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ORIGINAL PAPER

The content of antimicrobial peptides – human β-defensin 2 and cathelicidin – in the secretion of the mucous membrane of the upper respiratory tract of children with bronchial asthma and allergic rhinitis

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ABSTRACT

Aim of the study: To study the concentrations of human β-2 defensin (HβD-2) and cathelicidin (hCAP-18/LL-37) in the mucosal secretions of the upper respiratory tract (URT) in children with asthma and allergic rhinitis, and to identify factors potentially affecting the levels of given antimicrobial peptides (AMP).

Material and methods: We performed a clinical and laboratory examination of 76 children aged 7 to 17 years with a verified diagnosis of asthma and/or allergic rhinitis lasting at least one year. The control group consisted of 20 gender-matched, clinically healthy peers. Levels of HβD-2 and hCAP-18/LL-37 in the URT secretions were determined beyond the exacerbation of the allergic disease using ELISA.

Results: We registered a significant decrease in the AMP concentrations in the URT secretions in atopic children: when compared with the controls' values, levels of HβD-2 concentrations in children with allergic rhinitis were on average 1.2 times lower, in asthmatics – 1.6 times lower, and in children with a combination of asthma and rhinitis – 2 times lower. Similarly, hCAP-18/LL-37 concentrations in children with allergic rhinitis were 2 times lower, in asthmatics – 2.9 times lower, and in patients with both diseases they were 2.4 times lower than in controls.

The severity of allergic diseases, lack of symptom control, passive smoking, and the presence of family history burdened with atopic diseases seem to have a potential negative impact on the levels of AMPs in the URT secretions. In contrast, the concentrations of the AMPs were positively associated with breastfeeding duration, full symptom control, and adherence to maintenance therapy.

An inverse correlation between levels of AMPs and the frequency ($r = -0.65$, $p < 0.05$) and duration ($r = -0.48$, $p < 0.05$) of viral URT infections in asthmatic children was also found.

Conclusions: Asthma and allergic rhinitis are associated with an altered mucosal innate immune response in the upper airways.

KEY WORDS:

children, asthma, allergic rhinitis, antimicrobial peptides.

INTRODUCTION

Chronic allergic diseases of the respiratory tract remain a global problem. For several decades, the incidence of bronchial asthma (BA) and allergic rhinitis (AR) has been steadily rising. According to some recent studies, about 339 million people worldwide have asthma [1], and about 400 million people suffer from allergic rhinitis [2]. In addition to the sick children's quality of life impairment, allergic diseases of the respiratory tract cause

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significant financial losses, both for the patients' families and for states [3–5]. At the same time, AR and asthma coexist very often, leading to the effect of mutual burden [6]. A number of studies show that the comorbidity of AR and BA is associated with a hampered asthma control achievement, regardless of the level of allergenic sensitization [7].

It has been proven that allergic diseases are often accompanied by disruption of epithelial barriers, which become more permeable not only to allergens but also to infectious agents. Infection is considered to be an important trigger of allergic inflammation (for instance, respiratory viral infection in bronchial asthma, allergic rhinitis, urticaria, etc.). One of the crucial components of epithelial barriers are antimicrobial peptides (AMPs) – a broad class of natural molecules, which are an integral part of all the multicellular organisms' innate immunity. AMPs are shown to perform many functions and to deliver antiviral, antibacterial, antifungal, and even immune modulation effects [8, 9]. One of the most studied AMP families to date are cathelicidin and representatives of human defensins [10, 11].

Human β-defensins (HbDs) are expressed in all epithelial tissues, including the epithelium of the oral cavity: they are present in the gingival and glossal epithelium as well as epithelium of salivary glands and oral mucosa. HbDs demonstrate antiviral activity against miscellaneous viruses (adenovirus, influenza A viruses, herpes simplex viruses types 1 and 2, and respiratory syncytial virus) and act through the inhibition of viral penetration into a host cell, destruction of the virus envelope, and inactivation of a virion. HbDs are involved in the early immediate immune response, enhancing the migration of monocytes into the inflammatory focus and stimulating the production of epithelial growth factor [12, 13]. Human cathelicidin (hCAP-18/LL-37) is a cationic molecule consisting of 37 amino acid residues and being expressed in neutrophil granules and epithelial cells. Alike all the AMPs, it possesses a wide range of antimicrobial activity against gram-negative and gram-positive bacteria, and also demonstrates an antiviral activity – in particular, it induces direct damage to the viral envelope of respiratory syncytial virus and provides a synergistic antibacterial effect with HbDs [14].

