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Original Article

Significant Changes in Trans-Epithelial Barrier Proteins of Adenoid Tissue with Atopic Status in Children

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Abstract

OBJECTIVES: Adenoid tissue is important in local immune response and epithelial barrier dysfunction of this tissue may contribute to allergies. The aim of this study was to evaluate the relationship between the status of cross-epithelial barrier elements in adenoid tissue lymphoepithelium and inhalant allergen sensitization.

MATERIALS AND METHODS: Children aged 5-15 years, who underwent adenotonsillectomy, participated in this study. All subjects underwent skin prick testing with environmental inhalant allergens. Occludin, ZO1, e-cadherin, β-catenin, desmoglein, desmoplakin, and connexon-43 were stained immunohistochemically in the adenoid tissues obtained and scored by H-score.

RESULTS: We enrolled 76 children, 14 among whom were sensitized to environmental allergens. Among the zonula occludens proteins, median H-scores for occludin, claudin, and ZO-1 were significantly lower in the atopic compared to the nonatopic group respectively ($p<0.001$). Similarly, median H-scores for e-cadherin and β catenin proteins of the zonula adherens were significantly lower in the atopic group ($p<0.001$). Both desmoglein and desmoplakin H-scores were significantly lower in the atopic group [60 (50-100) vs 280 (260-300), $p<0.001$ and 105 (87.5-120) vs 280 (67.25-300), $p<0.001$ respectively]. Moreover, connexin-43 protein of the gap junction was significantly lower in the atopic group ($p<0.001$).

CONCLUSION: Adenoid tissue, which is the initial point of contact of inhalant allergens demonstrates epithelial barrier junctional protein, changes in children with inhalant allergen sensitization without clinical allergic disease symptoms. Therefore, it may be concluded that epithelial barrier function plays an important role in the development of allergen sensitization versus tolerance.

KEYWORDS: Adenoid tissue, allergy, epithelial barrier

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INTRODUCTION

Adenoid tissue is a nasopharyngeal component of the lymphoid tissue group known as the Waldeyer's ring and contributes to the development of initial defense mechanisms against antigenic substances due to its location at the entrance of the respiratory tract [1]. Adenoid tissue is lined by pseudostratified epithelium like the rest of the respiratory tract [2]. Moreover, it is a component of the mucosa associated lymphoid tissue (MALT), like the Peyer patches, and nasal associated lymphoid tissue (NALT) [3]. Functionally, it plays a role in the innate immune system by creating a barrier through the tight junctions in its lymphoepithelial structure. Additionally, it stimulates development of an adaptive immune response by the M cells and the dendritic cells that play a role in antigen uptake, processing, and presentation [2, 4]. Thus the adenoid tissue functions as an intermediary step for both the innate and adaptive immune responses. The localization and histological structure of the adenoid tissue, which lacks afferent lymphatics, is designed to sample antigenic material from the epithelial surface [4].

The epithelium has an anti-inflammatory function in tissue homeostasis and any damage to the epithelial barrier changes the adaptive immune response [5]. Epithelial barrier dysfunction plays a role in the pathogenesis of many diseases; it has been demonstrated that permeability of epithelial barrier is significantly increased in allergic diseases [6, 7]. There are junctional complexes between the sides of two neighboring cells throughout the epithelium; zonula occludens, located at the most proximally, is composed of transmembrane occluding, claudin, junctional adhesion molecule (JAM), and intracytoplasmic zonula occludens (ZO)-1, ZO-2, ZO-3, and MUPP1 proteins [8-10]. Zonula adherens is located right below the zonula occludens and contains the transmembrane e-cadherin molecule in the intercellular space. E-cadherin binds catenin on the cytoplasmic side and the resultant e-cadherin-catenin complex interacts with the actin filaments of the cell cytoskeleton. Macula adherens (desmosome) is composed of junction plaques that completely wrap the cell.

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These proteins are desmoplakins and plakoglobins that have the capacity to build intermediary filaments. There are proteins named desmoglein and desmocollin in the intercellular space [11]. Gap junction (nexus) is composed of tightly packed particles (connexin); these particles are composed of six subunits named connexins, which are the integral membrane proteins [12].

