for urine dilution.

**Data Analysis**

Based on our power analysis, enrollment of 128 children yields 81% power to detect statistically significant differences in urinary LTE4 levels. A 2-tailed independent samples t test was utilized to assess whether there was a difference in the mean urinary LTE4 levels and the degree of OSA severity. A multiple linear regression analysis was performed to determine if there was an impact of BMI, age, secondhand smoke exposure, or a history of allergic rhinitis and asthma on the association between urinary LTE4 levels and common PSG parameters, such as AHI and nadir oxygen saturation. Urinary LTE4 levels and AHI were both log transformed. P values <.05 were considered significant.

**Results**

Urine LTE4 levels and PSG data were available for 123 children. Ten children were excluded because their urinary LTE4 levels were >2 SD from the mean; thus, 113 children were included in the study. Table 1 depicts the baseline demographics of our patient population. The mean age was 7 years (range, 3-15 years). Of the 113 patients, 59 (52%) were male, and the majority were white (n = 58, 53%). Thirty-nine percent (n = 44) were obese (BMI >95th percentile). Common comorbid medical conditions as reported by caregivers included allergic rhinitis (47%), asthma (27%), and eczematous dermatitis (11%).

Eighty-seven children (77%) were diagnosed as having OSA (AHI >1) after undergoing PSG (Table 2). The majority (n = 43, 38%) had mild OSA (AHI, 1-5), and 27% (n = 30) had severe OSA (AHI >10). The mean obstructive AHI in the study population was 7.9 (range, 0-81). The mean nadir oxygen saturation was 88.9% (range, 54%-96%). The mean OSA-18 score was 61, indicating a moderate impact of SDB on quality of life for the majority of patients studied. Most children had tonsillar hypertrophy, as defined by grade 2 to 4 tonsil size on physical examination (n = 111, 98%).

Urine LTE4 levels (ng/mM) ranged from 9.1 to 219.3. The mean urinary LTE4 level was 91.3 ng/mM. There was no correlation in geometric mean urinary LTE4 levels between children with OSA (73.1 ng/mM; 95% CI, 62.2-85.9) and without OSA (69.6 ng/mM; 95% CI, 49.8-97.2; P = .78). Table 3 lists the mean urinary LTE4 levels for children with varying severities of SDB. There was a significant difference in mean
urinary LTE4 levels between patients with mild SDB (n = 68, AHI <5) and those with moderate to severe OSA (n = 45, AHI ≥5; P = .03). The geometric mean urinary LTE4 in children with mild SDB was 82.0 ng/mM (95% CI, 69.2-97.1), which was significantly higher than the level of children with moderate to severe OSA (59.8 ng/mM; 95% CI, 46.6-76.9). When controlling for multiple demographic factors, including BMI, age, history of asthma or allergic rhinitis, and second-hand smoke exposure, urinary LTE4 levels did not correlate with PSG parameters, such as AHI (P = .58) and nadir oxygen saturation (P = .64). Urinary LTE4 levels did not correlate with BMI (P = .35) or second-hand smoke exposure (P = .61) and were not significantly different among subjects with asthma (P = .82) or allergic rhinitis (P = .70).

Discussion

Limitations in pediatric PSG, such as expense and lack of access, have led to a search for alternative methods for the evaluation of SDB among children. To this end, recent research has focused on identifying potential biomarkers that could be utilized to diagnosis pediatric OSA. Biomarkers are biological molecules found in blood, urine, or other body fluids or tissues that can be used to diagnose and evaluate treatment response to a disease. The ideal biomarker for evaluation of pediatric OSA should be sensitive and specific, correspond to severity of disease, and be tolerable by children.

