

ORIGINAL ARTICLE

Local atopy is more relevant than serum sIgE in reflecting allergy in childhood adenotonsillar hypertrophy

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Abstract

Background: There was a lack of convincing evidence supporting the influence of allergy on the adenotonsillar hypertrophy (ATH). We investigated the presence of specific IgE (sIgE) in the serum and adenotonsillar tissues in children with ATH.

Methods: A Pharmacia ImmunoCAP system was used to test sIgE against 31 allergens in the serum in 83 children with ATH before adenotonsillectomy. sIgE against 15 representative allergens were detected in adenotonsillar tissues from 20 of those children.

Results: A total of 51 (61.45%) children had positive serum sIgE. 20 children with ATH had positive sIgE to more than two allergens at both tonsils and adenoids, although half of them were serum sIgE negative. The percentage of subjects with positive sIgE expression in adenoids and tonsils was 50.0% and 42.9%, respectively, among the subjects with positive serum sIgE expression. Of subjects with negative serum sIgE expression, local sIgE was detected in 36.0% of adenoids and 43.8% of tonsils, respectively. The rate of sIgE presence in local tissue (adenoids or tonsils) was significantly higher than that seen in the serum.

Conclusion: This study provided evidence that there was an important role for allergic inflammation in the ATH. The inconsistency in sIgE expression between adenotonsillar and serum suggests a role of local atopy in childhood ATH.

Sensitization to a variety of allergens is believed to be involved in the development of adenotonsillar hypertrophy (ATH), a common disease in children. Sadeghi-Shabestari et al. found significantly higher positive rate of skin prick test (SPT) in ATH children (70.3%) than in those without ATH (10%; 1). In their study, however, elevated levels of total IgE were noted in only 48% of the SPT-positive children. Many other studies also demonstrated that less than half of the children with ATH had atopy(2–4). The role of allergy in childhood ATH therefore remains inconclusive.

Anatomically, the adenoids and tonsils constitute the major part of Waldeyer's ring, with the tubal tonsils and lateral pharyngeal bands as less prominent components(5). These organs play an important immune-inductive role as part of mucosa-associated lymphoid tissue (MALT). The MALT also shows similarities with lymph nodes and may, in addition, contribute as an effector site of local systemic type and mucosal secretory type of adaptive humoral immunity(6).

Papatziamos et al. demonstrated the presence of IgE-bound plasma cells in the adenoids of atopic children. This suggests

that adenoids are involved in IgE-mediated sensitization, with local differentiation of IgE-producing plasma cells constituting a probable source of mucosal B cells for the upper airways(7). A few studies have also shown that adenoids in atopic children present with cellular and cytokine profiles different from those in non-atopic children and that eosinophils could be involved in adenoid inflammation(8, 9). Shin et al. have demonstrated the local production of total IgE and *D. pteronyssinus* (DP)-specific antibodies, as well as evidenced eosinophilic inflammation in adenoid tissues of atopic children. Moreover, locally produced DP-specific IgE, IgA, and IgG antibodies may contribute to eosinophilic inflammation(10). So far, very few studies in the literature have shed light on the local allergy in adenotonsillar tissues. We hypothesized that in ATH children, there might be a localized allergic reaction in the adenotonsillar tissues, which is not always reflected by an assay of systemic IgE. The aim of this study was to determine the specific IgE (sIgE) in the serum and adenotonsillar tissues using ImmunoCAP system and to compare the difference between local and systemic sIgE profiles in children with ATH.

Materials and methods

Study subjects

Between February 2010 and May 2012, there were 88 consecutive children undergoing adenotonsillectomy at the Department of Otolaryngology–Head and Neck Surgery, First Affiliated Hospital, Guangzhou Medical College, Guangzhou, China. Of these children, subjects who fulfilled the following inclusion criteria were enrolled in our study: (i) a pre-surgical confirmed diagnosis of obstructive sleep apnea (OSA), (ii) failed medical treatment for ATH during the previous 3 months, and (iii) grade 2 or higher enlargement of tonsils, plus grade 3 or higher enlargement of adenoids. Patients with cystic fibrosis, ciliary dyskinesia, known immunodeficiency, or systemic corticosteroid use during the previous 30 days were excluded. Those with recurrent upper respiratory infections were also excluded, because evidence regarding the outcome of upper respiratory infections after adenotonsillectomy is limited and no guidelines on the indications for this procedure have been so far accepted nationally or internationally(11). A contemporary cohort of 59 children who visited our hospital for routine health examinations without history and symptoms of ATH, allergic rhinitis, or asthma were included as controls.