It is known that hCAP-18/LL-37 and HβD-2 concentrations in the upper respiratory tract (URT) secretions tend to elevate in the case of acute inflammation, particularly that caused by human rhinovirus, influenza viruses, and respiratory syncytial virus [10, 15]. There is also evidence that the levels of AMPs in the upper airways decline in individuals with chronic inflammatory diseases of the oral cavity, including the carious process, as well as in atopic dermatitis [16, 17]. In contrast, only a small number of studies have focused on the specificity of airway mucosal AMP production in allergic diseases, and in asthma in particular [18, 19]. In addition, an association between the AMP content in the URT secretions and the intrinsic patterns of acute respiratory infections in children with respiratory allergy may also be of particular research interest.

We hypothesized that some allergic diseases in children, such as asthma or allergic rhinitis, may be associated with an altered mucosal innate immune response in the upper airways.

Considering the aforementioned, the study objectives were to explore the concentrations of human β-2 defensin (HβD-2) and cathelicidin (hCAP-18/LL-37) in the mucosal secretions of the upper airways in children with asthma and allergic rhinitis, and to identify factors potentially affecting the levels of the given AMPs.

MATERIAL AND METHODS

The observational study of a cross-sectional design was conducted in the Municipal Enterprise "Specialized Medical Rehabilitation Centre for Children and Adolescents" of the Dnipropetrovsk Regional Council, one of the healthcare secondary-level municipal children settings in Dnipro, Ukraine in the period from September 2018 to January 2020.

The study was pilot in nature, and the article discusses results obtained during the pilot stage.

In accordance with the aim of the study, we performed a clinical and laboratory examination of 76 children aged 7 to 17 years, who were subsequently subdivided into the 3 study subgroups. Twenty-four children were diagnosed with allergic rhinitis (AR subgroup), 28 with bronchial asthma (BA subgroup), and 24 with bronchial asthma and allergic rhinitis ([BA + AR] subgroup). Twenty clinically healthy children of comparable age and sex constituted the control group.

Children who met the following inclusion criteria were eligible for the recruitment: having a formal diagnosis of asthma and/or allergic rhinitis lasting for at least 12 months and verified in accordance with the national clinical recommendations; and being school age (7 to 17 years).

All the study subjects were screened for the criteria of exclusion: chronic hereditary and congenital bronchopulmonary diseases, acute exacerbation of chronic allergic or non-allergic pathology of the respiratory tract; ongoing acute infectious disease; and a history of systemic or local antimicrobial or probiotic medication use within one month immediately preceding the study.

Anamnestic data for each study subject were collected using a semi-structured questionnaire and interviews with parents, as well as through analysis of the children's medical records. All children underwent clinical and laboratory testing, including blood eosinophilia, total immunoglobulin E (IgE) measurement, and identification of the causal allergens (pollen allergens, animal dander, moulds, and other indoor allergens) with a skin prick testing or serum-specific IgE assay (Cobas 6000; Roche

Diagnostics (Switzerland). In addition, habitual patterns of respiratory morbidity were registered, comprising frequency, duration, and nature of acute respiratory infections during the last 3 years.

We used an enzyme-linked immunosorbent assay (ELISA) for quantitative determination of the studied AMPs levels in the upper airway secretion (Human LL-37 ELISA test kit, Human DEFβ2/DEFB2 ELISA test kit, Hycult biotech, Netherlands). The nasopharyngeal aspirate technique was used to collect the material. The test material was collected in the morning after awakening, before the hygienic procedures of the oral cavity. The material was stored frozen (at –70°C) in plastic tubes.

