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ORIGINAL ARTICLE

Adenotonsillectomy-related changes in systemic inflammation among children with obstructive sleep apnea

Chung-Guei Huang[a,b,c], Jen-Fu Hsu[d,e], Li-Pang Chuang[e,f,g], Hsueh-Yu Li[e,f,h], Tuan-Jen Fang[e,f,h], Yu-Shu Huang[e,f,i], Albert C. Yang[j,k], Guo-She Lee[l,m], Terry B.J. Kuo[k,n,o], Cheryl C.H. Yang[k,o], Li-Ang Lee[e,f,h,k,p,*], Hai-Hua Chuang[e,p,q,r,*]

[a]Department of Laboratory Medicine, Linkou Chang Gung Memorial Hospital, Taoyuan, Taiwan, ROC; [b]Department of Medical Biotechnology and Laboratory Science, College of Medicine, Chang Gung University, Taoyuan, Taiwan, ROC; [c]Research Center for Emerging Viral Infections, College of Medicine, Chang Gung University, Taoyuan, Taiwan, ROC; [d]Department of Pediatrics, Linkou Chang Gung Memorial Hospital, Taoyuan, Taiwan, ROC; [e]Faculty of Medicine, Graduate Institute of Clinical Medicine Sciences, College of Medicine, Chang Gung University, Taoyuan, Taiwan, ROC; [f]Sleep Center, Linkou Chang Gung Memorial Hospital, Taoyuan, Taiwan, ROC; [g]Department of Pulmonary and Critical Care Medicine, Linkou Chang Gung Memorial Hospital, Taoyuan, Taiwan, ROC; [h]Department of Otorhinolaryngology—Head and Neck Surgery, Linkou Chang Gung Memorial Hospital, Taoyuan, Taiwan, ROC; [i]Department of Child Psychiatry, Linkou Chang Gung Memorial Hospital, Taoyuan, Taiwan, ROC; [j]Department of Psychiatry, Taipei Veterans General Hospital, Taipei, Taiwan, ROC; [k]Institute of Brain Science, National Yang Ming Chiao Tung University, Taipei, Taiwan, ROC; [l]Faculty of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan, ROC; [m]Department of Otolaryngology, Taipei City Hospital, Ren-Ai Branch, Taipei, Taiwan, ROC; [n]Center for Mind and Brain Medicine, Tsaotun Psychiatric Center, Ministry of Health and Welfare, Nantou, Taiwan, ROC; [o]Sleep Research Center, National Yang Ming Chiao Tung University, Taipei, Taiwan, ROC; [p]School of Medicine, College of Life Science and Medicine, National Tsing Hua University, Hsinchu, Taiwan, ROC; [q]Department of Family Medicine, Chang Gung Memorial Hospital, Taipei Branch and Linkou Main Branch, Taoyuan, Taiwan, ROC; [r]Department of Industrial Engineering and Management, National Taipei University of Technology, Taipei, Taiwan, ROC

Abstract

Background: Adenotonsillar hypertrophy is the most common cause of pediatric obstructive sleep apnea (OSA). Although adenotonsillectomy considerably reduces OSA and systemic inflammation, whether and how systemic inflammation influences the effects of adenotonsillectomy on OSA has yet to be determined.

Methods: This study investigated the associations between changes in anatomical variables, % changes in subjective OSA-18 questionnaire scores, % changes in 11 polysomnographic parameters, and % changes in 27 systemic inflammatory biomarkers in 74 children with OSA.

Results: Fifty-six (75.6%) boys and 18 (24.4%) girls with the mean age of 7.4 ± 2.2 years and apnea-hypopnea index (AHI) of 14.2 ± 15.9 events/h were included in the statistical analysis. The mean period between before and after adenotonsillectomy was 5.6 ± 2.6 months. After adenotonsillectomy, the OSA-18 score, eight of 11 polysomnographic parameters, and 20 of 27 inflammatory biomarkers significantly improved (all $p < 0.005$). Notably, there were significant associations between change in tonsil size and % change in AHI ($r = 0.23$), change in tonsil size and % changes in interleukin-8 (IL-8) ($r = 0.34$), change in tonsil size and % change in and IL-10 ($r = -0.36$), % change in IL-8 and % change in C-C chemokine ligand 5 (CCL5) ($r = 0.30$), and % change in CCL5 and % change in AHI ($r = 0.38$) (all $p < 0.005$). Interestingly, % change in IL-8 and % change in CCL5 serially mediated the relationship between change in tonsil size and % change in AHI (total effect: $\beta = 16.672$, standard error = 8.274, $p = 0.048$).

Conclusion: These preliminary findings suggest that systemic inflammation is not only a complication of OSA but also that it mediates the surgical effects, which may open avenues for potential interventions to reduce tonsil size and OSA severity through the regulation of IL-8 and CCL5.

Keywords: Adenotonsillectomy; Apnea-hypopnea index; C-C chemokine ligand 5; Interleukin-8; Obstructive sleep apnea

** Address correspondence. Dr. Li-Ang Lee, Department of Otorhinolaryngology-Head and Neck Surgery, and Dr. Hai-Hua Chuang, Department of Family Medicine, Linkou Chang Gung Memorial Hospital, 5, Fu-Hsing Street, Taoyuan 333, Taiwan, ROC. E-mail address: 5738@cgmh.org.tw (L.-A. Lee); chhaihua@gmail.com (H.-H. Chuang).*

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1. INTRODUCTION

Obstructive sleep apnea (OSA) is one of the most common sleep disorders in children, occurring in 1% to 5% of children worldwide.[1] Recurrent hypopneas and apneas due to upper airway collapse and obstruction are a hallmark feature of OSA and induce intermittent hypoxemia, autonomic fluctuation, and sleep fragmentation.[2] Hypertrophy of the adenoids and tonsils and obesity are common etiologic factors in children with OSA.[3] The apnea-hypopnea index (AHI) has been shown to be significantly associated with adenoid grade, tonsil size, and body mass index (BMI) z-score in children and adolescents.[4] Persistent

OSA may increase the risks of systemic inflammation, metabolic syndrome, cardiovascular disease, and neurocognitive morbidities.[5,6] There are many treatment modalities for pediatric OSA; however, adenotonsillectomy remains the first-line treatment for pediatric OSA.[7]

Intermittent hypoxia and hypercapnia can induce systemic inflammation via hypoxia-inducible factor (HIF)-1[8] and nuclear factor κ beta[9] pathways. Children with OSA frequently have a higher level of systemic inflammation as measured by peripheral blood C-reactive protein,[10] interleukin (IL)-6,[11] IL-8,[12] and tumor necrosis factor-α (TNF-α),[13] which can be improved after adenotonsillectomy. Furthermore, the relationship between OSA and systemic inflammation is bi-directional. However, the causality between OSA and systemic inflammation is often confounded by comorbid obesity.[14] Chuang et al previously found significant differences in IL-6 and C-C chemokine ligand 5 (CCL5) among various weight status and OSA subgroups, and that these differences diminished along with a reduction in AHI in those with similar weight status.[15] Accordingly, some inflammatory biomarkers appear to be related to OSA severity independently of BMI.