Pathogenesis of an allergic disease has many different components, and Th2 type inflammation is not the sole mechanism. Recent research points to epithelial structural changes in biopsies from asthmatic patients, and these changes are proposed to precede inflammation and clinical findings [2, 13]. Epithelium has a central role in attaining immune homeostasis and suppression of inflammation [5, 14]. Comprehension of this central role of epithelium in allergic diseases might guide the development of novel diagnostic and therapeutic modalities.

The aim of this study was to evaluate the relationship between the status of intercellular junctions, zonula occludens, zonula adherens, and gap junctions in adenoid tissue lymphoepithelium and inhalant allergen sensitization.

MATERIALS AND METHODS

Study Design

This cross-sectional study was approved by the Ethics Committee of Celal Bayar University (2.1.2012-20278486004) and written informed consent was obtained from all the cases and their parents.

Study Population

Children who underwent adenotonsillectomy in the Otolaryngology Clinics of the two hospitals between 2013 and 2014 were enrolled consecutively in this study. Indications to be enrolled were age between 5-15 years, having an indication for adenoidectomy such as sleep related breathing disorders resistant to medical treatment, and adenoid tissue that obstructs more than 80% of the upper airway lumen as observed in nasopharyngoscopy.

Exclusion criteria were having a disease that might affect epithelial integrity of the adenoid tissue such as a collagen tissue disease or immunodeficiency, use of systemic or nasal topical steroids in the previous one-month period, or having had an upper respiratory tract infection in the previous week.

MAIN POINTS

- Levels of intra-epithelial tight junctional proteins such as occludin, claudin, and ZO-1 are lower in the lymphoepithelium of the adenoid tissue in the upper respiratory tract of subjects sensitized to inhalant antigens.
- Low levels of e-cadherin and catenin in adenoid tissue of atopic subjects without clinical allergy findings support the hypothesis that altered physical properties of zonula addherens, are associated with sensitization to allergens, which may or may not be associated with development of clinical allergic diseases.
- Decreased levels of desmoglein and desmoplakin in the adenoid samples from the subjects with allergen sensitization may denote that, along with the commonly investigated tight junctional proteins, desmosome structure and integrity is also important in the development of global epithelial barrier function.

Study Procedures

Age, sex, adenoidectomy indication, family history for allergic diseases, and personal history for allergic diseases were recorded. Skin prick tests were performed before adenoidectomy to test for inhalant allergen sensitization. Adenoid tissues extracted during the surgery were fixated in 10% formalin for histological examination.

Inhalant Allergen Skin Prick Test

Skin prick tests were performed according to the EAACI guidelines [15]. Positive control (histamine) and negative control solution (saline) as well as *Dermatophagoides farinea* and *Dermatophagoides pteronyssinus*, mold mix, *Alternaria*, grass pollen mix, *Olea europea* solutions (Allergopharma, Germany) were applied to the skin with the prick method. The tests were evaluated with induration response to histamine above 3 mm. Induration of more than 3 mm with any of the allergen solutions indicated that the child had allergic sensitization.

Immunohistochemical Evaluation

Adenoid tissue samples obtained were taken into 10% formaldehyde solution through brush biopsy. Tissue samples treated with alcohol series and xylene in accordance with routine paraffin tissue methods were embedded in paraffin, and 5 µm thick sections obtained from paraffin blocks were histologically evaluated with hematoxylin-eosin staining followed by immunohistochemical methods. Immunohistochemical staining was achieved by the use of indirect avidin-biotin peroxidase method with anti-occludin (sc-133256, Santa Cruz), anti-claudin (sc-166338, Santa Cruz), anti-ZO1 (617300, Zymed) antibodies for zonula occludens; anti-e-cadherin (sc-7870, sc-8246, Santa Cruz) and anti-β-catenin (sc-7963, sc-59737, Santa Cruz) for zonula adherens; anti-desmoglein (NBP1-45230, Novus Biologicals) and anti-desmoplakin (NBP1-49879, Novus Biologicals) for macula adherens and anti-connexon-43 (sc-59949, Santa Cruz) for gap junctions. Immunoreactivity scores were calculated with the semi-quantitative H-score; considering staining intensity as mild (+), moderate (++) and severe (+++) and the number of stained cells by a histologist blinded to the subject groups. The formula for H-score is total number of stained cells % X (staining intensity +1) [16].

