

Inflammation and asthma control in children with comorbid obstructive sleep apnea

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Abstract

Objectives: A bi-directional relationship exists between asthma and obstructive sleep apnea (OSA) in which presence of one is associated with increased prevalence and severity of the other. Our objective was to determine whether OSA accounted for differences in airway and systemic inflammation in asthmatic children and whether inflammation was associated with asthma control. We hypothesized that greater severity of SDB would correlate with increased upper airway and systemic inflammation and result in reduced asthma control.

Methods: Non-obese children aged 4-12 years with persistent asthma, with or without OSA were recruited. Asthma control was measured with the Childhood Asthma Control Test. Children underwent polysomnography and blood sampling, and children with OSA underwent clinically indicated adenotonsillectomy. Tonsils and sera were analyzed for 11 cytokines.

Results: Twenty-seven children (20 with OSA, seven without OSA) participated, mean age 7.9 years, 55.6% female, 92.6% African American. Levels did not differ for any cytokine between children with and without OSA. Lower nadir oxygen saturation was associated with higher levels of tonsil TNF- α ($P < 0.001$) and IL-10 ($P < 0.05$). Higher REM-related apnea-hypopnea index was associated with higher levels of tonsil TNF- α ($P < 0.05$). Children with uncontrolled asthma had significantly higher levels of serum IL-10, IL-13, and TNF- α , and tonsil TNF- α (all $P < 0.05$) than well-controlled asthmatic children. There was no association between OSA, or any polysomnography variable, and asthma control.

Conclusions: Despite the presence of OSA-associated airway inflammation, and asthma control-associated airway and systemic inflammation, OSA was not related to level of asthma control in this non-obese, largely minority, low income sample.

KEYWORDS

asthma, child, cytokines, inflammation, palatine tonsil

1 | INTRODUCTION

Asthma is a common chronic inflammatory disease of the lower airways, with a prevalence of 8.4% in children under 18 years of age in the United States.¹ Many risk factors for poor asthma control are known, yet despite this knowledge, the rate, and associated morbidities of asthma have changed little over time. Identifying additional modifiable risk factors for poor asthma control is essential to decrease the burden of asthma in children.

Sleep disordered breathing (SDB) is a modifiable risk factors for asthma control that has recently gained attention. SDB is a spectrum of disordered breathing during sleep ranging from habitual snoring without gas exchange abnormalities to obstructive sleep apnea (OSA). In OSA, partial or complete upper airway obstruction occurs repetitively during sleep resulting in oxygen desaturations and sleep fragmentation. Like asthma, pediatric OSA is common, with a prevalence of 1-5%.² Asthma and OSA commonly co-occur and share multiple risk factors including obesity, atopy, allergic rhinitis, race, and low socioeconomic status (SES).^{3,4} A systematic review by Sanchez et al⁵ found evidence for a bidirectional relationship between the two disorders. Children with asthma or wheezing were at higher risk for habitual snoring and OSA than children without these, while children with SDB were more likely to develop wheezing or asthma than children without SDB. Additionally, asthma was associated with more severe OSA, while SDB was associated with more severe asthma.⁵

Mechanisms of this relationship have not been fully elucidated. The unified airway hypothesis suggests that the respiratory tract functions as an integrated unit and inflammation in any part can promote airway disease diffusely.⁶ OSA is associated with inflammation in tonsils,^{7,8} so theoretically could lead to lower airway inflammation and asthma exacerbations. Systemic inflammation is also present in OSA, with evidence of alterations in the balance between CD4+ T lymphocyte subsets (eg, helper T-cells [Th]1, Th2, Th17, and T regulatory [Treg] cells)^{9,10} and elevation of cytokines of these T-cells, many of which are also elevated in children with asthma.^{11,12} Treatment of OSA by adenotonsillectomy improves systemic inflammation¹³ and asthma control.⁵ These findings suggest a role for OSA-associated inflammation in poor asthma control. The aim of this study in non-obese children with persistent asthma was: 1) To understand the relationship between the severity of SDB and airway and systemic inflammation; and 2) the impact of this inflammation on asthma control. We hypothesized that greater severity of SDB would correlate with increased upper airway and systemic inflammation and result in reduced asthma control.

