



Contents lists available at ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: www.elsevier.com/locate/ijporl



Th17/Treg balance in children with obstructive sleep apnea syndrome and the relationship with allergic rhinitis

Kun Ni, Limin Zhao, Jiali Wu, Wei Chen, HongyaYang, Xiaoyan Li *

Department of Otolaryngology-Head & Neck Surgery, Shanghai Children's Hospital, Shanghai Jiao Tong University, Shanghai, China

ARTICLE INFO

Article history:

Received 20 January 2015

Received in revised form 19 June 2015

Accepted 20 June 2015

Available online xxx

Keywords:

Children

Obstructive sleep apnea hypopnea syndrome

Adenoidal hypertrophy

Th17/Treg

Allergic rhinitis

ABSTRACT

Objective: This study aims to explore the role of the Th17 to Treg cell ratio in children with OSA and its relationship with allergic rhinitis.

Methods: The study included 127 children diagnosed with OSA by polysomnography (PSG) testing and 29 children without OSA. The 127 children with OSA were divided into the following groups: OSA with moderate adenoidal hypertrophy ($n = 47$), OSA with severe adenoidal hypertrophy ($n = 49$), and OSA complicated by allergic rhinitis (AR) ($n = 31$). The adenoids of the 29 children without OSA were mildly hypertrophic. We measured the number of Th17 and Treg cells, the levels of related serum cytokines in cellular secretions, and the expression of key transcription factors in both the peripheral blood and adenoid tissue. The Th17/Treg ratio was calculated and analyzed between groups. The numbers of Th17 and Treg cells were measured by flow cytometry; the secreted IL-17, IL-10, and TGF- β were measured by ELISA; and the expression levels of ROR γ t and Foxp3 were measured by RT-PCR.

Results: Compared with the control group, OSA children exhibited a significant increase in the number of peripheral Th17 cells, Th17-related cytokine secretion (IL-17), and ROR γ t mRNA levels, whereas they exhibited a decrease in the number of Treg cells, Treg-related cytokine secretions (IL-10, TGF- β) and Foxp3 mRNA levels. The Th17/Treg ratio was higher ($p < 0.05$) in the OSA groups than in the control group. The Th17/Treg ratio was correlated with the size of the adenoids. We also found that the Th17/Treg balance in OSA patients was complicated by allergic rhinitis; the increase was significantly larger in the AR group ($p < 0.05$, $p = 0.021$) than in OSA groups without AR. These results were observed in both the peripheral blood and local adenoid tissue.

Conclusion: The Th17/Treg imbalance may increase the risk of developing OSA, and AR may promote the development of the disease. These results provide an alternative explanation for OSA pathogenesis that warrants additional research and presents new directions for the prevention and treatment of OSA in children.

© 2015 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

OSA in children is usually caused by structural or functional abnormalities or by the obstruction of the upper airway. The most

common cause of OSA in children is tonsil and/or adenoid hypertrophy. Adenoid tissue in the mucosa-associated lymphoid system of the upper respiratory tract is an important part of the Waldeyer ring. Inflammation in the nasopharynx and adjacent areas may cause repeated stimulation of the inflammatory response due to pathological adenoid hyperplasia [1]. Immune activation in the adenoids may occur following a variety of external stimuli, and the imbalance of local immune cells and an abnormal inflammatory response may lead to delayed healing, causing adenoidal hypotrophy. The adenoids' local immune status is closely linked to the incidence of OSA. The synchronization of the inflammatory states of both the adenoids and the peripheral blood of children with OSA can help us understand the pathogenesis of OSA.

Sleep is closely related to the body's immune system. Ganz [2] believed that sleep is associated with immune function, and this

Abbreviations: OSA, obstructive sleep apnea hypopnea syndrome; AHI, apnea-hypopnea index; AR, allergic rhinitis; PBMC, peripheral blood mononuclear cells; PSG, polysomnography; Foxp3, forkhead box P3; ROR γ t, retinoic acid related orphan receptor γ t; TGFBR3, transforming growth factor, beta receptor III.

* Corresponding author at: Department of Otolaryngology-Head & Neck Surgery, Shanghai Children's Hospital, Shanghai Jiaotong University, Shanghai, Luding Road, No. 355, Putuo District, Shanghai 200062, China.
Tel.: +86 2118917128276; fax: +86 2162790494.

E-mail addresses: nikun1008@163.com (K. Ni), zlmzmh@163.com (L. Zhao), wujiali540@hotmail.com (J. Wu), cwjsha@126.com (W. Chen), yaya041202@hotmail.com (HongyaYang), chshshent@163.com (X. Li).

<http://dx.doi.org/10.1016/j.ijporl.2015.06.026>

0165-5876/© 2015 Elsevier Ireland Ltd. All rights reserved.

relationship is partially based on the physiological basis of sleep, sleep architecture, the sleep-wake cycle, cytokines and the hypothalamic-pituitary axis. Many immune functions are dependent on circadian rhythms and regular sleep. Sleep disorders can cause changes in the number and function of immune cells, particularly T cells. Over the past decade, scholars have begun to focus research on subsets of T cells in OSA patients, including the Th1 and Th2 cell populations. Bollinger [3] found that there is a certain 24-h rhythm of the regulatory T cells (Treg) and that sleep deprivation seriously disrupts Treg cell function. Alert [4] studied patients with Th1-type active mode-based OSA, and Dyugovskaya [5] suggested that compared with wakefulness, early nocturnal sleep induced a shift in the Th1/Th2 cytokine balance towards increased Th1 activity; however, the Th1 shift was only moderate and was replaced by Th2 dominance during late sleep [6]. In recent years, Th17 and Treg T cell populations have also been discussed. Th17 cells are key effectors of the immune response. Th17 cells expressing retinoic acid-related orphan receptor γ t (ROR γ t) play critical roles in the development of autoimmunity and allergic reactions through IL-17 production. Treg cells expressing the forkhead/winged helix transcription factor (Foxp3) orchestrate the overall immune response and play a role in immune tolerance through contact-dependent suppression or by regulating the activity of the effector T cells or releasing anti-inflammatory cytokines, such as interleukin-10 (IL-10) and transforming growth factor β (TGF- β 1). Sade [7] found that the Th17/Treg ratio negatively correlates with the clinical score for OSA independent of age and gender. Jin Ye [8] found that the Th17/Treg ratio is elevated in patients with OSA and positively correlates with apnea–hypopnea index (AHI). Anderson [9] found that CD4 T cells are increased locally in the tonsils of children with OSA, whereas CD8 T cells are reduced, and that the Th17 cell population is affected. Although these results are varied and have yet to produce a unified conclusion, it is clear that T cell population imbalances, either Th1/Th2 or Th17/Treg imbalances, are observed in patients with OSA. This issue, however, requires further study and discussion.

Therefore, we have designed a research study of preschool-aged children (3–6 years) to measure the number of Th17 and Treg cells as well as the expression of related genes and cytokines in both the peripheral blood and adenoid tissue. Our aims are to investigate the significance of the Th17/Treg ratio in children with OSA, including the effect of changes in this ratio on the local and systemic inflammatory response, and to investigate how allergic rhinitis affects the Th17/Treg ratio in children with OSA.

2. Patients and methods

2.1. Patient information

Children (ages 3 to 6 years) with OSA newly confirmed by overnight PSG were initially recruited into the OSA group. Children diagnosed with chronic tonsillitis who underwent surgical treatment and were excluded from a diagnosis of OSA by PSG were recruited into the control group. All patients were determined to be free of other cardiovascular, endocrine, urinary system, metabolic and neuromuscular diseases and disorders. The selected patients had no specific response to aspirin, bronchiectasis, typical histories of autoimmune diseases, or severe respiratory infection within two weeks prior to enrollment. Any history of allergic rhinitis, asthma or other allergic diseases was recorded for all children. Skin prick tests were performed to assess allergens, and fiber nasopharyngoscopy was performed to assess adenoid size. Routine preoperative examinations were performed to exclude surgical contraindications.

The fiber nasopharyngoscopy was performed by the fixed endoscopist in the Endoscopy room.

Based on the results of the fiber nasopharyngoscopy, adenoid size was classified into four groups of differing degrees of blockage: level I (0–25% nostril blockage), level II (26–50% blockage), level III (51–75% blockage), and level IV (76–100% blockage) [2]. Levels I and II included patients who were mildly hypertrophic, which normally will not cause obstruction of the airway. Level III included patients who were moderately hypertrophic, and level IV included patients who were severely hypertrophic. According to the “children with obstructive sleep apnea syndrome treatment guidelines” published in 2007 by the Chinese Journal of Otorhinolaryngology Head and Neck, a diagnosis of OSA is established when the obstructive apnea index (AOI) is greater than 1/h or the obstructive apnea hypopnea index (AHI) is greater than 5 times/h and the lowest oxygen saturation is less than 92%.

Children with adenoid levels I and II hypertrophy had an AHI lower than 5, no complaints of sleep apnea and no allergic rhinitis, which is called group I (adenoid level I and level II) for short. The OSA children were divided into groups: group II was the adenoid III group (moderate hypertrophy group), group III was the adenoid IV group (severe hypertrophy group), and group IV was the OSA + AR group (OSHAS children with concurrent allergic rhinitis). The study was approved by the Institutional Review Board of Shanghai Children's Hospital. The parents of all of the children provided informed consent to take the children's blood and adenoid tissue for research.

2.2. ROR γ t and Foxp3 expression determined by real-time quantitative PCR

Total RNA was extracted from the peripheral blood PBMCs by TRIzol extraction (TRIzol Reagent, Ambion) according to the manufacturer's instructions. Total RNA extraction from the adenoids was performed as follows. Approximately 100 mg of tissue was placed into an EP tube, 1 mL of TRIzol was added, and RNA was extracted per the instructions. The concentration of the sample was measured, and the OD260/OD280 ratio was 1.91. The extracted RNA was of high purity, and there were no residual proteins or DNA. cDNA was synthesized using random hexamer primers and RNase H⁻ reverse transcriptase (Invitrogen). The mixture was prepared in 0.2 mL RNase-free EP tubes, and the reaction was performed at 37 °C for 15 min followed by 5 s at 85 °C. The primer pairs used are shown in Table 1. The resulting cDNA was diluted with 10 μ L ddH₂O and was frozen at –20 °C. The product could then be used for direct 2nd-strand cDNA synthesis. The recommended maximum volume of cDNA for use in PCR amplification was 1 μ L.

Relative gene expression was calculated using the comparative CT method. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene for normalization, and a no template sample was used as the negative control. All reactions were carried out in triplicate per sample.

Table 1
Primer sequences.

Gene	Primer	Product length (bp)	<i>T_m</i> (°C)
GAPDH	5-GGCTGTGGGCAAGGTCATCCCTG-3	101	67.62
	5-GACGGCAGGTCAGGTCCACCACTG-3		68.96
FOXp3	5-GGCAGCCAAGGCCCTGCTGTC-3	91	70.07
	5-GGCTACCCACAGGTGCTCCGG-3		70.87
ROR γ t	5-GTGCCCAACCTACCGAGGCC-3	148	71.51
	5-TAGGCCCGCACATCTAACCAGC-3		68.46

Table 2

Baseline characteristics of study population.

Variables	Group I	Group II	Group III	Group IV
Subjects (n)	29	47	49	31
Age (years)	6.51 ± 2.42	5.62 ± 4.20	4.86 ± 7.21	5.50 ± 3.98
AHI (episodes/h)	1.07 ± 1.56	6.95 ± 4.24 ^a	8.73 ± 8.52 ^b	8.36 ± 2.51 ^c
SaO ₂ < 90% (%TST)	95.01 ± 3.46	85.33 ± 11.70 ^a	82.94 ± 8.19 ^b	83.56 ± 12.54 ^c

Values are expressed as mean ± SD. Differences between the values were determined using Student's *t*-test.

^a *p* < 0.05, group I versus group II.

^b *p* < 0.05, group I versus group III.

^c *p* < 0.05, group I versus group IV.

2.3. Flow cytometry analysis of Treg and Th17 cells

2.3.1. Blood samples and PBMC isolation

Three milliliters of peripheral blood (PB) samples was collected into collection tubes containing sodium heparin. An equal volume of HBSS (Hank's balanced salt solution, pH 7.2–7.4) was added to the peripheral blood. The PBMCs were prepared by Ficoll–Hypaque density centrifugation and were resuspended at a density of 2×10^6 cells/mL for analysis by flow cytometry.

2.3.2. Preparation of PBMCs from adenoid tissue

The adenoid tissue was rinsed for 10 s in a 75% ethanol solution and then cleaned using PBS containing a 10% antimicrobial solution. After being rinsed for 10 s in RPMI-1640 medium, the tissue was cut into pieces. The broken tissue was ground in 40 mM mesh with a syringe needle core to give a single cell suspension in 10% FBS + RPMI-1640. Ficoll–Hypaque density centrifugation was used to purify the PBMCs, and the PBMCs were suspended at a density of 1×10^6 cells/mL.

2.3.3. Cell culture

The PBMC suspension (2×10^6 cells/mL) was put into T25 flasks, and 3 mL of media including PMA (50 ng/mL, Sigma, USA), ionomycin calcium salt (1 µg/mL, Sigma, USA) and BFA (10 µg/mL) was added. The incubator was set at 37 °C under a 5% CO₂ environment for 4 h of culture.

Cultured cells in solution were subjected to human Th17 and Treg analysis (the experimental procedure was performed in accordance with instructions from BD Inc. (560762)). The stained cells were analyzed by flow cytometry using a FACScan flow cytometer (Becton Dickinson) equipped with CellQuest Pro 5.2 software (BD Biosciences, USA).

Table 3

Summary of RT-PCR, flow cytometry, and ELISA results from peripheral in different groups.

Variables	Group I	Group II	Group III	Group IV
mRNA expression of transcription factors (RT-qPCR)				
RORγt/GADPHmRNA	5.37 ± 3.19	28.01 ± 6.65 ^a	38.53 ± 9.72 ^b	53.50 ± 7.49 ^{c,e}
Foxp3/GADPHmRNA	2.72 ± 0.34	1.82 ± 0.43 ^a	1.07 ± 0.59 ^{b,d}	0.21 ± 0.98 ^{c,e}
T-cell counts (flow cytometry)				
Th17 (% of CD4+)	0.97 ± 0.38	1.84 ± 0.25 ^a	2.87 ± 0.43 ^b	3.24 ± 1.07 ^{c,e}
Treg (% of CD4+)	1.91 ± 0.56	0.46 ± 0.38 ^a	0.36 ± 0.15 ^b	0.23 ± 0.26 ^{c,e}
Th17/Treg ratio	0.54 ± 0.32	4.22 ± 1.82 ^a	7.52 ± 2.36 ^{b,d}	13.21 ± 4.25 ^{c,e}
Levels of cytokines (pg/mL) (ELISA)				
IL-17 (pg/mL)	1.52 ± 1.43	2.58 ± 0.34 ^a	3.11 ± 1.07 ^b	4.66 ± 1.92 ^{c,e}
IL-10 (pg/mL)	53.87 ± 11.45	47.68 ± 3.45	45.35 ± 7.43	31.67 ± 13.59 ^c
TGF-β (pg/mL)	139.65 ± 23.68	80.42 ± 5.67 ^a	48.72 ± 10.35 ^{b,d}	30.11 ± 9.36 ^{c,e}

Values are expressed as mean ± SD. Differences between the values were determined using Student's *t*-test.

