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OTOLOGY

Peripheral blood immunological parameters of children with adenoid hypertrophy with otitis media with effusion: propensity score matching

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Abstract

Purpose To evaluate peripheral blood immunological parameters and the possible correlation with age, gender and adenoid size in children with adenoid hypertrophy with OME.

Methods A total of 664 children with adenoid hypertrophy were initially enrolled in our study, of which 83 had concomitant OME. To minimize selection bias, we performed one to two propensity score matching (PSM) between children with and without OME. After PSM, 80 children with OME (OME group) and 157 children without OME (adenoid hypertrophy [AH] group) were selected. The patients' peripheral blood samples were prepared prior to surgery and their immunological parameters were compared between groups.

Results Compared to the AH group, the serum level of C3 was significantly higher in the OME group (0.88 ± 0.01 g/L vs. 0.94 ± 0.02 g/L; $p = 0.014$), which was the only independent risk factor for OME (odds ratio 13.58, 95% confidence interval 1.25–147.99; $p = 0.032$). However, no such difference was seen for serum immunoglobulin (IgG, IgA, IgM, IgE), T cell subsets (CD3+, CD4+ and CD8+ T cells), or lymphocytes and monocytes. Further subgroup analyses showed that in children ≤ 5 years old, the C3 level was significantly higher in OME patients ($p = 0.023$). A subgroup analysis based on sex indicated that there was a significantly higher level of serum C3 ($p = 0.009$) and lower CD3+ and CD4+ T cells ($p = 0.010$ and $p = 0.021$, respectively) in girls with OME compared to those without OME. No association between immunological parameters and adenoid size was found.

Conclusions There were no significant differences in cellular immunology and humoral immune indicators in children with adenoid hypertrophy with or without OME. In children ≤ 5 years old, significantly higher serum C3 levels in patients with OME demonstrate excessively activated C3 in comparison to patients without OME. For girls, a higher serum level of C3 with a lower amount of CD3+ and CD4+ T cells may be associated with OME.

Keywords Otitis media with effusion · Adenoid hypertrophy · Immunological parameters · Propensity score matching

Introduction

Adenoid hypertrophy (AH) and otitis media with effusion (OME) are common diseases in children. Patients with OME have high-grade biofilm formation on their adenoid surfaces, indicating that adenoids may play crucial roles in the induction of OME [1]. However, the exact pathogenesis remains unclear. AH may obstruct the opening of eustachian tubes, leading to reservoirs for bacteria and dysfunction of eustachian tubes via ascending infection [2].

Nevertheless, researchers have observed that adenoid size does not appear to be related to the incidence of OME [3]. Sade and Luntz et al. found no eustachian tube lumen

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obstruction or significant differences in lumen size in healthy patients or patients with OME or acute otitis media [4, 5]. In addition, a previous study reported that bacteria were only detected in one-third of patients with middle ear effusion [6]. Mechanisms other than bacterial infection may be responsible for the occurrence of otitis media in children with AH.

Currently, immunological factors concomitant with ongoing complement activation and inflammation are considered one of the most important causes of OME [7]. Adenoids containing T and B lymphocytes, which secrete immunoglobulin (by B lymphocytes) and other immune factors in and around the nasopharynx, either occur spontaneously or in response to antigens [8, 9]. When stimulated by antigens, lymphocytes, immune factors, and inflammatory components aggregate in the mucous of the middle ear cavity where they can interact with pathogenic factors and cause persistent inflammation [5, 10–14]. Previous studies have researched the impact of immunoglobulin and lymphocyte subpopulations located in the adenoids and peripheral blood of AH patients with or without OME, complement and complement fragments in middle ear effusion patients, and serum specimens in OME children. However, no unanimous conclusion has been reached [9, 12–16]. Small sample sizes and lack of compensation for confounding factors have impacted these results.

Therefore, we retrospectively analyzed data from AH patients admitted to our hospital between May 2016 and April 2019. We used propensity score matching (PSM) to exclude confounding factors and then compared cellular (CD3+, CD4+, CD8+ T cell and CD4+/CD8+ T cell), humoral (IgG, IgA, IgM, IgE), and complement (C3, C4, PFB) immune functions between AH patients with or without OME.