Even though adenotonsillectomy considerably reduces pediatric OSA severity and systemic inflammation,[10,11,13,15] whether and how tonsil size and adenoidal-nasopharyngeal ratio (ANR) influence the effects of adenotonsillectomy on systemic inflammation, and whether and how systemic inflammation influences the effects of adenotonsillectomy on OSA have yet to be determined. Therefore, this study aimed to investigate adenotonsillectomy-related changes in systemic inflammation in association with other variables of interest. We hypothesized that changes in tonsil size and ANR may be related to % change in systemic inflammation, which may then mediate the association between adenotonsillectomy and % change in OSA severity.

2. METHODS

2.1. Study participants

This retrospective cohort study included consecutive pediatric patients who underwent adenotonsillectomy at our institute due to OSA from March 1, 2017, to September 30, 2021. This study was approved by the Institutional Review Board of our institute (No. 202201618B0). The requirement for written informed consent was waived. All procedures were conducted in compliance with the Declaration of Helsinki 1975. The Strengthening the Reporting of Cohort Studies in Surgery guidelines were followed.[16]

The inclusion criteria were: (1) age 5–12 years, (2) presence of OSA symptoms such as habitual snoring and sleep disturbed breathing, and/or comorbidities such as elevated blood pressure, daytime sleepiness, learning problems, growth failure, or enuresis, and (3) obstructive AHI (OAHI) ≥ 5.0 events/h or OAHI ≥ 1.0 event/h plus at least one OSA-related comorbidity.[15,17] The exclusion criteria were (1) patients with craniofacial, neuromuscular, or chronic inflammatory disorders requiring multimodality treatments,[18] or (2) no available polysomnographic data. Age, sex, tonsil size,[19] ANR,[20] and BMI z-score were recorded. The children with follow-up assessments of OSA and systemic inflammation, performed at least 3 months after adenotonsillectomy, were included in the statistical analysis (Fig. 1).

The tonsil size was directly inspected and graded with a size scale from 0–4 (0: s/p total tonsillectomy; 1: tonsils within the tonsillar; 2: tonsils visible outside the anterior pillars; 3: tonsils extending three-quarters of the way to the midline; 4: tonsils meeting at the midline).[19] The ANR (distance from the point of maximal convexity of the adenoid shadow/the distance between the posterior border of the hard palate and the anteroinferior edge of the sphenobasioccipital synchondrosis) were measured on neck lateral view.[20]

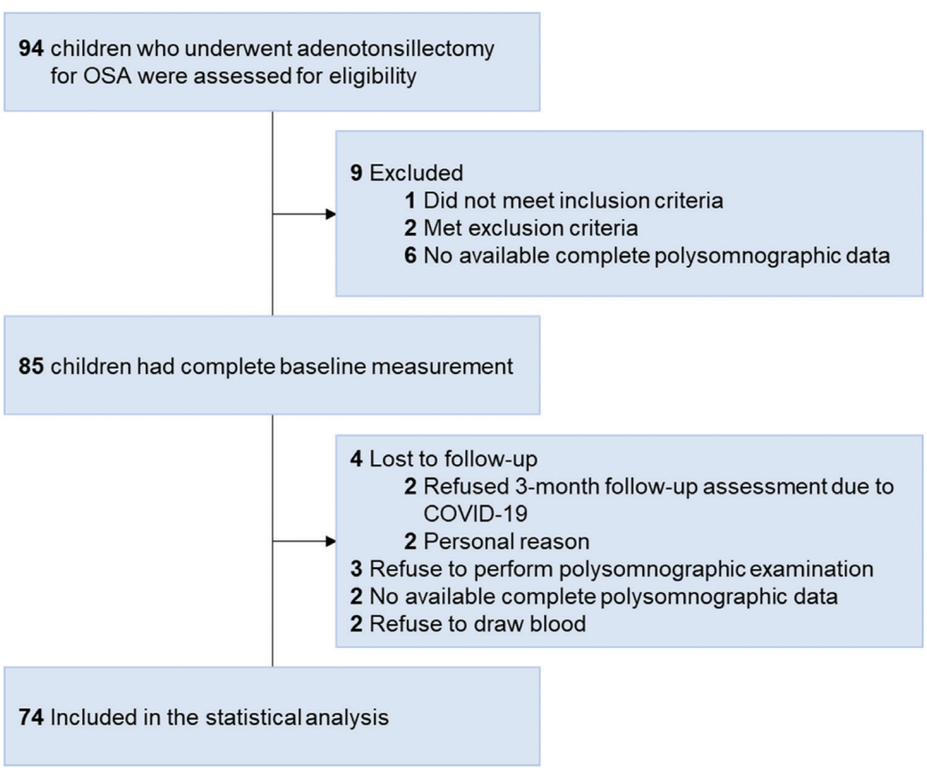

Fig. 1 Flowchart of the present study.