^a *p* < 0.05, group I versus group II.

^b *p* < 0.05, group I versus group III.

^c *p* < 0.05, group I versus group IV.

^d *p* < 0.05, group III versus group II.

^e *p* < 0.05, group IV versus group III.

2.4. Detection of cytokine levels

The PBMC plasma levels of IL-17, TGF-β1, and IL-10 were measured by enzyme-linked immunosorbent assay (ELISA) following the manufacturer's instructions (ELISA kits).

2.5. Statistical analysis

The values in all of the tables and figures are expressed as the means ± standard deviation (SD). Statistically significant differences between the values were determined using Student's *t*-tests. Grouped data were analyzed using a one-way analysis of variance (ANOVA). Statistical analyses were performed using a commercial software package (SPSS, version 16.0 for Windows; SPSS Inc., Chicago, IL). *p*-Values less than 0.05 indicate significance.

3. Results

3.1. Subject characteristics

A total of 156 children were assessed, with 29 in group I (the control group without OSA and with levels I and II adenoid), 47 in group II (the OSA group with level III adenoids), 49 in group III (the OSA group with level IV adenoids), and 31 in group IV (OSHAS children with allergic rhinitis). The characteristics of the study population are shown in Table 2.

Preschool children aged 3–6 years were enrolled, and no statistically significant differences in age were found between the four groups (*p* > 0.05; *p* = 0.701). Based on PSG monitoring, the AHI and oxygen saturation of the control group were deemed normal. The AHIs of the OSA groups and OSA + AR group were greater than 5, and the lowest oxygen saturations were below 92%. The differences in AHI values and oxygen saturation compared with the controls were significant in the OSA + AR group and the OSA groups without AR (*p* < 0.05; *p* = 0.023 and *p* = 0.027, respectively). However, there was no significant difference between the level III-adenoid OSA group and the level IV-adenoid OSA group.

Summaries of the RT-qPCR, flow cytometry, and ELISA results from the peripheral blood of the control group and the OSA groups are shown in Table 3. The results from the adenoid tissue are summarized in Table 4. The data for each group were statistically analyzed.

3.2. The expression of Foxp3 and RORγt mRNA in the PBMCs and adenoid tissue

RORγt is an important transcription factor for the differentiation of Th17 cells, whereas Foxp3 is the master transcription factor

Table 4

Summary of RT-PCR, flow cytometry, and ELISA results from adenoid tissue in different groups.

Variables	Group I	Group II	Group III	Group IV
mRNA expression of transcription factors (RT-qPCR)				
RORγt/GADPHmRNA	11.52 ± 4.97	82.78 ± 6.41 ^a	96.45 ± 10.47 ^{b,d}	112.64 ± 21.55 ^{c,e}
Foxp3/GADPHmRNA	3.81 ± 0.31	2.42 ± 0.87 ^a	1.56 ± 0.58 ^{b,d}	0.92 ± 0.63 ^{c,e}
T-cell counts (flow cytometry)				
Th17 (% of CD4+)	1.53 ± 0.39	2.64 ± 0.57 ^a	3.63 ± 1.01 ^{b,d}	3.74 ± 0.72 ^{c,e}
Treg (% of CD4+)	10.61 ± 2.62	1.12 ± 0.92 ^a	0.82 ± 0.53 ^{b,d}	0.39 ± 0.07 ^{c,e}
Th17/Treg ratio	0.20 ± 0.07	2.41 ± 0.99 ^a	4.49 ± 1.25 ^{b,d}	9.97 ± 2.10 ^{c,e}
Levels of cytokines (pg/mL) (ELISA)				
IL-17 (pg/mL)	42.15 ± 11.98	53.72 ± 8.38 ^a	55.70 ± 9.45 ^b	63.76 ± 8.52 ^{c,e}
IL-10 (pg/mL)	14.43 ± 5.68	7.69 ± 4.20 ^a	7.01 ± 2.58 ^b	3.67 ± 1.59 ^c
TGF-β (pg/mL)	875.32 ± 43.56	649.54 ± 28.64 ^a	433.09 ± 19.51 ^{b,d}	300.11 ± 19.54 ^{c,e}

Values are expressed as mean ± SD. Differences between the values were determined using Student's *t*-test.^a *p* < 0.05, group I versus group II.^b *p* < 0.05, group I versus group III.^c *p* < 0.05, group I versus group IV.^d *p* < 0.05, group III versus group II.^e *p* < 0.05, group IV versus group III.

in Treg cells. The results showed that in the peripheral blood and adenoid tissue, RORγt levels were higher in the OSA groups than in the control group, and the differences in RORγt expression were associated with adenoid size (Fig. 1A). Relative RORγt expression in the peripheral blood of group III (38.53 ± 9.72) was higher than in group II (28.01 ± 6.65) (*p* = 0.048, *p* < 0.05), and relative RORγt expression was 53.50 ± 7.49 in group IV, which was significantly higher than group I (5.37 ± 3.19) (*p* = 0.004, *p* < 0.05) and group III (*p* = 0.023, *p* < 0.05). Furthermore, relative Foxp3 expression was reduced in group II (*p* = 0.028, *p* < 0.05), group III (*p* = 0.025, *p* < 0.05), and group IV (*p* = 0.017, *p* < 0.05), and there was also a significant difference between the two OSA groups distinguished by adenoid size (*p* = 0.039, *p* < 0.05) (Fig. 1B).

3.3. Analysis of the Th17 cell population, Treg cell population and ratio of Th17/Treg cells in the peripheral blood and adenoid tissue

Compared with the control group, the percentages of Th17 cells in the peripheral blood and adenoid tissue were higher in the OSA groups, whereas the percentage of Treg cells was reduced. The Th17/Treg ratios in the OSA groups increased with adenoid size (*p* = 0.043, *p* < 0.05). The Th17/Treg ratio in the peripheral blood from group I was 0.54 ± 0.32, and the Th17/Treg ratios were 4.22 ± 1.82^a and 7.52 ± 2.36 in group II and group III, respectively. The Th17/Treg ratio in group IV was 13.21 ± 4.25, which was significantly higher than group I (*p* = 0.007, *p* < 0.01) and group III

(*p* = 0.038, *p* < 0.05) (Fig. 2A). We observed similar results from the adenoid tissue; namely, the Th17/Treg ratio was higher in the OSA groups than in the control group, and the increase was associated with both adenoid size and the co-diagnosis of AR (Fig. 2B).

3.4. Concentrations of the related cytokines IL-17, IL-10, and TGF-β

The levels of IL-17, IL-10, and TGF-β were detected in the four groups using ELISA tests (Table 3). The cytokine values were statistically analyzed, and we found that TGF-β levels were significantly different among the four groups both in the peripheral blood and the adenoid tissue samples (Fig. 3). According to the data from the peripheral blood and adenoid tissue, the IL-17 levels in group II and group III were significantly higher than in group I (*p* = 0.041, *p* = 0.035, *p* < 0.05), but there was no difference between group II and group III. IL-17 levels in group IV were higher than in group I and group III. IL-10 levels from the adenoid tissue in group II and group III were not different compared to group I, but IL-10 levels in group IV were lower than in group I (*p* = 0.033, *p* < 0.05). There was no difference between group I, group II, and group III with respect to the adenoid tissue IL-10 levels. However, we can see that the adenoid tissue IL-10 levels in group IV were higher than in group I and group III, relative to the peripheral blood levels. In both the peripheral blood and adenoid tissue samples, the IL-17 and IL-10 levels were also significantly different between group II and group III (Fig. 4A and B).

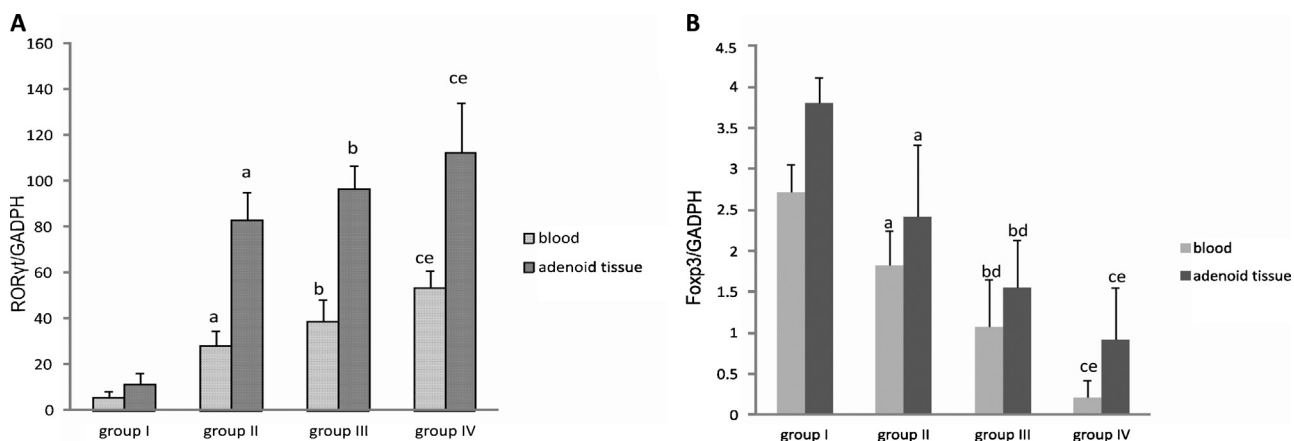


Fig. 1. Expression levels of RORγt in PBMCs from the peripheral blood and adenoid tissue. (A) RORγt/GADPH mRNA ratios were compared between the four groups. (B) Foxp3/GADPH mRNA ratios were compared between the four groups. ^a *p* < 0.05, group I versus group II; ^b *p* < 0.05, group I versus group II; ^c *p* < 0.05, group I versus group IV.

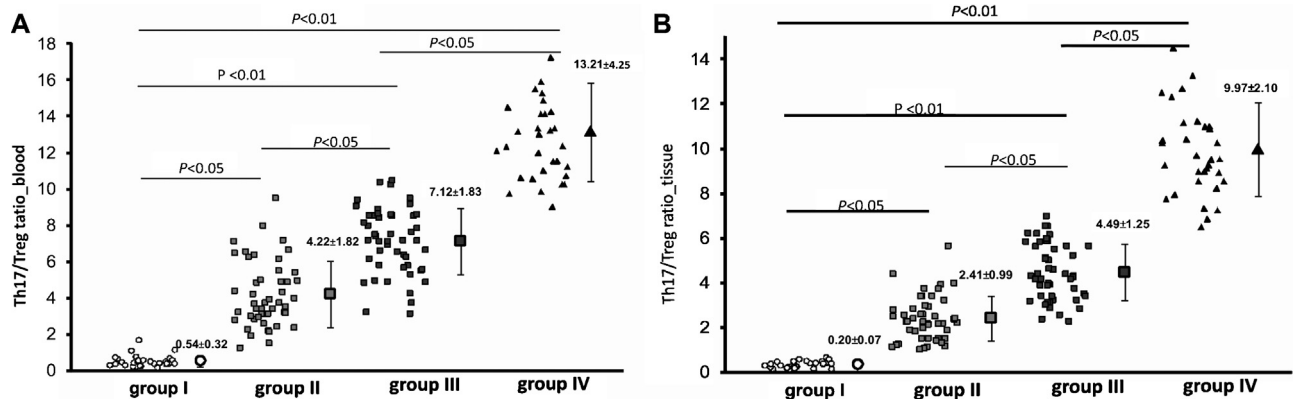


Fig. 2. The Th17/Treg ratio increased significantly between the four groups. (A) The Th17/Treg ratios in the OSA groups increased in the PBMCs from peripheral blood. (B) The Th17/Treg ratios in the OSA groups increased in the PBMCs from adenoid tissue.

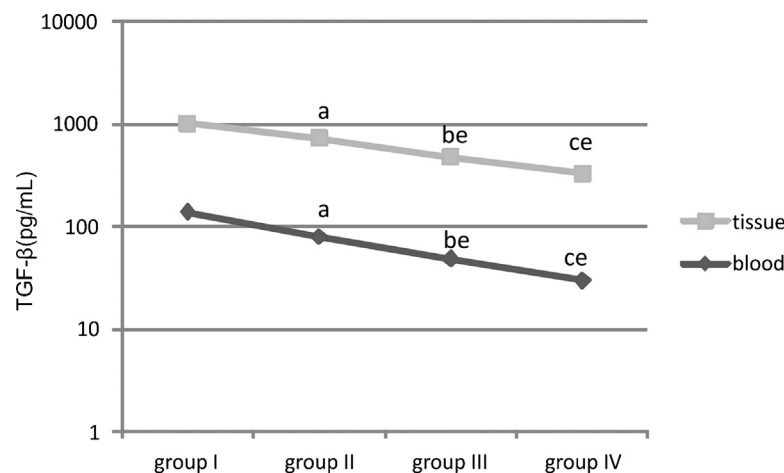


Fig. 3. TGF-β levels in the peripheral blood and adenoid tissue. The data represent the plasma TGF-β concentrations in the four groups. The data are expressed as the mean ± SD; ^a $p < 0.05$, group I versus group II; ^b $p < 0.05$, group I versus group II; ^c $p < 0.05$, group I versus group IV; ^d $p < 0.05$, group III versus group II; ^e $p < 0.05$, group IV versus group III.