Statistical data processing was performed using standard statistical analysis software Statistica for Windows v. 6.1 and Microsoft Excel. Shapiro-Wilks test for normality was run to evaluate the distribution of quantitative variables. Because the normality hypothesis for the data distribution had been declined, the analysis was grounded on nonparametric statistics: quantitative data were represented with medians (Me) and the interquartile range (IQR, [Q25; Q75]). The Mann-Whitney U-test was used to evaluate the differences between the independent groups for quantitative values, and Pearson's χ^2 test was run to compare the qualitative characteristics in the study groups. Bonferroni multiple-comparison correction was applied where relevant. We used the Spearman's rank correlation to determine the relationship between the quantitative and qualitative ordinal parameters. To determine the relationship between qualitative categorical variables, we used the odds ratio (OR) with a 95% confidence interval (95% CI) and *p*-value. The boundary for statistical significance was set as a *p*-value of 0.05 or less.

This study was conducted according to the declaration of Helsinki on Biomedical Research Involving Human Subjects. Ethical approval for the research protocol was granted by the Commission on Biomedical Ethics of the State Institution "Dnipropetrovsk Medical Academy of the Ministry of Health of Ukraine". According to the bioethical regulations, we obtained written informed consent for the participation in the study from the parents or legal representatives of all study subjects.

RESULTS

The median age of the study participants was 11 (9–12) years. Among the children enrolled into the study, the vast majority (94.7%) were between 7 and 15 years old. All the study samples demonstrated an uneven gender distribution: the BA and (BA + AR) subgroups showed a male sex predominance – 57.1% and 83.3%, respectively; a slight female predominance with 58.3% of girls was registered in the AR subgroup only.

Among the AR subgroup patients, the median duration of the disease was 5 (4–6) years, while AR duration in the group with the AR and BA comorbidity was 6 (5–7) years. The median asthma duration was 5.5 (5–6) and 6 (6–7) years in the BA and (BA + AR) subgroups, respectively. Additional characteristics of the studied groups are shown in Table 1.

Most patients demonstrated blood eosinophilia (86.8%) and elevated total serum IgE level (81.5%), which is confirmatory for the atopic nature of the disease. We did not find any significant differences in the median levels of eosinophilia and the median serum IgE concentrations between the study subgroups.

With the analysis of respiratory morbidity patterns, we revealed that, taken in total, children with allergic respiratory diseases had contracted 30% more viral upper respiratory tract infections (URTIs) over the preceding 3 years than their healthy peers in the control group. The highest median number of URTIs was registered in patients with a sole BA 4 (3–4 episodes per year). This number was almost twice the relevant number in the controls 2 (1–2.5 episodes per year; $p = 0.01$) and was significantly higher than in children with AR 3 (2–4 episodes per year; $p = 0.04$). The incidence of URTIs in the (AR + BA) subgroup was nearly as high 3 (3–5 episodes per year). Although the lowest URTI incidence was found in children with isolated AR, it was still significantly higher than in the control group. Routinely, during the URTI episodes, the intensity of allergic manifestations was raised in 81% of children in the asthmatic subgroup, in 83% of the (BA + AR) subgroup, and in 53% of AR subgroup patients who required a temporary increase in the volume of treatment. In addition, the median duration of the URTI episodes in children with allergic diseases was prolonged and amounted to 18 (17.5–19) days in children with BA and AR, which is nearly 2.6 times greater than the value of the control group (7 [7–8] days, $p = 0.01$). The aforementioned pattern was less expressed but still present in patients with isolated BA and isolated AR: the median duration of one URTI episode was 14 (13–14) and 10.5 (10–11.5) days, respectively ($p = 0.01$ and $p = 0.01$, compared with the controls).