Biomarkers for OSA have become an area of intense research focus over the past decade. Several studies found that inflammation is linked to the pathophysiology of pediatric OSA. Thus, markers of inflammation, such as C-reactive protein, interleukins, tumor necrosis factor alpha, and CysLTs, have been identified as potential targets for biomarkers in serum, urine, and exhaled breath condensate. CysLTs in particular have been recognized as important biomarker candidates due to their intricate involvement in the regulation of the immune system. These inflammatory mediators are produced by leukocytes and derived from the 5-lipoxygenase pathway.

Recent studies demonstrated increased systemic CysLT levels and increased local CysLT receptor expression in adenotonsillar tissues of children with OSA. For example, the addition of CysLT D4 to in vitro tonsillar tissue induced a proliferative response. Furthermore, 2 recent studies reported on elevated urinary excretion of CysLT in pediatric OSA. Shen et al documented increased urinary LTE4 levels in Chinese children with OSA versus nonsnorers. The authors reported that urine LTE4 levels correlated with OSA severity (defined by AHI). They also noted that even children with mild disease had evidence of significant inflammation as noted by elevated urinary LTE4 levels. In the current study, we did not identify a correlation between AHI and LTE4 levels. Differences in our findings may be related to our inclusion of children with primary snoring and inflammatory conditions, such as asthma and allergic rhinitis. However, similar to Shen et al, we did note

<table>
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<tr>
<th>Table 1. Baseline Subject Demographic Data.</th>
<th>Subjects, n (%)</th>
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| Sex | Male 59 (52)  
Female 54 (48) |
| Ethnicity | Black 46 (42)  
White 58 (53)  
Other 6 (5) |
| Age, mo | 88 (40-190)* |
| Obesity (BMI >95%) | 44 (39) |
| Tonsillar size | Grade 1 2 (1.7)  
Grade 2 38 (33.6)  
Grade 3 59 (52.2)  
Grade 4 13 (11.5) |
| Presence of comorbidities | Allergic rhinitis 53 (47)  
Asthma 30 (27)  
Ectopic dermatitis 12 (11)  
Chronic tonsillitis 5 (4) |
| Urinary LTE4, ng/mM | 91.3 (9.1-219.3)* |

Abbreviations: BMI, body mass index; LTE4, leukotriene E4.  
*Mean (range).

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<tr>
<th>Table 2. Subject Polysomnogram and QOL Data.</th>
<th>n (%) or Mean (Range)</th>
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<tbody>
<tr>
<td>Presence of obstructive sleep apnea</td>
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No 25 (22)  
Mild 43 (38)  
Moderate 15 (13)  
Severe 30 (27)  
Obstructive AHI 7.9 (0-81.5)  
Nadir oxygen saturation, % 88.9 (54-96)  
OSA-18 QOL score 61 (29-118) |

Abbreviations: AHI, apnea-hypopnea index; QOL, quality of life.

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<th>Table 3. Urinary LTE4 Levels Categorized by Subject OSA Severity.</th>
<th>LTE4 Level, ng/mM</th>
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<tbody>
<tr>
<td>Classification (AHI)</td>
<td>Subjects, n</td>
</tr>
<tr>
<td>Primary snoring (&lt;1)</td>
<td>25</td>
</tr>
<tr>
<td>OSA</td>
<td>Mild (1-5)</td>
</tr>
<tr>
<td>Moderate (5.1-10)</td>
<td>15</td>
</tr>
<tr>
<td>Severe (&gt;10)</td>
<td>30</td>
</tr>
</tbody>
</table>

Abbreviations: AHI, apnea-hypopnea index; LTE4, leukotriene E4; OSA, obstructive sleep apnea.
elevated urinary LTE4 levels among children with mild OSA. In fact, children with mild SDB had significantly higher LTE4 levels than those with more severe disease. While it is unclear why children with mild OSA had such high LTE4 levels, we hypothesize that children with mild OSA may have an increased inflammatory component affecting their disease process. To our knowledge, this finding has not been reported and will necessitate further research to confirm. We acknowledge that our study was underpowered and that confounding factors may have influenced our urinary LTE4 findings.