Diagnosis of OSA syndrome was established by overnight polysomnography in our sleep laboratory. Presence of OSA was defined as having an apnea index of 1 or greater (at least one episode of apnea per hour of sleep). The assessments of ATH were performed by two otolaryngologists (Shuai Li and Hui Jin) before the patient recruitment. Nasal endoscopy was performed to obtain the images of the choanal openings, which were divided into four segments from the upper choanal border to the nasal floor. The severity of adenoidal hypertrophy was graded as follows: grade 1: The adenoid tissue occupies only the upper segment of the rhinopharyngeal cavity (<25%) with free choanal openings; grade 2: The adenoid tissue is confined to the upper half (<50%) of the rhinopharyngeal cavity; grade

3: The adenoid tissue extends over the rhinopharynx (<75%) with obstruction of choanal openings and partial closure of tube ostium; and grade 4: The adenoid obstructs almost the whole choana. The severity of tonsillar hypertrophy was graded as follows: grade 1: The tonsils are in the tonsillar pillar; grade 2: The tonsils protrude out of the tonsillar pillar; grade 3: The tonsils reach the midpoint between the anterior tonsillar pillar and uvula; and grade 4: The tonsils reach the uvula.

This study was approved by the Ethics Committee of Guangzhou Medical College, and an informed consent was obtained from the legal caregiver of each participant.

Tissue processing and serum preparation

All freshly obtained adenoid or tonsillar specimens were immediately rinsed in normal saline to completely remove any secretions and weighed before being transferred into a plastic tube containing phosphate-buffered saline. The tissue was homogenized with a mechanical homogenizer at 2706 g on ice. After homogenization, the suspension was centrifuged at 1509 g for 10 min at 4°C before the supernatants were collected. Serum samples were obtained from the whole-blood samples after centrifugation and were frozen until analyzed.

Specific IgE Analysis

The levels of serum sIgEs against *D. pteronyssinus*, *D. farinae*, *Blomia tropicalis*, *dog dander*, *cat epithelia*, *German cockroach*, *mosquito*, *egg*, *milk*, *wheat flour*, *fish*, *peanut*, *soybean*, *crab*, *shrimp*, *mugwort sagebrush*, *common ragweed*, *plane*, *willow*, *poplar*, *Penicillium notatum*, *Cladosporium herbarum*, *aspergillus fumigatus*, *rice*, *tomato*, *onion*, *beef*, *chicken meat*, *mutton*, *mushroom*, and *carp* were measured with the ImmunoCAP 100 system according to the manufacturer's directions (Pharmacia Diagnostics, Clayton, NC, USA). sIgE levels against the first 15 of 31 aforementioned allergens in homogenates of adenotonsillar tissues were also measured by ImmunoCAP 100 system. A test of sIgE was defined as positive if the sIgE level was ≥ 0.35 kU/l. Atopy was defined as having a positive sIgE test to at least one of the allergens(12, 13).

Statistical analysis

Statistical analysis was performed using SPSS version 16 (SPSS Inc, Chicago, IL, USA). All descriptive statistics were presented as mean $\pm$ s.e.m. Differences between the two groups were tested by independent-samples Student's *t*-tests. One-way analysis of variance (ANOVA) was used to evaluate the differences among multiple groups. Univariate analysis by chi-square test was performed to examine the association between sIgE in adenoid/tonsillar tissues and sIgE in serum. A *p* value <0.05 was considered significant.