Methods

Protocol and registration

Written informed consent was obtained from all children's parents prior to surgery. This observational study was approved by the Ethics Committee of West China Hospital of Sichuan University (No. 2018-146). The clinical trial was registered on the Chinese Clinical Trial Registry (ChiCTR1900022630, https://www.chictr.org.cn/index.aspx).

Patients

Between May 2016 and April 2019, 664 children with AH, who were admitted to the West China Hospital of Sichuan University for adenoidectomy, were included in our study. Among them, 83 children were diagnosed with OME, and myringotomy was performed at the same time as the adenoidectomy. AH was diagnosed based on complaints of snoring and sleep apnea, lateral neck radiograph, and physical examination. OME was diagnosed by persistent middle ear effusion signs for more than 3 months, pneumatic otoscopy, and tympanometry [17]. Children with AH without OME had no prior history of recurrent middle ear disease or hearing impairment, and they all exhibited a normal tympanic membrane before surgery. Exclusion criteria included previous tonsillar or adenoid surgery, craniofacial malformation, suspicion of congenital or acquired immune deficiency, acute upper respiratory tract infection prior to surgery, or children with allergies or suspected allergic diseases. Demographic data such as sex, age, height, weight, and BMI were recorded. Prior to surgery, tonsils were graded as follows [8]: Grade 1, tonsil confined to tonsillar fossa; Grade 2, tonsils extending just outside the anterior pillars; Grade 3, tonsils extending outside the posterior pillars but not meeting in the midline; and Grade 4, tonsils meeting in the midline and almost completely obstructing the airway. The adenoid–nasopharyngeal (A/N) ratio derived from lateral neck radiographs was used to assess adenoid size [18]. To minimize patient selection bias and adjust for confounding factors, we performed PSM between patients with and without OME. The propensity score of all covariates (sex, age, BMI, A/N ratio, and tonsil grade) was calculated using logistic regression analyses for each patient, and one to two matched analyses using nearest-neighbor matching were performed with 0.1 standard deviations of the logit of the propensity score as the caliper value.

Complete blood count

Venous blood (2 mL) was obtained from each patient, and a complete blood count was performed within 1 h with the SYSMEX xe-2100D automatic blood cell analyzer (SYSMEX Corporation, Kobe, Japan).

Flow cytometry

Peripheral blood T lymphocytes and their subsets (CD3+, CD4+, CD8+ T lymphocyte) were detected via flow cytometry. Blood samples were collected in 4 mL tubes containing ethylenediaminetetraacetic acid (EDTA) and corresponding antibodies. After 30 min incubation at 25 °C, the samples were analyzed with the FACS Canto Flow Cytometer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Serum immune factors (immunoglobulin and complement) were detected with rate scatter nephelometry using the IMMAGE 800 immunochemistry analyzer (Beckman Coulter, Brea, CA, USA).

Statistical analyses

Baseline characteristics are expressed as the mean ± standard error of the mean (SEM) or *n* (%). The chi-square test or Fisher's exact test were used for comparisons of categorical variables, whereas the independent sample *t* test was used for continuous variables. The immune status of AH patients with OME and without OME were compared using the independent samples *t* test; further logistic regression analyses were used to explore the independent risk factors for occurrence of OME in children with AH. The independent sample *t* test was used for comparison between different subgroups. Linear regression analysis was used to examine the association between immunological parameters and the A/N ratio. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using SPSS ver. 24 for Windows (IBM, Armonk, NY, USA).

Results

Patient characteristics

Of the 664 children included in our study, 83 had AH with OME (OME group) and 581 had AH without OME (AH group). Age ($p = 0.010$), tonsil grade ($p = 0.031$), and A/N ratio ($p = 0.043$) were significantly different between the OME and AH groups. Compared to the AH group, the OME group was younger, and had a lower tonsil grade and greater adenoid size. There was no significant difference in sex or BMI between groups. After one to two nearest-neighbor matching PSM analyses, 80 patients from the OME group and 157 patients from the AH group were selected for further analyses, and no significant differences in age, tonsil grade, A/N ratio, sex, or BMI were found (Fig. 1, Table 1).

