

Relationship between tissue and serum eosinophilia in children undergoing adeno-tonsillectomy with allergic rhinitis

Abstract

Background: Previous reports suggested that allergic/eosinophilic inflammation affects the adenoid and tonsillar tissue. The aim of this study is to evaluate and compare the tissue and serum eosinophilia in children undergoing adeno-tonsillectomy with allergic rhinitis.

Materials and methods: The clinical registers of 125 children who underwent adenoidectomy/tonsillectomy due to adenoid/tonsil hypertrophy were examined and reviewed retrospectively. 57 children with skin prick test positivity and with symptoms of allergic rhinitis were included in the study as the atopic group, whereas 68 children with no allergic symptoms and skin prick test negativity were included as the non-atopic group. Consequently, the total immunoglobulin level E, serum and tissue eosinophilia of the atopic and non-atopic group was compared.

Results: Serum eosinophilia in atopic group was found to be significantly higher than the non-atopic group ($P= 0.045$). A significantly higher eosinophil was counted in adenoid/tonsil tissue of atopic group ($P< 0.001$; $P= 0.023$, respectively). However, no significant correlation between tissue and serum eosinophilia was found.

Conclusion: The inconsistency between tissue and serum eosinophilia in atopic children would particularly indicate a role of local atopy in adeno-tonsillar hypertrophy. Further studies are necessary to better understand the effect and usefulness of serum and tissue eosinophilia in children with allergic rhinitis.

Key words: Allergic rhinitis, adeno-tonsillar hypertrophy, local atopy, tissue eosinophilia, serum eosinophilia.

1. Introduction

Adeno-tonsillar hypertrophy (ATH) is the most common cause of upper respiratory tract obstruction in childhood. Although the etiology of ATH have not been elucidated, chronic and recurrent inflammatory events around the tissue may be significant because of the decisive role of subject tissues in immune response. Allergy is one of the most common inflammatory processes for upper respiratory tract [1]. Despite the fact that the prevalence of adeno-tonsillar disease is reported as 21.2% and 22.9% in some studies [2,3] in children with allergic rhinitis (AR), Griffin et al. [4] reported that the prevalence of AR is similar to age-matched controls.

AR is an IgE mediated type-1 hypersensitivity reaction of nasal mucosa which causes eosinophilic inflammation following allergen exposure of the mucous membranes. AR is clinically defined disease with four main symptoms such as rhinorrhea, nasal obstruction, nasal itching and sneezing. Skin prick tests are widely used to identify allergen that triggers allergy [5]. The adenoid and tonsil tissues are located at the entrance of respiratory tract and they function as the first contact points with inhalant allergens. Previous reports suggested that allergic diseases can initiate inflammatory processes affecting the adenoid and tonsil tissues, and that it can lead to formation of an allergic inflammation which causes a large number of IgE positive plasma cells/mast cells, allergen specific IgE and eosinophilic infiltration in the tissue [1,6-11]. Having found high numbers of IgE positive plasma cells in the adenoids of atopic children, Papatziamos et al. [11] argued that lymphatic tissue of adenoids could produce local specific IgE. Another study indicates that locally produced total IgE and specific IgE antibodies to *Dermatophagoides pteronyssinus* in adenoid tissue homogenate are significantly higher in atopic children than in non-atopic children, and reports that these

antibodies may lead to eosinophilic infiltration in adenoid tissue of atopic children [9]. Afore mentioned studies propose that allergic/eosinophilic inflammation occurs in adenoid and tonsil tissue of children with AR. Recent allergy studies suggest that a localized allergic response might occur without systemic atopy in adenoid and tonsil tissue [8, 12]. In a previous study of ours, we investigated the number of eosinophils in adenoid and tonsil tissue of sensitized children, where we found that the AR diagnosis may be supported by tissue eosinophilia [13]. Hereby, the current study aims to evaluate the serum and tissue eosinophilia in children with ATH and investigates the correlation of serum and tissue eosinophilia in children with AR.

2. Materials and methods

2.1 Study Population

Approval from the Ethics Committee of Adana Teaching and Research Hospital is maintained for the study (Ethics Committee No/date: 168 / 28.Feb.2018). Between March 2017 and February 2018, we reviewed and investigated the clinical registers of 125 children undergoing adenoidectomy/tonsillectomy due to ATH at the Department of Otorhinolaryngology, Adana City Training and Research Hospital. In patient registry files; gender, age, detailed histories of systemic disease and clinical visit notes, results of allergic tests (skin prick test and serum total IgE), complete blood cell count (CBC) within one week prior to surgery of patients were evaluated. Patients with asthma, immunodeficiency, autoimmune diseases, drug induced diseases, infectious diseases, cranial or genetic syndromes, vitamin D deficiency and insufficient file information were excluded from the study. The severity of adenoid and tonsil hypertrophy was classified in accordance with Brodsky criteria [14]. Surgical indications were $\geq$ grade 3 tonsil growth and adenoids which causes of obstructive sleep apnea. A total number of

125 patients between 3 and 10 years old were included in the study. Children with clinical symptoms as rhinorrhea, nasal obstruction, nasal itching and sneezing and also positive skin test were assigned to atopic group (n= 57), while children without clinical symptoms and negative skin test were assigned to non-atopic group (n= 68).