2 | MATERIALS AND METHODS

This was a cross-sectional, descriptive study approved by the Human Research Protections Office, University of Maryland Baltimore. Data were collected after obtaining written informed parental consent and child assent.

2.1 | Sample

The goal was to recruit a convenience sample of 30 non-obese children aged 4-12 years with persistent asthma (20 with OSA scheduled for adenotonsillectomy, 10 without OSA). This sample size was powered (2-tailed $\alpha=0.05$, $\beta=0.8$) to detect group differences on IL-2, IL-4, IL-12, INF- γ , and TNF- α and slightly underpowered to detect differences on IL-17, based on studies comparing exhaled breath condensate (IL-2, IL-4, INF- γ , and IL-12P70)¹¹ representing upper airway inflammation, and plasma cytokines (IL-10, TGF- β , IL-17)¹² between children with and without asthma, and tonsil TNF- α levels of children with OSA versus recurrent tonsillitis.⁷ Only non-obese children were included to minimize the effect of obesity on inflammation.¹⁴

Asthma was diagnosed by Pediatric Pulmonology or Allergy specialists, and asthma severity was determined by parent interview conducted by a pediatric nurse practitioner, based on National Asthma Education and Prevention Program-Expert Panel Report 3 (NAEPP-EPR3) criteria.¹⁵ Responses were verified by review of medical records by a Pediatric Allergist, based on most recent health assessment and/or level of pharmacotherapy.¹⁵

Children with OSA confirmed by clinically indicated polysomnography within the previous year were recruited from an urban academic Pediatric Otorhinolaryngology clinic. Control children with asthma but not OSA were recruited from Pediatric Pulmonary and Allergy clinics in the same academic center and screened for SDB.¹⁶ Those at low risk for SDB underwent polysomnography to confirm absence of OSA, unless they had undergone polysomnography within the past year (two children). Exclusions were immune disorders, gestational age <36 weeks, >5 episodes of tonsillitis in the past year, chronic lung disease other than asthma, neuromuscular or craniofacial disorder, body mass index (BMI) z-score >1.64 (corresponding to BMI 95th percentile for age/sex),¹⁷ acute illness, asthma exacerbation, or oral corticosteroid or antibiotic use within 2 weeks of data collection, and previous adenotonsillectomy.

2.2 | Study protocol

Following polysomnography, participants underwent the study protocol in the preoperative area of the surgery center prior to adenotonsillectomy, or the General Clinical Research Center for controls. Procedures included blood draw for cytokine and allergen sensitization analysis, questionnaires, and spirometry. Patients with OSA undergoing adenotonsillectomy had one tonsil harvested during surgery for cytokine analysis.

2.3 | Measures

Questionnaires captured data on demographics, healthcare utilization, symptom frequency, missed school, and work days due to asthma, medications, comorbidities, and pet and tobacco smoke exposure. Height and weight were collected from the most recent healthcare visit.

2.4 | Obstructive sleep apnea

The Pediatric Sleep Questionnaire, Sleep-Related Breathing Disorders subscale¹⁶ was used to screen participants for SDB. This 22-item parent-report questionnaire screens for sleep disorders in children aged 2–18 years. Scores range from zero to one, with scores ≥ 0.33 suggestive of SDB.¹⁶

