ORIGINAL ARTICLE

Systemic inflammation, tonsil microbiome, obstructive sleep apnea, and surgical outcome among children of different weight status

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Running title: Inflammation, Tonsil Microbiome, and OSA

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Abstract

Background: Systemic inflammation and tonsil microbiome have been linked to chronic intermittent hypoxia during sleep in children with obstructive sleep apnea (OSA). However, their relationships have not been comprehensively studied. Here, we investigated the associations between systemic inflammation, tonsil microbiome, OSA severity, and surgical outcome in pediatric OSA patients regarding different weight status.

Methods: We recruited 33 children with OSA and non-healthy-weight (cases) and 33 children with OSA and healthy-weight (controls) were prospectively recruited and matched by the proportion of chronic tonsillitis. Each patient underwent adenotonsillectomy and received at least 3-month follow-ups. Systemic inflammatory biomarkers (interleukin [IL]-6, IL-10) were detected in the blood sampled in the morning. Tonsil microbiome was identified with 16S ribosomal RNA gene sequencing. OSA severity was assessed by standard whole-night polysomnography.

Results: Differences in systemic inflammatory biomarkers, tonsil phyla, and apnea-hypopnea index were not statistically significant between both groups. After adenotonsillectomy, all OSA severity variables significantly improved; however, apnea-hypopnea index was significantly higher in the non-healthy-weight group and serum level of IL-6 significantly reduced in the healthy-weight group. The percentage

changes in IL-6 and minimal pulse oxygen saturation were respectively associated with *Deinococcus-Thermus* and *Eremiobacteraeota* in the non-healthy-weight group, whereas the percentage change in IL-6 was associated with *Patescibacteria* and *Cloacimonetes* in the healthy-weight group. Furthermore, chronic tonsillitis was related to *Chloroflexi*, *Acidobacteria*, *Euryarchaeota, Thermotogae*, *Hydrogenedentes*, and *Rokubacteria* in the non-healthy-weight group.

Conclusion: These preliminary findings are novel and provide insight for future research to understand the pathogenesis of the disease and to develop personalized treatments.

KEYWORDS

adenotonsillectomy, children, interleukin, microbiome, obstructive sleep apnea, surgical outcome, systemic inflammation, tonsil, weight status

Key Message

This study shows that the associations between systemic inflammation, tonsil microbiome, obstructive sleep apnea severity, and surgical outcome significantly differently interact with each other in children of different weight status, potentially influencing the pathogenesis and treatment outcome.

1 | INTRODUC TION

Pediatric obstructive sleep apnea (OSA) is a chronic sleep disorder characterized by intermittent partial or complete upper airway obstruction during sleep. It has a prevalence of 1%–4% in healthy children and can result in long-term adverse effects.[1] Adenotonsillar hypertrophy and overweight/obesity are the most common risk factors for pediatric OSA.[1] OSA causes chronic intermittent hypoxia and systemic inflammation,[2] and further interacts with neurobehavioral impairment[3] and other chronic conditions[4-6] in children. Inflammatory cytokines such as interleukin (IL)-6[4,7] and IL-10[6] are associated with the apnea-hypopnea index (AHI). However, the physiological roles of inflammatory biomarkers may differ and have opposing actions in maintaining cardiovascular homeostasis among populations.[4] Therefore, studies are warranted to investigate differences in IL-6 and IL-10 in pediatric OSA patients with different weight statuses.

Microbiomes of the adenoid and tonsils change with many diseases, such as chronic tonsillitis and OSA.[8,9] The two main methods to detect bacterial communities on the tonsil surface are swab cultures and culture-free molecular tests based on 16S ribosomal RNA or ribosomal DNA sequencing.[10] Molecular tests enable metagenomic studies with better detection of slow-growing, uncultivable, and rare bacteria.[11] However, to our best knowledge, differences in tonsil microbiota between non-

healthy-weight (cases) and healthy-weight children (controls) with OSA have not been elucidated.

The first aim of this study was to compare differences in systemic inflammation, tonsil microbiome, OSA severity, and surgical outcome in pediatric OSA patients with various weight statuses. The second aim was to correlate these variables of interest with each other in non-healthy-weight and healthy-weight children with OSA.

2 | MATERIAL S AND METHODS

2.1 | Study design and data collection

We prospectively recruited pediatric patients referred to the Department of Otorhinolaryngology-Head and Neck Surgery at Linkou-Chang Gung Memorial Hospital (Taoyuan, Taiwan) for adenotonsillectomy between March 1, 2017, and January 31, 2019. The Institutional Review Board of Chang Gung Medical Foundation approved this prospective case-control study (104-7279A3). Written informed consent was obtained from all parents and participants ≥ 6 years. The protocol has been previously published.[12] The inclusion criteria were: (1) age 5–12 years and (2) AHI ≥ 5.0 or AHI ≥ 1.0 plus at least one morbidity (such as elevated blood pressure, daytime sleepiness, learning problems, growth failure, or enuresis).[5] Patients with craniofacial, neuromuscular, or chronic inflammatory disorders were

excluded.[5,12] The subjects were further divided into two subgroups according to body mass index (BMI) z-score: ‘non-healthy-weight’ (≤ -2.0 and ≥ 1.0) group, and 'healthy-weight’ (> -2.0 and < 1.0) group.[13] Both groups were matched by the proportion of chronic tonsillitis. Figure E1 demonstrates flow diagram.