2.2 Skin Prick Test

Before the surgery, skin prick tests have been performed with multi-test applicator for thirty most common aeroallergens by standard Alyostal ST-IR (*Stallergenes SA, France*) allergen extracts (Table 1). Prior to application of skin prick test, parents of children were questioned about the status of drug use (antihistamines, antitussives, corticosteroids, H₂ receptor antagonists) in last 7 days. Histamine hydrochloride (10mg/ml) and physiological saline were used as positive and negative control, respectively. Skin reactions were measured 20 minutes immediately after the application and skin induration with ≥ 3 mm diameter or larger than negative control was considered as positive reaction.

2.3 Pathological Examination

The adenoid and tonsil tissue samples taken with the operation were examined with microscopy in hematoxylin-eosin stained sections. Sections were scored under 400 $\times$ magnification in a blinded fashion by the same pathologist. Eosinophils were counted in 10 random sections for all tissue samples.

2.4 Complete blood cell counts

Blood samples were obtained within one week prior to surgery. The eosinophil count ($10^3\mu\text{L}$) was examined with fully automated cell counter (Sysmex XN-9100™ Automated Hematology System, Kobe, Japan).

2.5 Statistical Analysis

The Shapiro-Wilk test was performed to test suitability of the numerical data's normal distribution. Descriptive analyses were presented using means $\pm$ standard deviations (SD) for normally distributed variables. Independent Sample t test was used for parametric variables comparison. The Chi-Square test was used for relationship between categorical variables. The Correlation Coefficient was calculated to investigate the conformity between two evaluation criteria in the categorical variables. ROC curve analysis was performed to find the cut off value for variables to predict the development of sensitivity. For all that, Sensitivity, Specificity and Area Under Curve were calculated. *P* value of less than 0.05 was deemed statistically significant.

3. Results

Demographic characteristics of 125 children between 3-10 years old included in the study were summarized and exhibited in table 2. There was no significant difference between atopic and non-atopic groups in the terms of gender and age distribution (*P*= 0.488 and *P*= 0.259 respectively).

In serum total IgE levels, no significance between atopic and non-atopic groups (*P*= 0.139) existed. There was found to be 41 adeno-tonsillectomy – 16 adenoidectomy in atopic group and 45 adeno-tonsillectomy - 23 adenoidectomy in non-atopic group. Totally 125 adenoid (57 adenoid from atopic group, 68 adenoid from non-atopic group) and 86 tonsil tissue (41 from atopic group, 45 from non-atopic group) were examined

on the basis of the number of eosinophils. There was a statistically significant difference between atopic and non-atopic groups with respect to tissue eosinophilia ($P < 0.001$ for adenoid tissue, $P = 0.023$ for tonsil tissue) (Table 2). Serum eosinophil count were significantly higher in atopic group ($P = 0.045$). A significant correlation was found between the number of eosinophils in adenoid and tonsil tissues of children who underwent adeno-tonsillectomy operation ($r = 0.676$, $P < 0.001$). On the other hand, there was no significant correlation between tissue eosinophilia and serum eosinophilia ($r = 0.064$ and $P = 0.588$ for adenoid and serum eosinophilia; $r = 0.017$ and $P = 0.906$ for tonsil and serum eosinophilia) (Table 3).

In order to find the optimal value of tissue eosinophilia and serum eosinophil count, ROC analysis was run. Associated results are depicted in table 4. The predictive value of eosinophil in the tissue was found to be $>4/10$ high powered fields for adenoid and to be $>2/10$ high powered fields for tonsil. Sensitivity and specificity value of these cut-off points were found as 75.8% - 88.1% for adenoid tissue and as 56.5% - 92.9% for tonsil tissue, respectively ($P = 0.0001$). The predictive value eosinophil in the serum was $>0.345 \times 10^3/\mu\text{L}$ for eosinophil count (Sensitivity value: 54.5 and specificity value: 78.6; $P = 0.028$). Sensitivity and specificity of tissue eosinophilia were found to be higher than serum eosinophilia particularly in adenoid tissue.