OSA was measured by polysomnography via overnight, attended study performed in an American Academy of Sleep Medicine-accredited sleep laboratory (Alice 2.8, Respiromics, Murrysville, PA). Recording montage included electroencephalography, submental and leg electromyography, electrocardiography, nasal air pressure (cannula) and oronasal airflow (thermistor), chest and abdominal excursion (respiratory inductance plethysmography), and pulse oximetry. Recording and scoring of respiratory events, sleep architecture and arousals were performed in accordance with American Academy of Sleep Medicine standards.^{18,19} Variables included the obstructive apnea-hypopnea index (OAHI = number of obstructive apneas plus hypopneas/hour sleep), apnea-hypopnea index during rapid eye movement sleep (REM_{AHI}), mean and nadir arterial oxygen saturation by pulse oximetry (SpO₂), percent of sleep time with SpO₂ <90% (T₉₀), end-tidal carbon dioxide (mmHg) maximum (ETCO_{2MAX}), and minutes of sleep with ETCO₂ >50 mmHg. OSA was defined as an OAHI ≥ 1.5 , based on data showing that the statistically significant OAHI in healthy children is 1.5.²⁰

2.5 | Asthma control and contributing factors

Asthma control was measured with the Childhood Asthma Control Test (cACT), a seven-item child- and parent-report questionnaire. Scores range from 5 to 27, with a score ≤ 19 indicating uncontrolled asthma, and >19 well-controlled asthma.²¹

Serum allergen-specific IgE serologic tests measured by fluorescent enzyme immunoassay were performed by a commercial laboratory (LabCorp, Inc., Baltimore, MD) to identify sensitization to 12 common environmental allergens. Atopy was defined as ≥ 1 positive allergen-specific IgE test. The International Study of Allergies and Asthma in Childhood allergic rhinitis module²² was used to screen for allergic rhinitis. Season of participation was collected, as asthma control can vary by season.²³

2.6 | Inflammation

Systemic inflammation was assessed by 11 serum cytokines: interleukin (IL) 2, IL-4, IL-5, IL-10, IL-12P70, IL-13, IL-17A, IL-22, interferon (IFN)- γ , tumor necrosis factor (TNF)- α , and transforming growth factor (TGF)- β . Blood was clotted at room temperature for 30 min, centrifuged at 1300 RCF at 4°C for 10 min, aliquoted, and stored at -80°C. Airway inflammation was assessed in tonsil supernatants by the same cytokines. Tonsils harvested during adenotonsillectomy were transported immediately to pathology, and tissue not required for diagnostic purposes was flash frozen on-site in liquid nitrogen and stored at -80°C. Processing and cytokine analyses were performed at three time points during the study. For processing, tonsils were thawed on wet ice, and a portion transferred into 1 mL buffer (50 mM Tris HCl,

150 mM NaCl, 10% glycerol, and 0.1% triton-100) and homogenized in a bead mill homogenizer (Precellys®). Supernatants were centrifuged at 13 000g for 10 min at 4°C. Cytokines were measured using Milliplex® Human Cytokine Multiplex Immunoassay kits (Millipore-Sigma, Temecula, CA), according to manufacturer protocol and read on a Luminex 100 System (Luminex Corp., Austin, TX). Data were analyzed in duplicate using BioRad Laboratories, Inc. BioPlex Software (Hercules, CA). Protein concentration was determined by the Bradford method²⁴ using a protein estimation kit (Bio-Rad Laboratories, Hercules, CA), and absorbance measured at 595 nm. Cytokine levels were normalized for protein concentration (pg or ng/mg protein). Lower limits of detection (LLD) of the test kit for each cytokine were: IL-2, 12 pg/mL; IL-4, 0.02 ng/mL; IL-5, 6 pg/mL; IL-10, 1 pg/mL; IL-12P70, 5 pg/mL; IL-13, 7 pg/mL; IL-17A, 12 pg/mL; IL-22, 0.04 ng/mL; IFN- γ , 10 pg/mL; TNF- α , 2.5 pg/mL and TGF- β , 3.2 pg/mL. For levels below the LLD, a value half the LLD was imputed, to avoid biasing values toward the null.

2.7 | Statistical analysis

Descriptive statistics, including frequencies, and mean (SD) were used to describe the sample and test results. Normality and skewness were checked prior to hypothesis testing. Group comparison of cytokines by presence or absence of OSA or level of asthma control were made using *t*-test (normally distributed variables) or Mann-Whitney test (non-normally distributed variables). Pearson or Spearman correlations were used to test bivariate associations. Analyses were performed with IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY). Statistical significance was a *P*-value <0.05.

