Evaluation of nasal fluid β-defensin 2 levels in children with allergic rhinitis

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Abstract

Aim: Knowledge about the role of the innate immune system in the pathogenesis of allergic diseases has been expanding in recent years. Defensins are antimicrobial peptides that are components of the innate immune system. Defensins have strong efficacy against bacterial, viral, and fungal infections. Moreover, they have regulatory functions in many physiologic processes such as antitumoral immunity, chemotaxis, inflammation, and wound healing. In this study, we aimed to investigate β-defensin 2 levels in the nasal fluids of children with allergic rhinitis.

Material and Methods: Study and control groups consisted of 28 patients with newly diagnosed allergic rhinitis who were not taking any medication, and 23 healthy children. Skin prick tests were performed on patients with allergic rhinitis and disease severity was assessed using the total symptom score. Nasal fluid samples were obtained using a modified polyurethane sponge absorption method from patients and control subjects. Nasal fluid β-defensin 2 levels were determined using an enzyme-linked immunosorbent assay (ELISA).

Results: The median value of nasal fluid β-defensin 2 levels were 173.8 pg/mL (interquartile range; 54.8-205.9 pg/mL) in allergic rhinitis group and 241.6 pg/mL (163.5-315.2 pg/mL) in the control group. There was a statistically significant difference between the two groups (p=0.01). Moreover, nasal fluid β-defensin 2 levels showed a significant negative correlation with total symptom scores (rho= -0.78, p<0.001).

Conclusions: Children with allergic rhinitis have reduced nasal fluid β-defensin 2 levels compared with controls, and β-defensin 2 levels were negatively correlated with disease severity. A more definite understanding of the roles of defensins and other antimicrobial peptides in allergic inflammation can open up new horizons in the management and treatment of these common diseases. (Turk Pediatri Ars 2017; 52: 79-84)

Keywords: Allergic rhinitis, children, defensin, disease severity, nasal fluid, polyurethane

Introduction

Allergic rhinitis (AR) is a common disease characterized by sneezing, nasal obstruction, and nasal pruritus, which affects 20-40% of the world population (1). When multi-center, large-scaled studies were evaluated, it was observed that the prevalence in childhood was reported to range between 8.5% and 14.6% worldwide, and was 17.2% in our country (2, 3). Type-1 hypersensitivity reactions developing against inhaled allergen are found in the background of the disease and regional immunoglobulin (Ig) Es in the nasal mucosa or systemically synthesized allergen-specific IgEs play a key role in the pathogenesis (1).

The innate immune system has for years been considered as a system that acts as a barrier only against pathogens in the external environment, which is relatively primitive compared with the adaptive immune system, and provides the first immune response against microbes, only until the adaptive immune system steps in (4). Therefore, studies related with the pathogenesis of AR only focused on the adaptive immune system for many years. In recent years, however, it has been recognized that the innate immune system has regulatory and directive effects on the adaptive immune system and many proinflammatory, antiinflammatory, and immunoregulatory roles, as well as antiinfectious characteristics (4).
Antimicrobial peptides (AMP) are low-molecular-weight, positively-charged molecules and significant elements of the innate immune system (5). Currently, more than 100 AMPs have been identified (6). The best known among these include lysozyme, cathelicidin and defensins (5). Defensins are AMPs that are found in all species and have biologic functions with an extraordinary variety (7). Defensins primarily show strong efficiency against bacterial, viral, and fungal infections. In addition, they have been shown to have a regulatory function in many physiologic processes including antitumor immunity, cytokine release, chemotaxis, histamine production, inflammation, and wound healing (7). Although there are a few studies conducted with adults in the literature, no studies have investigated the roles of defensins in the pathogenesis of AR in childhood (8, 9). In this study, we aimed to investigate the levels of β-defensin 2 (BD-2), which is AMP synthesized in the respiratory tract of pediatric patients with AR.