Results

Finally, a total of 142 subjects were recruited in this study, comprising 83 with ATH and 59 healthy controls. The

distribution of age and gender was comparable between patients with ATH and controls. All subjects were tested for the levels of sIgE in serum and 20 of the patients tested for the levels of sIgE in both serum and adenotonsillar tissues.

Of all recruited subjects, 4 (6.78%) healthy controls and 51 (61.45%) patients with ATH tested positive for serum sIgE to at least one of the examined allergens. The adjusted current prevalence of allergy rhinitis (AR) in children with ATH was 29 (34.94%) with the diagnostic criterion of nasal symptoms (+) and sIgE (+).

There were no statistical differences in gender distribution, age, AHI, size of adenoids or tonsils, and percentage of peripheral blood eosinophils between atopic and non-atopic children with ATH. However, the atopic children showed higher levels of total IgE than those seen in children without atopy (94.93 ± 90.14 vs. 20.97 ± 23.43 , $p < 0.01$) (Table 1).

According to serum test of specific IgEs, milk was the most prevalent sensitizing allergen in patients ($n = 32$, 38.55%), followed by DP, D farinae, Blomia tropicalis, dog dander, and cat epithelia.

In addition, 20 children with ATH underwent immunologic test of sIgEs to 15 allergens in both adenotonsillar tissues and serum. The results showed that all these children were locally sensitized to more than two allergens at the level of adenotonsillar tissues, although 10 of them were serum sIgE negative. In this subgroup, DP was the most prevalent locally sensitizing allergen, followed by milk, D farinae, Blomia tropicalis, and dog dander. Interestingly, 100% of the serum sIgE-negative subjects from these 20 patients were sensitized to DP, milk, Blomia tropicalis, and dog dander.

Of the subjects with positive serum sIgE, 50.0% and 42.9% were also positive for local sIgE in adenoids and tonsils, respectively. Of the subjects with negative serum sIgE, local sIgEs in adenoids and tonsils were detected in 36.0% and 43.8%, respectively. Overall, the positive rate of sIgE antibodies in local tissues (adenoids or tonsils) was significantly higher than that in the serum ($\chi^2 = 61.51$ and 77.07 , respectively; both $p < 0.01$) (Table 2).

Discussion

With ImmunoCAP method, 61.45% of the ATH children in the present study were found to be sensitized to more than

Table 1 General characteristics of atopic and non-atopic patients

	Atopy	Non-atopy	p value
Number of subjects	51	32	
Gender (male, %)	54.90	53.13	NS
Age (yr)	6.68 ± 3.30	6.13 ± 2.62	NS
AHI (n/h)	7.09 ± 6.48	8.06 ± 6.67	NS
Adenoid size (grade)	2.92 ± 0.48	2.81 ± 0.54	NS
Tonsil size (grade)	2.75 ± 0.48	2.66 ± 0.48	NS
Eosinophil count (%)	3.98 ± 3.13	3.63 ± 2.20	NS
in peripheral blood			
Serum total IgE (kU/L)	94.93 ± 90.14	20.97 ± 23.43	<0.01

NS, not significant.

Table 2 Profiles of specific IgE in tonsils, adenoids, and serum ($n^{\dagger}$)

	Serum sIgE (+)	Serum sIgE (–)	Total [‡]
Adenoid sIgE (+)	14	98	112
Adenoid sIgE (–)	14	174	188
$\chi^2 = 61.51$, $p < 0.001$			300
Tonsillar sIgE (+)	12	119	131
Tonsillar sIgE (–)	16	153	169
$\chi^2 = 77.07$, $p < 0.001$			300

[†]A test of specific IgE (sIgE) was defined as positive if the sIgE level was ≥ 0.35 kU/l using the ImmunoCAP method.

[‡]This subgroup analysis included 20 patients with ATH for whom the sIgE to 15 representative allergens was examined in serum and adenoid/tonsillar tissues simultaneously. Hence, there were 300 sIgE tests for each type of samples (serum, adenoids, or tonsils) in all 20 subjects (15×20).