3 | RESULTS

3.1 | Description of the sample

Participants were recruited from December, 2014 through February, 2017. Twenty-seven children completed the protocol—20 with and seven without OSA. Mean age of participants was 7.9 years, 55.6% were female and 92.6% were African American (Table 1). One child had only enough blood to test for cytokines but not allergen specific IgE levels. That child was considered atopic based on history of seasonal allergy symptoms.

Sensitization to at least one allergen was found in 80.8% of children. Uncontrolled asthma was identified in 48.1% of children. Atopy and number of positive allergen-specific IgE tests did not differ by asthma control (well-controlled vs uncontrolled). Season of participation was not associated with cACT scores or number of positive allergen-specific IgE tests.

3.2 | Inflammation and sleep disordered breathing severity

Mean lag time from tonsil harvest to cytokine analysis was 144.6 ± 102.1 days, excluding one tonsil supernatant that required

TABLE 1 Sample description, children with persistent asthma $N = 27$

Variable	Mean $\pm$ SD	N (%)
Age (years)	7.9 $\pm$ 2.2	
Sex, female		15 (55.6)
Race, African American		25 (92.6)
BMI z-score	0.33 $\pm$ 0.81	
Asthma severity classification ^a		
Mild persistent		14 (51.9)
Moderate persistent		5 (18.5)
Severe persistent		8 (29.6)
Households with pets		8 (29.6)
Households with smokers		6 (22.2)
Allergic rhinitis (current) ^b		13 (48.1)
Environmental allergies (parent report)		11 (40.7)
Positive allergen-specific IgE tests ($n = 26$)	4.9 $\pm$ 4.4	
Childhood asthma control test score	18.6 $\pm$ 4.6	
Missed school days due to asthma, past year	2.1 $\pm$ 5.1	
Acute healthcare visits for asthma, past year	3.6 $\pm$ 6.5	
Prescribed asthma medications		
Albuterol (as needed)		26 (96.3)
ICS only		9 (33.3)
ICS + LTRA		4 (14.8)
LABA/ICS		1 (3.7)
LABA/ICS + LTRA		8 (29.6)
Spirometry ($n = 13$)		% predicted
FEV ₁	1.4 $\pm$ 0.5	100.5 $\pm$ 18.8
FVC	1.7 $\pm$ 0.6	106.4 $\pm$ 17.6
FEV ₁ /FVC ratio	84.5 $\pm$ 10.6	94.8 $\pm$ 11.7

BMI, body mass index; FEV₁, forced expiratory volume in 1 s; FVC, functional vital capacity; ICS, inhaled corticosteroid; LABA, long-acting beta agonist; LTRA, leukotriene receptor antagonist.

^aBased on National Asthma Education and Prevention Program-Expert Panel Report 3 guidelines.

^bInternational Study of Asthma and Allergies in Childhood Allergic Rhinitis Module.

reprocessing with a subsequent batch (lag 621 days), a period considered acceptable for cytokine stability.²⁵ Mean lag time for serum analysis was 127.5 $\pm$ 92.8 days post-collection. Five serum cytokines (IL-4, IL-10, IL-12P70, IL-13, and TNF- α) and five tonsil cytokines (IL-4, IL-10, IL-17A, IFN- γ , and TNF- α) were included in analyses. Remaining cytokines were detectable in four or fewer samples and were dropped from analysis.

Polysomnography variables are shown in Table 2. Mean SpO₂ was normal ($\geq 96\%$) for all children, so was not included in analyses. There

were no differences in any serum cytokine between children with and without OSA (Table 3) and no serum cytokines were associated with any polysomnography variable. Among children with OSA, REM_{AHI} was significantly positively associated with tonsil TNF- α ($r = 0.472$, $P = 0.041$); and nadir SpO₂ was significantly negatively associated with tonsil TNF- α ($r = -0.579$, $P = 0.008$) and tonsil IL-10 ($r = -0.452$, $P = 0.046$).

