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Can the number of eosinophils in adenoid and tonsil tissue determine the allergy in children?



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ABSTRACT

Objective: Previous reports have indicated the association of allergy with Waldeyer's ring. The aim of this prospective study was to evaluate the correlation between the allergy and the number of eosinophils in the adenoid and tonsil tissue.

Methods: 101 children who were underwent adenoidectomy and/or tonsillectomy were prospectively enrolled in this study. 46 children who had positive skin-prick test for at least one of the allergen panel were sensitized and 55 children were nonsensitized as a result of skin-prick test. Hematoxylin-eosin stained sections were examined under 400× magnification in a blinded fashion in 10 random sections for all samples and compared the groups.

Results: The number of eosinophils in adenoid and tonsil tissue was significantly higher in sensitized patients. The number of eosinophils in the adenoid and tonsil samples also were interrelated too ($p < 0.001$, kappa coefficient: 0.617). As a result of ROC analysis, patients with a cut-off of ≥ 5 eosinophils was in the sensitized group for adenoid samples [Sensitivity value: 72.09 and specificity value: 91.84]. Patients with a cut-off of ≥ 3 eosinophils are in the sensitized group for tonsil samples [Sensitivity value: 52.94 and specificity value: 92.11].

Conclusion: Presence of sensitization can be distinguished by looking at the number of eosinophils in the adenoid and tonsil tissue. It can be used to determine whether the patient is allergic or not. Examination of the routine hematoxylin-eosin stained adenotonsillary specimen for eosinophilia will guide us the diagnosis and treatment of allergic rhinitis and also reduce the cost considerably.

1. Introduction

Allergic rhinitis (AR) which is an Ig E-mediated allergic reaction and cause of inflammatory reaction after allergen exposure is the most common disease in children. AR is also often associated with adenotonsillar disease and positive correlation between AR and adenotonsillar disease has been shown in many studies. The principal triggers of adenotonsillar disease include allergic and infectious stimuli [1–3]. Allergic reaction consists of two phases including early and late phase. Late phase reactions leading to clinical manifestations is characterized by the infiltration of various inflammatory cells such as neutrophils, basophils and above all eosinophils, and the release of various cytokine chemokines (IL-4, IL-5, IL-13) and adhesion molecules (Vascular Cell Adhesion Molecule-1 [VCAM-1], Intercellular adhesion molecule-1 [ICAM-1]). Recent studies have shown that VCAM-1 increase after 24 h following nasal allergen challenge. This molecule interacts with Very

Late Antigen-4 (VLA-4). The VLA-4/VCAM-1 interaction is known to be one of the mechanisms regulating the migration of specific eosinophils from the circulation to allergic inflammatory sites [4,5]. Mast cells and eosinophils are the two main effector cells in allergic inflammation. Tissue eosinophilia describe the allergic inflammation in the nasal and bronchial mucosa also adenoid and tonsil tissue [6].

Adenoid and tonsil tissues are the major constituent of Waldeyer's ring which are physiologically natural defense mechanism of the body against inhaled allergens and microorganisms. As a result of long-term exposure to antigens, adenotonsillar disease is often experienced due to their position of upper respiratory tract. Adenoidectomy and/or tonsillectomy are the most common surgical procedure with many indications such as hypertrophy, obstructive sleep apnea, frequent infections in children [1,2,7,8].

Adenotonsillar disease and AR have a major effect on quality of life and they also have considerable healthcare cost. Therefore, this

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prospective study aims to examine the eosinophilia of adenoid and tonsil tissue specimens, to look for sensitization, the major allergens involved, to compare the groups with sensitized to those nonsensitized children, to find the association between adenotonsillar tissue eosinophilia and allergy. We also aimed to determine whether we can put the diagnosis of sensitization by tissue eosinophilia.

2. Materials and methods

2.1. Patients

101 patients who were undergoing adenoidectomy and/or tonsillectomy from 2016 to 2017 was investigated for allergic rhinitis by the clinical visit, skin prick test and serum total Ig E at the ENT department of the Health Science University Adana Numune Education and Research Hospital. Approval of the Ethics Committee of our institution was received for the study (**Ethics Committee No/date: 36/24.Feb.2017**). Parents of children gave written informed consent for including the study. Exclusion criteria were poor cooperation, the presence of immunodeficiency, severe dermatographism, diffuse dermatological conditions, a craniofacial or genetic syndromes. Inclusion criteria were age between 3 and 9 years old children who were undergoing adenoidectomy and/or tonsillectomy. Adenoidectomy indication was adenoid hypertrophy (patients whose cavum obstruction greater than %70 with 3.2 mm flexible fiberoptic scope), frequent infection story (four or more episodes of recurrent purulent rhinorrhea in the last year), hyponasal speech, obstructive sleep apnea and adenoid face. Tonsillectomy indication was tonsillar hypertrophy (more than %75 obstruction) and frequent infection story (seven or more episodes of recurrent tonsillitis in last one year, five or more episodes of recurrent tonsillitis in each of the last two years, three or more such episodes in each of the last three years).

2.2. Skin prick test

The drugs used by the patient questioned before the skin prick test including antihistamines, antitussives, corticosteroids. Skin prick tests were performed with multitest applicator for thirty most common aeroallergens by the same Allergy and Clinical Immunology specialist. Histamine (10 mg/ml) was used as positive reference and physiological saline was used as negative reference. Skin reactions were evaluated 20 min after the application and at least 3 mm diameter of the induration or larger than that of the negative control was regarded as positive reaction. The patients were divided into 2 groups; sensitized group and nonsensitized group according to the skin prick test results and patients who were positive skin test for at least one of the allergens panel were considered as sensitized.