Statistical Analysis

Statistical Package for Social Sciences version 20.0 (IBM SPSS Corp.; Armonk, NY, USA) program was used for the statistical analysis of the data. Normal distribution of data was evaluated by histogram and stem-leaf graphs. Sociodemographic data was summarized and expressed as mean (standard deviation) while H-score data was summarized and expressed as median (interquartile range). H-scores of the atopic and nonatopic groups were compared with the nonparametric Mann-Whitney U test. Distribution of sex between the atopic and nonatopic groups was achieved by the Chi-squared test. Ages of the atopic and nonatopic groups were compared with the nonparametric Mann-Whitney U test. Statistical significance was defined as a p value <0.05.

RESULTS

Sociodemographic Characteristics

We enrolled 76 subjects (48 males) in this study. The mean age of the study population was 7.4±2.59 years. Skin prick tests were positive in 14 subjects (18.4%). Among these 14 subjects, three were sensitive to grass pollen allergens, one was sensitive to Olea allergen, and three were sensitive to Dermatophagoides allergens while seven were sensitized to a mixed group of allergens. Age and sex were not significantly different between the two groups (p=0.255 and p=0.79 respectively). Familial history of atopy was significantly more common in the skin prick test positive group (p=0.02).

Comparison of Epithelial Barrier Components between the Two Groups

Among the zonula occludens proteins, median (interquartile range) H-score for occludin was 84 (40-108) and 304 (280-350) in atopic and nonatopic groups, respectively (p<0.001). Median (interquartile range) for the other zonula occludens proteins, claudin, and ZO-1 were 84 (40-108) vs 304 (280-350) and 100 (84-192) vs 280 (266-320) in atopic and nonatopic groups, respectively (p<0.000) (Figure 1 and Figure 2).

Similarly, median (interquartile range) H-scores of e-cadherin and β catenin proteins of the zonula adherens were significantly lower in the atopic group compared to the that of the nonatopic group [84 (56-120) vs 280 (280-300), p=p<0.000 and 84 (56-120) vs 280(280-300), p=p<0.000, respectively] (Figure 2, 3).

Among the desmosomal proteins both desmoglein and desmoplakin H-scores were significantly lower in the atopic group [60 (50-100) vs 280 (260-300), p<0.000 and 105 (87.5-120) vs 280 (67.25-300), p<0.000, respectively] (Figure 4).

Moreover, connexin-43 protein of the gap junction was significantly lower in the atopic group compared to that in the nonatopic group [120 (87.5-150) vs 322.5 (284-355), respectively, p<0.000] (Figure 4).

DISCUSSION

The results of our study demonstrate that the levels of intraepithelial junctional proteins in the lymphoepithelium of the adenoid tissue in the upper respiratory tract are lower in subjects with sensitization to inhalant antigens. This difference does not exist only in occludin, claudin, and ZO-1 proteins that are components of the zonula occludens but also in the proteins of zonula adherens and gap junctions.

Epithelial barrier dysfunction has been implicated in the pathogenesis of many diseases given that epithelium is an element of innate immunity; both the barrier function and the cytokines secreted contribute to development of allergic diseases. Epithelial barrier permeability is increased in allergic diseases, and allergens and irritants reach the basal layer with ease [6, 7]. The genetic basis of epithelial injury has been demonstrated in allergic diseases and the genetically determined defects in structure and function of epithelial barrier results in augmented response to environmental insults such as viruses and allergens, Th2 mediated allergic inflammatory