Analyses of systemic immune function

In the propensity score-matched cohort, the serum level of C3 was significantly higher in the OME group (0.94 ± 0.02 g/L) compared to the AH group (0.88 ± 0.01 g/L) ($p = 0.014$). However, there were no significant differences in C4 or PFB between groups ($p = 0.201$ and $p = 0.067$, respectively). In addition, there were no significant differences in cell-mediated or humoral immunity, peripheral blood lymphocyte count, lymphocyte ratio, monocyte count, or monocyte ratio (Table 2). We performed further logistic regression analyses to confirm the independent risk factors for OME in children with AH. Corresponding to our earlier results, higher serum C3 level was the only significant factor associated with OME (odds ratio 13.58, 95% confidence interval 1.25–147.99; $p = 0.032$) (data not shown).

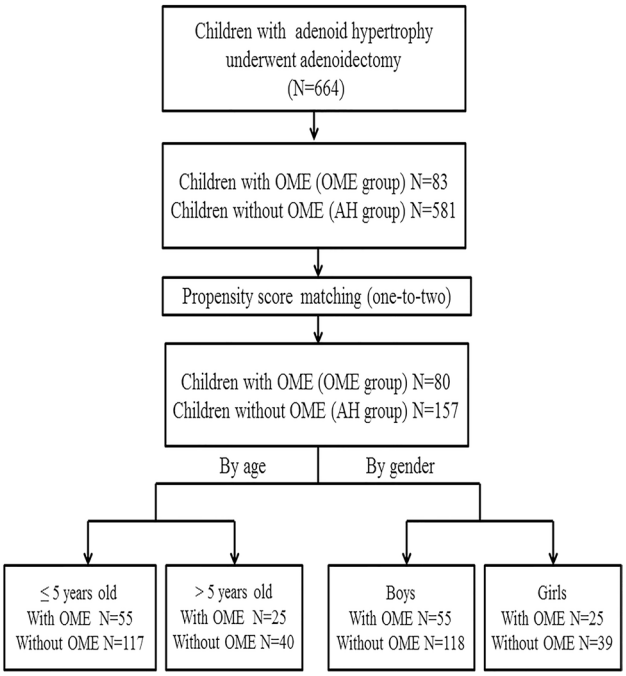

Fig. 1 Flow chart of matching process and patient groups

Systemic immune function of different age sub-groups

To analyze the impact of age on immune parameters, we divided the propensity score-matched cohort into two age groups: > 5 years ($n = 65$) and ≤ 5 years ($n = 172$). The older children had higher levels of serum IgG, IgA, C3, C4, and blood monocyte ratio, while the younger children had higher CD4+ T cells, lymphocyte count, and lymphocyte ratio. There were no significant differences in serum IgM, IgE, PFB, CD3+ T cells, CD8+ T cells, CD4+/CD8+ T cell, or monocyte count according to age (Table 3). Next, we analyzed the impact of age on the incidence of OME in each age group. The only significant difference was the higher serum C3 level in the younger OME group versus the younger AH group ($p = 0.023$). In the two older groups, there were no significant differences in any immunological parameter (Fig. 1, Table 4).

Systemic immune function of different sex sub-groups

Next, we divided the children into four groups: boys with or without OME ($n = 55$ and $n = 118$, respectively) and girls with or without OME ($n = 25$ and $n = 39$, respectively) (Fig. 1). In the two girl groups, the OME group had

Table 1 Baseline clinical characteristics of patients before and after propensity score matched analysis

Variables	Before propensity score matching		*p* value	After propensity score matching		*p* value
	AH group[a]	OME group[b]		AH group[a]	OME group[b]	
Patients number (*N*)	581	83		157	80	
Gender (*n*, %)			0.949			0.293
Male	401 (69.0%)	57 (68.7%)		118 (75.2%)	55 (68.8%)	
Female	180 (31.0%)	26 (31.3%)		39 (24.8%)	25 (31.3%)	
Age ($\overline{X} \pm$ SE, years)	5.51 ± 0.09	5.00 ± 0.17	0.010*	4.87 ± 0.13	5.08 ± 0.18	0.37
Body mass index ($\overline{X} \pm$ SE, kg/m^2)	15.96 ± 0.12	16.53 ± 0.61	0.156	15.75 ± 0.20	16.04 ± 0.30	0.422
Tonsils grade (*n*, %)			0.031*			0.368
I	4 (0.7%)	2 (2.4%)		0 (0.0%)	1 (1.3%)	
II	29 (5.0%)	9 (10.8%)		10 (6.4%)	8 (10.0%)	
III	302 (52.0%)	45 (54.2%)		89 (56.7%)	45 (56.3%)	
IV	246 (42.3%)	27 (32.5%)		58 (36.9%)	26 (32.5%)	
Adenoid (A/N, $\overline{X} \pm$ SE)	0.69 ± 0.00	0.71 ± 0.01	0.043*	0.72 ± 0.01	0.70 ± 0.01	0.365