2.2 | Systemic inflammatory biomarkers

Morning blood samples were taken within 1 week before adenotonsillectomy. In cases of acute systemic inflammation, blood tests were not performed until the condition had subsided.[14] The serum levels of IL-6 and IL-10 were determined using the Bio-Plex® Pro Human Cytokine assay (Bio-Rad Laboratories, Hercules, CA, USA) as described previously.[12] Duplicate samples were centrifuged, diluted, incubated with antibody-coupled beads, incubated with detection antibodies, and treated with streptavidin according to the manufacturer’s instructions.

2.3 | Tonsil microbiota

Tonsils with crypts were excised using sterile scissors during adenotonsillectomy. Genomic DNA was extracted from the specimen using an EasyPrep Genomic DNA Extraction Kit (Tools Biotechnology, Taiwan). Tonsil tissue was treated with 4 μL of RNase A (100 mg/mL) for 5 min at room temperature, 20 μL of Proteinase K at 56°C until completely lysed, and 200 μL ethanol (96–100%) for 15 sec.[15] The quality and

quantity of genomic DNA were measured on a NanoPhotometer P360 system (Implen, USA). Polymerase chain reaction (PCR) was used to amplify the V3–V4 regions of the gene that encoded for 16S rRNA in bacteria using composite primers including the forward primer 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTAYGGGRBGCASCAG-3' and the reverse primer 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACNNGGGTATCTAAT-3'.[16] Amplicons were purified using a QiaQuick PCR Purification Kit (Qiagen). PCR amplicons were sequenced using the Illumina HiSeq 2500 platform following the manufacturer's instructions to generate 250 bp paired-end reads.

2.4 | OSA severity variables

We assessed OSA severity variables (AHI, mean pulse oxygen saturation [SpO_2], and minimal SpO_2) by standard polysomnography, according to the 2012 American Academy of Sleep Medicine Manual.[17] A detailed protocol of polysomnography had been described previously.[12]

2.5 | Surgical outcomes

All patients underwent adenotonsillectomy and received at least 3-month follow-ups. Percentage change ([postoperative value - preoperative value] / [preoperative value] ×

100) was calculated for variables of interest.

2.6 | Sample size

The sample size was estimated using primary outcome effects (BMI z-score) based on a *priori* study[12] (healthy-weight group = 0.52 ± 1.12 and non-healthy-weight group = 1.53 ± 1.05). We used a two-tailed Wilcoxon-Mann-Whitney test to calculate the sample size (effect size = .93; type I error = .05; power = .95), which generated a sample size of 33 in each group.

2.7 | Bioinformatics and statistical analysis

The entire paired-end reads were assembled using FLASH (version 1.2.7) and reads with quality score < 20 were removed using QIIME (version 1.7). Sequences were chimera-checked using UCHIME and filtered from the data set before operational taxonomic unit (OTU) picking of 97% sequence identity using USEARCH (version 7). Taxonomy classification was annotated according to the Greengenes Database (version 13.8). To identify relationships between different OTU, multiple sequences were aligned using PyNAST (version 1.2) against the Greengenes core set database. We used Graphical Phylogenetic Analysis for visualizations of microbial genomes and metagenomes. Detailed protocols of our bioinformatics had been described previously.[16,18] During data collection and analysis, the investigators were blinded to

group allocation.

The D'Agostino and Pearson normality test showed that most variables had non-normal distributions. Therefore, descriptive statistics were expressed as the median, interquartile range (IQR), or frequency. Differences in variables of interest between the 'healthy-weight' and 'non-healthy-weight' groups were determined using the Mann-Whitney *U* test or chi-square test as appropriate. Spearman's correlation test was used to determine associations among major (> 0.1% abundance and present in >90% of samples[18]) or minor phyla with the patient characteristics and inflammatory biomarkers. Overall taxonomic or phylum-level abundances were included to determine the most discriminatory taxa between two groups. Analysis of similarity was performed to compare bacterial communities. For reducing the effect of multiple comparisons of the tonsil phyla, we performed the *Bonferroni* correction. All statistical analyses were conducted using R software (versions 2.15.3 and 3.6.1, R Foundation for Statistical Computing, http://www.r-project.org/) and Graph Pad Prism software (version 7.00; Graph Pad Software Inc., San Diego, CA, USA). A two-tailed *P* value of < .05 was considered to be statistically significant.