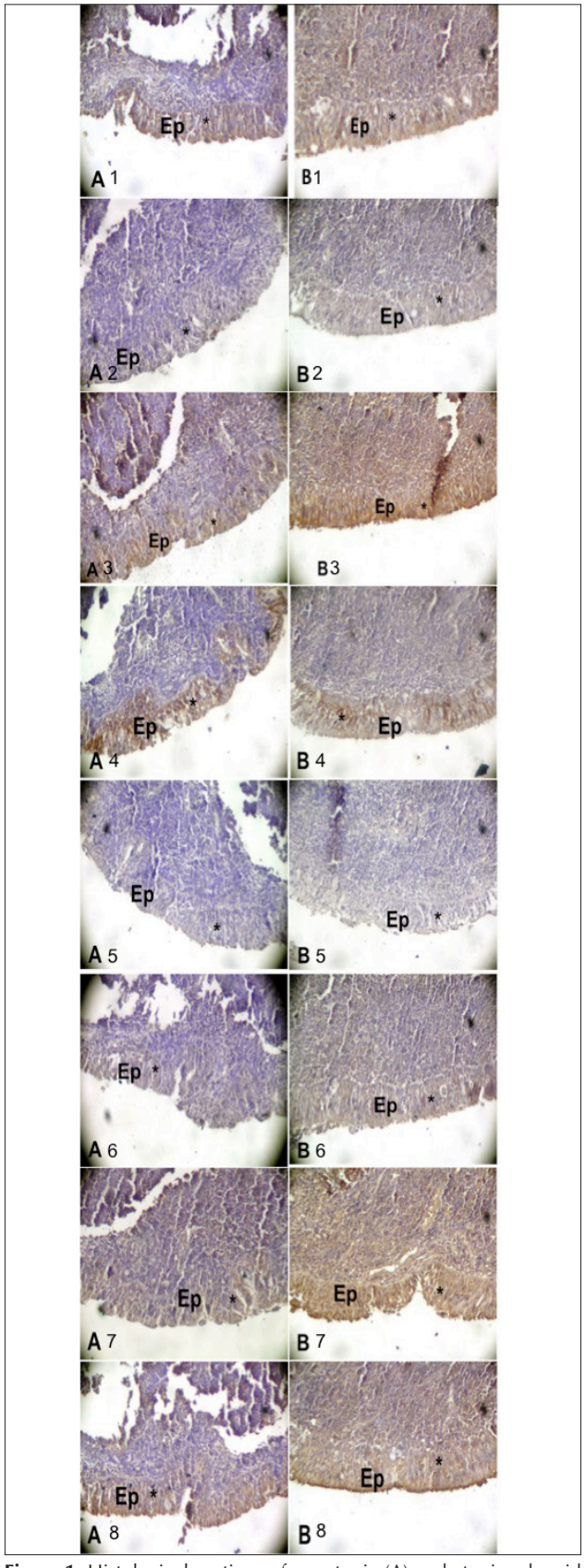

Figure 1. Histological sections of nonatopic (A) and atopic adenoid epithelium (B) processed to visualize anti-interepithelial junctional proteins immunoreactivity using avidin-biotin peroxidase method. (1: Occludin, 2: Claudin, 3: Zo-1, 4: E-cadherin, 5: Beta-catenin, 6: Desmoglein, 7: Desmoplakin, 8: Connexin-43). Brown staining shows the presence of interepithelial junctional proteins positivity between the epithelial cells (*) Ep: Epithelium (original x200)

response due to interaction of epithelial cells with immune and mesenchymal cells, and finally in abnormal healing-remodeling. Thus, these findings suggest that treatment modalities enhancing epithelial structure and function should be the focus of further research [17].

Tight junction molecules form a strong barrier on the luminal side of the epithelium and play a role in the development of mucosal diseases. Many research studies have investigated the role of tight junction proteins in epithelial barrier dysfunction in the development of asthma [1-12, 14, 18]. Moreover, tight junctions have dynamic structures in allergic rhinitis and viral infections [19]. In concordance with this information, the results of our study indicated that ZO-1 and occludin immunoreactivities are significantly lower in sensitized children compared to the non-atopics. Considering that the epithelial barrier prevents passage of allergens to the submucosa, this finding suggests that increased permeability of adenoid epithelium may be associated with inhalant allergen sensitization.