The figures denote the numbers of tests according to sample type and positive/negative findings.

one allergen in serum, with milk being the most prevalent sensitizing allergen, followed by DP, D farinae, Blomia tropicalis, dog, and cat. Similarly, a number of studies have noted the frequent presence of certain allergens, such as house dust mites and pollens, in children with adenoid hypertrophy (1, 14–16). All these suggested that patients with ATH are more likely to have concomitant allergy compared with the general population.

The most important finding in the present study was that all ATH children were locally sensitized to more than two allergens at both tonsils and adenoid tissues as reflected by positive sIgE test of the adenotonsillar homogenate, even though half of them did not test positive for sIgE antibodies in serum. This pointed to the presence of local IgE production, a phenomenon first observed by Tse et al. in sinonasal tissues (17). Recently, local atopy was detected from mucosal surfaces of several organs including the lung, eye, and gut (18, 19). The local regulation and production of IgE in target organs were considered as factors controlling the possible manifestations and severity of allergic diseases (13). Nevertheless, few studies addressed local atopy in ATH. Shin et al. found that local production of total IgE and sIgE antibodies to house dust mites was more prominent in adenoid tissues in the atopic subjects (10). In the present study, DP was also the most prevalent allergen, followed by milk, D. farinae, Blomia tropicalis, and dog dander, in both tonsillar and adenoid tissues of ATH children, which was independent of the serum sIgE profiles. To the best of our knowledge, this was the first report regarding multiple local IgE production in adenotonsillar tissues in childhood ATH. Interestingly, the positive rate of sIgE antibodies in local tissues (adenoids or tonsils) was significantly higher than that in the serum. These findings supported our hypothesis that there may be a potential localized allergic reaction in the adenotonsillar tissues even if not accompanied by systemic atopy in ATH children. For the first time, our results clearly demonstrated that local atopy is more relevant than systemic atopy in reflecting allergy status in childhood ATH.

By testing sIgE, we found that 62.75% of atopic children with ATH were sensitized to milk in serum, and all 20 patients were sensitized to milk both in tonsillar and adenoid tissues. This was not surprising because adenotonsillar tissues are part of an important immune system that produces immunoglobulins to protect the body against potentially antigenic substances in inhaled or ingested materials(20). Allergy to milk may 'open the gates' to an outbreak of other atopic manifestations, such as atopic dermatitis, asthma, and allergic rhinitis(21–24). In a previous study conducted to investigate the cause of asthma after food challenge, this process was suggested to result from sensitization through inhalation(25), meaning that children may inhale food particles through the airway. In addition, several studies indicated that certain aeroallergens may share homologous epitope proteins with food allergens(26–28). It is generally assumed that the first challenging allergens for younger children are the proteins in foods such as milk or egg, followed by the indoor allergens to which they are likely to be exposed at a young age(29). As the tolerance to sensitizing foods develops(30), respiratory allergy would manifest. The prevalence of AR was similar to the prevalence of milk-positive serum sIgE in children with ATH, but it was lower than the prevalence of milk-positive sIgE in tonsillar and adenoid tissues. However, further studies are needed to clarify whether milk as an alimentary antigen may play a role in the pathogenesis of ATH.

Evidence of a pathophysiologic link between ATH and localized allergic reaction suggests a promising role for topical anti-inflammatory medication and specific immunotherapy in ATH, even after adenotonsillectomy where residual adenoids may remain and systemic/local allergy may frequently persist.

Several limitations in the present study should be acknowledged. This was a single-center study with a relative small sample size. The age of subjects in our study was 2 to 13 yr. However, this age range was too broad for the study of ATH. Certain allergens, such as pollens, were not tested in this study because they are not common allergens in southern China. Owing to considerations of medical ethics, there was no age- and gender-matched control of tonsillar and adenoid tissues. Future studies with multicenter collaborations are warranted to validate our findings and to shed light on the mechanisms by which allergens interact with development and progression of ATH.

In conclusion, the present study provided evidence for the important role of allergic inflammation in ATH. The inconsistency in sIgE expression between adenotonsillar and serum suggests a role of local atopy in childhood ATH.

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Disclosure

None of the authors have any conflict of interest related to this manuscript.

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