Table 2 shows the median AMPs levels in the upper respiratory tract secretions in the study subjects with allergic diseases versus those in healthy controls. All the allergic subgroups demonstrated significantly lower HbD-2 concentrations in comparison with the control group values: on average, the HbD-2 levels were 1.2 times lower in children from the AR subgroup, 1.6 times lower in children from the asthmatic subgroup, and 2 times lower in children with a combination of asthma and allergic rhinitis, compared with the control values. Simultaneously, in the paired comparisons, differences in the HbD-2 levels between the allergic subgroups were also statistically significant ($p = 0.03$ for the AR versus BA subgroup comparison; $p = 0.05$ for the AR versus [BA + AR] subgroup comparison; $p = 0.005$ for the BA versus [BA + AR] comparison). Patients with a sole AR demonstrated

TABLE 1. The basic characteristics of the studied groups

Characteristics	Study Groups		
	AR ($n = 24$)	BA ($n = 28$)	BA + AR ($n = 24$)
Manifestations of allergic rhinitis:			
seasonal	58.3%	–	41,6%
year-round	41.7%	–	58.4%
Severity of allergic rhinitis:			
mild	45.9%	–	50.0%
moderate	54.1%	–	50.0%
Treatment for allergic rhinitis (at the time of enrollment):			
received:	75.0%	–	70.8%
- good symptom control	94.4%	–	88.2%
- poor symptom control	5.6%	–	11.8%
didn't receive:	25.0%	–	29.2%
- good symptom control	83.3%	–	85.7%
- poor symptom control	16.7%	–	14.3%
The course of bronchial asthma:			
intermittent	–	53.5%	66.6%
mild persistent	–	28.7%	33.4%
moderate	–	17.8%	–
Treatment for bronchial asthma (at the time of enrollment):			
received:	–	17.8%	12.5%
- good symptom control	–	80.0%	66.6%
- poor symptom control	–	20.0%	33.4%
didn't receive:	–	82.2%	87.5%
- good symptom control	–	91.3%	85.7%
- poor symptom control	–	8.7%	14.3%
Causal allergens:			
pollen allergens	54.0%	62.0%	29.0%
animal dander	37.0%	33.0%	33.0%
molds	25.0%	20.0%	12.0%
other indoor allergens	8.0%	25.0%	37.0%

the highest levels of HbD-2 among all subgroups, while in patients with asthma and rhinitis comorbidity the lowest HbD-2 concentrations were detected.

Similarly, we found a significant decline in the hCAP-18/LL-37 concentrations in the upper respiratory tract secretions in all allergic subgroups as compared to the control group: median hCAP-18/LL-37 levels in the AR, BA, and (BA + AR) subgroups were 2 times, 2.9 times, and 2.4 times lower than in controls, respectively. The lowest levels of hCAP-18/LL-37 were registered in asthmatic patients.

Table 3 presents the results of a more detailed analysis of the selected markers of the mucosal innate immunity characteristics performed for the allergic individuals. We found that both studied AMP concentrations in children with partially controlled or uncontrolled asthma course were significantly lower than in individuals with a complete control of asthma symptoms. However, with Bonferroni correction restricting the boundary of statistical significance to the mining of p-values less than 0.017 here, we can reliably consider the difference as significant only for hCAP-18/LL-37. Probably the small sample size had an influence on the results. In particular, median HbD-2 and hCAP-18/LL-37 levels in the latter subgroup were 1.5 times and 1.1 times greater than the former's cohort values, respectively. In contrast, for AR patients in the absence of symptom control the decline in AMP concentrations was statistically insignificant. However, even in the case of fully controlled course of the allergic diseases in the study subjects, i.e. in the subjects with a minimal

TABLE 2. Median concentrations of antimicrobial peptides in the upper respiratory tract secretions in the study participants; Me (Q25; Q75)

Study Groups	HbD-2 (pg/ml)	hCAP-18/LL-37 (ng/ml)
AR ($n = 24$) (p-value in comparison with the controls*)	195 (190; 197) ($p = 0.0281$)	0.43 (0.41; 0.46) ($p = 0.0318$)
BA ($n = 28$) (p-value in comparison with the controls*)	146 (129; 179) ($p = 0.0266$)	0.33 (0.28; 0.34) ($p = 0.0263$)
BA + AR ($n = 24$) (p-value in comparison with the controls*)	126 (119; 136) ($p = 0.0257$)	0.36 (0.33; 0.38) ($p = 0.0318$)
Control ($n = 20$)	251 (248; 253)	0.89 (0.83; 0.92)

** p-value is derived from the Mann-Whitney U-test*

TABLE 3. Median concentrations of antimicrobial peptides in the upper respiratory tract secretions in the study subjects depending on the level of symptom control; Me (Q25; Q75)