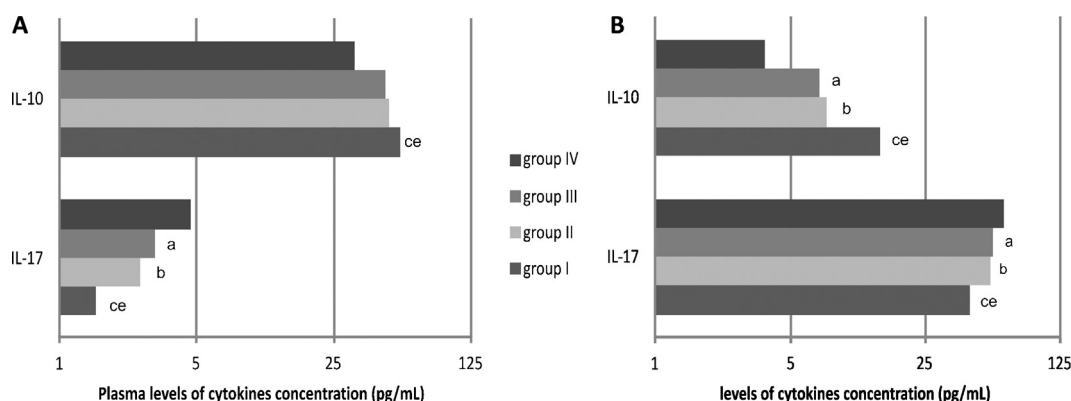


Fig. 4. IL-17 and IL-10 concentrations in the peripheral blood (A) and adenoid tissue (B). IL-17 concentrations were significantly higher in group IV, and the IL-10 concentrations were lower in group IV. The concentrations of IL-17 and IL-10 were not different between group II and group III. The data are expressed as the mean ± SD; ^a $p < 0.05$, group I versus group II; ^b $p < 0.05$, group I versus group II; ^c $p < 0.05$, group I versus group IV; ^e $p < 0.05$, group IV versus group III.

4. Discussion

The newly discovered Th17 cells are a class of cells independent of the Th1 and Th2 T cell subsets. Th17 cells express RORγt and mainly secrete interleukin-17A (IL-17A). Th17 cells are an important class of effector T cells that regulate the body's fight against extracellular pathogens while also promoting the occurrence of autoimmune and inflammatory diseases. Th17 cells play

an important role in the development of immunity, mainly by secreting IL-17. TNF-α and IL-6 also have a role in this process. Unlike Th17 cells, human regulatory T cells (Treg) maintain peripheral tolerance. Treg cells express FoxP3 and complete the immunosuppressive function primarily through direct contact inhibition or by inhibiting cytokine IL-10 and TGF-β and adjusting the activity of effector T cells. Treg and Th17 cells have opposite effects on autoimmunity and inflammation. Both can arise from

T0 cell differentiation. T cell development is plastic and mutual differentiation may occur depending on the local cytokine microenvironment. Some studies [10] have shown that Treg cells are able to differentiate into IL-17-producing cells and that human peripheral blood and lymphoid tissue contain a significant number of CD4⁺ Foxp3⁺ T cells that have the capacity to produce IL-17 upon activation. In addition to the human Th1/Th2 balance, the balance between Treg and Th17 cells [11] is critical for controlling the development of autoimmune and inflammatory responses. The Th17/Treg balance has an important role in the development of a variety of human diseases, including cancer, inflammation, and autoimmune diseases [12–15]. Galon [16] suggested that an elevated Th17/Treg ratio may be a severe marker of autoimmune disease, inflammatory disease, and allergic disease, indicating that the equilibrium of different types of immune cells can be used to better predict clinical prognosis in patients. In summary, in consideration of the role that the Th17/Treg ratio has in other types of disease, we studied the relationship between changes in the Th17/Treg ratio in children with OSA and different adenoid sizes and co-diagnosis with allergic rhinitis. This research is founded in evidence and has important clinical significance.

Our results showed that children with OSA exhibited significant increases in the number of Th17 cells and the levels of Th17-related transcription factors (ROR γ t), as well as dramatic decreases in the number of Treg cells and the levels of Treg-related transcription factors (Foxp3) when compared with controls. The Th17/Treg ratios were also elevated in the OSA groups. These results were obtained from both the peripheral blood and adenoid tissue samples. Further, the Th17/Treg ratios were associated with adenoid size. Our data show a significant difference in the Th17/Treg ratio between group II and group III ($p < 0.05$), as well as the levels of ROR γ t and Foxp3. However, the levels of IL-17 and IL-10 were independent of adenoid size. Previous studies have demonstrated that the Th17/Treg ratio positively correlates with the severity of OSA [8,19]. It is known that adenoid hypertrophy is the main cause of OSA in children, and our results illustrate that the Th17/Treg ratio is associated with adenoid hypertrophy. Thus, the Th17/Treg ratio may be a factor in the development of OSA in children. We also found that the Th17/Treg ratio was higher in group IV compared to group III, and this difference was statistically significant. These positive results suggest that the Th17/Treg ratio is elevated in children with OSA and that the elevation is higher with the co-diagnosis of allergic rhinitis. Therefore, we believe that the Th17/Treg balance plays an important role in the pathogenesis of OSA in children and may be a co-factor in the development of OSA and/or a prognostic predictor of children with OSA. Aho V. reported [25] that the expression levels of the 10 most up-regulated and the 10 most down-regulated transcripts were correlated with a subjective assessment of insufficient sleep in a population cohort and that the 10 most down-regulated genes, including TGFBR3, were positively correlated with insufficient sleep. Thus, sleep disorders may down-regulate TGFBR3, which causes the levels of TGF- β to decrease, which in turn is associated with OSA severity. Our results show that the levels of TGF- β from the peripheral blood and adenoid tissue increased in the OSA groups and were even much higher in the OSA + AR group. These differences were associated with the adenoid size, which further illustrates this point.

As described above, sleep disorders impact the number and function of human immune cells [3] and lead to changes in Treg and effector T cells. Early experiments found [17,18] that in the micro-environment of OSA patients, the elevation of pro-inflammatory cytokines IL-17 and IL-6 and the reduction of TGF- β potentially promote the continued growth of Th17 cells while inhibiting Treg development, leading to Th17/Treg imbalance disorders. This disorder can enhance the inflammatory cytokines in

the microenvironment, eventually resulting in a positive feedback loop that amplifies the proinflammatory response. In a study of OSA in adults [8], the Th17/Treg ratio was higher in the peripheral blood of patients with OSA and was positively correlated with the severity of OSA. Studies of children with OSA have also yielded some results. Kim [19] studied 47 cases of children with OSA and found that the FOXP3 gene is more likely to display increased methylation in children with OSA who exhibit increased systemic inflammatory responses. FOXP3 DNA methylation levels are positively correlated with hsCRP levels and AHI, and high FOXP3 DNA methylation may favor the downregulation of Foxp3 protein expression, thus reducing the number of Treg cells. Anderson [9] also confirmed the presence of T cell balance disorders in children with OSA, and these imbalances were characterized by Th17 cell dominance. The findings of our study are consistent with these conclusions. We found that in the peripheral blood and adenoid tissue of children with OSA, the expression of Foxp3 is decreased and the number of Treg cells is reduced, whereas the number of Th17 cells is increased and Th17/Treg imbalance was apparent. We propose a possible mechanism that involves the fact that the children's adenoids, which are located in the nasopharynx, suffered long-term stimulation by various microorganisms. Upon acute infection, the adenoids experienced a local inflammatory response under the influence of various inflammatory factors. One of the inflammatory factors, IL-17, is a principal inflammatory factor that can promote CD4⁺ T cells to differentiate into Th17 cells and, in conjunction with reduced TGF- β , inhibit the development of Treg cells, causing a Th17/Treg imbalance. An increase in the Th17/Treg ratio contributes to the formation of a new microenvironment, and ultimately results in the formation of a positive feedback mechanism that amplifies the immune response. Repeated inflammatory stimuli result in the proliferation of local lymphoid tissue, which is expressed as adenoid hyperplasia. Hypertrophic adenoid tissues block the airway, causing apnea and hypopnea during sleep and resulting in OSA. Furthermore, sleep disorders will affect the normal balance of Th17 and Treg cells. Bollinger demonstrated that sleep deprivation severely disturbs the functional rhythm of Treg cells [3]. Aho et al. [25] demonstrated that partial sleep restriction affects the regulation of signaling pathways related to the immune system and that some of these changes appear to be long-lasting and may at least partly contribute to inflammation-associated pathological states. In other words, OSA may lead to persistent or worsening systemic inflammatory immune functioning. Thus, this mechanism explains our findings regarding the Th17/Treg ratio imbalances in both the peripheral blood and local adenoid tissue.

Previous studies have demonstrated [16,20–23] that Th17 has an important role in the pathogenesis of allergic rhinitis, and the Th17/Treg balance can be used to assess allergic rhinitis. In our study, allergic rhinitis was considered an influential factor in the analysis of the Th17/Treg ratio. Studies have shown [24] that adult OSA might trigger the development of autoimmune phenomena. Under these circumstances, a higher Th17/Treg ratio may result in a loss of tolerance and regulation and ultimately a persistent, low-degree systemic inflammatory reaction characterized by autoimmune or allergic diseases. We found that the Th17/Treg ratio was also significantly increased in patients with OSA and allergic rhinitis compared with the control group and the OSA groups without allergic rhinitis. Therefore, we believe that the Th17/Treg imbalance caused by AR and the Th17/Treg imbalance due to OSA disorder may mutually promote one another and cause further imbalance, which can lead to more obvious clinical symptoms. Therefore, regardless of which pathological changes appear first, AR or OSA, both will cause changes in the Treg and Th17 cells, leading to Th17/Treg imbalance disorders, and these disorders will have roles in promoting the development of other diseases.

Therefore, such patients should be treated simultaneously for both diseases. In cases where we are assessing the severity of a disease, treatment and prognosis, we should not ignore the impact of other concurrent diseases. The measurement and analysis of the Th17/Treg ratios in OSA children with or without concomitant allergic rhinitis are of great significance for the assessment of these conditions.

Inflammation caused by an immune response is a complex, dynamically updated process. Do differences in the duration of disease and differences in the age of patients affect the imbalance of T cell subsets? After surgery, medical treatment or positive pressure ventilation therapy in OSA children, is there a change in the Th17/Treg ratio? What should be done about the development of concomitant allergic diseases? Our research also needs to refine intervention factors based on further studies and a comprehensive analysis of the results.

5. Conclusion

Our data confirm that the Th17/Treg ratio is increased in the peripheral blood and local adenoid tissue of OSA children. In cases of OSA of similar severity, changes in the Th17/Treg ratio are associated with adenoid size. The Th17/Treg ratio is also elevated in OSA children with allergic rhinitis to a greater extent than in children without AR. The Th17/Treg ratio may play a critical role in the pathogenesis of OSA in children, and AR may promote the process.

Conflict of interest statement

None of the participating institutions and authors have conflicts of interest regarding the study.

Acknowledgments

This study was supported by the Youth Research Fund of the Shanghai Municipal Health Bureau Item, China (no. 20124Y058), and by a project of the Shanghai Committee of Science and Technology, China (Grant No. 12411952407).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ijporl.2015.06.026>.

References

- [1] (a) X. Huang, J. Wang, Practical Otolaryngology Head and Neck Surgery, second ed., People's Health Press, Beijing, 2008;
(b) F.D. Ganz, Sleep and immune function, Crit. Care Nurse 32 (2) (2012) e19–e25.
- [2] F.D. Ganz, Sleep and immune function, Crit. Care Nurse 32 (2012) 19–25.
- [3] T. Bollinger, A. Bollinger, L. Skrum, S. Dimitrov, T. Lange, W. Solbach, Sleep-dependent activity of T cells and regulatory T cells, Clin. Exp. Immunol. 155 (2009) 231–238.
- [4] A. Alberti, P. Sarchielli, E. Gallinella, A. Floridi, A. Floridi, G. Mazzotta, et al., Plasma cytokine levels in patients with obstructive sleep apnea syndrome: a preliminary study, J. Sleep Res. 12 (2003) 305–311.
- [5] L. Dyugovskaya, P. Lavie, M. Hirsh, L. Lavie, Activated CD8+ T-lymphocytes in obstructive sleep apnoea, Eur. Respir. J. 25 (2005) 820–828.
- [6] S. Dimitrov, T. Lange, S. Tieken, H.L. Fehm, J. Born, Sleep associated regulation of T helper 1/T helper 2 cytokine balance in humans, Brain Behav. Immun. 18 (2004) 341–348.
- [7] K. Sade, G. Fishman, S. Kivity, A. Derowe, S. Langier, Expression of Th17 and Treg lymphocyte subsets in hypertrophied adenoids of children and its clinical significance, Immunol. Invest. 40 (2011) 657–666.
- [8] J. Ye, H. Liu, G. Zhang, P. Li, Z. Wang, S. Huang, et al., The Treg/Th17 imbalance in patients with obstructive sleep apnoea syndrome, Mediators Inflamm. 2012 (2012) 815308.
- [9] M.E. Anderson Jr., Z.S. Buchwald, J. Ko, R. Aurora, T. Sanford, Patients with pediatric obstructive sleep apnea show altered T-cell populations with a dominant Th17 profile, Otolaryngol. Head Neck Surg. 150 (2014) 880–886.
- [10] K.S. Voo, Y.H. Wang, F.R. Santori, C. Boggiano, Y.H. Wang, K. Arima, et al., Identification of IL-17-producing FOXP3+ regulatory T cells in humans, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 4793–4798.
- [11] E. Bettelli, Y. Carrier, W. Gao, T. Korn, T.B. Strom, M. Oukka, et al., Reciprocal developmental pathways for the generation of pathogenic effector Th17 and regulatory T cells, Nature 441 (2006) 235–238.
- [12] Z.D. Liu, L. Wang, F.H. Lu, H. Pan, Y.X. Zhao, S.J. Wang, et al., Increased Th17 cell frequency concomitant with decreased Foxp3+ Treg cell frequency in the peripheral circulation of patients with carotid artery plaques, Inflamm. Res. 61 (2012) 1155–1165.
- [13] C. Zeng, X. Shi, B. Zhang, H. Liu, L. Zhang, W. Ding, et al., The imbalance of Th17/Th1/Tregs in patients with type 2 diabetes: relationship with metabolic factors and complications, J. Mol. Med. 90 (2012) 175–186.
- [14] A. Alunno, E. Bartoloni, O. Bistoni, G. Nocentini, S. Ronchetti, S. Caterbi, et al., Balance between regulatory T and Th17 cells in systemic lupus erythematosus: the old and the new, Clin. Dev. Immunol. 2012 (2012) 823085.
- [15] B. Afzali, G. Lombardi, R.I. Lechler, G.M. Lord, The role of T helper 17 (Th17) and regulatory T cells (Treg) in human organ transplantation and autoimmune disease, Clin. Exp. Immunol. 148 (2007) 32–46.
- [16] J. Galon, A. Costes, F. Sanchez-Cabo, A. Kirilovsky, B. Mlecnik, C. Lagorce-Pagès, et al., Type, density, and location of immune cells within human colorectal tumors predict clinical outcome, Science 313 (2006) 1960–1964.
- [17] M. Oukka, Interplay between pathogenic Th17 and regulatory T cells, Ann. Rheum. Dis. 66 (2007) 87–90.
- [18] N. Vgontzas, Does obesity play a major role in the pathogenesis of sleep apnoea and its associated manifestations via inflammation, visceral adiposity, and insulin resistance? Arch. Physiol. Biochem. 114 (2008) 211–223.
- [19] J. Kim, R. Bhattacharjee, A. Khalyfa, L. Kheirandish-Gozal, O.S. Capdevila, Y. Wang, et al., DNA methylation in inflammatory genes among children with obstructive sleep apnea, Am. J. Respir. Crit. Care Med. 85 (2012) 330–338.
- [20] K. Oboki, T. Ohno, H. Saito, Th17 and allergy, Allergol. Int. 57 (2008) 121–134.
- [21] C. Zhang, S. Hong, G. Hu, The expression of Treg/Th17 cells related transcription factors and cytokines in PBMCs and plasma in patients with allergic rhinitis, Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 26 (2012) 209–211.
- [22] J.H. Mo, Y.J. Chung, J.H. Kim, T cell transcriptional factors in allergic rhinitis and its association with clinical features, Asia Pac. Allergy 3 (2013) 186–193.
- [23] X. Huang, Y. Chen, F. Zhang, Peripheral Th17/Treg cell-mediated immunity imbalance in allergic rhinitis patients, Braz. J. Otorhinolaryngol. 80 (2014) 152–155.
- [24] B. Abrams, Long-term sleep apnea as a pathogenic factor for cell-mediated autoimmune disease, Med. Hypotheses 65 (2005) 1024–1027.
- [25] V. Aho, H.M. Ollila, V. Rantanen, Partial sleep restriction activates immune response-related gene expression pathways: experimental and epidemiological studies in humans, PLoS ONE 8 (2013) e77184.