The most common allergens were found to be dermatophagoides farinae [71.93% (41/57)] and dermatophagoides pteronyssinus [63.16% (36/57)] (Table 1). 15 children demonstrated single sensitization, whereas 42 children proved poly-sensitization. No conspicuous association was distinguished between the poly-sensitization and single sensitization groups with respect to adenoid eosinophilia, tonsil eosinophilia and serum eosinophilia ($P = 0.699$, $P = 0.794$, $P = 0.208$, respectively).

4. Discussion

Several authors suggested that allergic/eosinophilic inflammation affects the homeostasis of adeno-tonsillar tissue. Increased expression of IgE positive cells/specific IgE antibodies, CD1a⁺ Langerhans cells, IL-4 and IL-5 mRNA positive cells was indicated in adenoid tissue of children with AR [6,9-11]. Recently, the pathophysiology of local allergic rhinitis (LAR) was defined as presence of allergen specific IgE in the nasal mucosa being absence of skin prick test and serum specific IgE test positivity in patients with symptoms of allergic rhinitis [15,16]. LAR is known as a localized nasal allergic response without systemic atopy. After exposure to aeroallergens, nasal TH2 IgE mediated inflammation occurs in the tissue. Eosinophil cationic protein, eosinophils, mast cells, basophils, CD3⁺ T cells and CD4⁺ T cells increased in the tissue [16]. LAR can be identified with multiple nasal provocation tests, local produced specific IgE or other local inflammation markers of allergic response. Most recently, allergy studies began to draw attention to local atopy in children with ATH [7,9,11]. Correspondingly, Zhang et al. [12] compared the sensitization type of serum and local tissue in children with ATH. Among 20 children with ATH, specific IgE is locally produced in both adenoid and tonsil tissue homogenate, but almost half of them is found to be negative for serum specific IgE. Cho et al. [8] demonstrated that 68.6% of children with ATH were sensitized to more than one allergen in adenoid and tonsil tissues and 53.9% of children with ATH were sensitized to serum. 36.2% children with specific IgE negative in the serum had positive specific IgE in adenoid and tonsil tissue. Also they reported more severe nasal symptoms in children with local atopy [8]. Discrepancy between adenoid/tonsil tissue and serum specific IgE may indicate that local allergic inflammation plays an important role in ATH [8,12].

Due to the primary effector cells of allergic diseases, eosinophil is often found to be increased in serum and related tissues. We have previously reported that the number of eosinophils in tissues of sensitized children who underwent adeno-tonsillectomy due to hypertrophy and/or recurrent infection was significantly higher, and that presence of eosinophils $\geq 5/10$ high powered fields in adenoid tissue and $\geq 3/10$ high powered fields in tonsil tissue may indicate sensitization [13]. The present study finds similar results in children with ATH. It must be noted that findings about the relationship between serum eosinophilia and AR are conflicting in the literature. For instance, while Chen et al. [17] suggested that serum eosinophil count supports the diagnosis and prediction of severity of allergic rhinitis, Yenigün et al. [18] found a relationship between serum eosinophilia and AR children with positive skin prick test results. However, latter did not find any significance with symptomatology of allergic rhinitis. These reports may indicate that allergic response is not only limited to local inflammation, but it also causes an increase in systemic inflammation. Reasonably, locally produced specific IgE might contribute to serum eosinophilia.

In our study, both tissues (adenoid and tonsil) and serum eosinophilia were found to be higher in atopic children. Yet, there was no statistically significant relationship between tissue and serum eosinophilia. This inconsistency may well support the presence of local atopy in childhood ATH. Additionally, we found that eosinophilia in the tonsil and adenoid tissue has a significant correlation ($p < 0,001$, correlation coefficient: 0,676). This result may be interpreted by the anatomical proximity of these tissues to each other.

According to ROC analyses, cut-off value of serum eosinophil count was $>0.345 \cdot 10^3/\mu\text{L}$ (sensitivity value 54.5%, specificity value 78.6%) in atopic group. As depicted in this

study, sensitivity and specificity of tissue eosinophilia was higher than serum eosinophilia. Although all these findings demonstrated the diagnostic significance of serum eosinophilia in AR, serum eosinophilia is still less diagnostic by itself. Because, apart from allergic diseases, it is known that immune deficiencies, autoimmune diseases, drug induced diseases and infectious diseases (especially parasitic helminth infections) are associated with serum eosinophilia [19].

Very much alike to previous studies, in our study, the most common allergen detected in skin prick test results was *dermatophagoides farinae* and *dermatophagoides pteronyssinus* [8,13,19]. 42 children (73.68%) had poly-sensitization and 15 children (26.32%) had single sensitization. Conforming the literature, poly-sensitization was found to be more common in children with ATH [3]. Because of the high poly-sensitization ratio and small size of study group, we did not perform statistical analyses as to determine which allergen is more eosinophilic. However, we found that poly-sensitization was not more significant than single sensitization on the basis of tissue and serum eosinophilia.