2.2. Measurements of pediatric OSA

2.2.1. Subjective measurements

In the present study, the subjective severity of pediatric OSA was assessed by parents using the Chinese version of the OSA-18 questionnaire.[21] This validated instrument includes 18 items on OSA-related quality of life which are scored using a Likert-type scoring system, and collects information about five subscales: sleep disturbance, physical suffering, emotional distress, daytime problems, and caregiver concerns. The total score ranges from 18 (no effect on the quality of life) to 126 (major negative effect). The OSA-18 questionnaire has been shown to have excellent test-retest reliability.[22]

2.2.2. Objective measurements

The objective severity of pediatric OSA was evaluated by standard, in-laboratory polysomnography (Nicolet Biomedical Inc., Madison, WI, USA) to measure multiple sleep parameters.[23] AHI, apnea index, arousal index, mean peripheral oxygen saturation (SpO_2), minimal SpO_2, snoring index, total sleep time, and sleep stages were scored and manually verified by the study investigators according to the 2012 protocol of the American Academy of Sleep Medicine.[24] In this study, we defined pediatric OSA as an OAHI ≥ 2.0 events/h or an obstructive apnea index ≥ 1.0 events/h.[17]

2.3. Measurements of systemic inflammation biomarkers

Systemic inflammation biomarkers, including adaptive immunity cytokines, proinflammatory cytokines, and anti-inflammatory cytokines, were measured using a single multiplex kit (Bio-Plex Pro Human Cytokine 27-plex panel, Bio-Rad Laboratories, Hercules, CA, USA). Patients with acute systemic infections or inflammation were not tested until the conditions had subsided.[25] Whole blood was obtained in the morning, and serum was separated from each sample. The serum specimens were immediately stored at −80°C until assay. The commercial kit allowed for the measurement of concentrations of 27 cytokines and chemokines, including IL-1β, IL-1 receptor antagonist (IL-1ra), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, eotaxin, basic fibroblast growth factor (basic-FGF), eotaxin, granulocyte-colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-γ, interferon gamma-induced protein-10 (IP-10), monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein (MIP)-1α, MIP-1β, platelet-derived growth factor-BB (PDGF-BB), CCR5, TNF-α, and vascular endothelial growth factor. All of the procedures followed the recommendations of the manufacturer. Duplicate measurements of all samples were assessed using a Bio-Rad Bio-Plex Luminex 200 instrument and Bio-Rad Bio-Plex Manager software (v6.0).

2.4. Adenotonsillectomy

Under general anesthesia, extracapsular tonsillectomy, adenoidectomy, and tonsillar pillar suturing were performed in a single stage by the principal investigator. All of the children received routine inpatient care for three days and usually recovered within three weeks. The surgical intervention has been detailed elsewhere.[26]

2.5. Statistical analysis

Changes in scores were calculated as postoperative minus preoperative values, and % change ([change in score/preoperative value] × 100) was calculated for variables of interest. Descriptive statistics were expressed as mean (standard deviation) for continuous variables and number (proportion) for categorical variables. For continuous variables, the paired Student's *t*-test was used to assess within-group changes. A two-sided *p*-value of < 0.05 was considered statistically significant.

Relationships between variables of interest were assessed using Pearson correlation analysis. To control for *p*-value inflation, a two-sided *p*-value of < 0.01 was considered statistically significant.

Multivariable linear regression models including variables with a *p*-value < 0.200 and manual selection based on a probability of $F < 0.05$ were used to identify independent associations between % changes in inflammatory biomarkers and % changes in variables of interest using a parsimonious approach. The variance inflation factor of each predictor was calculated to adjust for intervariable relationships within the model. The regression model was repeated after removing all variables with a variance inflation factor ≥ 5 to reduce multicollinearity.[27] A two-sided *p*-value of < 0.05 was considered statistically significant.

Conditional process analysis was further performed to evaluate the mediators between change in tonsil size and % change in AHI using the SPSS PROCESS macro (version 4.1).[28] Bias-corrected 95% CIs were estimated via bootstrapping (10,000 runs) to verify mediation. Data were analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 9.0 for Windows (Graph Pad Software Inc., San Diego, CA, USA). A significant effect was indicated when the bootstrap 95% CI did not include zero or a two-sided *p*-value of < 0.05.

3. RESULTS

3.1. Demographics before and after adenotonsillectomy

Ninety-four Taiwanese children with OSA underwent adenotonsillectomy during the study period; however, 20 children were excluded as summarized in Fig. 1. Therefore, a total of 74 participants (56 [75.6%] boys and 18 [24.4%] girls) with a mean age of 7.4 ± 2.2 years were included in the statistical analysis (Table 1). The mean period between before and after adenotonsillectomy was 5.6 ± 2.6 months. After adenotonsillectomy, the difference in BMI z-score was not statistically significant. During the follow-up period, none of the participants had regrowth of the adenoid and tonsils after adenotonsillectomy. Therefore, we arbitrarily defined the postoperative tonsil size of "0" (the lowest value of tonsil size[19]) and the postoperative ANR of "0.499" (the lowest value of ANR[20]) for each participant.

3.2. Measurements of pediatric OSA before and after adenotonsillectomy

3.2.1. Subjective measurements

The mean total score of the OSA-18 questionnaire significantly decreased from 81.5 ± 16.0 to 52.2 ± 13.6 after adenotonsillectomy ($p < 0.001$).

3.2.2. Objective measurement

The mean AHI significantly decreased from 14.2 ± 15.9 to 3.3 ± 2.9 events/h after adenotonsillectomy ($p < 0.001$). All other polysomnographic variables except for the arousal index and total sleep time significantly improved after adenotonsillectomy (Table 1).

3.3. Measurements of systemic inflammatory biomarkers

After adenotonsillectomy, the mean IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-8, IL-9, IL-10, IL-15, IL-17, eotaxin, basic-FGF, GM-CSF, interferon-γ, IP-10, MCP-1, MIP-1β, PDGF-BB, CCL5, and TNF-α levels significantly reduced (Table 2).

3.4. Associations of changes in biomarkers of systemic inflammation over time with changes in other variables of interest

Change in tonsil size was positively associated with % change in OSA-18 score, % change in AHI, and % change in IL-8, and

Table 1

Demographics and polysomnographic variables before and after adenotonsillectomy

Variables	Preoperation	Postoperation	% Change	*p*[a]
Demographics				
Male sex (%)	56 (75.6%)			
Age (y)	7.4 ± 2.2			
Tonsil size (grade)	3.3 ± 0.6			
ANR (ratio)	0.769 ± 0.116			
BMI (kg/m^2) z-score (score)	0.459 ± 1.992	0.628 ± 1.536	15.8 ± 58.9	0.194
OSA-18 (score)	81.5 ± 16.0	52.2 ± 13.6	–34.1 ± 18.9	<0.001
Polysomnography				
AHI (events/h)	14.2 ± 15.9	3.3 ± 2.9	–55.6 ± 40.5	<0.001
Apnea index (events/h)	4.3 ± 8.0	1.3 ± 1.4	–38.3 ± 62.2	0.001
Arousal index (events/h)	14.3 ± 11.1	10.5 ± 20.3	–23.2 ± 42.5	0.170
Mean SpO_2 (%)	97.2 ± 1.3	97.7 ± 0.8	0.5 ± 1.5	0.002
Minimal SpO_2 (%)	87.7 ± 6.9	91.2 ± 3.4	4.7 ± 10.2	<0.001
Snoring index (events/h)	280.9 ± 242.0	163.4 ± 184.6	–21.2 ± 70.7	<0.001
Total sleep time (min)	333.5 ± 42.3	328.0 ± 35.1	–0.1 ± 17.2	0.334
N1 sleep (%)	13.0 ± 10.4	10.1 ± 5.3	–0.1 ± 59.4	0.018
N2 sleep (%)	38.7 ± 9.3	41.6 ± 8.6	11.1 ± 29.7	0.025
N3 sleep (%)	29.4 ± 10.9	26.0 ± 7.6	–2.3 ± 39.5	0.018
REM sleep (%)	18.7 ± 5.8	22.3 ± 6.4	24.1 ± 41.4	<0.001

Results are presented as mean ± standard deviation for continuous variables and as absolute number (relative frequency) for categorical variables.