E-cadherin and catenin immunoreactivities were low in patients with inhalant allergen sensitization. E-cadherin-catenin complex is essential in the formation of zonula adherens junction. E-cadherin also plays a role in the integrity of zonula occludens through interacting with ZO-1 and actin filaments. Yuksel et al. [18] has demonstrated a decrease in exhaled breath e-cadherin levels in asthmatic children. Similarly, Boer et al. [20] has shown that Z0-1 and e-cadherin levels in bronchial biopsy samples were lower in atopic asthmatics compared to those in healthy nonatopic subjects. The decrease in e-cadherin expression is associated with increased epithelial permeability [21]. Thus, in our study, low levels of e-cadherin and catenin in adenoid tissue of atopic subjects without clinical allergy findings support the hypothesis that altered physical properties of intercellular adhesion complexes are associated with sensitization to allergens, which may or may not be associated with development of clinical allergic diseases.

Desmosome junctions (macula adherens) bind intermediate filaments of neighboring cells and provide the attachment of intermediate filaments with the basal layer in case of hemidesmosomes junctions. Desmosomal junctional structural proteins are decreased in asthma as well as the nasal samples of patients with chronic rhinosinusitis and nasal polyposis [10, 22]. Desmoglein and desmoplakin of the desmo-

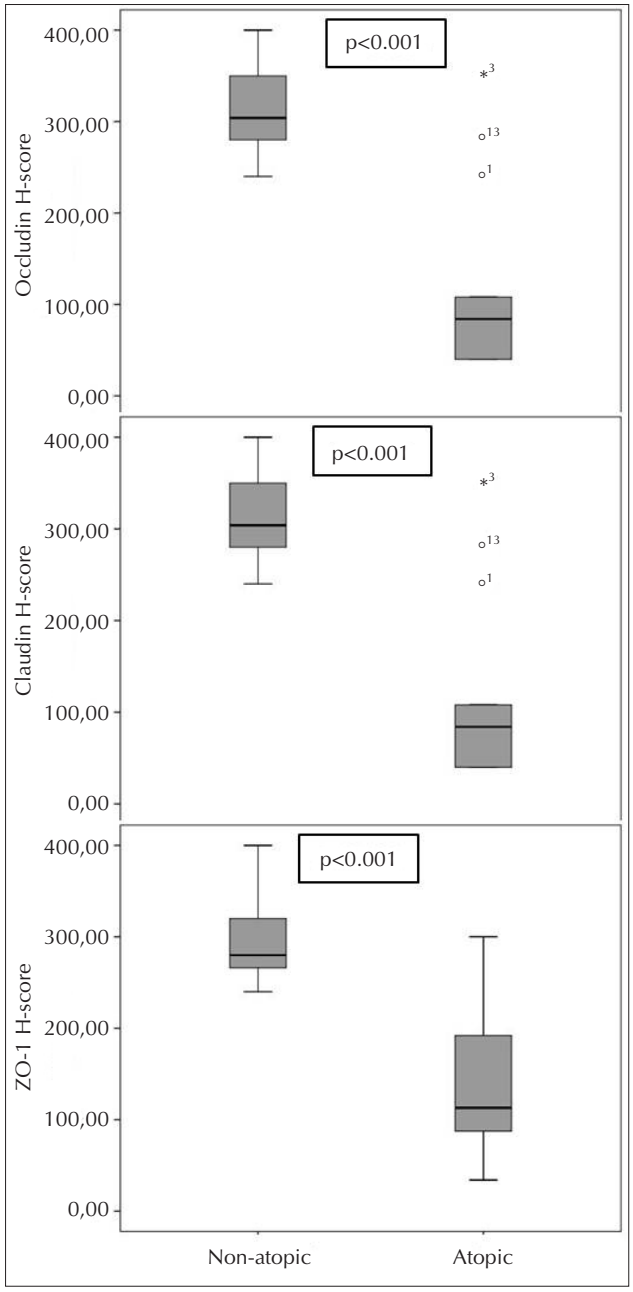

Figure 2. Box-plot of H-scores of the zonula occludens interepithelial junctional proteins in atopic and nonatopic subjects