3.3 | Inflammation and asthma control

Children with well-controlled and uncontrolled asthma did not differ on any polysomnography variable, therefore, the entire sample was included in analyses. There were significant differences between asthma control groups for serum cytokines IL-10 ($t_{(25)} = -2.08$, $P = .048$), IL-13 ($t_{(25)} = -2.43$, $P = 0.023$) and TNF- α ($t_{(25)} = -2.35$, $P = 0.027$), and among children with OSA for tonsil TNF- α ($t_{(18)} = -2.32$, $P = 0.033$). Children with uncontrolled asthma had higher levels of serum IL-10, IL-13, and TNF- α , and tonsil TNF- α compared to children with well-controlled asthma (Table 4).

4 | DISCUSSION

This is one of the first studies to investigate a range of inflammatory cytokines in a sample of non-obese children with persistent asthma and evaluate their relationship with SDB severity and asthma control. Although cytokine levels did not differ between children with and without OSA, among children with OSA higher REM_{AHI} was associated with higher tonsil TNF- α levels, and lower nadir SpO₂ was associated with higher tonsil IL-10 and TNF- α levels. Children with uncontrolled asthma had significantly higher levels of serum IL-10, IL-13 and TNF- α , and among children with OSA those with uncontrolled asthma had higher tonsil TNF- α than children with well-controlled asthma.

OSA-associated inflammation is thought to be caused locally by mechanical vibration of upper airway tissue during snoring and obstructed ventilation, and locally and systemically by intermittent hypoxemia during sleep causing ischemia-reperfusion injury.^{26,27} Our finding that higher levels of tonsil TNF- α —a cytokine that promotes inflammatory response—was associated with higher REM_{AHI} and lower nadir SpO₂ lends support for these proposed mechanisms. Lower nadir SpO₂ was also associated with higher tonsil IL-10—a cytokine that downregulates hyperactive immune responses and inhibits synthesis of inflammatory cytokines. In children with OSA and chronically inflamed tonsils, IL-10 might well be elevated in response. In comparison, Kim et al⁷ found that concentrations of IL-1 α , IL-6, and TNF- α were significantly higher in tonsils of children with OSA than in children undergoing adenotonsillectomy for recurrent tonsillitis without OSA. Similarly, Israel et al⁸ found higher expression of IL-1 α and classical subunits p50 and p65 of nuclear factor kappa B (NF- κ B)—a transcription factor implicated in initiation and progression of inflammation—in tonsils of children with OSA compared to matched children with recurrent tonsillitis without OSA. Although these studies did not evaluate relationships between inflammatory markers and

TABLE 2 Polysomnography variables by presence or absence of OSA

Variable	Mean $\pm$ SD		
	Overall	No OSA (n = 7)	OSA (n = 20)
Total sleep time (min)	390.9 $\pm$ 40.8	401.6 $\pm$ 36.3	386.8 $\pm$ 42.7
N1 (%)	0.3 $\pm$ 0.5	0.1 $\pm$ 0.2	0.3 $\pm$ 0.6
N2 (%)	49.9 $\pm$ 9.8	54.5 $\pm$ 5.8	48.2 $\pm$ 10.6
N3 (%)	24.5 $\pm$ 8.8	23.8 $\pm$ 4.9	24.7 $\pm$ 10.1
REM%	9.5 $\pm$ 5.5	7.0 $\pm$ 3.4	10.5 $\pm$ 6.0
AHI	9.9 $\pm$ 10.0	0.8 $\pm$ 0.3	13.1 $\pm$ 9.8^a
OAHI	9.6 $\pm$ 9.9	0.8 $\pm$ 0.3	12.8 $\pm$ 9.8^a
REM _{AHI}	21.9 $\pm$ 24.7	2.8 $\pm$ 3.4	28.9 $\pm$ 25.5^a
Mean SpO ₂	98.3 $\pm$ 0.8	98.7 $\pm$ 0.5	98.1 $\pm$ 0.9
SpO ₂ nadir	88.4 $\pm$ 8.0	90.6 $\pm$ 6.3	87.7 $\pm$ 8.5
Percent sleep time with SpO ₂ <90%	0.13 $\pm$ 0.5	0.0	0.18 $\pm$ 0.53
ETCO ₂ (min $\geq$ 50 mm Hg)	74.2 $\pm$ 131.3	1.9 $\pm$ 2.2	101.3 $\pm$ 145.7^{b,c}
ETCO ₂ maximum (mm Hg)	55.1 $\pm$ 11.0	56.5 $\pm$ 20.1	54.6 $\pm$ 5.7