3 | RESULTS

3.1 | Participants' characteristics

Sixty-six children with OSA (16 girls and 50 boys; median age, 6.5 years [IQR, 6.0–9.0]; BMI z-score, 0.91 [IQR, -0.38–2.03]; median AHI, 8.5 events/hour [IQR, 4.1–19.5]) were enrolled (Table 1). The non-healthy-weight group (n = 33) had significantly higher age and BMI z-score, and lower mean and minimal SpO_2 than the healthy-weight group (n = 33). There were no significant differences in the other characteristics including the proportions of male sex and chronic tonsillitis, serum levels of IL-6 and IL-10, and AHI before adenotonsillectomy.

3.2 | Correlations of systemic inflammation and participants' characteristics

Although there were some weak associations between IL-6 and AHI and mean SpO_2 in the healthy-weight group before adenotonsillectomy, serum levels of IL-6 and IL-10 were not associated with patient characterises, system inflammation, and obstructive sleep apnea severity after the *Bonferroni* correction (Table 2).

3.3 | Difference in tonsil microbiome between different weight status groups

Figures 1A and 1B demonstrate OTU trees of the non-healthy-weight group and healthy-weight groups, respectively. The difference in tonsil bacterial communities between the two groups was not statistically significant. For simplified clinical comparisons, we analyzed the difference in the phyla level of the tonsil microbiota in this study. Fifty-seven phyla were identified from the tonsil samples. There were six

major phyla, including *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Fusobacteria*, *Actinobacteria*, and *Epsilonbacteraeota*, and 51 minor phyla. In descending order of median relative abundance, the 10 most common phyla were *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Fusobacteria*, *Actinobacteria*, *Epsilonbacteraeota*, *Spirochaetes*, *Chloroflexi*, *Acidobacteria*, and *Euryarchaeota*. The relative abundances of these phyla in the non-healthy-weight group (Figure 1C) were equivalent to those of these phyla in the healthy-weight group (Figure 1D).

3.4 | Associations of participants' characteristics, systemic inflammation, and tonsil phyla

There were some weak associations between participants' characteristics, systemic inflammation, and the major tonsil phyla (Figure 2). However, chronic tonsillitis was significantly related to high relative abundances of *Chloroflexi*, *Acidobacteria*, and *Euryarchaeota* after the *Bonferroni* correction in the non-healthy-weight group (Figure 2A), whereas the corrected associations were not statistically significant in the healthy-weight group (Figure 2B).

Table 3 further summarizes uncorrected associations among the patient characteristics and the other minor phyla of the tonsil. After the *Bonferroni* correction, chronic tonsillitis was significantly associated with high relative abundances of *Thermotogae*, *Hydrogenedentes*, and *Rokubacteria* in the non-healthy-weight group,

whereas the patient characteristics were not associated with the other minor tonsil phyla in the healthy-weight group.

There were several weak associations between systemic inflammation, OSA severity variables, and the minor tonsil phyla in the non-healthy-weight group (detail see in Table E1) and the healthy-weight group (detail see in Table E2). The corrected association between high baseline serum level of IL-6 and *Eremiobacteraeota* (WPS-2) was still significant. However, there were no significantly corrected associations in the healthy-weight group.

3.5 | Main surgical outcomes

Figure 3 demonstrates changes in six main outcomes after adenotonsillectomy in detail. Percentage changes in BMI z-score, IL-6 level, IL-10 level, AHI, mean SpO_2, and minimal SpO_2 were comparable between both groups (Table 1). After the *Bonferroni* correction, the percentage change in IL-6 was inversely associated with the baseline IL-6 level in the healthy-weight group (Table 2). Furthermore, percentage changes in IL-10 were inversely associated with the baseline IL-10 level in both the groups.

3.6 | Associations of main surgical outcomes and tonsil phyla

Percentage changes in variables of interest were not significant associated with the top 10 tonsil phyla in the non-healthy-weight group (Figure 2A) and the healthy-weight

group (Figure 2B) after the *Bonferroni* correction. The percentage change in BMI z-score was inversely associated with the relative abundance of *Planctomycetes* in the non-healthy-weight group but not in the healthy-weight group after the *Bonferroni* correction (Table 3). The corrected associations between the percentage change in IL-6 and the relative abundance of *Deinococcus-Thermus*, and the percentage change in minimal SpO_2 and the relative abundance of *Eremiobacteraeota* (WPS-2) were significant in the non-healthy-weight group (Table E1). In the healthy-weight group, the percentage changes in IL-6 was related to the relative abundance of *Patescibacteria*, and inversely associated with the relative abundance of *Cloacimonetes* after the *Bonferroni* correction (Table E2).

4 | DISCUSSION

The advent of metagenomics has led to an increase in investigations on human microbiota. However, information on the tonsil microbiomes in pediatric OSA patients and their relationships with patient characteristics, systemic inflammation, and OSA severity are still lacking. Furthermore, our findings have suggested IL-10 plays a minor role in systemic inflammation in children with OSA regardless of BMI. In the following paragraphs, we address several novel findings in this study, regarding the weight status.