	BA		P-value (Mann-Whitney U-test)	AR		*P*-value (Mann-Whitney U-test)	Control group
	Partial control ($n = 14$)	Full control ($n = 26$)		Partial control ($n = 13$)	Full control ($n = 23$)		
HbD-2 (pg/ml)	119 (110; 129) *	148 (136; 179)*	0.0316	189 (119; 189)*	195 (136; 196)*	0.4366	251 (248; 253)
hCAP-18/LL-37 (ng/ml)	0.30 (0.23; 0.33)*	0.34 (0.33; 0.36)*	0.0157	0.43 (0.35; 0.43)*	0.40 (0.38; 0.44)*	0.6953	0.89 (0.83; 0.92)

** $p < 0.05$ in comparison with the control group*

level of allergic inflammation in the airways, the AMP levels in the upper airway secretions were significantly lower than in healthy individuals.

Our study analysed variables that may be associated with the mucosal innate immunity function of the upper airways. Several variables reflecting the medical history of the study subjects were identified by the Spearman's rank correlation test and were found to be statistically significant. Thus, we elicited a significant negative relationship between the levels of the studied AMPs and the severity of allergic pathology. In particular, the inverse relationship of medium strength was shown between the hCAP-18/LL-37 concentration in the upper respiratory tract secretions and the severity of AR ($R = -0.68$, $p = 0.001$) and the severity of asthma ($R = -0.54$, $p = 0.001$). Similarly, the HβD-2 concentrations demonstrated a medium strength inverse association with the severity of AR ($R = -0.64$, $p = 0.001$). Also, a strong negative relationship was observed between the concentration of HβD-2 in the upper respiratory tract secretions and the severity of asthma ($R = -0.86$, $p = 0.001$).

Regarding factors of personal and family history, with the Spearman's rank correlation test we identified a positive association between the AMP concentrations in the upper airway secretions and the total duration of breastfeeding in infancy. There was a weak relationship between the HβD-2 level and the total duration of breastfeeding in asthmatic children ($R = 0.33$, $p = 0.04$). Slightly more expressed the relationship was in the AR subgroup, both for the HβD-2 ($R = 0.40$, $p = 0.02$) and for hCAP-18/LL-37 ($R = 0.41$, $p = 0.02$) concentrations. The strongest relationship between breastfeeding duration and the level of HβD-2 was observed in the (BA + AR) subgroup ($R = 0.80$, $p = 0.001$).

Subsequently, the study analysed the link between AMP levels and preceding acute respiratory morbidity patterns in children with AR and asthma. For the asthmatic cohort of patients we revealed the presence of a medium-strength relationship between the incidence of viral upper respiratory tract infections (URTI) over the past 3 years and the level of hCAP-18/LL-37 ($R = -0.65$, $p = 0.0001$), as well as a weak inverse correlation between the concentrations of both studied AMPs in the upper airway secretion and the average duration of one episode of URTI ($R = -0.31$, $p = 0.05$ and $R = -0.48$, $p = 0.008$ for hCAP-18/LL-37 and HbD-2, respectively).

A summary of the estimates for the association between various levels of AMPs in the URT secretions and some categorical variables reflecting medical and family history of the study subjects is given below. In this case, because there are no standards for antimicrobial peptides, the distribution at high and low levels was conditional. We focused on the median in the atopic group because the lowest levels of antimicrobial peptides were observed there, i.e. all values that were below the median in atopic patients were considered by us as low levels. The threshold for low AMP level was estimated as "lower than 120 pg/ml" for HbD-2 and "less than 0.25 ng/ml" for hCAP-18/LL-37, according to the median of atopic patients, because the concentration of antimicrobial peptides in this group was the lowest; otherwise the AMP concentrations were considered high. Hereby, we identified the major predictors of an altered respiratory mucosal innate immunity function in allergic patients.

The presence of asthma symptoms at the time of enrolment was associated with increased odds of finding both AMP levels reduced (for HβD-2: OR = 45; 95% CI: 2.34–869; $p = 0.01$; for hCAP-18/LL-37: OR = 7.2; 95% CI: 1.14–45.1, $p = 0.03$).