Group means compared by *t*-tests unless otherwise indicated.

Bolded values indicate significant group differences.

AHI, apnea-hypopnea index; ETCO₂, end-tidal carbon dioxide; N, non-rapid eye movement (REM) sleep stage; OAHI, obstructive AHI; REM_{AHI}, AHI during REM sleep; SpO₂, arterial oxygen saturation by pulse oximetry.

^a*P* < 0.001.

^b*P* < 0.05.

^cMann-Whitney test.

individual polysomnography variables, our findings corroborate those of other studies demonstrating increased upper airway inflammation in pediatric OSA.

Asthma of all children was considered stable (no recent asthma exacerbations) at the time of participation, yet nearly half had uncontrolled asthma based on cACT scores. Children with well-controlled asthma had significantly lower levels of serum IL-13—a

cytokine associated with induction of allergic airway disease—compared to children with uncontrolled asthma. Well-controlled asthmatics also had lower levels of serum and tonsil TNF- α . In comparison, Wei et al¹² found that children with asthma had higher levels of plasma IL-6, IL-10, IL-17, and IL-23 compared to control children without asthma, although found no difference in TGF- β between groups. Brown et al²⁸ reported that plasma TNF- α , and

TABLE 3 Serum and tonsil cytokine levels, overall, and by OSA status

	Mean $\pm$ SD		
	Overall	No OSA	OSA
Serum cytokines (pg/mL unless otherwise indicated)	(N = 27)	(n = 7)	(n = 20)
IL-4 (ng/mL)	0.04 $\pm$ 0.04	0.04 $\pm$ 0.04	0.03 $\pm$ 0.03
IL-10	1.79 $\pm$ 1.25	2.03 $\pm$ 1.76	1.70 $\pm$ 1.06
IL-12P70	5.01 $\pm$ 3.24	5.37 $\pm$ 4.15	4.88 $\pm$ 2.97
IL-13	67.68 $\pm$ 66.56	85.26 $\pm$ 86.79	61.53 $\pm$ 59.42
TNF- α	4.69 $\pm$ 3.15	5.24 $\pm$ 3.25	4.50 $\pm$ 3.18
Tonsil cytokines ^a (pg/mg unless otherwise indicated)		(n = 0)	(n = 20)
IL-4 (ng/mg)	NA	NA	0.002 $\pm$ 0.003
IL-10	NA	NA	0.21 $\pm$ 0.23
IL-17A	NA	NA	7.90 $\pm$ 17.12
IFN- γ	NA	NA	1.31 $\pm$ 2.47
TNF- α	NA	NA	13.04 $\pm$ 5.65

IL, interleukin; NA, not applicable; ng, nanogram; OSA, obstructive sleep apnea; pg, picogram; TNF- α , tumor necrosis factor-alpha.

There were no differences by OSA status for any cytokine.

^aNormalized for protein content.