Rubriq scorecard

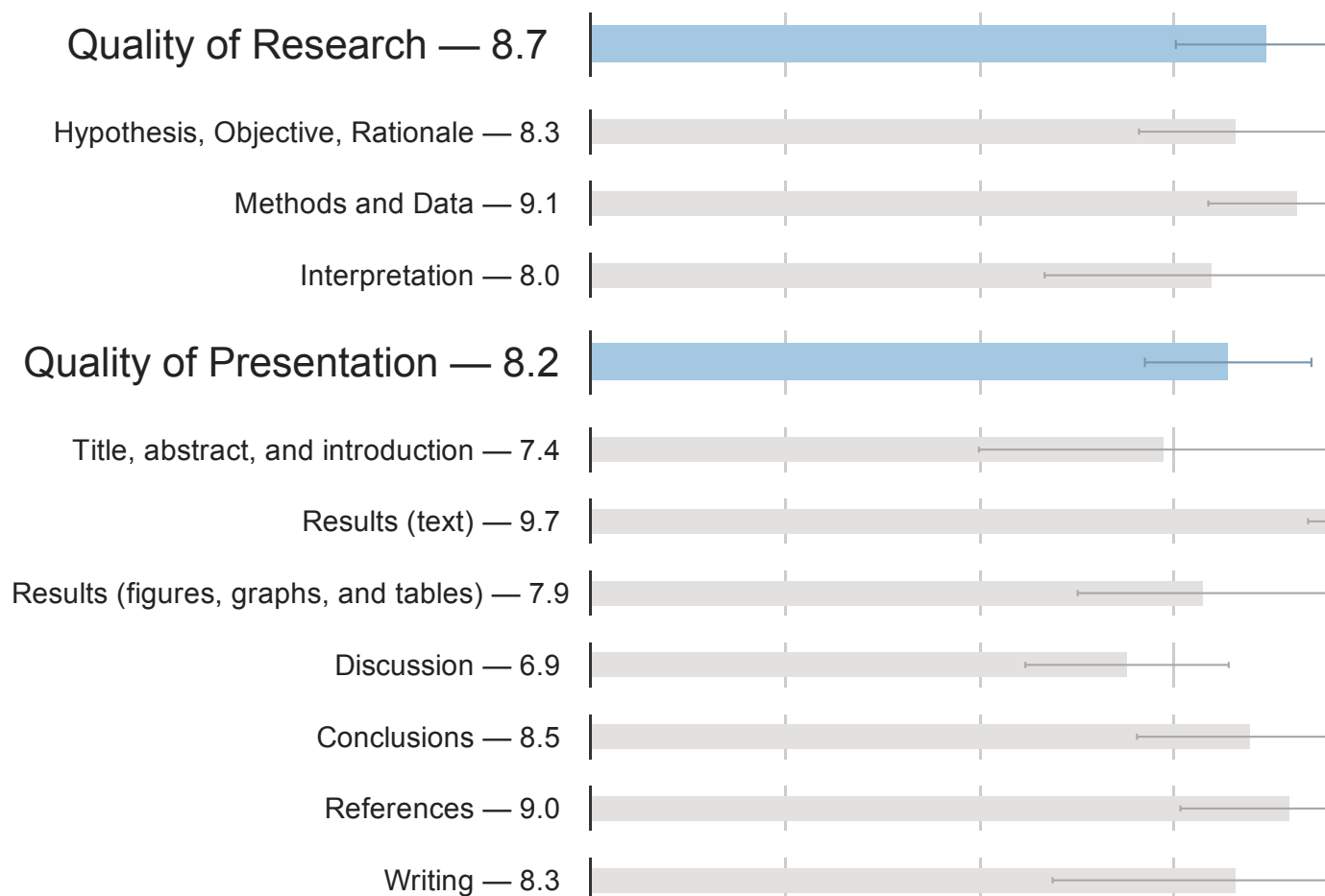
Compiled on January 12th, 2015

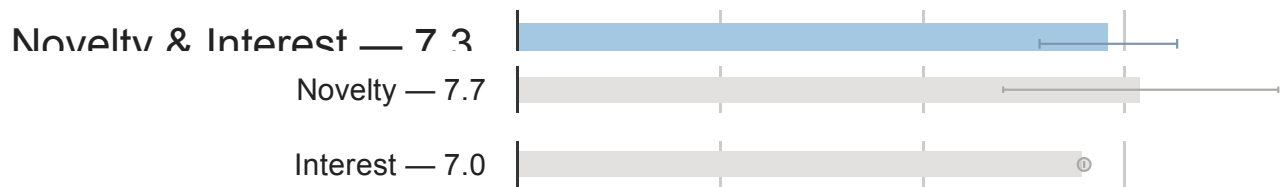
Manuscript: Th17 / Treg balance in children with obstructive sleep apnea syndrome and the relationship with allergic rhinitis

Client: AJE Rubriq



R-Score: **7.4** / 10





Reviewer 1

I think this work is relevant in the fields of ENT and pediatrics, but also to those doing basic science work in inflammation and immunity regulation. Th17/Treg ratios had been a new area of interest in basic science, but in particular as it relates to sleep apnea. I think these results will have significant impact.

Reviewer 2

I simply have a hard time believing the group assignments are clean enough: positive allergy testing is highly prevalent in children, and can be especially so in those with adenoid disease that is severe enough to warrant adenoidectomy for OSA. Conversely, it is possible that OSA patients without allergy (the OSAHS-adenoid groups) do indeed have some underlying inflammatory disorder like allergies.

Reviewer 3

This manuscript has the potential to be published in (at least) a mid-level journal. It aims at elucidating the the Th17 / Treg ratio in both adenoids and peripheral blood in children with OSA, children with OSA and AR, and normal controls. This has never been done before and is important for better understanding the pathogenesis of OSA in children. If the authors revise the introduction, so that they show the reader the rationale and novelty of their work, they can offer a worth-publishing manuscript. The measurements they have done both in adenoidal tissue and peripheral blood (cytokines, cells, mRNA) are of great importance.

Sound Research

Disregarding any consideration of novelty, does this work represent technically sound research?

Reviewer 1

Yes, with or without minor revisions

No specific recommendations for revision. Excellent manuscript.

Reviewer 2

Maybe with major revisions

I simply have a hard time believing the group assignments are clean enough: positive allergy testing is highly prevalent in children, and can be especially so in those with adenoid disease that is severe enough to warrant adenoidectomy for OSA. Conversely, it is possible that OSA patients without allergy (the OSAHS-adenoid groups) do indeed have some underlying inflammatory disorder like allergies.

Reviewer 3

Yes, with or without minor revisions

Yes, with revisions. It includes measurements that are difficult to do and are important for the elucidation of the pathogenesis of a common disorder in children.

Quality of Research

Hypothesis, Objective, Rationale – 8.3

Key points

	Meets all criteria
	Rationale is unclear
	Objective/hypothesis is not supported by background
	Objective/hypothesis is not stated
	Other - see comments



Reviewer 1

This is a very well written manuscript on an interesting new topic in immunity and in OSA in particular. I think the question is interesting and the study is well designed and executed.



Reviewer 2

The objective is stated, but it would be more helpful if the authors specifically stated a hypothesis, e.g., what they expected to observe and why they designed the study the way they did to test it.



Reviewer 3

The rationale is vague because the structure of the introduction is bad. The authors should state the current knowledge, and then make apparent that no one has studied before the Th17 / Treg (as well as cytokine levels and mRNA expressions) in both adenoids and peripheral blood in children with OSA, children with OSA and AR, and normal controls. This information is important because it adds to the elucidation of the pathogenesis of OSA in children.

Methods and Data – 9.1

Key points

	Meets all criteria
---	--------------------



Reviewer 1

	Missing essential references
	Design/techniques not up-to-date
	Missing important details for reproducibility
	Missing some experimental controls
	Inappropriate statistical analyses
	Missing an important experiment
	Approach/data not consistent with objectives/hypothesis
	Other - see comments

Nice, clear presentation of methods and results



good

Reviewer 2

It is unclear whether the authors excluded allergy in the OSAHS+adenoid group. They themselves report that inflammatory disease may be a significant underlying factor in adenoid enlargement and some inflammatory factor could therefore be implicated in the adenoid enlargement. Similarly, it also seems inconsistent that so many children in the OSAHS + AR group presumably had no significant adenoid tissue for purposes of group assignment, yet underwent adenoidectomy for tissue analysis.



excellent

Reviewer 3

The authors state that they recruited children aged 3-6 years, but if one looks at table 2, it appears that they included older children also.

Interpretation – 8.0

Key points

	Meets all criteria
	Does not adhere closely to the data
	Biased or overstated interpretation
	Leads to inaccurate conclusions



excellent

Reviewer 1

The authors did a pretty good job of discussing how these inflammatory changes (i.e., an increase in the Th17/Treg ratio) might related to OSA and allergic rhinitis in the discussion. However, it does sounds like it may be hard to know whether this finding is a cause or an effect. As they state, these results provide a rationale for more study.



fair

Reviewer 2

	Not supported by the data
	Other - see comments

I am concerned the conclusions are biased by the group selections and assignments. It is more likely that inflammatory disease, rather than sleep apnea, is a more significant factor impacting Tcell populations, and it is not clear that the group selection enables resolution of the effects of these two factors.



Reviewer 3

The interpretation should be more structured. Highlight the novel results of your research from the beginning in order to increase the interest of the reader.

Quality of Presentation

Title, abstract, and introduction – 7.4

Key points

	Meets all criteria
	Title is inappropriate
	Background/rationale is missing from abstract or introduction
	Methods are missing from abstract
	Conclusions are missing from abstract
 	Hypothesis/objective is missing from abstract or introduction
	Results are missing from abstract
	Other - see comments



Reviewer 1

Excellent and well written abstract and introduction. No significant problems with grammar.



Reviewer 2

The objective is stated, but it would be more helpful if the authors specifically stated a hypothesis. eg what they expected to observe and why they designed the study the way they did to test it.



Reviewer 3

The rationale of the article is poorly presented. The introduction needs major revision. I would suggest to start with a paragraph describing basic knowledge about sleep apnea in children and its connection with adenoids and allergic rhinitis. A second paragraph should include the already known data regarding the Th17 / Treg and their importance in the pathogenesis of OSA. A third paragraph should be devoted to the paucity of data regarding Th17 / Treg in children with OSA and AR. This structure will make clear which gap this article is aiming to fill (4th paragraph). The hypothesis based on the data stated in paragraph 2 of the introduction should also be included in part 4.

The abstract includes details about the measurements (e.g., ELISA or RT-PCR) that should be omitted. The conclusion of the abstract should be more concise.

Include in the first paragraph of your abstract the gap in the literature that your research is aiming to fill.

Results (text) – 9.7

Key points

	Meets all criteria
	Excessive repetition of data (e.g., from tables or figures)
	Poorly organized or not succinctly presented
	Missing significance indicators (e.g., p-values)
	Not focused on objectives
	Other - see comments



Reviewer 1

Good, clear presentation of results.



Reviewer 2

The results are presented in orderly fashion.



Reviewer 3

The authors repeat in the text the numbers that can be seen in the tables or figures. Only the significance of the differences should be included. For the rest, the reader can look at the figures/tables.

Results (figures, graphs, and tables) – 7.9

Key points

	Meets all criteria
	Problems with ordering, numbering, titles, or labels
	Data presentation is inappropriate for the experiments performed
	Images are of poor



Reviewer 1

Figures, tables and graphs all clear and easy to understand.



Reviewer 2

Table 2: SaO2 < 90% as % TST presumably means percent of total sleep time where saturation was less than 90. If so, do the authors mean this to be percent of total sleep time where saturation was above 90? In other words controls should be above 90 for the majority of the sleep

	quality
	Figures are too complex, confusing, or unclear
	Captions, footnotes, or legends are incomplete or missing
	Missing important data
	Other - see comments

time. A footnote explaining abbreviations would be helpful.



Reviewer 3

Figure 1: show the differences.

Figure 2: it repeats data that is already shown in Tables 3 and 4 and is also cited in the results section. Authors must consolidate.

Figure 3: add TGF-b on the axis.

Figure 3 and 4: they repeat the data already shown in Tables 3 and 4.

Figure 3 legend ", and the lower concentrations positively correlated with the degree of adenoid hypertrophy": authors must explain in the body of the manuscript what they mean.

Tables 2, 3 and 4: I would suggest to use the sign *: $p < 0.05$ versus controls as you compare the 3 OSAHS groups with the controls subjects, in order to avoid the use of # and §.

Discussion – 6.9

Key points

	Meets all criteria
	Missing concise and accurate summary of results
	Missing discussion of potential limitations
	Biased commentary



Reviewer 1

The authors did a pretty good job of discussing how these inflammatory changes (i.e., an increase in the Th17/Treg ratio) might related to OSA and allergic rhinitis in the discussion. However, it does sounds like it may be hard to know whether this finding is a cause or an effect. As they state, these results provide a rationale for more study.



Reviewer 2

The authors propose an interesting mechanism on pages

	Insufficient comparison to relevant literature/previous results
	Not consistent with objective/hypothesis
	Other - see comments

9 and 10 which would be strengthened by references to similar observations in other disease states, for without that, it is only speculation.



fair

Reviewer 3

The interpretation should be more structured. Highlight the novel results of your research from the beginning in order to increase the interest of the reader. No study has measured the Th17 / Treg in both adenoids and peripheral blood in children with OSA, children with OSA and AR, and normal controls.