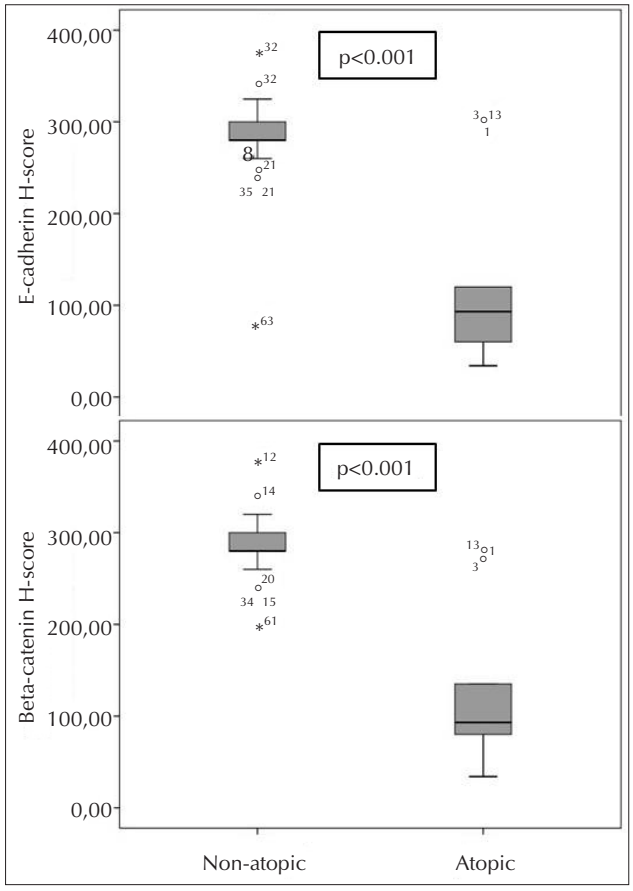

Figure 3. Box-plot of H-scores of the zonula adherens interepithelial junctional proteins in atopic and nonatopic subjects

some junction have been demonstrated to decrease in the adenoid samples from our subjects with allergen sensitization. This may denote that, along with the commonly investigated tight junctional proteins, desmosome structure and integrity is also important in the development of epithelial barrier function globally.

Connexins, which are the gap junction components, provide a direct connection between the cytoplasm of neighboring cells. The decrease in connexin expression in mucosal inflammatory conditions has been demonstrated [23]. Thus, eosinophilic inflammation was found to be associated with the decrease in connexin 43 in subjects with nasal polyposis [24]. The main function of connexin junctions is signal transduction between the cells, and the inhibition of this leads to increased leukocyte adhesion and inflammation [25]. Connexin 37 was found to be decreased in murine model of asthma, which negatively correlates with inflammation severity [25]. In our study, connexin 43 was decreased in the adenoid tissue from the atopic subjects. Cross-sectional design of our study prevents us from drawing a direct inference on causal relationships; however, we may interpret this finding in favor of an association between allergic sensitization and the decrease in connexin 43 expression.