[a]AH group = adenoid hypertrophy without otitis media with effusion

[b]OME group = adenoid hypertrophy with otitis media with effusion

*$p < 0.05$

Table 2 Comparison of peripheral blood immunology parameters between AH group and OME group

Variables ($\overline{X} \pm$ SE)	AH group[a] *N* = 157	OME group[b] *N* = 80	*P* value
IgG (g/L)	10.60 ± 0.19	10.52 ± 0.27	0.804
IgA (mg/L)	1358.63 ± 52.44	1372.19 ± 70.68	0.880
IgM (mg/L)	1285.55 ± 37.77	1353.82 ± 58.86	0.315
IgE (IU/ml)	120.45 ± 19.79	97.87 ± 18.75	0.476
C3 (g/L)	0.88 ± 0.01	0.94 ± 0.02	0.014*
C4 (g/L)	0.19 ± 0.01	0.21 ± 0.01	0.201
PFB (mg/L)	324.34 ± 7.28	353.19 ± 13.79	0.067
CD3+ T cell (%)	66.64 ± 0.59	64.95 ± 0.81	0.099
CD4+ T cell (%)	33.85 ± 0.59	32.52 ± 0.69	0.144
CD8+ T cell (%)	24.45 ± 0.47	24.47 ± 0.67	0.975
CD4+/CD8+ T cell	1.49 ± 0.04	1.42 ± 0.06	0.364
Lymphocyte count (10^9/L)	3.55 ± 0.09	3.30 ± 0.11	0.111
Lymphocyte ratio (%)	46.75 ± 0.89	44.25 ± 1.19	0.100
Monocytes count (10^9/L)	0.50 ± 0.01	0.49 ± 0.02	0.565
Monocytes ratio (%)	6.58 ± 0.13	6.45 ± 0.19	0.555

[a]AH group = adenoid hypertrophy without otitis media with effusion

[b]OME group = adenoid hypertrophy with otitis media with effusion

*$p < 0.05$

Table 3 Comparison of peripheral blood immunology parameters in different age sub-groups

Variables ($\overline{X} \pm$ SE)	≤ 5 years old *N* = 172	> 5 years old *N* = 65	*p* value
IgG (g/L)	9.98 ± 0.17	12.14 ± 0.27	0.000*
IgA (mg/L)	1188.26 ± 41.32	1820.49 ± 84.23	0.000*
IgM (mg/L)	1311.19 ± 35.78	1300.43 ± 67.93	0.881
IgE (IU/ml)	98.20 ± 16.87	152.70 ± 29.44	0.098
C3 (g/L)	0.87 ± 0.01	0.98 ± 0.02	0.000*
C4 (g/L)	0.19 ± 0.01	0.22 ± 0.01	0.036*
PFB (mg/L)	325.77 ± 7.95	353.28 ± 11.74	0.064
CD3+ T cell (%)	66.37 ± 0.54	65.39 ± 1.02	0.363
CD4+ T cell (%)	34.01 ± 0.52	31.87 ± 0.93	0.036*
CD8+ T cell (%)	24.27 ± 0.45	24.94 ± 0.76	0.438
CD4+/CD8+ T cell	1.51 ± 0.04	1.37 ± 0.06	0.081
Lymphocyte count (10^9/L)	3.60 ± 0.09	3.13 ± 0.13	0.005*
Lymphocyte ratio (%)	47.44 ± 0.87	41.83 ± 1.11	0.000*
Monocytes count (10^9/L)	0.49 ± 0.01	0.52 ± 0.02	0.165
Monocytes ratio (%)	6.40 ± 0.12	6.91 ± 0.21	0.031*

*$p < 0.05$

a significantly higher level of serum C3 ($p = 0.009$), and a significantly lower CD3+ and CD4+ T cell count ($p = 0.010$ and $p = 0.021$, respectively). There were no significant differences in any immunological parameter in the two groups of boys (Table 5).