Conclusions – 8.5

Key points

	Meets all criteria
	Missing take-away statements
	Vague, overstated, or understated applicability
	Not consistent with discussion or objectives
	Not supported by the data
	Other - see comments



excellent

Reviewer 1

The conclusion section is well written. I agree that further study is needed to illuminate just how this imbalance in Th17/Treg relates to OSA risk, severity and the presence of allergic rhinitis.

I'm not sure if it can be concluded, as the authors suggest, that this is a step in OSAHS pathogenesis, or whether it might be a result of the disease process.

I certainly agree that these results support further research on the topic and that these findings may have implications for treatment and disease prevention in children with OSAHA and/or allergic rhinitis.



good

Reviewer 2

The only conclusion I can draw from this data is that inflammatory disease, especially AR, increases Th17/Treg ratio. The group selections do not permit strong assessments of what roles OSAHS or adenoid enlargement specifically have,



Reviewer 3

The questions just before the conclusions are confusing the reader. They are apparently nice ideas for future research but what do they add to the current conclusions of the study?

References – 9.0

Key points

	Meets all criteria
	Problems with ordering or numbering
	Not focused on current/latest literature
	Do not provide informative context for Introduction/Discussion
	Do not support objectives
	Missing key references
	Other - see comments



Reviewer 1

Adequate references.



Reviewer 2

The authors propose an interesting mechanism on pages 9 and 10, which would be strengthened by references to similar observations in other disease states, for without that, it is only speculation.



Reviewer 3

Reference 8 and 14: are the pages correct?

Writing – 8.3

Key points

	Meets all criteria
--	--------------------



Reviewer 1

	Incorrect level of depth in some or all sections (too brief or too lengthy)
	Poor/unscientific word choice or grammar
	Writing lacks clarity, focus, or organization
	Other - see comments

Excellent writing - clear with no significant grammatical errors.



Reviewer 2

The quality of the English is fairly good especially given that the origin is presumably China, and English may not be the native language. The report follows a logical progression.



Reviewer 3

"in children with OSAHS combined with allergic compared with OSAHS children without AR"

"Children with obstructive sleep apnea hypopnea syndrome (OSAHS) suffer from sleep apnea"

The first paragraph of the introduction needs rephrasing.

Novelty & Interest

Novelty – 7.7

Key points

New technique, method, or approach (proof of principle)	
High	
Med	
Low	
N/A	

New question, theory or hypothesis (totally new idea)	
High	

High	-
Med	■ ■
Low	
N/A	

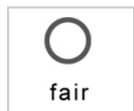
New result, discovery, or perspective/synthesis (proves an established idea)	
High	
Med	■ ■
Low	■
N/A	



Reviewer 1

This is a new and emerging topic in OSA.

A previous study on Th17/Treg has been done by Ye and Liu et al., 2012 in adults, but this would represent some of the earliest work in regard to children with OSAHS.



Reviewer 2

Attempts to decipher associations between AR, adenoids, and OSA at a cytokine level are admirable.



Reviewer 3

Although there are novel findings in this manuscript, the rationale behind them is not well presented and there is no hypothesis. The results are consistent with previous findings, which corroborated their importance.

Interest – 7.0

Key points

	of broad interest to researchers in this field and other fields
 	of broad interest within the field
	of moderate interest within the field
	of interest to a small group within the field
	of limited interest



Reviewer 1

I think this work is relevant in the fields of ENT and pediatrics, but also to those doing basic science work in inflammation and immunity regulation.



Reviewer 2

Potential interest in sleep medicine, immunology, and pediatrics.



Reviewer 3

The manuscript includes many measurements (cell count, mRNAs, cytokines) in both adenoidal tissue and peripheral blood which make it of great interest.



Th17 / Treg balance in children with obstructive sleep apnea syndrome and the relationship with allergic rhinitis

By AJE Rubriq

Th17 / Treg balance in children with obstructive sleep apnea syndrome and the relationship with allergic rhinitis

Authors: [REDACTED]

Affiliations: [REDACTED]

Corresponding author: [REDACTED]

Abstract

Objective: Obstructive sleep apnea hypopnea syndrome (OSAHS) is a serious sleep disorder that occurs in children and can cause a variety of complications. This study aims to explore the role of the Th17 to Treg cell ratio in children with OSAHS and its relationship with allergic rhinitis.

Methods: The study included 29 children diagnosed with chronic tonsillitis and the exclusion of OSAHS and 104 children diagnosed with OSAHS by polysomnography (PSG) testing. The 104 children with OSAHS were divided into the following groups: OSAHS with moderate adenoidal hypertrophy (n=40), OSAHS with severe adenoidal hypertrophy (n=33), and OSAHS complicated by allergic rhinitis (AR) (n=31). We measured the number of Th17 and Treg cells, the levels of related serum cytokine in cellular secretions, and the expression of key transcription factors in both peripheral blood and adenoid tissue. The Th17 / Treg ratio was calculated and analyzed between the different groups. The numbers of Th17 and Treg cells were measured by flow cytometry; secreted IL-17, IL-10, and TGF- β were measured by ELISA; and the expression levels of ROR γ t and Foxp3 were measured by RT-PCR.

Results: Compared with the control group, OSAHS children exhibited a significant increase in peripheral Th17 cell number, Th17-related cytokine secretion (IL-17), and ROR γ t mRNA levels, whereas they exhibited a decrease in Treg cell number, Treg-related cytokine secretions (IL-10, TGF- β) and Foxp3 mRNA levels. The Th17 / Treg ratio was higher ($p < 0.05$) in the OSAHS groups than in the control group. The Th17 / Treg ratio did not correlate with the size of the adenoids. We also found that the Th17 / Treg balance in OSAHS patients was complicated by allergic rhinitis; the increase was significantly larger in the AR group ($p < 0.05$) than in OSAHS groups without AR. These results were observed in both the peripheral blood and local adenoid tissue.

Conclusion: Th17 / Treg imbalance plays an important role in children with OSAHS. In children with OSAHS of similar severity, the Th17 / Treg ratio was related to AR but independent of adenoid size. Th17 / Treg imbalance may cause changes in the inflammatory cytokine microenvironment and increase the risk of developing OSAHS. These results provide an alternative explanation for OSAHS pathogenesis that warrants additional research and open new directions for the prevention and treatment of OSAHS in children.

Keywords: children, OSAHS, adenoidal hypertrophy, Th17 / Treg, allergic rhinitis

1. Introduction

Children with obstructive sleep apnea hypopnea syndrome (OSAHS) suffer from sleep apnea and ventilatory abnormalities caused by structural or functional abnormalities and the obstruction of the upper airway. These abnormalities are accompanied by snoring, sleep disorders, frequent oxygen desaturation, daytime sleepiness and other symptoms. The most common cause of OSAHS in children is tonsil or/and adenoid hypertrophy. Adenoid tissue in the mucosa-associated lymphoid system of the upper respiratory tract is an important part of the Waldeyer ring. Inflammation in the nasopharynx and adjacent areas may cause repeated the stimulation of the inflammatory response to pathological adenoid hyperplasia [1]. Immune activation in the adenoids may occur following a variety of external stimuli, and the imbalance of local immune cells and abnormal inflammatory response may lead to delayed healing, causing adenoidal hypotrophy. The adenoids' local immune status is closely linked to the incidence of OSAHS. The synchronization of the inflammatory states of both adenoids and peripheral blood of children with OSAHS can help us to understand the pathogenesis of OSAHS.

Sleep is closely related to the body's immune system. Ganz [2] believed that sleep is associated with immune function, and this relationship is partially based on the physiological basis of sleep, sleep architecture, the sleep-wake cycle, cytokines and the hypothalamic-pituitary axis. Many immune functions are dependent on circadian rhythms and regular sleep. Many changes in immune cells, particularly T cells, can cause sleep disorders. Over the past decade, scholars have begun to focus research on subsets of T cells in OSAHS patients, including the Th1 and Th2 cell populations. Bollinger [3] found that there is a certain 24-hour rhythm of the regulatory T cells (Treg) and that sleep deprivation seriously disrupts Treg cell function. Alert [4] studied patients with Th1-type active mode-based OSAHS, and Dyugovskaya [5] suggested that, compared with wakefulness, early turnal sleep induced a shift in the Th1 / Th2 cytokine balance towards increased Th1 activity; however, the Th1 shift was only moderate and was replaced by Th2 dominance during late sleep ($p < 0.05$).

In recent years, Th17 and Treg T cell populations have also been discussed. Th17 cells are key effectors in the immune response. Th17 cells expressing retinoic acid-related orphan receptor γ (ROR γ t) play critical roles in the development of autoimmunity and allergic reactions through IL-17 production. Treg cells expressing the forkhead/winged helix transcription factor (Foxp3) orchestrate the overall immune response and play a role in immune tolerance through contact-dependent suppression or by regulating the activity of the effector T cells or releasing anti-inflammatory cytokines, such as interleukin-10 (IL-10) and transforming growth factor β (TGF- β 1). Sade [7] found that the Th17 / Treg ratio negatively correlates with the clinical score for OSAHS independent of age

and gender. Jin Ye [8] found that the Th17 / Treg ratio is elevated in patients with OSAHS and positively correlates with AHI. Anderson [9] found that CD4 T cells are increased locally in the tonsils of children with OSAHS, whereas CD8T cells are reduced, and that the Th17 cell population is affected. Although these results are varied and have produced no unified conclusion, it is clear that T cell population imbalance, either Th1 / Th2 or Th17 / Treg imbalance, is observed in patients with OSAHS. This issue, however, requires further study and discussion.

Therefore, we have designed a research study of preschool-aged children (3-6 years) to measure the number of Th17 and Treg cells as well as the expression of related genes and cytokines in both peripheral blood and adenoid tissue. Our aims are to investigate the significance of the Th17 / Treg ratio in children with OSAHS, including the effect of changes in this ratio on the local and systemic inflammatory response, and to investigate how allergic rhinitis affects the Th17 / Treg ratio in children with OSAHS.

2. Materials and Methods

2.1 Patient information

Children (ages 3 to 6 years) with OSAHS newly confirmed by overnight PSG were initially recruited into the OSAHS group. Children diagnosed with chronic tonsillitis who underwent surgical treatment and were excluded from a diagnosis of OSAHS by PSG were recruited as the control group. All patients were determined to be free of other cardiovascular, endocrine, urinary system, metabolic and neuromuscular diseases and disorders. The selected patients had no specific response to aspirin, bronchiectasis, typical histories of autoimmune diseases, or severe respiratory infection within two weeks prior to enrollment. History of allergic rhinitis, asthma or other allergic diseases were recorded for all children. Skin prick tests were performed to assess allergens, and fiber nasopharyngoscopy was performed to assess adenoid size. Routine preoperative examinations were performed to exclude surgical contraindications.

Based on the results of the fiber nasopharyngoscopy, adenoid size was classified into four groups of differing degrees: level I (0-25% nostril blockage), level II (26% -50% blockage), level III (51% -75% blockage), and level IV (76% -100% blockage) [2]. According to the "child with obstructive sleep apnea syndrome treatment guidelines" published in 2007 by the Chinese Journal of Otorhinolaryngology Head and Neck, a diagnosis of OSAHS is established when the obstructive apnea index (AOI) is greater than 1 / hour or the obstructive apnea hypopnea index (AHI) is greater than 5 times / hour and the lowest oxygen saturation is less than 92%.

The OSAHS children were divided into groups: the adenoid III group (moderate hypertrophy group), adenoid IV group (severe hypertrophy group), and OSAHS + AR group (OSHAS children with

concurrent allergic rhinitis). The control subjects were children with chronic tonsillitis who were undergoing tonsillectomy and adenoidectomy after informed consent. Children in the control group had an AHI lower than 5, no complaints of sleep apnea and no allergic rhinitis.

The study was approved by the Institutional Review Board of Shanghai Children's Hospital. The parents of all the children provided informed consent to take the children's blood and adenoid tissue for research.

2.2 ROR γ t and Foxp3 expression determined by real-time quantitative PCR

Total RNA was extracted from the peripheral blood PBMCs by TRIzol extraction (TRIzol Reagent, Ambion) according to the manufacturer's instructions. Total RNA extraction from the adenoids was performed as follows: Approximately 100 mg of tissue was placed into an EP tube, 1 mL of TRIzol was added and RNA was extracted following the instructions. The concentration of the sample was measured and the OD260 / OD280 ratio was 1.91. The extracted RNA was of high purity, and there were no residual proteins or DNA.

cDNA was synthesized using random hexamer primers and RNase H⁻ reverse transcriptase (Invitrogen). The mixture was prepared in 0.2 mL RNase-free EP tubes, and the reaction was performed at 37 °C for 15 min followed by 5 sec at 85 °C. The primer pairs used are shown in Table 1. The resulting cDNA was diluted with 10 μ L ddH₂O and froze at -20 °C. It could then be used for direct 2nd-strand cDNA synthesis. The recommended maximum volume of cDNA for use in PCR amplification was 1 μ L.

Relative gene expression was calculated using the comparative CT method. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene for normalization, and a no template sample was used as a negative control. All reactions were carried out in triplicate per sample.

2.3 Flow cytometric analysis of Treg and Th17 cells

2.3.1 Blood samples and PBMC isolation

Three milliliters of peripheral blood (PB) samples were collected into collection tubes containing sodium heparin. An equal volume of HBSS (Hank's balanced salt solution, pH7.2-7.4) was added to the peripheral blood; PBMCs were prepared by Ficoll-Hypaque density centrifugation and resuspended at a density of 2×10^6 cells/mL for analysis by flow cytometry.

2.3.2 Preparation of PBMCs from adenoid tissue

The adenoid tissue was rinsed for 10 s in a 75% ethanol solution and then cleaned using PBS containing 10% antimicrobial solution. After being rinsed for 10 s in RPMI-1640 medium, the tissue was cut into pieces. The broken tissue was ground in 40 mesh with a syringe needle core to give a single cell suspension in 10% FBS + RPMI-1640. Ficoll-Hypaque density centrifugation was used to purify the PBMCs, and the PBMCs were suspended at a density of 1×10^6 cells/mL.

2.3.3 Cell culture

The PBMC suspension (2×10^6 cells/mL) was put into T25 flasks and 3 mL of media including PMA (50 ng / mL, Sigma, USA), Ionomycin calcium salt (1 μ g / mL, Sigma, USA) and BFA (10 μ g / mL) were added. The incubator was set at 37°C under a 5% CO₂ environment for 4 h of culture.

Cultured cells in solution were subjected to human Th17 and Treg analysis. The experimental procedure was performed in accordance with instructions from BD Inc. (560762)). Stained cells were analyzed by flow cytometry using a FACScan flow cytometer (Becton Dickinson) equipped with the CellQuest Pro 5.2 software (BD Biosciences, USA).