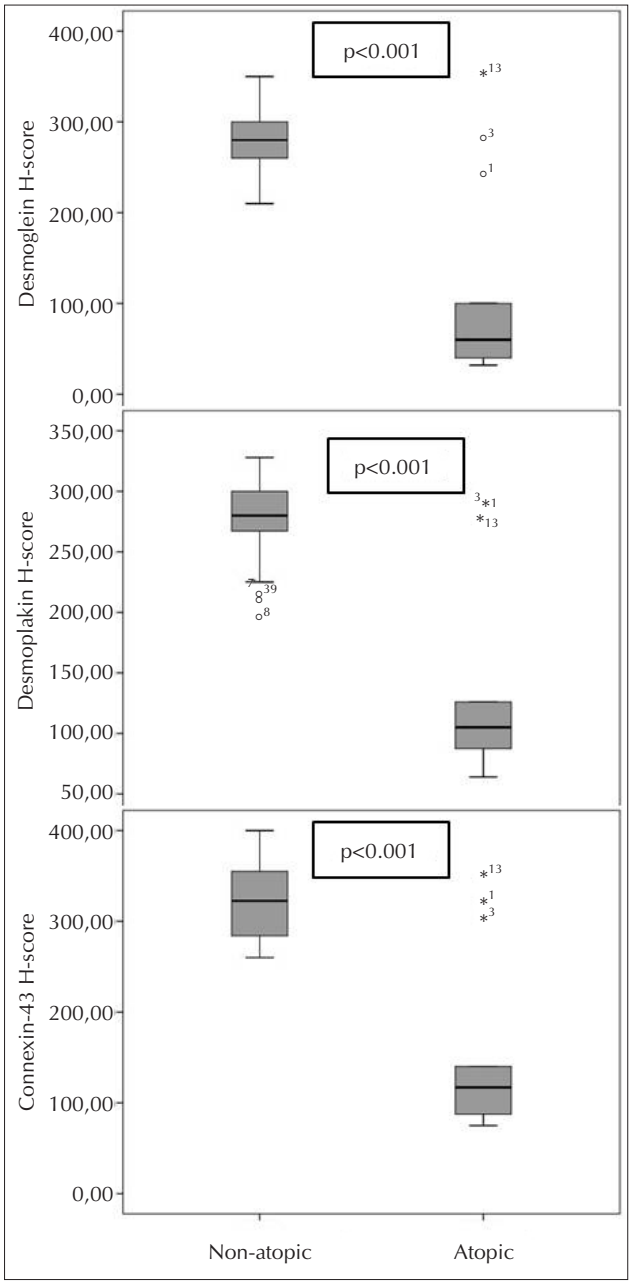

Figure 4. Box-plot of H-scores of the desmosome and gap junction interepithelial junctional proteins in atopic and nonatopic subjects

The major limitations of our study are the semi-quantitative evaluation of immunohistochemical H-scores. Moreover, the lack of functional evaluation prevents us from drawing direct inferences about the results.

Our findings are unique in demonstrating structural changes in all junctional complexes including tight junctions, adherens junctions, and desmosomes in an important lymphoid tissue of the upper respiratory tract in subjects with allergen sensitization. These results point to an association between atopy and these proteins.

In conclusion, adenoid tissue that is the initial point of contact with inhalant allergens, demonstrates epithelial barrier junctional protein changes in children with inhalant allergen sensitization without clinical allergic disease symptoms. These changes are prominent not only in zonula occludens but also in zonula adherens, desmosomes, and gap junction proteins. Therefore, it may be concluded that interepithelial barrier and communication plays an important role in development of allergen sensitization versus tolerance. However, direct causal inferences are not possible since this is not a cohort study. Future treatment modalities that target epithelium may need to be centralized in our effort to treat allergic diseases.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethical Committee of Celal Bayar University (Date: 02.01.2012 Number: 20478486-004).

Informed Consent: Written informed consent was obtained from the patients and parents' of the patients who participated in this study.

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Author Contributions: Concept - Ö.Y., H.Y., G.E., S.İ.; Design - Ö.Y., H.Y., S.İ., G.E., E.P.; Supervision - Ö.Y., H.Y., Ö.B.; Resources - Ö.B., Ö.Y., H.Y.; Materials - G.E., E.P., S.İ., Ö.B., Y.Ş., E.K.; Data Collection and/or Processing - G.E., E.P., S.İ., Ö.B., Y.Ş., E.K.; Analysis and/or Interpretation - Ö.Y., H.Y., S.İ.; Literature Search - Ö.Y., H.Y., Ö.B.; Writing Manuscript - Ö.Y., H.Y.; Critical Review - Ö.Y., H.Y., G.E., S.İ., Ö.B., Y.Ş., E.P., E.K.

Conflict of Interest: The authors have no conflicts of interest to declare.

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