Despite no significant difference in systemic inflammatory biomarkers or tonsil microbiome observed between two body weight groups, the associations between these variables were different in each group. Notably, the baseline serum level of IL-6 was weakly associated with AHI, but not BMI z-score, in the non-healthy-weight group; furthermore, the baseline serum level of IL-6 was not associated with AHI and BMI in the healthy-weight group. These novel findings are not entirely consistent with data in adult patients and suggest that the pathogenesis of OSA differs between adults and children, as well as in children with different weight status. However, these findings warrant further large-scale investigations.

IL-6 is a cytokine acting as both inflammatory and anti-inflammatory effects and produced by monocytes, endothelial cells, and adipocytes.[19] Although OSA can result in elevated IL-6 levels independent of BMI in adolescents[20] and adults[21], whether OSA further reinforces systemic inflammation in obese children or not is not conclusive in non-healthy-weight children[22]. However, the percentage change in IL-6 was not associated with the percentage change in AHI, as a previous report[23], suggests that the IL-6 level maybe not directly to AHI in the non-healthy-weight group.

Interestingly, the relative abundance of *Eremiobacteraeota* (WPS-2) of the tonsil was associated with both the baseline serum level of IL-6 and the percentage change in minimal SpO_2, and the percentage change in IL-6 was associated with

Deinococcus-Thermus in the non-healthy-weight group. The candidate division WPS-2 has been firstly described in the Wittenberg-polluted soil[25] and identified as a lesser-known phylum in human oral taxa[26]. *Deinococcus-Thermus* is a bacterial phylum featuring with high resistance to environmental hazards[27], and has been linked to allergic rhinoconjunctivitis[28]. Furthermore, the percentage change in IL-6 was associated with *Patescibacteria* and *Cloacimonetes* in the healthy-weight group. *Patescibacteria* exists in groundwater, seawater, and soil[29], whereas *Cloacimonetes* exists in an anaerobic sludge digester[30]. To our best knowledge, these lesser-known phyla have not been reported in the human tonsils; surgical removal of these tonsil phyla may involve in the changes of serum level of IL-6 and minimal SpO_2 in children with OSA. Therefore, these novel host-microbe associations warrant further investigations in the future.

Also, some OSA severity variables were weakly associated with some phyla (*Bacteroidetes*, *Deferribacteres*) in the healthy-weight group, but with other ones in the non-healthy-weight group. Tonsil microbiome seemed to be involved in OSA severity in healthy-weight than in non-healthy-weight pediatric patients. This novel finding suggests a possible underlying link between tonsil microbiome and OSA severity in pediatric patients. Future studies are warranted to elucidate the pathophysiological mechanisms.

Furthermore, two of the top 10 tonsil phyla (*Epsilonbacteraeota* and *Acidobacteria*) in both groups, four of chronic tonsillitis-related phyla (*Acidobacteria*, *Thermotogae*, *Hydrogenedentes*, and *Rokubacteria*) and one BMI-related phylum (*Planctomycetes*) in the non-healthy-weight group have not been listed in the Human Oral Taxa according to the expanded Human Oral Microbiome Database.[24] To date, these lesser-known phyla have not been reported to be involved in OSA, chronic tonsillitis, and BMI. Nevertheless, these preliminary findings indicate there are different interactions between tonsil microbiome, chronic tonsillitis, or body status in children with OSA and need future studies to confirm their pathogenetic effects.

Several limitations should be addressed in this study. First, the subject group contained a single ethnicity and was predominantly male, which may limit the generalizability of the results. However, the proportions of sex and chronic tonsillitis were comparable in both body weight groups to minimize confounding effects from baseline characteristics. Second, there may have been the co-existence of chronic tonsillitis, which would interfere with the analysis of inflammatory biomarkers and tonsil microbiome. Third, many of these phyla cannot or are very difficult to culture and have only been identified very recently with the advent of metagenomics. Future investigations on the effect of OSA treatment on systemic inflammatory biomarkers and tonsil microbiome with a larger sample size are warranted.

331

332 5 | CONCLUSION

333 The systemic inflammation and tonsil microbiome play roles in pediatric OSA, and
334 they seem to have different effects regarding various weight statuses. We found
335 several novel associations among systemic inflammatory biomarkers, tonsil phyla,
336 disease severity variables, and surgical outcomes in different body weight groups.
337 Future studies to investigate associations among tonsil microbiome and pathogenesis
338 and exacerbation of the disease are warranted.