2.4 Detection of cytokine levels

The plasma levels of IL-17, TGF- β 1, and IL-10 were measured for the PBMC by enzyme-linked immunosorbent assay (ELISA) following the manufacturer's instructions (ELISA kits).

2.5 Statistical analysis

Values in all tables and figures are expressed as the mean $\pm$ standard deviation (SD). Statistically significant differences between the values were determined using Student's t-test. Grouped data were analyzed using a one-way analysis of variance (ANOVA). Statistical analyses were performed using a commercial software package ((SPSS, version 16.0 for Windows; SPSS Inc., Chicago, IL). A P-value less than 0.05 indicates significance.

3. Results

3.1 Basic characteristics of studied subjects

A total of 133 children were analyzed, with 40 in the OSAHS group with level III adenoids (moderate hypertrophy group), 33 in the OSAHS group with level IV adenoids (severe hypertrophy group), 31 in the OSAHS + AR group (OSAHS children with allergic rhinitis) and 29 in the control group. The characteristics of the study population are shown in Table 2.

Preschool children of 3-6 years of age were enrolled, and no statistically significant differences in age were found between the four groups ($P > 0.05$). Based on PSG monitoring, the AHI and oxygen saturation of the control group were deemed normal. The AHIs of the OSAHS groups and OSAHS + AR group were greater than 5, and the lowest oxygen saturations were below 92%. The differences in AHI and oxygen saturation compared with controls were more significant in the OSAHS + AR group than in the OSAHS groups without AR ($p < 0.05$). However, there was no significant difference between the level III-adenoid OSAHS group and the level IV-adenoid OSAHS group.

Summaries of RT-qPCR, flow cytometry, and ELISA results from peripheral blood of the control group and the OSAHS groups are shown in Table 3. The results from adenoid tissue are summarized in Table 4. The data for each group were analyzed statistically.

2.2 Expression of *Foxp3* and *RORyt* mRNA in PBMCs from peripheral blood and adenoid tissue

RORyt is an important transcription factor for the differentiation of Th17 cells, whereas *Foxp3* is the master transcription factor in Treg cells. The results showed that in the peripheral blood and adenoid tissue, *RORyt* levels were higher in the OSAHA groups than in the control group, and differences in *RORyt* expression were independent of adenoid size (Figure 1A). Relative *RORyt* expression in the peripheral blood of the level IV adenoids group (32.50 ± 9.83) was not significantly different than that of the level III adenoids group (30.11 ± 11.32), and relative *RORyt* expression was 53.50 ± 7.49 in the OSAHS combined with AR group, significantly higher than that in control group (5.37 ± 3.19) and the OSAHS groups. Furthermore, relative *Foxp3* expression was reduced in the OSAHS groups and the OSAHS combined with AR group ($p < 0.05$), but there was no significant difference between the two OSAHS groups distinguished by adenoid size (Figure 1B).

3.3 Analysis of the Th17 cell population, Treg cell population and ratio of Th17 / Treg cells in the peripheral blood and adenoid tissue

Compared with the control group, the percentage of Th17 cells in the peripheral blood and adenoid tissue was higher in the OSAHS groups, whereas the percentage of Treg cells was reduced. The Th17 / Treg ratio in the OSAHS groups increased with adenoid size, whereas the Th17 / Treg ratio did not differ significantly between the level III adenoids and level IV adenoids groups ($p > 0.05$). The Th17 / Treg ratio in the peripheral blood from the control group was 0.05 ± 0.32 , and the Th17 / Treg ratios in OSAHS groups without AR were 4.32 ± 1.94 and 4.71 ± 1.76 in the level III- and level IV-adenoid groups, respectively. The Th17 / Treg ratio in the OSAHS combined with AR group was 13.21 ± 4.25 , which was significantly higher than the control group ($p < 0.01$) and the OSAHS groups without AR (Figure 2A). We observed similar results from adenoid tissue; namely, the Th17 / Treg ratio was

higher in the OSAHS groups than in the control group, and the increase was independent of adenoid size but positively correlated with the co-diagnosis of AR (Figure 2B).

3.4 Concentrations of the related cytokines IL-17, IL-10, and TGF- β

The levels of IL-17, IL-10, and TGF- β were detected in normal controls and OSAHS patients using ELISA tests (Table 3). The cytokine values were statistically analyzed, and we found that TGF- β levels were significantly different among the four groups both in the peripheral blood and adenoid tissue samples (Figure 3). In both peripheral blood and adenoid tissue samples, IL-17 and IL-10 levels were also significantly different between the OSAHS groups and the OSAHS with AR group. However, there were no significant differences between the groups with different levels of adenoid hypertrophy (level III and level IV) (Figure 4A and B).

4. Discussion

The newly discovered Th17 cells are a class of cells independent of the Th1 and Th2 T cell subsets. Th17 cells express ROR γ t and mainly secrete interleukin-17A (IL-17A). Th17 cells are an important class of effector T cells that regulate the body's fight against extracellular pathogens while also promoting the occurrence of autoimmune and inflammatory diseases. Th17 cells play an important role in the development of immunity, mainly by secreting IL-17; TNF- α and IL-6 also have a role in this process. Unlike Th17 cells, human regulatory T cells (Treg) maintain peripheral tolerance. Treg cells express FoxP3 and complete the immunosuppressive function primarily through direct contact inhibition or by inhibiting the cytokine IL-10 and TGF- β and adjusting the activity of effector T cells. Treg and Th17 cells have opposite effects on autoimmunity and inflammation. Both can arise from T0 cell differentiation. T cell development is plastic and mutual differentiation may occur depending on the local cytokine microenvironment. Some studies [10] have shown that Treg cells are able to differentiate into IL-17-producing cells and that human peripheral blood and lymphoid tissue contain a significant number of CD4+Foxp3+ T cells that have the capacity to produce IL-17 upon activation. In addition to the human Th1 / Th2 balance, the balance between Treg and Th17 cells [11] is critical to control the development of autoimmune and inflammatory responses. The Th17 / Treg balance has an important role in the development of a variety of human diseases, including cancer, inflammation, and autoimmune diseases [12-15]. Galon [16] suggested that an elevated Th17 / Treg ratio may be a severe marker of autoimmune disease, inflammatory disease, and allergic disease, indicating that the equilibrium of different types of immune cells can be used to better predict clinical prognosis in patients. In summary, because the Th17 / Treg ratio is significant in the pathogenesis of disease, we studied the relationship of changes in the Th17 / Treg ratio in children

with OSAHS with adenoid size and with co-diagnosis of allergic rhinitis. This research is based in a clear foundation and has important clinical significance.

Our results showed that children with OSAHS exhibited significant increases in Th17 cell number and levels of Th17-related cytokines (IL-17) and transcription factors (ROR γ t) as well as dramatic decreases in Treg cell number and levels of Treg-related cytokines (TGF- β and IL-10) and transcription factors (Foxp3) when compared with controls. The Th17 / Treg ratios were also elevated in the OSAHS groups. These results were obtained for both the peripheral blood and adenoids. Further, the Th17 / Treg ratios were not dependent on adenoid size. We also found that the Th17 / Treg ratio was also elevated in children with OSAHS combined with allergic compared with OSAHS children without AR, and this difference was statistically significant. These positive results suggest that the Th17 / Treg ratio is elevated in children with OSAHS and that elevation is higher with co-diagnosis of allergic rhinitis. Therefore, we believe that the Th17 / Treg balance plays an important role in the pathogenesis of OSAHS in children and may be a factor in OSAHS development and / or a prognostic predictor in OSAHS patients.

As described above, sleep disorders impact the numbers and functions of human immune cells [3], leading to changes in Treg and effector T cells. Early experiments found [17,18] that in the micro-environment of OSAHS patients, the elevation of the pro-inflammatory cytokines IL-17 and IL-6 and reduction of TGF- β potentially promote the continued growth of Th17 cells while inhibiting Treg development, leading to Th17 / Treg imbalance disorders. This disorder can enhance the inflammatory cytokines in the microenvironment, eventually resulting in a positive feedback loop that amplifies the proinflammatory response. In a study of OSAHS in adults [8], the Th17 / Treg ratio was higher in the peripheral blood of patients with OSAHS and positively correlated with the severity of OSAHS. Studies of children with OSAHS have also yielded some results. Kim [19] studied 47 cases of children with OSAHS and found that the FOXP3 gene is more likely to display increased methylation in children with OSAHS who exhibit increased systemic inflammatory responses. FOXP3 DNA methylation levels positively correlated with hsCRP levels and AHI, and high FOXP3 DNA methylation may favor the downregulation of Foxp3 protein expression, thus reducing the number of Treg cells. Anderson [9] also confirmed the presence of T cell balance disorders in children with OSAHS and these imbalances were characterized by Th17 cell dominance. Our study is consistent with these conclusions. We found that in the peripheral blood and adenoid tissue of children with OSAHS, the expression of Foxp3 is decreased and the number of Treg cells is reduced, whereas the number of Th17 cells is increased and Th17 / Treg imbalance was apparent. We propose a possible mechanism that involves the fact that the children's adenoids, which are located in the nasopharynx, suffered long-term stimulation by various microorganisms. Upon acute infection, the adenoids

experienced a local inflammatory response under the influence of various inflammatory factors. One of the inflammatory factors, IL-17, is a principal inflammatory factor that can promote CD4 + T cells to differentiate into Th17 cells and, in conjunction with reduced TGF- β , inhibit the development of Treg cells, causing a Th17 / Treg imbalance. An increase in the Th17 / Treg ratio contributes to the formation of a new microenvironment, and ultimately, this results in the formation of a positive feedback mechanism that amplifies the immune response. Repeated inflammatory stimuli result in the proliferation of local lymphoid tissue, which is expressed as adenoid hyperplasia. Hypertrophic adenoid tissue blocks the airway, causing apnea and hypopnea during sleep and resulting in OSAHS. Furthermore, sleep disorders will affect the normal balance of Th17 and Treg cells, leading to a persistent or worsening systemic inflammatory immune status. Thus, this mechanism explains our findings regarding the Th17 / Treg ratio imbalances in both the peripheral blood and local adenoid tissue.

The difference in the Th17 / Treg ratio between the level III adenoids group and the level IV adenoids group was not obvious, possibly because children in these groups have OSAHS with similar severity although they differ based on adenoid size. There was no significant difference between the AHI index and lowest oxygen saturation ($p > 0.05$) between these two OSAHS groups, suggesting that the severity of OSAHS was similar between the two groups. Previous studies have demonstrated that the Th17 / Treg ratio positively correlates with the severity of OSAHS [8,19], and our results further illustrate that absolute size itself does not affect the Th17 / Treg ratio. In other words, similar AHIs in OSAHS result in similar effects on the Th17 / Treg ratio. Therefore, the Th17 / Treg ratios, which reflect the systemic inflammatory immune status, can be used to assess the condition, but adenoid size is not valuable to assess the condition or prognosis. Interestingly, the level of cytokine TGF- β differed with varying degrees of adenoid hypertrophy, suggesting that TGF- β may play an important role in adenoid hyperplasia. This result may be useful for research in adenoid pathogenesis.

Previous studies have demonstrated [16,20-23] that Th17 has an important role in the pathogenesis of allergic rhinitis, and Th17 / Treg balance can be used to assess allergic rhinitis. In our study, allergic rhinitis was considered an influential factor in the analysis of the Th17 / Treg ratio. Studies have shown [24] that adult OSAHS might trigger the development of autoimmune phenomena. Under the circumstances, a higher Th17/Treg ratio may result in loss of tolerance and regulation and ultimately a persistent, low-degree systemic inflammatory reaction characterized by autoimmune or allergic diseases. We found that the Th17 / Treg ratio was also significantly increased in patients with OSAHS and allergic rhinitis compared with the control group and the OSAHS groups without allergic rhinitis. Therefore, we believed that the Th17 / Treg imbalance caused by AR and the Th17 / Treg imbalance due to OSAHS disorder may mutually promote one another and cause further

imbalance, which can lead to more obvious clinical symptoms. Therefore, regardless of which pathological changes appear first, AR or OSAHS, both will cause changes in Treg and Th17 cells, leading to Th17 / Treg imbalance disorders, and this disorder will have a role in promoting the development of other diseases. Therefore, such patients should be treated simultaneously for both diseases. In cases where we are assessing the severity of a disease, treatment and prognosis, we should not ignore the impact of other concurrent diseases. The measurement and analysis of the Th17 / Treg ratios in OSAHS children with or without concomitant allergic rhinitis are of great significance for the assessment of these conditions.

Inflammation caused by an immune response is a complex, dynamically updated process. Do differences in the duration of disease and differences in the age of patients affect the imbalance of T cell subsets? After surgery, medical treatment or positive pressure ventilation therapy in OSAHS children is there a change in the Th17 / Treg ratio? What should be done about the development of concomitant allergic diseases? Our research also needs to refine intervention factors based on further study and a comprehensive analysis of the results.

5. Conclusion

Our data confirm that the Th17 / Treg ratio is increased in the peripheral blood and local adenoid tissue of OSAHS children. In cases of OSAHS with similar severity, changes in the Th17 / Treg ratio are independent of adenoid size. The Th17 / Treg ratio is also elevated in OSAHS children with allergic rhinitis to a greater extent than that observed in OSAHS patients without AR. These results provide data to further study the pathogenesis of childhood OSAHS and adenoidal hypertrophy and provide direction for research assessments, treatment of children with OSAHS, and disease prevention.