The allergen panel of skin prick test (*Alyostal ST-IR, Stallergenes SA, France*) were *Dermatophagoides farina*, *Dermatophagoides pteronyssimus*, *Betulaceae* (*Betula alba*, *Alnus Glutinosa*, *Carpinus betulus*, *Corylus avellana*), *Salicaceae* (*Populus alba*, *Salix caprea*), mixture of 12 grasses (*Lolium perenne*, *Dactylis glomerata*, *Phleum pratense*, *Anthoxanthum odoratum*, *Poa pratensis*, *Festuca eliator*, *Agrostis vulgaris*, *Holcus lanatus*, *Cynodon dactylon*, *Avena sativa*, *Avena fatua*, *Lotus corniculatus*), *Oleaceae* (*Olea europaea*, *Ligustrum vulgare*, *Fraxinus excelsior*), *Compositae* (*Solidago canadensis*, *Taraxacum officinale*, *Chrysanthemum leucanthemum*, *Pittrak*) and *aspergilli mix* (*Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus nidulans*).

2.3. Pathological examination

Adenoid and tonsil tissues were examined on the basis of the number of eosinophils with microscopy in hematoxylin-eosin stained sections (Fig. 1). Sections were scored under 400× magnification in a blinded fashion. Eosinophils were counted in 10 random sections for all samples [9].

2.4. Statistical analysis

The Shapiro-Wilk test was performed to test the suitability of the numerical data's normal distribution. Descriptive analyses were presented using median (minimum-maximum) for variables not distributed normally and means ± standard deviations (SD) for normally distributed variables. The Chi-Square test was used for relationship between categorical variables. The kappa coefficient was calculated to investigate the fit between two evaluation criteria in the categorical variables. Roc curve analysis was performed to find the cut off value for adenoid and tonsil tissue eosinophils count to predict the development of atopy. At the same time, Sensitivity, Specificity and Area Under Curve were calculated. A p value of less than 0.05 was considered statistically significant.

3. Results

One hundred and one children who were underwent adenoidectomy and/or tonsillectomy were prospectively enrolled in the study. There were 46 children (25 girls and 21 boys) in sensitized group and 55 children (29 girls and 26 boys) in nonsensitized group. The mean age of the patients were $6,02 \pm 1.77$ (range 3–9) in sensitized group and 5.64 ± 2.05 (range 3–9) in nonsensitized group. The results did not reveal any significant differences between two groups in the terms of sex and age ($p = 0.785$ and $p = 0.554$ respectively).

In sensitized group, 35 children showed polysensitization and 11 had single sensitization. 37 children had sensitization to *Dermatophagoides farina* (valid percent %80.4), 39 to *Dermatophagoides pteronyssimus* (valid percent %84.8), 13 to grasses (valid percent %28.3), 3 to *Oleaceae* (valid percent %6.5), 3 to *salicaceae* (valid percent %6,5), 2 to *aspergilli mix* (valid percent %4.3). There were no children to sensitization of *betulaceae* and *compasitae*. *Dermatophagoides pteronyssimus* and *Dermatophagoides farina* was the most common sensitizing allergen in sensitized patients. There were no significant differences between the poly-sensitization and single-sensitization children according to the number of eosinophils in adenoid tissue and tonsil tissue ($p = 0.203$ and $p = 0.984$ respectively) (Table 1).

Comparing the serum total Ig E levels in both the sensitized (range: 311.90 (25.44–2370.00)) and nonsensitized groups (range: 715.60 (12.90–2500.00)) were not significant differences ($p = 0,381$).

Pathologically, 92 adenoid tissue and 72 tonsil tissue were examined for eosinophilia. Of these, 63 patients had both adenoid and tonsil tissue. In total, there were 43 adenoids and 34 tonsils in sensitized group, 49 adenoids and 38 tonsils in nonsensitized group. There was a statistically significant relationship between the number of eosinophil in adenoid tissue and prick test positivity ($p < 0,001$) (Fig. 2). When looking at tonsil tissue, there was a statistically significant relationship between the number of eosinophil in tonsil tissue and sensitization too ($p = 0,047$) (Fig. 2). The number of eosinophil in tissue was significantly higher in sensitized patients.

Adenotonsillectomy was performed in 63 patients and 31 of these patients were in sensitized group while 32 were in the nonsensitized group. In these groups, there was a significant relationship between the adenoid and tonsil tissue significance ($p < 0,001$). At the meaningful finding of them, kappa coefficient was found as 0,617.

Patients with a cut-off of 5 and over number of eosinophils were higher in the adenoid tissue with the sensitization. The area under curve (AUC = 0.850; $p = 0.0001$) was found as the result of ROC analysis. Sensitivity value of this cut-off point was found as 72.09 (56.3–84.7) and specificity value 91.84 (80.4–97.7) (Fig. 3). The number of tonsil tissue eosinophil was significantly higher in sensitized patients. Patients with a cut-off of 3 and over number of eosinophils were in the sensitized group. The area under curve (AUC = 0.762; $p = 0.0001$) was found as the result of ROC analysis. Sensitivity value of this cut-off point was found as 52.94 (35.1–70.2) and specificity value 92.11

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