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REFERENCES

1. Marcus CL, Brooks LJ, Draper KA, et al. Diagnosis and management of childhood obstructive sleep apnea syndrome. *Pediatrics*. 2012;130(3):576-584.
2. Eltzschig HK, Carmeliet P. Hypoxia and inflammation. *The New England journal of medicine*. 2011;364(7):656-665.
3. Madaeva I, Berdina O, Polyakov V, Kolesnikov S. Obstructive Sleep Apnea and Hypertension in Adolescents: Effect on Neurobehavioral and Cognitive Functioning. *Can Respir J*. 2016;2016:3950914.
4. Smith DF, Hossain MM, Hura A, et al. Inflammatory Milieu and Cardiovascular Homeostasis in Children With Obstructive Sleep Apnea. *Sleep*. 2017;40(4).
5. Huang YS, Guilleminault C, Hwang FM, et al. Inflammatory cytokines in pediatric obstructive sleep apnea. *Medicine*. 2016;95(41):e4944.
6. Leon-Cabrera S, Arana-Lechuga Y, Esqueda-Leon E, et al. Reduced systemic levels of IL-10 are associated with the severity of obstructive sleep apnea and insulin resistance in morbidly obese humans. *Mediators of inflammation*. 2015;2015:493409.
7. Shalitin S, Deutsch V, Tauman R. Hepcidin, soluble transferrin receptor and IL-6 levels in obese children and adolescents with and without type 2 diabetes mellitus/impaired glucose tolerance and their association with obstructive sleep apnea. *Journal of endocrinological investigation*. 2018;41(8):969-975.
8. Johnston J, Hoggard M, Biswas K, et al. The bacterial community and local lymphocyte response are markedly different in patients with recurrent tonsillitis compared to obstructive sleep apnoea. *International journal of pediatric otorhinolaryngology*. 2018;113:281-288.
9. Xu H, Li X, Zheng X, et al. Pediatric Obstructive Sleep Apnea is Associated With Changes in the Oral Microbiome and Urinary Metabolomics Profile: A Pilot Study. *Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine*. 2018;14(9):1559-1567.
10. Johnston JJ, Douglas R. Adenotonsillar microbiome: an update. *Postgraduate medical journal*. 2018;94(1113):398-403.
11. Verma D, Garg PK, Dubey AK. Insights into the human oral microbiome. *Archives of microbiology*. 2018;200(4):525-540.
12. Chuang HH, Huang CG, Chuang LP, et al. Relationships among and predictive values of obesity, inflammation markers, and disease severity in pediatric patients with obstructive sleepapnea before and after adenotonsillectomy. *J Clin Med*. 2020;9:579.

13. de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. *Bulletin of the World Health Organization*. 2007;85(9):660-667.
14. Lee LA, Huang CG, Chen NH, Wang CL, Fang TJ, Li HY. Severity of obstructive sleep apnea syndrome and high-sensitivity C-reactive protein reduced after relocation pharyngoplasty. *Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery*. 2011;144(4):632-638.
15. Chang CC, Huang YS, Lin YM, et al. The role of sentrin-specific protease 2 substrate recognition in TGF-beta-induced tumorigenesis. *Scientific reports*. 2018;8(1):9786.
16. Wu TR, Lin CS, Chang CJ, et al. Gut commensal Parabacteroides goldsteinii plays a predominant role in the anti-obesity effects of polysaccharides isolated from Hirsutella sinensis. *Gut*. 2019;68(2):248-262.
17. Berry RB, Budhiraja R, Gottlieb DJ, et al. Rules for scoring respiratory events in sleep: update of the 2007 AASM Manual for the Scoring of Sleep and Associated Events. Deliberations of the Sleep Apnea Definitions Task Force of the American Academy of Sleep Medicine. *Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine*. 2012;8(5):597-619.
18. Wu IW, Lin CY, Chang LC, et al. Gut Microbiota as Diagnostic Tools for Mirroring Disease Progression and Circulating Nephrotoxin Levels in Chronic Kidney Disease: Discovery and Validation Study. *International journal of biological sciences*. 2020;16(3):420-434.
19. Rattazzi M, Puato M, Faggin E, Bertipaglia B, Zambon A, Pauletto P. C-reactive protein and interleukin-6 in vascular disease: culprits or passive bystanders? *J Hypertens*. 2003;21(10):1787-1803.
20. Gaines J, Vgontzas AN, Fernandez-Mendoza J, et al. Inflammation mediates the association between visceral adiposity and obstructive sleep apnea in adolescents. *Am J Physiol Endocrinol Metab*. 2016;311(5):E851-E858.
21. De Luca Canto G, Pacheco-Pereira C, Aydinoz S, Major PW, Flores-Mir C, Gozal D. Diagnostic capability of biological markers in assessment of obstructive sleep apnea: a systematic review and meta-analysis. *J Clin Sleep Med*. 2015;11(1):27-36.
22. Van Eyck A, Van Hoorenbeeck K, De Winter BY, Van Gaal L, De Backer W, Verhulst SL. Sleep-disordered breathing, systemic adipokine secretion, and metabolic dysregulation in overweight and obese children and adolescents. *Sleep Med*. 2017;30:52-56.