TABLE 4 Serum and tonsil cytokine levels by asthma control group

	Mean $\pm$ SD	
	Well-controlled	Uncontrolled
Serum cytokines (pg/mL unless otherwise indicated)	(n = 14)	(n = 13)
IL-4 (ng/mL)	0.02 $\pm$ 0.02	0.04 $\pm$ 0.03
IL-10	1.33 $\pm$ 0.64	2.27 $\pm$ 1.56^a
IL-12P70	4.39 $\pm$ 2.86	5.68 $\pm$ 3.60
IL-13	40.16 $\pm$ 43.50	97.32 $\pm$ 75.59^a
TNF- α	3.42 $\pm$ 2.48	6.06 $\pm$ 3.31^a
Tonsil cytokines ^b (pg/mg unless otherwise indicated)	(n = 11)	(n = 9)
IL-4 (ng/mg)	0.003 $\pm$ 0.004	0.001 $\pm$ 0.001
IL-10	0.23 $\pm$ 0.21	0.19 $\pm$ 0.26
IL-17A	11.65 $\pm$ 22.80	3.32 $\pm$ 1.80
IFN- γ	1.31 $\pm$ 2.02	1.32 $\pm$ 3.07
TNF- α	10.65 $\pm$ 5.96	15.96 $\pm$ 3.75^a

IL, interleukin; ng, nanogram; pg, picogram; TNF- α , tumor necrosis factor-alpha.

Bolded values indicate significant difference.

^aP < 0.05.

^bNormalized for protein content.

mRNA expression of TNF- α , NF- κ B1, and several genes downstream of TNF- α activation and binding, were significantly higher in children with uncontrolled asthma than children with mild asthma or healthy non-asthmatic children.

No previous published studies have evaluated inflammatory markers in tonsils of children with asthma, but several evaluated airway inflammation via analysis of sputum or exhaled breath condensate. Vizmanos-Lamotte et al²⁹ measured cytokine profiles in induce sputum samples of atopic and non-atopic asthmatic children and healthy controls. They found that IL-2, IL-4, IL-5, IL-12p70, and IFN- γ concentrations were significantly higher in children with atopic asthma than in non-atopic asthmatic or control children, while IL-8 was higher in asthmatic children regardless of atopy status compared to controls. In contrast, sputum IL-10 was significantly lower in atopic asthmatic children compared to non-atopic asthmatic or control children.²⁹ Robroeks et al¹¹ found significantly higher levels of IL-2, IL-4, IL-13, IFN- γ , and TNF- α in exhaled breath condensate of children with asthma compared to healthy controls. On the contrary, Rosias et al³⁰ were unable to demonstrate the presence of C-reactive protein, IL-6, IL-8, or TNF- α in exhaled breath condensate of children with mild to moderate persistent asthma.

Our finding that poorer asthma control was associated with greater systemic inflammation measured by serum cytokines IL-10, IL-13, and TNF- α , and greater upper airway inflammation measured by tonsil TNF- α largely support the findings of other studies. Our findings did not support the hypothesis that greater SDB severity is associated with poorer asthma control. There were no significant associations between the OAHl or other polysomnography variables reflecting SDB severity and asthma control in this sample. These findings seem to

differ from current literature linking SDB and asthma. Part of the explanation may be that few studies examining this relationship have objectively measured SDB using polysomnography, the gold standard measure of OSA. A systematic review of SDB in children with asthma by Sanchez et al⁵ reported that of 32 qualifying studies, eight verified OSA by polysomnography. Only two of these examined the relationship between SDB severity and asthma, with conflicting results, but neither excluded obese children. It could also be that OSA is different among children with asthma, regardless of asthma control, than it is in non-asthmatic children. Supporting this concept, Gutierrez et al³¹ found that asthmatic children with moderate-severe OSA had a significantly higher prevalence of REM-related OSA and maximal percentage of SpO₂ desaturation during REM sleep relative to children with only OSA, independent of BMI, sex, age, atopy or asthma control.³¹ If true, we might be unlikely to find significant differences in SDB-related variables between asthmatic children based on level of asthma control, and we did not have a comparison group without asthma.