Association of immune parameters and A/N ratio

Linear regression analysis was performed to compare the immunological parameters and A/N ratio. However, no significant associations were revealed between any immunological parameter and A/N ratio among the propensity score-matched cohort. Furthermore, there were no

Table 4 Comparison of immunology parameters between AH group and OME group in different age sub-groups

Variables ($\overline{X} \pm SE$)	≤ 5 years old		*p* value	> 5 years old		*p* value
	AT group[a]	OME group[b]		AT group[a]	OME group[b]	
Patients number (*N*)	117	55		40	25	
IgG (g/L)	10.03 ± 0.22	9.87 ± 0.26	0.673	12.29 ± 0.26	11.90 ± 0.57	0.534
IgA (mg/L)	1179.32 ± 48.43	1208.00 ± 78.99	0.749	1883.13 ± 115.35	1720.28 ± 117.97	0.351
IgM (mg/L)	1309.77 ± 41.40	1314.32 ± 70.05	0.953	1214.73 ± 85.51	1437.56 ± 108.19	0.111
IgE (IU/ml)	110.95 ± 23.39	69.40 ± 14.73	0.257	147.28 ± 37.07	162.56 ± 49.51	0.806
C3 (g/L)	0.85 ± 0.01	0.92 ± 0.03	0.023*	0.96 ± 0.03	1.01 ± 0.05	0.275
C4 (g/L)	0.18 ± 0.01	0.21 ± 0.01	0.095	0.22 ± 0.01	0.20 ± 0.01	0.427
PFB (mg/L)	315.56 ± 8.48	349.15 ± 17.22	0.085	348.48 ± 13.64	362.90 ± 22.65	0.567
CD3+ T cell (%)	66.89 ± 0.68	65.18 ± 0.83	0.144	65.94 ± 1.21	64.47 ± 1.82	0.489
CD4+ T cell (%)	34.36 ± 0.67	33.22 ± 0.74	0.255	32.38 ± 1.23	31.03 ± 1.44	0.49
CD8+ T cell (%)	24.38 ± 0.55	24.03 ± 0.78	0.718	24.65 ± 0.95	25.43 ± 1.28	0.623
CD4+/CD8+ T cell	1.52 ± 0.05	1.49 ± 0.08	0.765	1.42 ± 0.09	1.28 ± 0.08	0.285
Lymphocyte count (10^9/L)	3.68 ± 0.11	3.43 ± 0.14	0.185	3.19 ± 0.16	3.02 ± 0.19	0.521
Lymphocyte ratio (%)	48.56 ± 1.04	45.05 ± 1.52	0.059	41.48 ± 1.43	42.42 ± 1.81	0.688
Monocytes count (10^9/L)	0.49 ± 0.01	0.48 ± 0.02	0.859	0.54 ± 0.03	0.50 ± 0.03	0.405
Monocytes ratio (%)	6.50 ± 0.15	6.19 ± 0.21	0.245	6.84 ± 0.25	7.04 ± 0.37	0.643

[a]AH group = adenoid hypertrophy without otitis media with effusion

[b]OME group = adenoid hypertrophy with otitis media with effusion

*$p < 0.05$

Table 5 Comparison of immunology parameters between AH group and OME group in different sex sub-groups