23. Chu L, Li Q. The evaluation of adenotonsillectomy on TNF-alpha and IL-6 levels in obese children with obstructive sleep apnea. *Int J Pediatr Otorhinolaryngol*. 2013;77(5):690-694.
24. Escapa IF, Chen T, Huang Y, Gajare P, Dewhirst FE, Lemon KP. New Insights into Human Nostril Microbiome from the Expanded Human Oral Microbiome Database (eHOMD): a Resource for the Microbiome of the Human Aerodigestive Tract. *mSystems*. 2018;3(6).
25. Nogales B, Moore ER, Llobet-Brossa E, Rossello-Mora R, Amann R, Timmis KN. Combined use of 16S ribosomal DNA and 16S rRNA to study the bacterial community of polychlorinated biphenyl-polluted soil. *Appl Environ Microbiol*. 2001;67(4):1874-1884.
26. Camanocha A, Dewhirst FE. Host-associated bacterial taxa from Chlorobi, Chloroflexi, GN02, Synergistetes, SR1, TM7, and WPS-2 Phyla/candidate divisions. *J Oral Microbiol*. 2014;6.
27. Griffiths E, Gupta RS. Identification of signature proteins that are distinctive of the Deinococcus-Thermus phylum. *Int Microbiol*. 2007;10(3):201-208.
28. Yau JW, Hou J, Tsui SKW, et al. Characterization of ocular and nasopharyngeal microbiome in allergic rhinoconjunctivitis. *Pediatr Allergy Immunol*. 2019;30(6):624-631.
29. Herrmann M, Wegner CE, Taubert M, et al. Predominance of Cand. Patescibacteria in Groundwater Is Caused by Their Preferential Mobilization From Soils and Flourishing Under Oligotrophic Conditions. *Front Microbiol*. 2019;10:1407.
30. Chouari R, Le Paslier D, Daegelen P, Ginestet P, Weissenbach J, Sghir A. Novel predominant archaeal and bacterial groups revealed by molecular analysis of an anaerobic sludge digester. *Environ Microbiol*. 2005;7(8):1104-1115.

Tables

Table 1. Patient characteristics, systemic inflammation, and obstructive sleep apnea severity before and after adenotonsillectomy

Variables	Non-healthy-weight group	Healthy-weight group	*P* Value[a]
Before adenotonsillectomy	n = 33	n = 33	
Patient characteristics			
Age (years)	**7.0 (6.0–8.0)**	**6.0 (5.0–7.5)**	**0.02**
Male sex, *n* (%)	28 (85)	22 (67)	0.09
Chronic tonsillitis	6 (18)	10 (30)	0.25
BMI (kg/m^2) z-score	**2.01 (1.46–2.38)**	**-0.36 (-1.16–0.18)**	**<0.001**
Systemic inflammation			
IL-6 (pg/mL)	1.37 (0.32–2.44)	1.37 (0.13–2.18)	0.84
IL-10 (pg/mL)	0.50 (0.39–0.51)	0.39 (0.39–0.51)	0.43
Obstructive sleep apnea severity			
AHI (events/h)	9.6 (5.0–25.2)	5.4 (3.9–16.5)	0.11
Mean SpO_2 (%)	**97 (96–98)**	**98 (97–98)**	**0.04**
Minimal SpO_2 (%)	**89 (83–91)**	**91 (88–93)**	**0.02**
After adenotonsillectomy	n = 31	n = 32	
Patient characteristics			
BMI (kg/m^2) z-score	**2.08 (1.63–2.43)**	**-0.06 (-0.82–0.52)**	**<0.001**
Systemic inflammation			
IL-6 (pg/mL)	1.14 (0.76–1.83)	0.65 (0.13–1.40)	0.07
IL-10 (pg/mL)	0.46 (0.15–0.73)	0.73 (0.18–0.85)	0.65

Obstructive sleep apnea severity			
AHI (events/h)	**2.7 (2.2–6.4)**	**2.2 (1.3–3.6)**	**0.03**
Mean SpO_2 (%)	98 (97–98)	98 (97–98)	0.12
Minimal SpO_2 (%)	**90 (88–93)**	**93 (90–94)**	**0.01**
Percentage change	n = 31	n = 32	
Patient characteristics			
Percentage change in BMI z-Score	-1 (-7–25)	-37 (-100–42)	0.21
Systemic inflammation			
Percentage change in IL-6	-36 (-64–84)	-64 (-91–35)	0.15
Percentage change in IL-10	0 (-24–28)	28 (-46–28)	0.17
Obstructive sleep apnea severity			
Percentage change in AHI	-67 (-89–-25)	-70 (-86–-42)	0.85
Percentage change in mean SpO_2	0 (0–1)	0 (0–1)	0.55
Percentage change in minimal SpO_2	2 (-1–9)	2 (-2–6)	0.50

Note: Data are expressed as median (interquartile range) or number (%).

AHI, apnea–hypopnea index; BMI: body mass index, IL, interleukin; SpO_2, pulse oxygen saturation.