References

1. X. Huang, J. Wang, Practical Otolaryngology Head and Neck Surgery, 2nd ed., People's Health Press, Beijing, 2008.
2. F.D. Ganz, Sleep and immune function, *Crit. Care Nurse* 32 (2012) e19–e25.
3. T. Bollinger, A. Bollinger, L. Skrum, S. Dimitrov, T. Lange, W. Solbach, Sleep-dependent activity of T cells and regulatory T cells, *Clin. Exp. Immunol.* 155 (2009) 231–238.
4. A. Alberti, P. Sarchielli, E. Gallinella, A. Floridi, A. Floridi, G. Mazzotta, V. Gallai, Plasma cytokine levels in patients with obstructive sleep apnea syndrome: a preliminary study, *J. Sleep Res.* 12 (2003) 305–311.
5. L. Dyugovskaya, P. Lavie, M. Hirsh, L. Lavie, Activated CD8+ T-lymphocytes in obstructive sleep apnoea, *Eur. Respir. J.* 25 (2005) 820–828.
6. S. Dimitrov, T. Lange, S. Tieken, H. L. Fehm, J. Born, Sleep associated regulation of T helper 1/T helper 2 cytokine balance in humans, *Brain Behav. Immun.* 18 (2004) 341–348.
7. K. Sade, G. Fishman, S. Kivity, A. Derowe, S. Langier, Expression of Th17 and Treg lymphocyte subsets in hypertrophied adenoids of children and its clinical significance, *Immunol. Invest.* 40 (2011) 657–666.
8. J. Ye, H. Liu, G. Zhang, P. Li, Z. Wang, S. Huang, Q. Yang, Y. Li, The treg/th17 imbalance in patients with obstructive sleep apnoea syndrome, *Mediators Inflamm.* 2012 (2012) 815308,
9. M.E. Anderson Jr., Z.S. Buchwald, J. Ko, R. Aurora, T. Sanford, Patients with pediatric obstructive sleep apnea show altered T-cell populations with a dominant TH17 profile, *Otolaryngol. Head Neck Surg.* 150 (2014) 880–886.
10. K.S. Voo, Y.H. Wang, F.R. Santori, C. Boggiano, Y.H. Wang, K. Arima, L. Bover, S. Hanabuchi, J. Khalili, E. Marinova, B. Zheng, D.R. Littman, Y.J. Liu, Identification of IL-17-producing FOXP3+ regulatory T cells in humans, *Proc. Natl. Acad. Sci. U.S.A.* 106 (2009) 4793–4798.
11. E. Bettelli, Y. Carrier, W. Gao, T. Korn, T.B. Strom, M. Oukka, H.L. Weiner, V.K. Kuchroo, Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells, *Nature* 441 (2006) 235–238.
12. Z.D. Liu, L. Wang, F.H. Lu, H. Pan, Y.X. Zhao, S.J. Wang, S.W. Sun, C.L. Li, X.L. Hu, Increased Th17 cell frequency concomitant with decreased Foxp3+ Treg cell frequency in the peripheral circulation of patients with carotid artery plaques, *Inflamm. Res.* 61 (2012) 1155–1165.
13. C. Zeng, X. Shi, B. Zhang, H. Liu, L. Zhang, W. Ding, Y. Zhao, The imbalance of Th17/Th1/Tregs in patients with type 2 diabetes: relationship with metabolic factors and complications, *J. Mol. Med.* 90 (2012) 175–186.

14. A. Alunno, E. Bartoloni, O. Bistoni, G. Nocentini, S. Ronchetti, S. Caterbi, V. Valentini, C. Riccardi, R. Gerli. Balance between regulatory T and Th17 cells in systemic lupus erythematosus: the old and the new, *Clin. Dev. Immunol.* 2012 (2012) 823085.
15. B. Afzali, G. Lombardi, R.I. Lechler, G.M. Lord, The role of T helper 17 (Th17) and regulatory T cells (Treg) in human organ transplantation and autoimmune disease, *Clin. Exp. Immunol.* 148 (2007) 32-46.
16. J. Galon, A. Costes, F. Sanchez-Cabo, A. Kirilovsky, B. Mlecnik, C. Lagorce-Pagès, M. Tosolini, M. Camus, A. Berger, P. Wind, F. Zinzindohoué, P. Bruneval, P.H. Cugnenc, Z. Trajanoski, W.H. Fridman, F. Pagès, Type, density, and location of immune cells within human colorectal tumors predict clinical outcome, *Science* 313 (2006) 1960–1964.
17. M. Oukka, "Interplay between pathogenic Th17 and regulatory T cells," *Ann. Rheum. Dis.* 66 (2007) iii87-90.
18. N. Vgontzas, Does obesity play a major role in the pathogenesis of sleep apnoea and its associated manifestations via inflammation, visceral adiposity, and insulin resistance?, *Arch. Physiol. Biochem.* 114 (2008) 211-223.
19. J. Kim, R. Bhattacharjee, A. Khalyfa, L. Kheirandish-Gozal, O.S. Capdevila, Y. Wang, D. Gozal, DNA methylation in inflammatory genes among children with obstructive sleep apnea, *Am. J. Respir. Crit. Care Med.* 85 (2012) 330–338.
20. K. Oboki, T. Ohno, H. Saito, Th17 and allergy, *Allergol. Int.* 57 (2008) 121-134.
21. C. Zhang, S. Hong, G. Hu, The expression of Treg/Th17 cells related transcription factors and cytokines in PBMCs and plasma in patients with allergic rhinitis, *Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi* 26 (2012) 209-211.
22. J.H. Mo, Y.J. Chung, J.H. Kim, T cell transcriptional factors in allergic rhinitis and its association with clinical features, *Asia Pac. Allergy* 3 (2013) 186-193.
23. X. Huang, Y. Chen, F. Zhang, Peripheral Th17/Treg cell-mediated immunity imbalance in allergic rhinitis patients, *Braz. J. Otorhinolaryngol.* 80 (2014) 152-155.
24. B. Abrams, Long-term sleep apnea as a pathogenic factor for cell-mediated autoimmune disease, *Med. Hypotheses* 65 (2005) 1024–1027.

Figure Legends

Figure 1: Expression levels of ROR γ t in PBMCs from peripheral blood and adenoid tissue are shown.

A: ROR γ t/GAPDH mRNA ratio was compared among four groups. B: Foxp3/GAPDH mRNA ratio was compared among four groups.

Figure 2: The ratio of Th17/Treg increased in OSAHS patients compared with controls.

A: The ratio of Th17/Treg in the OSAHS groups and control group in PBMCs from peripheral blood.

B: The ratio of Th17/Treg in the OSAHS groups and control group in PBMCs from adenoid tissue.

Figure 3: TGF- β levels in peripheral blood and adenoid tissue.

The data represent the plasma levels of TGF- β concentration in the four groups (controls, level III-adenoid OSAHS group, level IV-adenoid OSAHS group, and OSAHS+AR group). TGF- β concentrations were significantly lower in the OSAHS groups than in the control group, and the lower concentrations positively correlated with the degree of adenoid hypertrophy.

Figure 4: IL-17 and IL-10 concentrations in peripheral blood (A) and adenoid tissue(B).

IL-17 concentrations were significantly higher in patients with OSAHS than in the control group. IL-10 concentrations were higher in the OSAHS and OSAHS +AR groups. The concentrations of IL-17 and IL-10 were comparable between the two OSAHS groups without AR.

Table 1: Primer sequences

Gene	Primer	Product length (bp)	Tm (°C)
GAPDH	5- GGCTGTGGGCAAGGTCATCCCTG -3	101	67.62
	5- GACGGCAGGTCAGGTCCACCACTG -3		68.96
FOXP3	5- GGCAGCCAAGGCCCTGTCGTCC -3	91	70.07
	5- GGCTACCCACAGGTGCCTCCGG -3		70.87
RORyt	5- GTGCCCACCACCTACCGAGGCC -3	148	71.51
	5- TAGGCCC GG CACATCCTAACCAGC -3		68.46

Table 2: Baseline characteristics of study population

Variables	OSAHS			
	Controls	Level III Adenoids	Level IV Adenoids	OSAHS+AR
Subjects, n	29	40	33	31
Age (years)	6.51±2.42	5.62±4.20	4.86±7.21	5.50±3.98
AHI (episodes/h)	1.07±1.56	6.95±4.24*	8.73±8.52#	8.36±2.51§
SaO ₂ < 90% (%TST)	95.01±3.46	85.33±11.70*	82.94±8.19#	83.56±12.54§

Values are expressed as the mean ± SD.

*P < 0.05, Controls versus OSAHS with level III Adenoids;

#P < 0.05, Controls versus OSAHS with level IV Adenoids;

§P < 0.05, Controls versus OSAHS with AR

Table 3: Summary of RT-PCR, flow cytometry, and ELISA results from peripheral blood in different groups

Variables	Control	OSAHS		OSAHS+AR
		Level III Adenoids	Level IV Adenoids	
mRNA expression of transcription factors (RT-qPCR)				
ROR γ /GADPH mRNA	5.37 $\pm$ 3.19	30.01 $\pm$ 11.32*	32.50 $\pm$ 9.83#	53.50 $\pm$ 7.49§*
Foxp3/GADPH mRNA	2.72 $\pm$ 2.34	1.08 $\pm$ 2.15*	1.25 $\pm$ 1.98#	0.21 $\pm$ 0.98§*
T-cell counts (Flow cytometry)				
Th17 (% of CD4+)	0.97 $\pm$ 0.38	2.24 $\pm$ 0.54*	2.77 $\pm$ 0.81#	3.24 $\pm$ 1.07§*
Treg (% of CD4+)	1.91 $\pm$ 0.56	0.61 $\pm$ 0.28*	0.59 $\pm$ 0.10#	0.23 $\pm$ 0.26§*
Th17/Treg ratio	0.05 $\pm$ 0.32	4.32 $\pm$ 1.94*	4.71 $\pm$ 1.76#	13.21 $\pm$ 4.25§*
Levels of cytokines (pg/mL) (ELISA)				
IL-17 (pg/mL)	1.52 $\pm$ 1.43	2.61 $\pm$ 1.08*	3.06 $\pm$ 2.05#	4.66 $\pm$ 1.92§*
IL-10 (pg/mL)	53.87 $\pm$ 11.45	45.77 $\pm$ 9.32*	42.47 $\pm$ 10.35#	31.67 $\pm$ 13.59§*
TGF- β (pg/mL)	139.65 $\pm$ 23.68	75.68 $\pm$ 10.42*	44.21 $\pm$ 12.73#▽	30.11 $\pm$ 9.36§*

Values are expressed as the mean $\pm$ SD.

*P < 0.05, Controls versus OSAHS with Level III Adenoids;

#P < 0.05, Controls versus OSAHS with Level IV Adenoids;

§P < 0.05, Controls versus OSAHS combined with AR;

*P < 0.05, OSAHS combined with AR versus OSAHS with Level III Adenoids and OSAHS with Level IV Adenoids;

▽ P < 0.05, OSAHS with Level III Adenoids versus OSAHS with Level IV Adenoids

Table 4: Summary of RT-PCR, flow cytometry, and ELISA results from adenoid tissue in different groups

Variables	Control	OSAHS 组		OSAHS+AR
		Level III Adenoids	Level IV Adenoids	
mRNA expression of transcription factors (RT-qPCR)				112.64±21.55§
RORyt/GADPH mRNA	11.52±4.97	78.82±9.23*	85.64±12.54#	*
Foxp3/GADPH mRNA	3.81±2.31	2.21±4.10	1.92±2.61#	0.92±1.63§*
T-cell counts (Flow cytometry)				
Th17 (% of CD4+)	1.53±0.39	2.53±0.58*	2.94±0.33#	3.74±0.72§*
Treg (% of CD4+)	10.61±2.62	1.13±0.47*	1.09±0.07#	0.39±0.07§*
Th17/Treg ratio	0.20±0.07	2.49±1.01*	2.79±3.44#	9.97±2.10§*
Levels of cytokines (pg/mL) (ELISA)				
IL-17 (pg/mL)	42.15±11.98	54.63±9.63*	52.47±10.38#	63.76±8.52§*
IL-10 (pg/mL)	14.43±5.68	8.66±4.01*	7.33±4.28#	3.67±13.59§*
TGF-β(pg/mL)	875.32±43.5	611.34±29.53*	450.65±18.43#▽	300.11±9.36§*
	6			

Values are expressed as the mean ± SD.

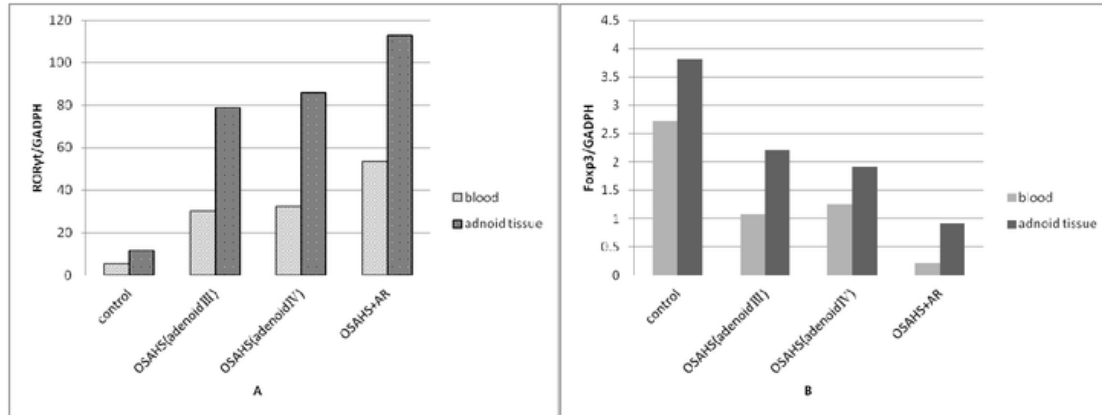
*P < 0.05, Controls versus OSAHS with Level III Adenoids;

#P < 0.05, Controls versus OSAHS with Level IV Adenoids;

§P < 0.05, Controls versus OSAHS combined with AR;

*P < 0.05, OSAHS combined with AR versus OSAHS with Level III Adenoids and OSAHS with Level IV Adenoids;

▽ P < 0.05, OSAHS with Level III Adenoids versus OSAHS with Level IV Adenoids



31
Figure 1

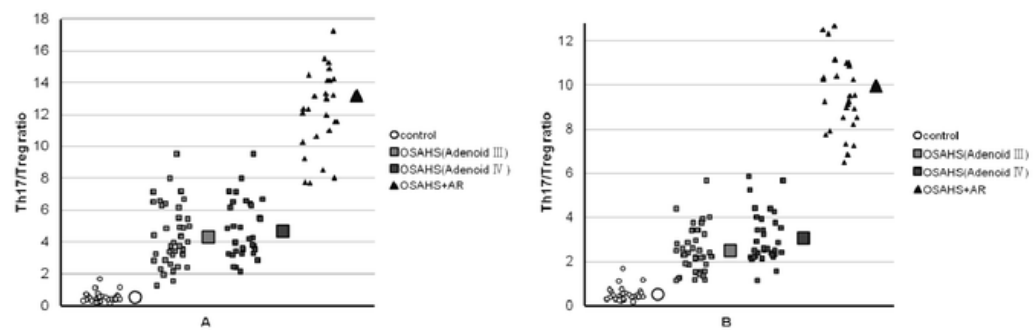


Figure 2

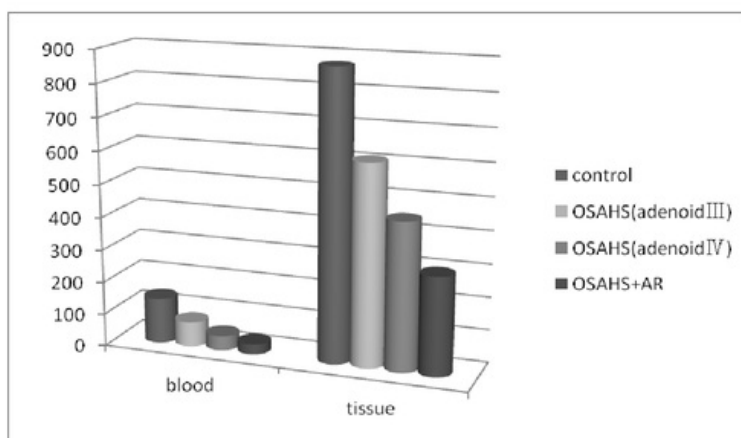


Figure 3

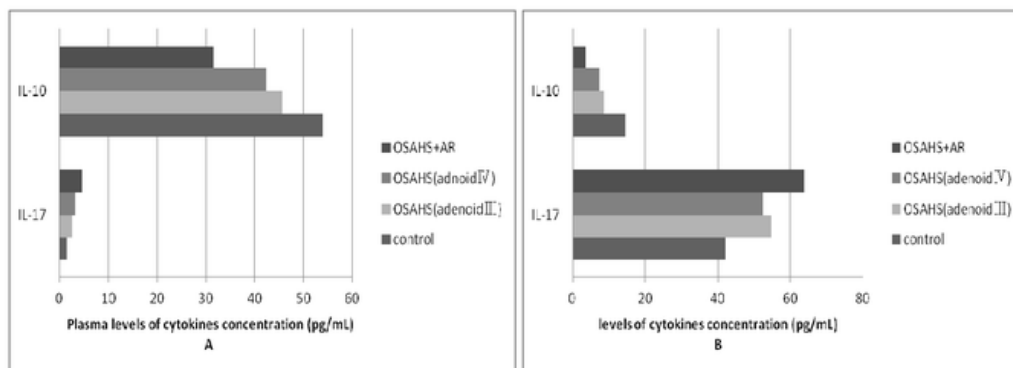


Figure 4

Th17 / Treg balance in children with obstructive sleep apnea syndrome and the relationship with allergic rhinitis