Although it has been reported in the literature that local allergic inflammation may play a role in ATH and cause allergic respiratory symptoms in children, few data are available about the clinical implications and management modalities of local allergic rhinitis in these patients [8, 12, 1]. It may develop into systemic classical allergic disease or these patients remain stable over long period of time. Further randomized clinical studies are needed to conclude more reliably on this clinical issue of paramount importance.

Among the limitations of our study, it might be stated that it is conducted at a single center due to lack of symptomatology of clinical allergy and that it has a retrospective

character. Additionally, the study population was relatively small and solely inhaled allergens were evaluated. Further researches are required to describe the value of eosinophilia in tissue and serum, such as large prospective series and profiles with cytokine, specific IgE and other allergic mediators.

To our knowledge, this was the first study to demonstrate no correlation between serum and tissue eosinophilia in AR children. The incoherence in eosinophilia between tissue and serum may indicate a role of local atopy in adenoid and tonsil tissue. At the same time, tissue eosinophilia may be utilized for the diagnosis of LAR in children who underwent adeno-tonsillectomy due to ATH. Examination of tonsil and adenoid specimens in terms of eosinophilia is a simple and cost effective method.

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Tables and figures

Table 1. Allergen Sensitization

<i>Allergen panel</i>	<i>Sensitized Group, (n/total) %</i>
Dermatophagoides farinae	71,93 (41/57)
Dermatophagoides pteronyssinus	63,16 (36/57)
Betulaceae (<i>Betula alba</i> , <i>Alnus Glutinosa</i> , <i>Carpinus betulus</i> , <i>Corylus avellana</i>)	-
Salicaceae (<i>Populus alba</i> , <i>Salix caprea</i>)	3,51 (2/57)
Mixture of 12 grasses (<i>Lolium perenne</i> , <i>Dactylis glomerata</i> , <i>Phleum pratense</i> , <i>Anthoxanthum odoratum</i> , <i>Poa pratensis</i> , <i>Festuca eliator</i> , <i>Agrostis vulgaris</i> , <i>Holcus lanatus</i> , <i>Cynodon dactylon</i> , <i>Avena sativa</i> , <i>Avena fatua</i> , <i>Lotus corniculatus</i>)	8,77 (5/57)
Oleaceae (<i>Olea europaea</i> , <i>Ligustrum vulgare</i> , <i>Fraxinus axcelior</i>)	-
Compasitae (<i>Solidago candensis</i> , <i>Taraxacum officinale</i> , <i>Chrysanthemum leucanthemum</i> , <i>Pittrak</i>)	-
Aspergilli mix (<i>Aspergillus fumigatus</i> , <i>Aspergillus niger</i> , <i>Aspergillus nidulans</i>)	3,51 (2/57)

Table 2. Demographic variables of the groups

<i>Variables</i>	<i>Atopic group</i>	<i>Non-atopic group</i>	<i>p</i>
Sex;			<i>0.488</i>
Boys, n	33 / 57	40 / 68	
Girls, n	24 / 57	28 / 68	
Age, mean $\pm$ SD, y	6.394 $\pm$ 2,207	5.786 $\pm$ 2.364	<i>0.259</i>
Total IgE, mean $\pm$ SD	2.538 $\pm$ 0.471	2.355 $\pm$ 0.538	<i>0.139**</i>

Tissue eosinophilia;			
Adenoid, mean $\pm$ SD	0.798 $\pm$ 0.293	0.378 $\pm$ 0.336	<0.001**
Tonsil, mean $\pm$ SD	0.472 $\pm$ 0.257	0.237 $\pm$ 0.321	0.023**
Serum measurements;			
Eosinophil, mean $\pm$ SD, $10^3\mu\text{L}$	0.416 $\pm$ 0.342	0.269 $\pm$ 0.283	0.045*
IgE= Immunoglobulin E			
*P Independent Sample t-test			
**P log transformation			

Table 3. Correlation of variables between tissue and serum

Variables	Study group; n	Correlation Coefficient; r	P value
Correlation of tonsil and adenoid eosinophilia	86	0.676	<0.001**
Correlation of adenoid eosinophilia and serum eosinophilia	125	0.064	0.588
Correlation of tonsil eosinophilia and serum eosinophilia	86	0.017	0.906
Pearson's correlation coefficient (rho)			
**Correlation is significant at the 0.01 level (2-tailed).			

Table 4. Results of ROC Analysis

Variables	Cut off value	AUC	Sensitivity	Specificity	P value
Adenoid eosinophilia	>4	0.816	75.8	88.1	0.0001
Tonsil eosinophilia	>2	0.786	56.5	92.9	0.0001
Serum eosinophilia, $10^3\mu\text{L}$	>0.345	0.649	54.5	78.6	0.028
AUC= Area Under Curve					

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