AHI = apnea-hypopnea index; ANR = adenoidal-nasopharyngeal ratio; BMI = body mass index; OSA = obstructive sleep apnea; REM = rapid eye movement; SpO_2 = peripheral oxygen saturation.

[a]Data were compared using the paired *t*-test for continuous variables.

Table 2

Biomarkers of systemic inflammation before and after adenotonsillectomy

Variables	Preoperation	Postoperation	% Change	*p*[a]
IL-1β (pg/mL)	1.2 ± 1.1	0.5 ± 0.7	–18.5 ± 72.1	<0.001
IL-1ra (pg/mL)	150.1 ± 96.2	99.6 ± 77.2	–24.0 ± 55.8	<0.001
IL-2 (pg/mL)	2.8 ± 2.1	1.8 ± 1.3	–14.2 ± 66.3	<0.001
IL-4 (pg/mL)	2.4 ± 1.2	1.6 ± 1.0	–24.4 ± 50.4	<0.001
IL-5 (pg/mL)	14.5 ± 18.5	7.1 ± 11.3	–31.7 ± 61.9	0.001
IL-6 (pg/mL)	1.9 ± 3.6	1.4 ± 2.7	–13.9 ± 74.1	0.318
IL-7 (pg/mL)	8.4 ± 6.4	7.5 ± 5.6	–3.7 ± 66.5	0.222
IL-8 (pg/mL)	5.8 ± 3.6	3.8 ± 2.2	–23.2 ± 48.8	<0.001
IL-9 (pg/mL)	131.0 ± 93.5	108.1 ± 71.0	–11.3 ± 54.4	0.022
IL-10 (pg/mL)	1.0 ± 1.8	0.4 ± 0.3	–4.5 ± 53.4	0.010
IL-12 (pg/mL)	1.8 ± 3.9	1.2 ± 2.7	7.3 ± 74.0	0.064
IL-13 (pg/mL)	1.0 ± 1.7	0.7 ± 0.8	–1.6 ± 73.8	0.193
IL-15 (pg/mL)	40.0 ± 47.2	26.3 ± 40.0	–16.3 ± 64.3	0.029
IL-17 (pg/mL)	16.7 ± 9.1	11.5 ± 7.1	–17.1 ± 52.0	<0.001
Eotaxin (pg/mL)	62.4 ± 34.9	44.7 ± 21.2	–16.6 ± 39.7	<0.001
Basic-FGF (pg/mL)	36.6 ± 20.1	29.5 ± 19.5	–4.3 ± 64.7	0.010
G-CSF (pg/mL)	67.0 ± 79.9	64.6 ± 8.9	–7.6 ± 64.7	0.974
GM-CSF (pg/mL)	1.7 ± 3.2	0.8 ± 1.8	–3.6 ± 71.6	0.011
Interferon-γ (pg/mL)	5.7 ± 4.9	4.0 ± 5.1	–12.5 ± 69.9	0.017
IP-10 (pg/mL)	848.6 ± 659.0	570.3 ± 397.0	–21.8 ± 39.8	<0.001
MCP-1 (pg/mL)	33.0 ± 16.3	16.7 ± 9.9	–40.4 ± 40.7	<0.001
MIP-1α (pg/mL)	1.6 ± 1.3	1.3 ± 1.3	–9.7 ± 55.5	0.106
MIP-1β (pg/mL)	157.8 ± 70.9	126.9 ± 56.5	–12.2 ± 28.1	<0.001
PDGF-BB (pg/mL)	6018.6 ± 3159.7	4328.5 ± 2518.3	–19.4 ± 50.6	<0.001
CCL5 (pg/mL)	21403.5 ± 8698.9	15850.4 ± 6155.0	–13.3 ± 66.6	<0.001
TNF-α (pg/mL)	88.4 ± 93.6	67.7 ± 65.1	5.2 ± 63.3	0.017
VEGF (pg/mL)	34.4 ± 48.7	33.3 ± 43.2	11.4 ± 68.9	0.858

Results are presented as mean ± standard deviation for continuous variables.

CCL5 = C-C chemokine ligand 5; FGF = fibroblast growth factor; G-CSF = granulocyte-colony-stimulating factor; GM-CSF = granulocyte-macrophage colony-stimulating factor; IL = interleukin; IP = interferon gamma-induced protein; MCP = monocyte chemotactic protein; MIP = macrophage inflammatory protein; PDGF = platelet-derived growth factor; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.

[a]Data were compared using the paired *t*-test for continuous variables.

inversely related to % change in IL-10 (Table 3). Change in ANR was positively correlated with % change in G-CSF. % Change in BMI z-score was positively correlated with % changes in MIP-1β and PDGF-BB, and inversely related to % change in OSA-18 score. % Change in AHI was further positively correlated with % change in CCL5, and inversely associated with % changes in mean SpO_2 and minimal SpO_2.

3.5. Associations between changes in biomarkers of systemic inflammation over time

Significant associations between the biomarkers of systemic inflammation are shown in Fig. 2. Notably, changes in the biomarkers were frequently and closely related. To reduce overestimation due to multiple comparisons, we further evaluated significant associations with a p-value of < 0.01. Since % change in CCL5 was significantly related to % change in AHI, we further found that % change in CCL5 was significantly associated with % changes in the other 17 biomarkers except for IL-10, IL-12, IL-15, GM-CSF, interferon-γ, IP-10, TNF-α, and vascular endothelial growth factor.