Variables ($\overline{X} \pm SE$)	Boys		*p* value	Girls		*p* value
	AT group[a]	OME group[b]		AT group[a]	OME group[b]	
Patients number (*N*)	118	55		39	25	
IgG (g/L)	10.59 ± 0.21	10.27 ± 0.32	0.408	10.65 ± 0.44	11.08 ± 0.52	0.546
IgA (mg/L)	1410.84 ± 61.17	1393.72 ± 81.72	0.872	1200.68 ± 98.55	1323.75 ± 139.94	0.463
IgM (mg/L)	1234.72 ± 39.85	1236.44 ± 66.03	0.981	1439.36 ± 89.17	1617.92 ± 103.49	0.206
IgE (IU/ml)	136.48 ± 25.34	99.22 ± 23.36	0.374	71.93 ± 20.07	94.98 ± 31.82	0.521
C3 (g/L)	0.88 ± 0.01	0.92 ± 0.03	0.191	0.86 ± 0.03	0.99 ± 0.05	0.009*
C4 (g/L)	0.19 ± 0.01	0.21 ± 0.01	0.296	0.19 ± 0.01	0.21 ± 0.02	0.458
PFB (mg/L)	318.87 ± 8.15	339.96 ± 14.84	0.217	340.47 ± 15.70	382.81 ± 29.43	0.169
CD3+ T cell (%)	65.83 ± 0.72	65.03 ± 0.99	0.529	69.06 ± 0.91	64.79 ± 1.40	0.010*
CD4+ T cell (%)	32.17 ± 0.59	31.63 ± 0.81	0.605	38.84 ± 1.26	34.42 ± 1.20	0.021*
CD8+ T cell (%)	24.96 ± 0.56	25.35 ± 0.87	0.705	22.93 ± 0.81	22.62 ± 0.89	0.804
CD4+/CD8+ T cell	1.38 ± 0.04	1.34 ± 0.06	0.568	1.81 ± 0.10	1.60 ± 0.12	0.193
Lymphocyte count (10^9/L)	3.53 ± 0.11	3.34 ± 0.14	0.308	3.61 ± 0.19	3.22 ± 0.21	0.176
Lymphocyte ratio (%)	46.03 ± 1.08	43.93 ± 1.38	0.259	48.95 ± 1.41	44.96 ± 2.35	0.126
Monocytes count (10^9/L)	0.52 ± 0.02	0.50 ± 0.02	0.44	0.43 ± 0.02	0.46 ± 0.03	0.554
Monocytes ratio (%)	6.84 ± 0.15	6.55 ± 0.23	0.297	5.81 ± 0.17	6.24 ± 0.32	0.247

[a]AH group = adenoid hypertrophy without otitis media with effusion

[b]OME group = adenoid hypertrophy with otitis media with effusion

*$p < 0.05$

significant associations between immunological parameters and A/N ratio in either the OME group or AH group (data not shown).

Discussion

We excluded confounding factors by PSM, and compared immunological parameters in peripheral blood between children with adenoid hypertrophy with or without OME. This approach, which aimed to clarify the relationship of systemic immune function with OME, revealed significantly higher serum C3 levels in OME children, particularly those under 5 years of age. No such difference was seen for cellular or humoral immunity-related parameters or lymphocyte and monocyte counts in peripheral blood. In addition, a higher level of serum C3 combined with lower CD3+ and CD4+ T cell numbers were seen in the girl's OME group compared with girls without OME. The immunological parameters were related to age, yet there was no correlation between immunological parameters and adenoid size.

The complement system is considered the first line of defense against invading pathogens, and it contributes to immunological and inflammatory processes [19]. C3 is a major component of the complement system, and is involved in the classic and alternative complement activation pathway. He and Meri et al. found a substantially lower level of C3 but higher levels of C3 breakdown products (C3a and C3b) in middle ear effusion compared to serum [13]. Furthermore, Allen et al. discovered that in a keyhole-limpet-hemocyanin-induced OME guinea pig model, serum decomplemented through treatment with cobra venom factor exhibited less effusion and inflammation in the middle ear [20]. These results suggest that C3 activation in the middle ear plays a crucial role in the induction of OME. However, there is a lack of research comparing the serum level of C3 in OME patients with those without OME, particularly in children with AH with or without OME.

Our study showed a significantly higher serum C3 level in children with AH with OME compared to those without OME. In addition, this difference was mainly seen in children under 5 years of age. Previous studies have reported that the peak incidence of OME happens at 2 and 5 years [21]. Thus, the relatively increased serum C3 level in OME children and the time of highest prevalence of OME seem to be correlated.

C3 is mainly produced in the liver, but is also produced by other cells such as epithelial cells, macrophages, or even human middle ear epithelial tissue (HMEE) [13]. HMEE stimulated by pathogens leads to local C3 synthesis, while the liver, adenoids, and other lymphoid tissues continue to produce systemic C3, which can coordinate with locally produced C3 participating in complement activation and the inflammatory response [10].