Factors common to both disorders that we could not control for may also be important in mediating the relationship between SDB and asthma. Our sample included only non-obese children, yet obesity is highly prevalent in both pediatric OSA and asthma, and is associated with increased systemic inflammation,³² more severe asthma,³³ and more severe OSA.³⁴ Allergic asthma is the most common asthma phenotype in children, and allergies are also associated with SDB. In our study, 80% of children demonstrated allergic sensitization, similar to other studies of asthmatic, minority children from low SES backgrounds,³⁵ so allergies could have played an important role in our findings. Less commonly considered, but potentially important factors include health disparities, and cumulative childhood adversity, which lead to a dysregulated stress response, altered immune function and physiologic changes that increase risk for chronic illnesses including asthma and sleep disorders.³⁶ Likewise, early viral infections can lead to long-term upper airway inflammation³⁷ and chronic adenotonsillar hypertrophy, predisposing to development of OSA, and serving as a potential inflammatory trigger in asthma.

Several limitations should be considered in interpreting these findings. Percent REM sleep in our sample was lower than the 12-23% found in normative child and adolescent samples,³⁸ which may have affected our estimate of OSA severity. This is not unusual for in-laboratory sleep studies where sleep structure is not representative of normal sleep due to first-night effect. Most apneas occur during REM sleep in children, so decreased REM sleep would likely underestimate OSA severity. The significant association we found between REM_{AHI} and tonsil TNF- α level despite low REM sleep may have been stronger with higher percent REM sleep. Some cytokines that were significantly elevated in other studies of children with asthma or OSA were largely undetectable in our study. Cytokine results vary widely across studies, based on multiple technical issues (eg, brand of test kit, processing methods, storage) and differences in the sample collected (eg, airway inflammation measured in tonsil, fractional exhaled nitric oxide or sputum; systemic inflammation measured in serum or plasma). We found no asthma studies measuring cytokines in tonsils, so were

unable to make direct comparisons of airway inflammation. For systemic inflammation, we measured cytokines in serum, which results in some loss of protein in the coagulation process and yields lower cytokine levels relative to plasma, as was more commonly tested in other studies. Our samples were analyzed in batches, resulting in a freeze-thaw cycle that can affect cytokine levels. However, serum is less affected by freeze-thaw cycles than plasma.³⁹ As our intent was to compare relative difference between groups, our results should not be affected. Our sample included largely African American children of low SES. These demographics limit the generalizability of our findings to other races and children of higher SES. However, given the disparities in morbidity and mortality of children with asthma or OSA among impoverished and African American children,⁴⁰ we believe this is an important group to study. Despite attempts to verify asthma-related healthcare visits and pharmacotherapy against the medical record, this information is imperfect. Thus, recall bias may affect our findings. Finally, this was a pilot study, and our sample size was small, particularly the group without OSA. Notably, it was difficult to find children with asthma who were not obese and did not have SDB. As a result, we could not control for confounders such as age, sex, allergies, and anti-inflammatory medications such as inhaled corticosteroids that may have influenced our outcomes.

This study also had several strengths. All participants had overnight, attended polysomnography in a sleep laboratory to verify their SDB status. Additionally, our sample, composed of non-obese children, should limit the confounding effect of obesity on inflammation, SDB or asthma. We also included only children with persistent asthma as defined using NAEPP-EPR3 criteria, so were less likely to include children experiencing viral-related wheezing that might not have been true asthma.

5 | CONCLUSION

In this pilot study of non-obese, largely African American children of low SES aged 4-12 years with persistent asthma, higher REM_{AHI}, and lower nadir SpO₂ were associated with greater upper airway inflammation measured by higher tonsil TNF- α and IL-10 among children with OSA. Children with uncontrolled asthma had greater systemic inflammation measured by higher serum IL-10, IL-13, and TNF- α and in children with OSA, greater upper airway inflammation measured by higher tonsil TNF- α , than children with well-controlled asthma. Level of asthma control was not associated with OSA presence or severity. Pediatric healthcare practitioners should be aware of the association between asthma and SDB and the importance of recognizing, assessing, and appropriately managing these comorbid disorders to provide optimal control of both. Further study is needed to understand the interplay between these two common disorders in children to improve health outcomes.

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CONFLICTS OF INTEREST

All authors declare no conflict of interest related to this manuscript.

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