[a]Data were compared using the Mann-Whitney *U* test or chi-square test as appropriate.

The significant correlations are marked in bold.

Table 2. Correlations of systemic Inflammation with patient characteristics and obstructive sleep apnea severity in children with obstructive sleep apnea

	Non-Healthy-weight group				Healthy-weight group			
Variables	**IL-6**	**IL-10**	**Percentage change in IL-6**	**Percentage change in IL-10**	**IL-6**	**IL-10**	**Percentage change in IL-6**	**Percentage change in IL-10**
Before adenotonsillectomy								
Patient characteristics	n = 33	n = 33	n = 31	n = 31	n = 33	n = 33	n = 32	n = 32
Age	0.10	0.06	-0.21	-0.05	-0.14	-0.17	0.01	0.21
Male	-0.27	0.03	0.24	-0.12	0.13	-0.03	0.02	0.22
Chronic tonsillitis	0.11	0.12	0.06	-0.01	-0.03	0.14	0.24	-0.39[a]
BMI z-score	0.21	-0.03	0.01	0.11	0.01	-0.05	-0.08	0.06
Systemic inflammation	n = 31	n = 31	n = 31	n = 31	n = 32	n = 32	n = 32	n = 32
IL-6	1.00	0.06	-0.31	-0.15	1.00	0.05	**-0.45**[a]	-0.13
IL-10	0.06	1.00	0.08	**-0.76**[c]	0.05	1.00	0.19	**-0.90**[c]
Obstructive sleep apnea severity	n = 31	n = 31	n = 31	n = 31	n = 32	n = 32	n = 32	n = 32

AHI	0.41[a]	-0.22	-0.21	0.16	-0.14	-0.09	-0.05	0.19
Mean SpO_2	-0.36[a]	-0.04	0.26	0.02	-0.01	0.16	0.11	-0.12
Minimal SpO_2	-0.33	0.12	0.13	0.05	-0.31	0.14	-0.04	-0.26
Percentage change	n = 31	n = 31	n = 31	n = 31	n = 32	n = 32		
Patient characteristics								
Percentage change in BMI z-score	-0.35	0.30	-0.08	-0.20	-0.04	-0.20	0.04	0.12
Systemic inflammation								
Percentage change in IL-6	-0.31	0.08	1.00	-0.29	-0.45[a]	0.19	1.00	-0.32
Percentage change in IL-10	-0.15	**-0.76[c]**	-0.29	1.00	-0.13	**-0.90[c]**	-0.32	1.00
Obstructive sleep apnea severity								
Percentage change in AHI	-0.34	0.35	0.21	-0.21	0.17	0.11	-0.15	-0.23
Percentage change in mean SpO_2	0.24	-0.25	-0.20	0.14	0.09	0.03	-0.01	-0.01
Percentage change in minimal SpO_2	0.23	-0.11	-0.15	0.08-	-0.44[a]	-0.06	0.09	0.27

Note: Spearman correlation coefficients are tabulated.

AHI, apnea–hypopnea index; BMI, body mass index; IL, interleukin; SpO_2, pulse oxygen saturation.

The significantly level is indicated as follows: [a] $P \geq .01$–$< .05$, [b] $P \geq .001$ –$< .01$, [c] $P < .001$.

After the *Bonferroni* correction, the significant correlations are marked in bold.

Table 3. Correlations of patient characteristics with the other minor tonsil phyla in children with obstructive sleep apnea

	Non-Healthy-weight group					Healthy-weight group				
Phyla	**Age**	**Male**	**Chronic tonsillitis**	**BMI z-score**	**Percentage change in BMI z-score**	**Age**	**Male**	**Chronic tonsillitis**	**BMI z-Score**	**Percentage change in BMI z-score**
	n = 33	n = 33	n = 33	n = 33	n = 31	n = 33	n = 33	n = 33	n = 33	n = 32
Synergistetes	0.35[a]	-0.02	0.27	0.01	0.06	0.22	0.20	0.12	-0.22	-0.17
Coprothermobacteraeota	-0.04	-0.13	0.54[b]	-0.06	-0.21	0.38[a]	0.17	-0.31	0.12	-0.17
Thermotogae	-0.01	-0.29	**0.66[c]**	0.04	-0.31	0.37[a]	0.07	-0.24	0.08	0.05
Entotheonellaeota	0.08	-0.42[a]	0.38[a]	0.24	-0.25	-0.02	-0.25	-0.12	-0.22	-0.05
Hydrogenedentes	0.04	-0.46[a]	**0.67[c]**	0.09	-0.37[a]	0.19	0.13	-0.12	-0.26	0.03
Rokubacteria	-0.01	-0.29	**0.66[c]**	0.04	-0.19	-0.05	-0.50[b]	-0.06	0.26	0.08
Dependentiae	-0.04	-0.17	0.51[b]	-0.03	-0.17	0.12	-0.26	-0.03	0.14	-0.22
Zixibacteria	-0.04	-0.13	0.54[b]	-0.06	-0.21	0.26	0.06	-0.24	0.23	-0.12
Margulisbacteria	0.03	-0.15	0.12	-0.20	-0.43[a]	0.01	0.01	-0.21	0.35[a]	0.01
Deinococcus Thermus	-0.12	-0.07	0.02	-0.25	-0.37[a]	0.20	0.09	0.05	-0.22	0.03