Material and Methods

Thirty-two patients with newly diagnosed AR aged between 6 and 18 years who had not used medication before and whose AR was diagnosed in the Division of Pediatric Allergy and Immunology Outpatient Clinic between September 2014 and April 2015 were included in the study. The diagnosis of AR was made according to the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines (10). Patients who had chronic disease including doctor-diagnosed asthma, whose family members were smokers, and who were using medication including vitamins were not included in the study, because some publications reported that use of vitamins and exposure to smoking affected BD-2 levels (11, 12). Twenty-four healthy children who were being followed up in the Outpatient Clinic of Healthy Children and had no known problems, whose parents did not smoke, and who were not using medication were included as the control group. Two patients from the study group and one child from the control group were excluded from the study because they could not tolerate the procedure. Two patients with AR were excluded from the study because nasal fluid could not be obtained, even though the procedure was performed in accordance with the protocol. Approval was obtained from the University Clinical Studies Ethics Committee (17.12.2014/No.71306642-050.01.04); the patients and their families were informed in detail and written consent was obtained for the study.

Skin prick tests with 10 different aeroallergens including house dust mites, grass pollens, weed pollens, tree pollens, and animal epithelium were performed in all patients. Standard allergens and a skin lancing device were used for these tests (Stallergenes® and Stallergent® Paris, France). An allergen response showing an induration of 3 mm above the negative control was considered positive (13). The severity of allergic rhinitis was evaluated using a total symptom score (TSS). The patient or family was asked to score between 0 and 3 for each of four main symptoms of AR (nasal obstruction, sneezing, discharge, pruritus) (Table 1). Thus, TSS scores ranged between 0 and 12 (14).

A modified version of a polyurethane sponge absorption method described by Lü et al (15) was used for obtaining nasal fluid. In this method, a piece of polyurethane sponge (0.5x1x2 cm) was prepared and sterilized in an autoclave at 121°C for 20 minutes. Subsequently, it was affixed to a disposable metal stick, which had a clamp holding the sponge, and inserted into the patient’s nasal cavity for 5 minutes, as shown in Figure 1. The polyurethane sponge containing nasal fluid was removed from the patient’s nose and placed in a device prepared as two interwoven tubes. The inner tube was an Eppendorf tube (Eppendorf AG, Hamburg, Germany) with 15 standard holes on the top so that the nasal fluid could pass and the outer tube was a standard 10-cc blood collection tube. The device was centrifuged at 3000 g for 10 minutes, and it was ensured that the nasal fluid had collected in the outer tube. The fluid obtained was stored at -80°C until required for analysis.

The measurement of BD-2 levels in nasal fluids was performed using a commercial kit (Human β-defensin 2 ELISA kit, Eastbiopharm, Hangzhou, China) in accordance with the recommendations of the manufacturer. The results were obtained as pg/mL. The lowest concentration of detection of the kit used was 16 pg/mL.

Statistical analysis

Statistical analyses were performed using the IBM SPSS 22 (IBM, NY, USA) program. The Shapiro–Wilk test was used to evaluate the compatibility of the data to normal distribution. Data that were compatible with normal distribution are expressed as median and interquartile ranges (IR). The Mann-Whitney U test was used to evaluate the compatibility of the data to normal distribution. Data that were not compatible with normal distribution are expressed as median and interquartile ranges (IR). The Mann-Whitney U test was used to evalu-

### Table 1. Calculation of total symptom score (14)

<table>
<thead>
<tr>
<th>Score</th>
<th>Severity of symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No symptoms</td>
</tr>
<tr>
<td>1</td>
<td>Symptoms present, but easily tolerated</td>
</tr>
<tr>
<td>2</td>
<td>Cause discomfort, but do not disrupt daily activities or sleep</td>
</tr>
<tr>
<td>3</td>
<td>Severe symptoms affecting daily activities and sleep</td>
</tr>
</tbody>
</table>
ulate the differences between the two groups and Spearman's test was used in the correlation analysis. Categorical variables were evaluated using the Chi-square test and a p values of <0.05 were considered statistically significant.