The activated complement pathway results in C3 cleavage and a sequential reactions, leading to the generation of the terminal components and the assembly of the membrane attack complex (MAC), which could eliminate invasive pathogens, or cause persistent inflammation reaction even lead to OME while the complement is excessively activated [7, 13, 19].

Immune dysfunction in the peripheral blood and adenoids may induce and even aggravate OME. In previous studies, specific immunological deficiencies were suggested to occur in otitis-prone children [22, 23]. Shin et al. discovered a correlation between OME and a lower level of serum IgA and IgG subclasses [24]. Nevertheless, in a large, well-defined patient population study, Selma et al. confirmed there were no differences in serum antibodies in children with persistent OME compared to healthy controls [25], and another study reported that a level of IgA and IgG that was within normal limits in children with or without OME [26]. ln addition, in accordance with our findings, no statistical differences in B cell (CD19+) or T cell subpopulations (CD3+, CD4+, CD8+) in peripheral blood in children with and without coexisting OME have been reported [15].

In our study, we saw no significant differences in immunoglobulin level, T cells, or lymphocyte and monocyte ratios in the peripheral blood of children with and without OME, which suggests that both groups had similar immune functions. When analyzing the effects of age on immunological parameters, we found higher serum immunoglobulin and complement levels and an increased monocyte ratio in older children. These results are similar to previous studies on the correlation between age and immunological parameters [9, 27]. However, there were no significant differences in immunological parameters of older children with or without OME. Therefore, the children's immune function developed with age, yet without an influence on OME.

In this study, no obvious differences in any immunological parameter were discovered in boys with or without OME. However, in accordance with other studies [28], we found that the majority of patients in the OME group were males, which may be explained by the defective pneumatization and higher degree of middle ear pathology resulting from more frequent and severe episodes of upper respiratory tract infections in boys [29]. However, the significantly higher level of serum complement and lower blood T cell subsets found in girls with OME compared to girls without OME suggest that excessively activated complement and decreased humoral immune function may be associated with OME in girls. Because of the difference in sex hormones and chromosomes, females mount higher innate and adaptive immune responses than males, and more likely to

trigger immune response after being stimulated by inflammation [30]. Consequently, differences of immune function in adenoid hypertrophy children with or without otitis media with effusion were affected by gender.

As a part of Waldeyer's ring, adenoids associate with the mucosal defense response of the nasopharynx, which contains T cells and B cells and are able to synthesize immunoglobulin (by B lymphocytes) and other immune factors. In a study by Chunyan et al., a higher proportion of follicular helper T cells were found in children with mild AH compared to healthy controls, whereas there were no significant differences in the moderate and severe AH groups [12]. Musiatowicz et al. revealed that despite decreasing in size with age, the adenoids maintain their function and the percentage of adenoid T cells and B cells does not change [27]. Similarly, we found there were no significant associations between serum immune parameters and adenoid size in children with AH. Consequently, the immune function of hypertrophic adenoids was not influenced by adenoid size, but by the proportion of activated lymphocytes.

Our study had several limitations. We did not compare the serum subclasses of immunoglobulin (e.g., IgG1, IgG2, sIgA), T cells with different antigen phenotypes, or complement fragment level between groups, which may be necessary to determine the effects, if any, of immunological parameters on the pathogenesis of OME. Previous studies have indicated that there is increased plasma IgG1 and decreased plasma IgG2 in children with secretory otitis media compared to healthy controls [31]. However, no significant differences in CD25+ and CD69+ T cells has been identified in those two groups [15]. Another limitation of our study is that there was no healthy control group, so we could not compare the direct influences of AH on immune function, although our study did not find any connection between adenoid size and serum immunological parameters.

Conclusion

It seems no significant differences in cellular or humoral immunity in children with AH with or without OME. OME children may have significantly higher serum C3 level, particularly in children under 5 years of age. For girls, a higher level of serum C3 with decreased blood CD3+ and CD4+ T cells may be associated with OME.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval This study was approved by the Ethics Committee of West China Hospital of Sichuan University (No. 2018-146).

Informed consent Written informed consent was obtained from all children's parents prior to surgery.

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