3.6. Multivariable linear regression models of % changes in inflammatory biomarkers over time

Using multivariable linear regression models with a parsimonious approach, significant and independent associations between "% change in IL-1ra and % change in BMI z-score," "% change in IL-8 and change in tonsil size," "% change in IL-10 and change in tonsil size," "% change in G-CSF and change in ANR," "% change in PDGF-BB and % change in BMI z-score," and "% change in CCL5 and % change in AHI" were noted (Table 4).

3.7. Mediation analysis

To further test whether the relationship between change in tonsil size and % change in AHI was mediated by % changes in inflammatory biomarkers, mediation analysis was performed. Only a conceptual serial multiple mediation model was found: change in tonsil size (independent variable), % change in IL-8 (first mediator), % change in CCL5 (second mediator), and % change in AHI (dependent mediator) (Fig. 3). The direct paths from change in tonsil size to % change in AHI, change in tonsil size to % change in IL-8, % change in IL-8 to % change

Table 3

Associations of changes in biomarkers of systemic inflammation with changes in other variables of interest[a]

Variables	Change in tonsil size	Change in ANR	% Change in BMI z-score	% Change in OSA-18 score	% Change in AHI	% Change in mean SpO_2	% Change in minimal SpO_2
Change in tonsil size		−0.01 (0.992)	0.06 (0.599)	0.33 (0.004)	0.23 (0.048)	−0.03 (0.814)	−0.02 (0.863)
Change in ANR	−0.01 (0.992)		−0.03 (0.834)	0.15 (0.207)	0.19 (0.105)	−0.01 (0.916)	0.04 (0.737)
% Change in BMI z-score	0.06 (0.599)	−0.03 (0.834)		−0.29 (0.012)	−0.05 (0.688)	0.03 (0.820)	−0.01 (0.918)
% Change in OSA-18 score	0.33 (0.004)	0.15 (0.207)	−0.29 (0.012)		0.16 (0.172)	−0.03 (0.813)	0.06 (0.618)
% Change in AHI	0.23 (0.048)	0.19 (0.105)	−0.05 (0.688)	0.16 (0.172)		−0.28 (0.019)	−0.37 (0.001)
% Change in mean SpO_2	−0.03 (0.814)	−0.01 (0.916)	0.03 (0.820)	−0.03 (0.813)	−0.28 (0.019)		0.62 (<0.001)
% Change in minimal SpO_2	−0.02 (0.863)	0.04 (0.767)	−0.01 (0.918)	0.06 (0.618)	−0.37 (0.001)	0.62 (<0.001)	
% Change in IL-1β	−0.09 (0.461)	0.04 (0.754)	0.10 (0.387)	0.01 (0.954)	0.01 (0.970)	0.04 (0.769)	−0.01 (0.929)
% Change in IL-1ra	−0.02 (0.871)	−0.02 (0.881)	0.21 (0.074)	0.01 (0.989)	0.19 (0.111)	−0.01 (0.932)	−0.12 (0.300)
% Change in IL-2	−0.07 (0.581)	0.15 (0.207)	0.07 (0.562)	−0.16 (0.168)	0.08 (0.498)	−0.08 (0.520)	−0.14 (0.242)
% Change in IL-4	0.07 (0.547)	0.04 (0.751)	0.10 (0.395)	−0.02 (0.838)	0.07 (0.570)	−0.07 (0.544)	−0.12 (0.296)
% Change in IL-5	0.17 (0.145)	−0.12 (0.322)	0.04 (0.746)	−0.03 (0.797)	−0.03 (0.835)	−0.13 (0.279)	−0.10 (0.376)
% Change in IL-6	−0.01 (0.915)	−0.02 (0.897)	−0.03 (0.819)	0.10 (0.384)	−0.05 (0.657)	−0.13 (0.295)	−0.01 (0.968)
% Change in IL-7	−0.19 (0.107)	0.07 (0.569)	−0.04 (0.742)	−0.02 (0.855)	−0.02 (0.843)	0.02 (0.876)	0.01 (0.933)
% Change in IL-8	0.34 (0.004)	0.17 (0.147)	0.02 (0.889)	0.06 (0.611)	0.12 (0.321)	−0.05 (0.680)	−0.04 (0.720)
% Change in IL-9	0.03 (0.811)	−0.09 (0.429)	0.01 (0.980)	−0.15 (0.211)	0.01 (0.992)	−0.09 (0.442)	−0.06 (0.633)
% Change in IL-10	−0.36 (0.001)	0.16 (0.175)	0.001 (0.999)	0.07 (0.549)	−0.17 (0.142)	0.09 (0.472)	0.18 (0.135)
% Change in IL-12	0.01 (0.964)	−0.04 (0.756)	0.04 (0.710)	0.03 (0.814)	0.02 (0.845)	−0.01 (0.976)	0.05 (0.681)
% Change in IL-13	−0.02 (0.851)	0.08 (0.506)	0.01 (0.921)	0.02 (0.863)	−0.03 (0.797)	0.01 (0.966)	0.13 (0.267)
% Change in IL-15	0.07 (0.546)	0.04 (0.765)	−0.05 (0.671)	0.12 (0.331)	0.02 (0.842)	−0.12 (0.306)	0.01 (0.915)
% Change in IL-17	0.001 (0.998)	−0.09 (0.437)	0.10 (0.385)	−0.14 (0.251)	−0.02 (0.837)	−0.11 (0.380)	−0.10 (0.384)
% Change in eotaxin	−0.05 (0.675)	0.11 (0.350)	0.04 (0.715)	−0.05 (0.675)	0.09 (0.436)	−0.07 (0.548)	−0.05 (0.656)
% Change in basic-FGF	−0.10 (0.390)	−0.05 (0.680)	0.09 (0.452)	−0.06 (0.615)	−0.03 (0.818)	0.06 (0.630)	−0.01 (0.929)
% Change in G-CSF	0.06 (0.624)	0.26 (0.026)	−0.11 (0.333)	0.01 (0.906)	0.15 (0.190)	−0.09 (0.463)	−0.03 (0.779)
% Change in GM-CSF	0.06 (0.610)	−0.06 (0.607)	−0.05 (0.669)	0.19 (0.102)	−0.06 (0.638)	0.03 (0.813)	0.16 (0.178)
% Change in interferon-γ	−0.07 (0.584)	−0.08 (0.516)	0.12 (0.314)	0.08 (0.519)	0.05 (0.695)	0.15 (0.226)	0.17 (0.156)
% Change in IP-10	−0.07 (0.568)	−0.07 (0.542)	0.149 (0.205)	−0.01 (0.931)	−0.08 (0.476)	0.14 (0.259)	−0.01 (0.955)
% Change in MCP-1	−0.09 (0.449)	−0.01 (0.928)	0.17 (0.159)	−0.20 (0.085)	0.04 (0.729)	−0.03 (0.836)	−0.01 (0.969)
% Change in MIP-1α	−0.04 (0.757)	0.09 (0.454)	0.11 (0.346)	−0.12 (0.308)	0.02 (0.845)	0.13 (0.267)	0.07 (0.529)
% Change in MIP-1β	−0.12 (0.291)	−0.13 (0.271)	0.25 (0.030)	−0.16 (0.182)	−0.05 (0.701)	0.01 (0.924)	−0.05 (0.683)
% Change in PDGF-BB	−0.09 (0.42)	0.07 (0.583)	0.24 (0.041)	−0.17 (0.151)	−0.01 (0.920)	−0.04 (0.714)	−0.15 (0.213)
% Change in CCL5	−0.61 (0.608)	0.12 (0.296)	0.04 (0.707)	0.04 (0.721)	0.38 (0.001)	−0.16 (0.187)	−0.16 (0.174)
% Change in TNF-α	−0.14 (0.246)	0.01 (0.962)	0.15 (0.211)	0.03 (0.782)	−0.01 (0.983)	0.12 (0.304)	0.019 (0.873)
% Change in VEGF	−0.03 (0.832)	0.02 (0.876)	0.05 (0.702)	0.04 (0.740)	−0.05 (0.663)	0.10 (0.388)	0.18 (0.124)