Results

The mean age was 11.1±3.1 years among patients with AR and 10.2±3.3 years in the control group. The patient group was composed of 17 boys and 11 girls, whereas the control group comprised 13 boys and 10 girls. No statistically significant differences were found between the two groups in terms of age and sex (p>0.05). Some demographic and clinical properties of the patient and control groups are shown in Table 2.

The mean nasal fluid BD-2 level was found as 173.8 pg/mL (54.8-205.9 pg/mL) in patients with AR and 241.6 pg/mL (163.5-315.2 pg/mL) in the control group. There was a statistically significant difference between the groups (p=0.01, Figure 2). In addition, a statistically significant correlation was found between the nasal fluid BD-2 levels and TSS values in the patient group (rho: -0.78, p<0.001; Figure 3).

Discussion

Our results showed that the nasal fluid BD-2 levels were significantly lower in patients with AR compared with healthy controls. In addition, there was a negative correlation between BD-2 levels and disease severity. Our study is the first study to evaluate nasal BD-2 levels and the correlation of these levels with disease severity in pediatric patients with AR.

The role of AMPs in the pathogenesis of AR has not been sufficiently studied in the literature and is not fully understood. In previous studies, lysozyme and cathelicidin levels were shown as low in patients with AR (16, 17). In the study conducted by Bogefors et al. (8), it was found that BD-1 and BD-3 expression in tonsillar tissues was lower in patients with AR compared with healthy controls. In another study, it was shown that BD-1 and BD-3 expressions significantly increased compared with the pre-treatment period in nasal biopsy samples of patients with AR after allergen-specific immunotherapy was completed (9). Similarly, Choi et al. (18) found that BD-2 mRNA expression and protein levels in tonsillar and adenoid tissues of patients with AR were significantly lower compared with healthy controls. As is seen, studies conducted in this area have mostly been conducted with tissue samples. Only in the study conducted by Kalfa et al. (16) were nasal fluids of adult patients collected by way of microaspiration and BD-2 levels were evaluated. In their study, it was reported that the nasal fluid BD-2 levels in patients with AR were lower compared with controls, but the differ-

![Figure 1. Appearance of nasal fluid collector device](image)

### Table 2. Some demographic and clinical characteristics of the patient and control groups

<table>
<thead>
<tr>
<th></th>
<th>Patients with AR (n =28)</th>
<th>Control group (n =23)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, mean± SD)</td>
<td>11.1±3.1</td>
<td>10.2±3.3</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>17/11</td>
<td>13/10</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Total Ig E (IU/L, median, IR)</td>
<td>200.1 [53.3-471.3]</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>Symptom period (years, mean ± SD)</td>
<td>1.6± 0.8</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Aeroallergen sensitivity [n (%)]</td>
<td></td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>Mite</td>
<td>18 (64%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grass</td>
<td>1 (4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fungus</td>
<td>1 (4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Numerous</td>
<td>8 (28%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSSP (mean ± SD)</td>
<td>6.1±2.3</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>BD-2 (pg/mL, median, IR)</td>
<td>173.8 (54.8-205.9)</td>
<td>241.6 (163.5-315.2)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

AR: Allergic rhinitis IR: interquartile range; D: not evaluated; SD: Standard deviation; TSS: total symptom score
ence was not statistically significant. The investigators related this to the reduction of BD-2 expression in the mucosa due to nasal steroid used by the majority of the patients; the BD-2 levels of the patients who did and did not receive nasal steroid were not evaluated comparatively (16). Thus, the investigators could not clearly determine if the cause of the reduction in BD-2 levels was the caused by the disease itself or the nasal steroids used by the patients. When our findings and the other studies in the literature are evaluated together, we concluded that the reduction in BD-2 levels in the nasal fluid in patients with AR was mainly caused by the disease itself, not steroid treatment (8, 18). On the other hand, only 16 patients with AR and 16 controls were included in the present study. The fact that the numbers of subjects in the groups were low might have complicated demonstrating a statistical significance.