As described here, there is a growing body of evidence linking elevated CysLT levels to pediatric OSA. However, less is known about what happens to this inflammatory linking elevated CysLT levels to pediatric OSA. However, less is known about what happens to this inflammatory marker following treatment of OSA among children. Satdhabudha et al reported a decrease in urinary LTE4 marker following treatment of OSA among children. While it is unclear why children with mild OSA continued our urinary LTE4 findings.

While the body of literature linking pediatric OSA to elevated levels of inflammatory markers is growing, it remains unknown if inflammation is the cause or result of pediatric OSA. The link between CysLT and pediatric OSA requires further investigation. In our study, we found that urinary LTE4 levels were surprisingly higher among children with mild SDB. LTE4 levels did not correlate with PSG parameters, such as AHI or nadir oxygen saturation. There are several explanations for the lack of correlation between OSA severity and LTE4 levels. First, unlike prior studies, we included children with allergic rhinitis and asthma who were taking topical inhaled corticosteroids. Perhaps, this affected LTE4 levels. We chose to include such patients because many children who present for evaluation of SDB have these common comorbid medical conditions. We thought that an adequate biomarker for pediatric OSA should be able to accurately detect obstruction in this group of children. Another potential confounding factor is the variability in the urinary proteome of healthy children. A recent study demonstrated sex and diurnal effects on urinary proteome. Finally, previous studies demonstrated a poor correlation between quality of life and AHI among children with mild OSA. Perhaps the pathophysiology of mild SDB is different from that of severe disease, with inflammation being more pronounced in children with mild disease. As this has not been previously reported, future research is needed to confirm this hypothesis. Mild OSA in children is not fully understood; further studies are necessary to determine pathophysiology, natural history, and outcomes.

The strengths of our study include the prospective design, the utilization of PSG to diagnose OSA, and the measurement of urinary creatinine to correct for LTE4 dilution. Limitations include failure to document utilization of inhaled steroids and lack of objective assessment of second-hand smoke exposure. To our knowledge, this is the first prospective study to examine urinary LTE4 levels in children with SDB. Prior studies in this area excluded children with primary snoring and common comorbid medical conditions, such as asthma and allergic rhinitis. We chose to include such children in our study to further evaluate whether urinary LTE4 could be a potential biomarker for the diagnosis of OSA. While other authors demonstrated a difference in urinary LTE4 levels between nonsnoring controls and children with OSA, we sought to determine if urinary LTE4 levels were different between children with primary snoring and mild OSA and those with more severe disease. Certainly, a biomarker that could reliably distinguish children with mild disease from those with severe OSA could have a significant impact on the diagnostic paradigm for OSA. Further research is needed to identify if a threshold urinary LTE4 level exists that can differentiate between mild SDB and severe OSA. In addition, further studies are needed to assess for changes in urinary LTE4 levels following OSA treatment and to identify whether this inflammatory marker may be an indicator of persistent disease.

**Conclusion**

Urinary LTE4 levels do not correlate with AHI or nadir oxygen saturation in children with SDB. As compared with children with severe OSA, children with mild SDB have higher urinary LTE4 levels. Further research is needed to determine whether urinary LTE4 is a satisfactory biomarker for pediatric OSA.

**Author Contributions**

Sneh Biyani, study design, acquisition of data, authorship of manuscript, final review of manuscript; M. Jedorah Benson, study design, acquisition of data, execution of methodology, interpretation of data, critical review of manuscript, final review of manuscript; Sarah C. DeShields, analysis and interpretation of data, initial drafting of manuscript, offered revisions for manuscript and final review of manuscript; Tina D. Cunningham, analysis and interpretation of data, initial drafting of manuscript, offered revisions for manuscript and final review of manuscript; Cristina M. Baldassarri, study design, acquisition and interpretation of data, authorship of manuscript, final review of manuscript.

**Disclosures**

**Competing interests:** None.

**Sponsorships:** None.

**Funding source:** MEDARVA Foundation.

**References**