Planctomycetes	-0.21	-0.18	0.27	0.17	**-0.61**[c]	0.01	-0.32	-0.09	0.01	0.24
Deferribacteres	0.32	0.16	0.09	0.19	-0.10	0.48[b]	0.13	-0.28	0.21	-0.18
Gemmatimonadetes	-0.16	-0.13	0.25	-0.01	-0.29	-0.26	-0.36[a]	0.08	0.27	-0.23
Halanaerobiaeota	0.24	0.08	-0.08	0.17	0.16	-0.17	-0.36[a]	0.12	-0.01	0.15
Eremiobacteraeota (WPS-2)	-0.07	-0.30	0.26	0.16	-0.23	-0.14	-0.20	0.40[a]	0.09	-0.04
Caldiserica	0.02	-009	0.25	0.11	-0.09	0.25	0.13	-0.37[a]	0.20	-0.16

Note: Spearman correlation coefficients are tabulated.

Notably, the phyla without statistically significant association with any patient characteristic and inflammatory biomarkers are not listed.

AHI, apnea–hypopnea index; BMI, body mass index; IL, interleukin; SpO_2, oxygen saturation measured by pulse oximetry.

The significantly level is indicated as follows: [a] $P \geq .01$–$< .05$, [b] $P \geq .001$ –$< .01$, [c] $P < .001$.

After the *Bonferroni* correction, the significant correlations are marked in bold.

Figure legends

Figure 1. Tonsil microbiome in children with obstructive sleep apnea. A, The operational taxonomic unit (OTU) tree of the non-healthy-weight group (n = 33) included 9318 OTUs, assessed by Graphical Phylogenetic Analysis. B, The OTU tree of the healthy-weight group (n = 33) included 9886 OTUs. C, The relative abundances of top 10 tonsil phyla in the non-healthy-weight group. D, The relative abundances of top 10 tonsil phyla in the healthy-weight group.

Figure 2. Correlations between the top 10 tonsil phyla, baseline variables of interest, and corresponding percentage changes. A, In the non-healthy-weight group, one minor phylum of top 10 tonsil phyla was associated with age, and three minor phyla were associated with chronic tonsillitis at baseline. B, In the healthy-weight group, there were significant associations among systemic inflammatory biomarkers (IL-6, IL-10), severity variables of obstructive sleep apnea (AHI, mean SpO_2, minimal SpO_2), and the top 10 tonsil phyla (two major and two minor phyla). Abbreviations: AHI, apnea-hypopnea index; BMI, body mass index; IL, interleukin; SpO_2, pulse oxygen saturation. [a] $P < .05$.

Figure 3. Comparisons of variables of interest before and after adenotonsillectomy (AT). A, Body mass index (BMI) z-scores in the non-healthy-weight group were higher than those in the healthy-weight group before and after AT. A significant increase in BMI z-score was noted after AT in the healthy-weight group. B, Serum levels of interleukin (IL)-6 in the non-healthy-weight group were comparable with

those in the healthy-weight group before and after AT. The serum level of IL-6 reduced after AT in the healthy-weight group. C, Serum levels of IL-10 in both groups were comparable before and after AT; their changes were not statistically significant. D, Baseline apnea-hypopnea index (AHI) in the non-healthy-weight group was comparable with that in the healthy-weight group. After AT, AHIs in the two groups significantly reduced. However, residual AHI in the non-healthy-weight group was significantly higher than that in the healthy-weight group. E, Differences and changes in mean pulse oxygen saturation (SpO_2) were parallel to those in AHI. F, Baseline minimal SpO_2 in the non-healthy-weight group was significantly lower than that in the healthy-weight group. After AT, minimal SpO_2 in the two groups significantly increased. However, postoperative minimal SpO2 in the non-healthy-weight group was still lower than that in the healthy-weight group. [a] $P \geq .01$–$< .05$, [b] $P \geq .001$ – $< .01$, and [c] $P < .001$.

Appendices

Table E1. Correlations of variables of interest with the tonsil phyla in the non-healthy-weight group (n = 31)

Table E2. Correlations of variables of interest with the tonsil phyla in the healthy-weight group (n = 32)

Figure E1. Flow diagram. The 'non-healthy-weight' group included 33 children with obstructive sleep apnea and the healthy-weight group included 33 children with obstructive sleep apnea. Both groups were matched by the proportion of chronic tonsillitis. All participants underwent adenotonsillectomy and 3 lost to follow-up. Therefore, 66 participants were included in the primary analysis and 63 were included in the outcome analysis.