Harder et al. (19) showed that airway epithelium cells synthesized BD-2 when they came into contact with *Pseudomonas aeruginosa*. In addition, it has been reported that defensin levels were increased in bronchoalveolar lavage fluids in many inflammatory lung diseases including cystic fibrosis, pneumonia, tuberculosis, and bronchiolitis obliterans (18-22). On the other hand, exposure of airway epithelium to Th2 cytokines including interleukin 4 and 13 significantly reduces BD-2 mRNA synthesis (23). In addition, Th2-type inflammation inhibits BD-2 synthesis in the skin (24, 25). Although the molecular mechanism of this has not been fully elucidated at the present time, it is thought that Th2 cytokines reduce defensin expression by leading to inhibition in the tumor necrosis factor (TNF)-α/ nuclear factor (NF)-κB system by way of signal transducer and activator of transcription (STAT) 6 activation (23, 26).

One of the reasons underlying the fact that immunotherapy increases BD-2 expression in the nasal mucosa may be the inhibition of Th2-type immune response by immunotherapy (9, 27).

The best way to evaluate cytokine levels in an inflammatory disease is through direct measurement of cytokine levels in biopsy samples. However, mucosal biopsy is an invasive procedure and causes severe pain and discomfort. In addition, it may lead to tissue injury and scarring (28). Undoubtedly, nasal fluid collection is a procedure that has fewer potential risks and causes much less discomfort in patients compared with nasal mucosal biopsy. In addition, clinical studies in the literature have shown that nasal fluid cytokine levels and clinical courses in patients with rhinitis were correlated with biopsy results (28, 29). The rates of nasal fluid and lavage fluid cannot be determined accurately in the fluid aspirated because it is not known how much of the nasal lavage fluid given in the nose is ingested and how much is aspirated back during the nasal lavage method, which is the most commonly used method in the collection of nasal fluids (30). Thus, there is always an unpredictable and uncontrollable dilution rate of the nasal fluid in the lavage material aspirated (30). In the method described by Lü et al. (15), which we used in this study, nasal fluid is collected by absorption by a polyurethane sponge. It has been shown that the cytokine levels are 8-fold higher and antibody levels are 6-290-fold higher in nasal fluid obtained using polyurethane sponges compared with nasal lavage. The polyurethane sponge method should be preferred in the collection of nasal fluid because it is a standardized, easily applicable, reproducible and minimally invasive method (15).

A reliable and practical biomarker that has been shown to be beneficial in routine use in order to determine the clinical severity of AR has not yet been defined (31). In
our study, we found that nasal fluid BD-2 levels showed a significant negative correlation with TSS. In the literature, no other studies have evaluated the correlation between nasal fluid BD-2 levels and severity of AR. If this correlation can be demonstrated with further studies, use of nasal fluid BD-2 levels as a practical, objective, and minimally invasive biomarker in the evaluation of AR severity may become current.

Understanding of the strong immunomodulator properties of antimicrobial peptides led to the development of a new drug class called “innate defense regulators,” which are synthetic analogs of these peptides (32). Phase 2 and phase 3 clinical studies of at least 11 of these drugs, which are greatly promising, are ongoing (32). As the roles of antimicrobial peptides in the pathogenesis of allergic diseases become clearer, studies related with the use of this new drug group in AR and other allergic diseases will probably come to the fore in the near future.

In conclusion, BD-2 levels in nasal fluid are significantly lower in children with AR compared with healthy children, and these levels decrease as the severity of disease increases. The roles of defensins and other AMPs in the pathogenesis of AR should be examined with further studies. Accumulation of knowledge in this area may present new biomarkers and treatment options in the near future.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Bezmialem Vakif University School of Medicine (17.12.2014/ No.71306642-050.01.04).

Informed Consent: Written informed consent was obtained from all study participants who participated in this study.

Peer-review: Externally peer-reviewed.


Conflict of Interest: No conflict of interest was declared by the authors.

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