Results are presented as rho (p-value).

AHI = apnea-hypopnea index; ANR = adenoidal-nasopharyngeal ratio; BMI = body mass index; CCL5 = C-C chemokine ligand 5; FGF = fibroblast growth factor; G-CSF = granulocyte-colony-stimulating factor; GM-CSF = granulocyte-macrophage colony-stimulating factor; IL = interleukin; IP = interferon gamma-induced protein; MCP = monocyte chemotactic protein; MIP = macrophage inflammatory protein; OSA = obstructive sleep apnea; PDGF = platelet-derived growth factor; SpO_2= peripheral oxygen saturation; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.

[a]Data were compared using the Pearson correlation test.

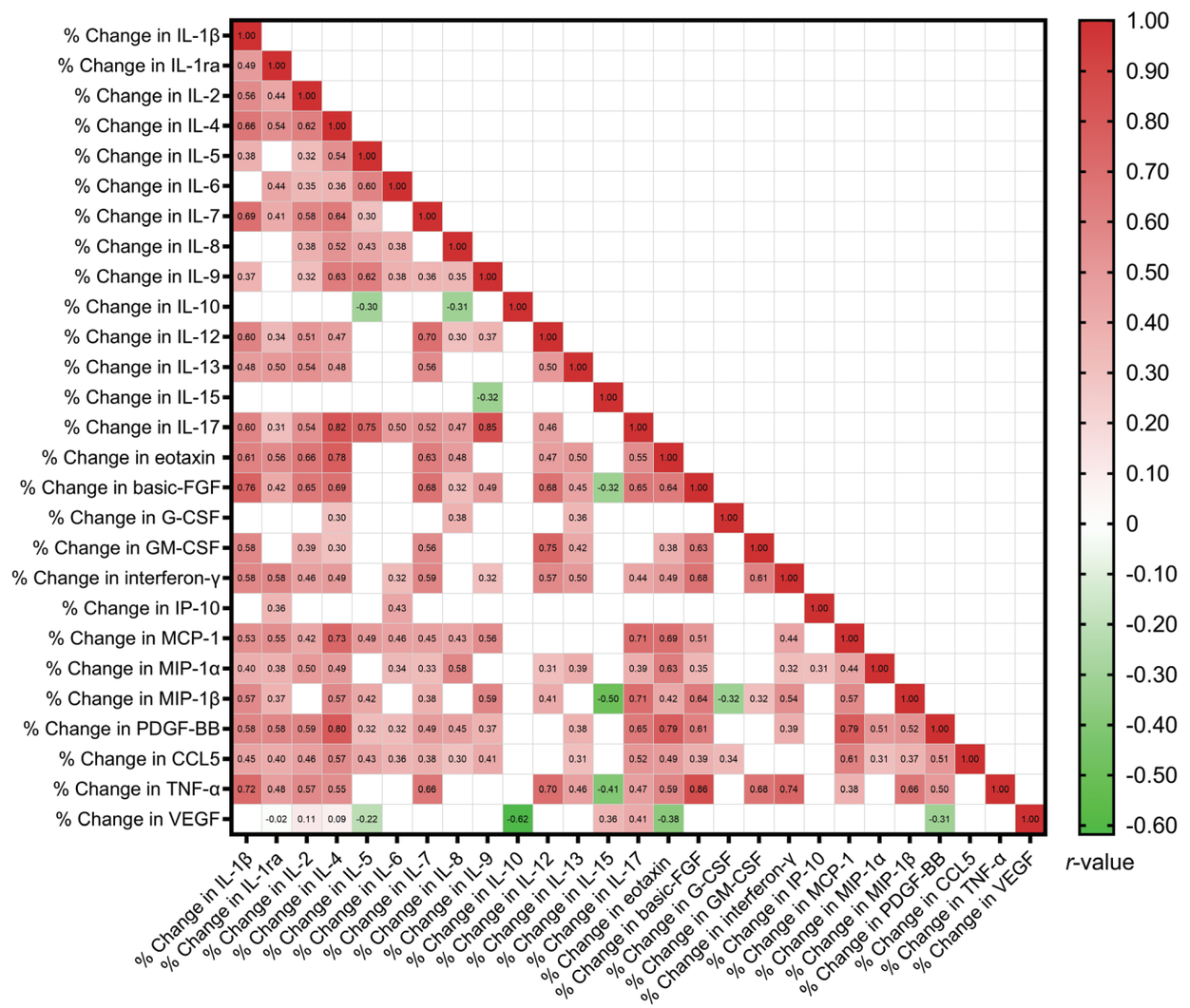

Fig. 2 Associations of % changes in biomarkers of systemic inflammation. Data are summarized as Pearson's rho. Blank spaces mean two-sided *p*-values ≥ 0.01. CCL5 = C-C chemokine ligand 5; FGF = fibroblast growth factor; G-CSF = granulocyte-colony-stimulating factor; GM-CSF = granulocyte-macrophage colony-stimulating factor; IL = interleukin; IP = interferon gamma-induced protein; MCP = monocyte chemotactic protein; MIP = macrophage inflammatory protein; PDGF = platelet-derived growth factor; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.

in CCL5, and % change in CCL5 to % change in AHI were significant. The serial mediation model revealed a positive total effect (β = 16.672, standard error = 8.274, $p = 0.048$). The direct effect of change in tonsil size on % change in AHI (β = 21.217, standard error = 8.224, $p = 0.012$) was significant. For the indirect effects, the path through % change in IL-8 and % change in CCL5 (effect = 3.740, 95% confidence interval: 0.079–12.828) was significant.