ORIGINALITY REPORT

22%

SIMILARITY INDEX

PRIMARY SOURCES

1	Ye, Jin, Hui Liu, Gehua Zhang, Peng Li, Zhiyuan Wang, Shaotong Huang, Qintai Yang, and Yuan Li. "The Treg/Th17 Imbalance in Patients with Obstructive Sleep Apnoea Syndrome", Mediators of Inflammation, 2012.	355 words — 6%
2	Xiao Shan Shao. "The prevalence of Th17 cells and FOXP3 regulate T cells (Treg) in children with primary nephrotic syndrome", Pediatric Nephrology, 06/05/2009	60 words — 1%
3	www.discoverymedicine.com	51 words — 1%
4	"ABSTRACTS OF THE XXIV CONGRESS OF THE INTERNATIONAL SOCIETY ON THROMBOSIS AND HAEMOSTASIS", Journal of Thrombosis and Haemostasis, 2013.	49 words — 1%
5	JIANBIN ZHANG. "Regulatory T cells/T-helper cell 17 functional imbalance in uraemic patients on maintenance haemodialysis: A pivotal link between microinflammation and adverse cardiovascular events", Nephrology, 02/2010	46 words — 1%
6	"Abstracts", Respiriology, 11/2011	44 words — 1%
7	Huang, Hui, Zhiwei Lu, Chunguo Jiang, Jia Liu, Yanxun Wang, and Zuojun Xu. "Imbalance between Th17 and Regulatory T-Cells in Sarcoidosis", International	36 words — 1%

-
- 8 ccn.aacnjournals.org 30 words — < 1%
Internet
-
- 9 Wang, Huaying, Weidong Peng, Yuesong Weng, Huajuan Ying, Hequan Li, Dajing Xia, and Wanjun Yu. "Imbalance of Th17/Treg cells in mice with chronic cigarette smoke exposure", International Immunopharmacology, 2012.
CrossCheck
-
- 10 www.ncbi.nlm.nih.gov 27 words — < 1%
Internet
-
- 11 www.jimmunol.org 25 words — < 1%
Internet
-
- 12 trinity.engr.uconn.edu 25 words — < 1%
Internet
-
- 13 Su, Zhi-Jun, Xue-Ping Yu, Ru-Yi Guo, De-Song Ming, Lv-Ye Huang, Mi-Long Su, Yong Deng, and Zhen-Zhong Lin. "Changes in the balance between Treg and Th17 cells in patients with chronic hepatitis B", Diagnostic Microbiology and Infectious Disease, 2013.
CrossCheck
-
- 14 Chen, Ou, Xiao-bo Zhu, Hui Ren, Yi-biao Wang, and Ruopeng Sun. "The imbalance of Th17/Treg in Chinese children with Henoch–Schonlein purpura", International Immunopharmacology, 2013.
CrossCheck
-
- 15 www.rheumatology.org.tw 23 words — < 1%
Internet
-
- 16 TUXUN, T., J.-H. WANG, R.-Y. LIN, J.-Y. SHAN, Q.-W. TAI, T. LI, J.-H. ZHANG, J.-M. ZHAO, and H. WEN. "Th17/Treg imbalance in patients with liver cystic echinococcosis :", Parasite Immunology, 2012.
CrossCheck
-

17	www.jlr.org Internet	22 words — < 1%
18	www.trianglealumni.org Internet	21 words — < 1%
19	"Posters", Journal of Gastroenterology and Hepatology, 10/2004 CrossCheck	21 words — < 1%
20	www.science.gov Internet	20 words — < 1%
21	www.obesityresearch.org Internet	18 words — < 1%
22	"3 rd GEBIN Symposium", European Archives of Psychiatry and Clinical Neuroscience, 10/01/2003 CrossCheck	18 words — < 1%
23	Terry Young. "The Occurrence of Sleep-Disordered Breathing among Middle-Aged Adults", New England Journal of Medicine, 04/29/1993 CrossCheck	17 words — < 1%
24	annals.org Internet	16 words — < 1%
25	ajrccm.atsjournals.org Internet	15 words — < 1%
26	Wu, Li, Li-Hua Luo, Ying-Xin Zhang, Qing Li, Bo Xu, Gui-Xiang Zhou, Hong-Bing Luan, and Yu-Sheng Liu. "Alteration of Th17 and Treg cells in patients with unexplained recurrent spontaneous abortion before and after lymphocyte immunization therapy", Reproductive Biology and Endocrinology, 2014. CrossCheck	15 words — < 1%
27	Cheng, X.. "The Th17/Treg imbalance in patients with acute coronary syndrome", Clinical Immunology, 200804	11 words — < 1%

28 G.-Y. Wang. "IL-17 Eliminates the Therapeutic Effects of Myelin Basic Protein-Induced Nasal Tolerance in Experimental Autoimmune Encephalomyelitis by Activating IL-6 : IL-17 Eliminated Nasal Tolerance in EAE by IL-6", *Scandinavian Journal of Immunology*, 10/29/2008

CrossCheck

11 words — < 1%

29 Li, Xia, Meng Zhang, Bin Wang, Yuzhu Li, Li Wang, Xiangzhong Zhao, Xianbin Zhou, Yuqi Guo, Guosheng Jiang, and Chengfang Yao. "Shenghua Decoction reduces uterine bleeding and regulates T-cell paradigm in human deciduas of RU486 medical abortion", *Journal of Ethnopharmacology*, 2013.

CrossCheck

11 words — < 1%

30 image.campushomepage.com

Internet

11 words — < 1%

31 www.kuulaire.com

Internet

10 words — < 1%

32 eurheartj.oxfordjournals.org

Internet

10 words — < 1%

33 Keiji Hirota. "Development, regulation and functional capacities of Th17 cells", *Seminars in Immunopathology*, 01/27/2010

CrossCheck

10 words — < 1%

34 Wisanti Laohaudomchok. "Toenail, Blood and Urine as Biomarkers of Occupational Exposure to Manganese :", *Epidemiology*, 01/2011

CrossCheck

10 words — < 1%

35 Brüstle, Anne, Dirk Brenner, Christiane B. Knobbe, Philipp A. Lang, Carl Virtanen, Brian M. Hershenfield, Colin Reardon, Sonja M. Lacher, Jürgen Ruland, Pamela S. Ohashi, and Tak W. Mak. "The NF-κB regulator MALT1 determines the encephalitogenic potential of Th17 cells", *Journal of Clinical Investigation*, 2012.

CrossCheck

9 words — < 1%

36 Felonato, Maíra, Adriana Pina, Eliseu Frank de Araujo, Flávio V. Loures, Silvia B. Bazan, Cláudia Feriotti, and Vera L. G. Calich. "Anti-CD25 Treatment Depletes Treg Cells and Decreases Disease Severity in Susceptible and Resistant Mice Infected with *Paracoccidioides brasiliensis*", PLoS ONE, 2012.

CrossCheck

9 words — < 1%

37 Sereshki, Nasrin; Gharagozloo, Marjan; Ostadi, Vajihe; Ghahiri, Ataollah; Roghaei, Mohammad Ali; Mehrabian, Ferdos; Andalib, Alireza; Hassanzadeh, Akbar; Hosseini, Habibolla and Rezaei, Abbas. "Variations in T-helper 17 and Regulatory T Cells during The Menstrual Cycle in Peripheral Blood of Women with Recurrent Spontaneous Abortion", International Journal of Fertility & Sterility, 2014.

Publications

9 words — < 1%

38 Liu, L.I.. "Th17/Treg imbalance in adult patients with minimal change nephrotic syndrome", Clinical Immunology, 201106

CrossCheck

9 words — < 1%

39 www.jove.com

Internet

9 words — < 1%

40 Kent, B.D.. "Obstructive sleep apnea and inflammation: Relationship to cardiovascular co-morbidity", Respiratory Physiology & Neurobiology, 20110930

CrossCheck

9 words — < 1%

41 Li, Ai-Li, Xiang-chen Meng, Gui-cheng Huo, Cui-cui Duan, Quan-ling Zheng, Dan Li, and Yan Wang. "The Th17/Treg imbalance in bovine β -lactoglobulin-sensitised mice", International Dairy Journal, 2013.

CrossCheck

9 words — < 1%

42 Chu, S.. "The expression of Foxp3 and ROR gamma t in lung tissues from normal smokers and chronic obstructive pulmonary disease patients", International Immunopharmacology, 201111

CrossCheck

8 words — < 1%

-
- 43 www.ajcei.us 8 words — < 1%
Internet
-
- 44 Liu, Chun-Yun, Shang-De Guo, Jie-Zhong Yu, Yan-Hua Li, Hui Zhang, Ling Feng, Zhi Chai, Hai-Jun Yuan, Wan-Fang Yang, Qian-Jin Feng, Bao-Guo Xiao, and Cun-Gen Ma. "Fasudil mediates cell therapy of EAE by immunomodulating encephalomyelitic T cells and macrophages : Clinical immunology", European Journal of Immunology, 2014. 8 words — < 1%
CrossCheck
-
- 45 Rana, A., R. Minz, R. Aggarwal, S. Anand, N. Pasricha, and S. Singh. "Gene expression of cytokines (TNF- α , IFN- α), serum profiles of IL-17 and IL-23 in paediatric systemic lupus erythematosus", Lupus, 2012. 8 words — < 1%
CrossCheck
-
- 46 Dai, Heling, Tiansheng Sun, Zhi Liu, Jianzheng Zhang, and Meng Zhou. "The imbalance between regulatory and IL-17-secreting CD4+T cells in multiple-trauma rat", Injury, 2013. 8 words — < 1%
CrossCheck
-
- 47 Kim, J.T.. "Tear cytokines and chemokines in patients with Demodex blepharitis", Cytokine, 201101 8 words — < 1%
CrossCheck
-
- 48 Gozal, D.. "Systemic inflammation in non-obese children with obstructive sleep apnea", Sleep Medicine, 200803 8 words — < 1%
CrossCheck
-
- 49 www.newmicrobiologica.org 8 words — < 1%
Internet
-
- 50 Anderson, M. E., Z. S. Buchwald, J. Ko, R. Aurora, and T. Sanford. "Patients with Pediatric Obstructive Sleep Apnea Show Altered T-Cell Populations with a Dominant TH17 Profile", Otolaryngology - Head and Neck Surgery, 2014. 8 words — < 1%
CrossCheck

51 Chevalier, Mathieu F., Gaël Petitjean, Catherine Dunyach-Rémy, Céline Didier, Pierre-Marie Girard, Maria Elena Manea, Pauline Campa, Laurence Meyer, Christine Rouzioux, Jean-Philippe Lavigne, Françoise Barré-Sinoussi, Daniel Scott-Algara, and Laurence Weiss. "The Th17/Treg Ratio, IL-1RA and sCD14 Levels in Primary HIV Infection Predict the T-cell Activation Set Point in the Absence of Systemic Microbial Translocation", PLoS Pathogens, 2013.

CrossCheck

8 words — < 1%

52 Qian Niu. "Disturbed Th17/Treg balance in patients with rheumatoid arthritis", Rheumatology International, 08/02/2011

CrossCheck

8 words — < 1%

53 Li, Y.. "CNI induced Th17/Treg imbalance and susceptibility to renal dysfunction in renal transplantation", International Immunopharmacology, 201112

CrossCheck

8 words — < 1%

54 Kim, J.. "Inflammatory pathways in children with insufficient or disordered sleep", Respiratory Physiology & Neurobiology, 20110930

CrossCheck

8 words — < 1%

55 parasitol.or.kr

Internet

8 words — < 1%

56 Chen Lina. "Combined Treatment of Etanercept and MTX Reverses Th1/Th2, Th17/Treg Imbalance in Patients with Rheumatoid Arthritis", Journal of Clinical Immunology, 05/12/2011

CrossCheck

8 words — < 1%

57 www.websciences.org

Internet

8 words — < 1%

58 Martin-Saavedra, F.M.. "Beta interferon restricts the inflammatory potential of CD4⁺ cells through the boost of the Th2 phenotype, the inhibition of Th17 response and the prevalence of naturally occurring T regulatory cells", Molecular Immunology, 200809

7 words — < 1%

-
- 59 Xie, J.j.. "The Th17/Treg functional imbalance during atherogenesis in ApoE^{0/0} mice", Cytokine, 201002 7 words — < 1%
CrossCheck
-
- 60 Zhang, Y.. "The imbalance of Th17/Treg in patients with uterine cervical cancer", Clinica Chimica Acta, 20110512 7 words — < 1%
CrossCheck
-
- 61 Zhang, Qibo, Linda Bagrade, Jolanta Bernatoniene, Ed Clarke, James C. Paton, Tim J. Mitchell, Desmond A. Nunez, and Adam Finn. "Low CD4 T Cell Immunity to Pneumolysin Is Associated with Nasopharyngeal Carriage of Pneumococci in Children", The Journal of Infectious Diseases, 2007. 6 words — < 1%
CrossCheck
-

EXCLUDE QUOTES OFF
EXCLUDE BIBLIOGRAPHY ON

EXCLUDE MATCHES OFF