4. DISCUSSION

As first-line treatment, adenotonsillectomy significantly improved the quality of life, polysomnographic parameters, and systemic inflammatory biomarkers in the enrolled children with OSA. After adenotonsillectomy, eight of eleven polysomnographic parameters and 20 of 27 inflammatory biomarkers significantly changed. However, in analysis of adenotonsillectomy-related changes in inflammatory biomarkers, % changes in IL-8 and IL-10 were associated with change in tonsil size, % change in G-CSF was related to change in ANR, and % change in CCL5 was correlated with % change in AHI. Furthermore, % changes in mean SpO_2 and minimal SpO_2 were not significantly related to the inflammatory biomarkers. Notably, % change in IL-8 and % change in CCL5 serially mediated the relationship between change in tonsil size and % change in AHI. These findings suggest that systemic inflammation is not only a result of OSA but that it can also mediate the surgical effects, which may open avenues for potential interventions to relieve the detrimental consequences of OSA. Accordingly, in the following paragraphs, we primarily focus on the two crucial mediators, CCL5 and IL-8.

CCL5 is also known as regulated upon activation T cell expressed and secreted (RANTES), and it belongs to the CC subfamily of chemokines, which is a chemoattractant for many immune cells.[29] CCL5 is expressed by T cells and monocytes, epithelial cells, fibroblasts, and thrombocytes, and plays an essential role in the defense system.[30] It can bind to C-C chemokine receptor type 5 (CCR5) and involve cell proliferation, migration, and angiogenesis.[31] Notably, CCL5/CCR5 can contribute to stabilizing and accumulating HIF-α and then activating the HIF-α

Table 4

Multivariable linear regression models of % changes in inflammatory biomarkers in the children with obstructive sleep apnea[a]

Variables	Change in tonsil size	Change in ANR	% Change in BMI z-score	% Change in OSA-18 score	% Change in AHI	% Change in mean SpO_2	% Change in minimal SpO_2
% Change in IL-1ra	NI	NI	0.25 (0.02–0.47), $p = 0.031$	NI	NS	NI	NI
% Change in IL-2	NI	NI	NI	NS	NI	NI	NI
% Change in IL-5	NS	NI	NI	NI	NI	NI	NI
% Change in IL-7	NS	NI	NI	NI	NI	NI	NI
% Change in IL-8	29.28 (10.06–48.49), $p = 0.003$	NS	NI	NI	NI	NI	NI
% Change in IL-10	-34.99 (–56.45 to –13.54), $p = 0.002$	NS	NI	NI	NS	NI	NS
% Change in G-CSF	NI	129.93 (2.33–257.54), $p = 0.046$	NI	NI	NS	NI	NI
% Change in GM-CSF	NI	NI	NI	NS	NI	NI	NS
% Change in interferon-γ	NI	NI	NI	NI	NI	NI	NS
% Change in MCP-1	NI	NI	NS	NS	NI	NI	NI
% Change in MIP-1β	NI	NI	NS	NS	NI	NI	NI
% Change in PDGF-BB	NI	NI	0.22 (0.04–0.42), $p = 0.039$	NS	NI	NI	NI
% Change in CCL5	NI	NI	NI	NI	0.65 (0.27–1.02), $p = 0.001$	NS	NS
% Change in TNF-α	NI	NI	NI	NI	NI	NI	NI
% Change in VEGF	NI	NI	NI	NI	NI	NI	NS

Results are presented as β (95% confidence interval).

AHI = apnea-hypopnea index; ANR = adenoidal-nasopharyngeal ratio; BMI = body mass index; CCL5 = C-C chemokine ligand 5; G-CSF = granulocyte-colony-stimulating factor; GM-CSF = granulocyte-macrophage colony-stimulating factor; IL = interleukin; MCP = monocyte chemotactic protein; MIP = macrophage inflammatory protein; NI = not included; NS = not significant; OSA = obstructive sleep apnea; PDGF = platelet-derived growth factor; SpO_2 = peripheral oxygen saturation; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.

[a]Data were compared using the Pearson correlation test.

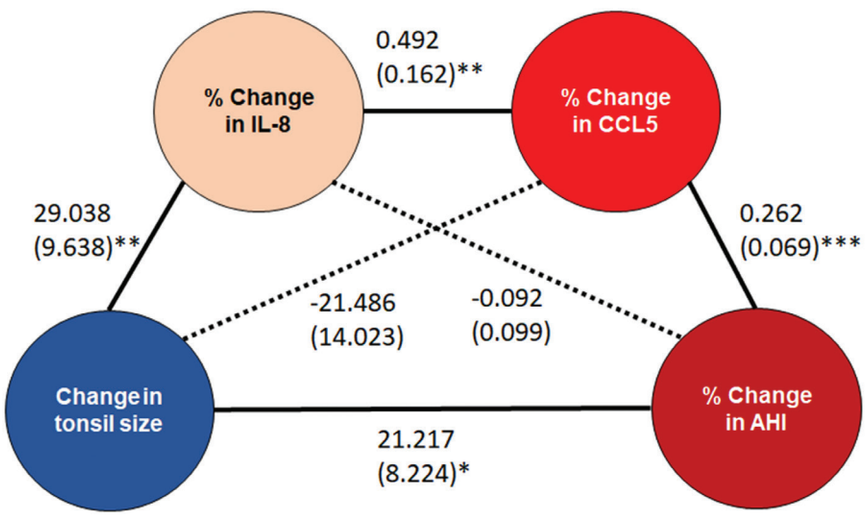

Fig. 3 A model of the effect of change in tonsil size. The change in tonsil size (independent variable) through the mediators: % change in interleukin-8 (IL-8) (first mediator), and % change in C-C chemokine ligand 5 (CCL5) (second mediator) in a serial multiple mediation model, on % change in apnea-hypopnea index (AHI) (dependent variable). Data are summarized as β and standard error. A serial multiple mediation model was performed. *$p < 0.05$ and ≥ 0.01; **$p < 0.01$ and ≥ 0.001; ***$p < 0.001$.

pathway.[32] Under low oxygen conditions, HIF-α may accumulate to induce HIF response element formation and initiate the process of angiogenesis.[33] In addition, intermittent hypoxia can trigger monocytic CCR5 gene expression and strengthen CCL5-mediated chemotaxis and adhesion.[34] Increased CCL5 levels can further attract inflammatory cells and promote preatherosclerotic remodeling.[35] Therefore, parallel reductions in AHI and serum levels of CCL5 after adenotonsillectomy may be beneficial for children with OSA, particularly regarding disease severity, systemic inflammation, and cardiovascular complications.

IL-8 is also called chemokine (C-X-C motif) ligand 8 (CXCL8), and it belongs to the CXC chemokine family. It is the primary chemokine involved in the recruitment and degranulation of neutrophils, and it also attracts many immune cells.[36] IL-8 is secreted by macrophages, monocytes, epithelial cells, airway smooth muscle cells, and endothelial cells, and plays a crucial role in innate immunity.[37] It binds to CXC receptor type 1 (CXCR1) and type 2 (CXCR2)[38] and stimulates phagocytosis of target cells.[39] In addition to innate immune responses, IL-8 also promotes angiogenetic responses in endothelial cells.[40] Children

with OSA have been shown to have significantly elevated serum levels of IL-8[41] and tonsillar concentrations of IL-8 and IL-10[42] compared with controls. In addition, hypoxia has been shown to activate the SUMO-1-HIF-1α signaling pathway to upregulate the production of IL-8 and permeability in human tonsil epithelial cells.[43] After total excision of the tonsils in our patients, % change in IL-8 was positively correlated with change in tonsil size (ie, the larger the preoperative tonsil size, the greater the postoperative reduction in the IL-8 serum level), suggesting that a hypertrophic tonsil is the primary origin of IL-8 production. Moreover, although previous studies have reported a higher concentration of IL-8 in OSA patients and that this may contribute to weight gain[44] and % body fat,[45] we found that % change in IL-8 after adenotonsillectomy was not related to % change in AHI or % change in BMI z-score.

Although the precise mechanism of the indirect pathway from change in tonsil size to % change in AHI through % change in IL-8 and % change in CCL5 among children with OSA remains to be determined, we hypothesize that tonsil monocytes, macrophages, and dendritic cells may play a pivotal role. Upon hypoxic tissue damage or infection, human peripheral blood monocytes are rapidly recruited to the tonsils, where they can differentiate into tissue macrophages or dendritic cells.[46] An in vitro study showed that a hypoxic environment could promote the onset of a highly proinflammatory gene expression profile in dendritic cells, including strong upregulation of the IL-8 gene and CCL5,[47] probably via modulation of nuclear factor κ beta and mitogen-activated protein kinase pathways.[48]

In addition, allergens may also provoke respiratory tissue eosinophilia and significantly increase IL-8 and CCL5 mRNA-positive cells.[49] IL-8 and CCL5 have been shown to regulate histamine and serotonin generation and cell function in mast cells.[50] Therefore, allergic airway inflammation considerably increases collapsibility of the upper airway and contributes to the development and worsening of OSA.[51] In contrast, excision of hypertrophic tonsils can reduce IL-8, CCL5, and airway inflammation, and subsequently mitigate AHI in children with OSA. Corticosteroids can reduce cellular proliferation rates of the adenoids and tonsils and the production of IL-8 using in vitro human whole tissue cell cultures[52] and intranasal corticosteroids may reduce the tonsil size and AHI[53] in children with mild OSA. Furthermore, montelukast has been shown to markedly reduce tonsil size and adenoid size[54] and suppress the release of IL-8 and CCL5 by nasal airway epithelial cells,[55] and also to have short-term beneficial treatment effects for pediatric OSA.[56] On the other hand, serum from continuous positive airway pressure-treated[57] or sleep surgery-treated[58] adults with OSA had lower inflammatory gene expressions than serum from OSA patients without treatment. Therefore, both continuous positive airway pressure and sleep surgery not only increases the upper airway size to improve intermittent hypoxia and systemic inflammation but also reduces the overall inflammatory potential in the circulation to alleviate upper airway collapse. Furthermore, a previous study reported that microRNA-200c, delivered using polyethyleneimine nanoparticles, could effectively reduce the production of IL-8 and CCL5 in primary human periodontal ligament fibroblasts.[59] Thus, microRNA-200c may have potential in the treatment of adenotonsillar hypertrophy and OSA. Taken together, based on these observations, future research directions can be proposed to find novel anti-inflammatory agents that both reduce IL-8 and CCL5 levels can also alleviate the severity of pediatric OSA.

Although our findings and implications are interesting, this study has some limitations. First, the sample size was not as large as we expected because of the impact of COVID-19 in the past 2 years. Second, changes in systemic inflammatory biomarkers were assessed 3 months after adenotonsillectomy, which may not be long enough to represent long-term effects. The research team is continuing with our efforts to recruit more subjects and collect more samples, and hopefully will be able to address the longer-term effects of adenotonsillectomy on systemic inflammation. Furthermore, we will explore the associations between systemic inflammation and autonomic function as well as systemic inflammation and gut microbiome in children with OSA. Third, some of the children may have had co-existing chronic adenoiditis/tonsillitis and gut dysbiosis before the operation, which would interfere with the analysis of systemic inflammation.[18,60] However, in the present study, we focused on adenotonsillectomy-related changes in inflammatory biomarkers. Therefore, the significant postoperative changes in inflammatory biomarkers may be the result of confounding effects of chronic adenoiditis and tonsillitis at baseline. Nevertheless, the effects of IL-8 and CCL5 on disease severity warrant future investigations in children with OSA.

In conclusion, the preliminary results of this study provide the first empirical evidence that systemic inflammation can modulate the effects of adenotonsillectomy on disease severity in children with OSA. Moreover, % changes in IL-8 and CCL5 may act as serial mediators of the association between change in tonsil size and % change in AHI. These findings may expand the existing biological knowledge concerning the adenotonsillar hypertrophy–OSA relationship from the perspective of circulatory inflammatory biomarkers. In addition, they may inform a translational conceptualization of how to improve tonsil size and pediatric OSA through the regulation of systemic inflammation.

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