In contrast, ongoing basic controller treatment for allergic disease at the time of enrolment was associated with significantly higher levels of both hCAP-18/LL-37 and HβD-2 in the URT secretions: OR = 17.2; 95% CI: 3.47–85.5; $p = 0.006$ for hCAP-18/LL-37 and OR = 8.25; 95% CI: 2.35–29.3; $p = 0.001$ for HβD-2. Both corticosteroid agents and leukotriene inhibitors used were related to a significant increase in mucosal AMP levels in upper airways.

Among the analysed environmental factors, passive smoking seems to contribute to the reduced levels of HβD-2 (OR = 4; 95% CI: 1.5–10; $p = 0.03$) and hCAP-18/LL-37 in the URT secretions (OR = 3.2; 95% CI: 1.2–8.4; $p = 0.02$). Personal history of atopic dermatitis in the study subjects was similarly associated with the increased odds of detection of the decreased levels of HβD-2 (OR = 8.1; 95% CI: 1.4–47; $p = 0.03$) and hCAP-18/LL-37 (OR = 9; 95% CI: 1.5–50; $p = 0.02$) in the secretion of the upper airways.

The aforementioned estimates were determined for individuals with allergic disease in total, regardless of the specific pathology (asthma or AR or asthma and AR comorbidity). Additionally, the maternal branch family history burdened with atopic diseases was found to be related to a decline in levels of HβD-2 in the respiratory mucosal secretions in children with asthma and AR comorbidity (OR = 12; 95% CI: 0.97–148; $p = 0.04$) and in children with a sole AR (OR = 19.9; 95% CI: 0.97–408; $p = 0.03$).

Simultaneously, neither the place of residence (urban or rural area) nor the presence of concomitant chronic non-allergic pathology demonstrated statistically significant association with the studied AMP levels. Likewise, we did not detect any relationship between the AMP concentrations in upper respiratory tract secretions and the duration of allergic disease, eosinophilia level, serum IgE concentrations, the type of sensitization, and the age at allergic disease onset.

DISCUSSION

Our study revealed a clear decline in the hCAP-18/LL-37 and HβD-2 levels in the URT secretions in children with allergic respiratory tract diseases in comparison with their healthy peers. The AMP concentrations in the upper airway secretions in children with asthma and AR were mostly linked to the severity of the disease, to the level of symptom control, and to the adherence to the basic controller therapy, while no association with the duration of allergic disease, the type of sensitization, and conventional biomarkers of atopy was revealed. Considering the aforementioned, we assume that the presence and intensity of chronic allergic airway inflammation contributes to a decrease in the concentrations of hCAP-18/LL-37 and HβD-2 in the URT secretions. Similarly, Xia *et al.* and Bogefors *et al.* [5, 8] suggested that the presence of chronic inflammation of the mucous membranes regardless of the aetiology contributes to the reduction of AMP levels. Instead, Marcinkiewicz argues that AMP levels may be elevated in rosacea and psoriasis, and Persson notes that high levels of some AMPs have been observed in chronic obstructive pulmonary disease [20, 21].

However, the presence of atopy could in itself be accompanied by abnormal production of AMP. This cannot be ruled out given that the AMP levels in the airway secretions in allergic individuals were still significantly lower than in healthy peers, even providing that the control of allergic disease symptoms was complete, and considering that a family or individual history of atopic disease was also associated with reduced mucosal AMP concentrations. Thus, it remains to be determined whether atopy is a major predictor of altered mucosal innate immunity function. Prospects for further research include the study of AMP levels in the mucosal secretions in atopic children prior to the development of a specific allergic disease.

High incidence and protracted duration of URTIs were additional factors closely tied to a decline in the AMP levels in the upper airway secretions, in asthmatic children in particular. We speculate that low levels of AMPs in children with allergic disease of the respiratory tract could be responsible for the increased susceptibility to respiratory infections, which usually contribute to amplification of inflammatory process, which, in turn, leads to a further depletion of AMPs in the URT mucosal discharge. According to van der Does *et al.*, this phenomenon can be explained by the following mechanism: chronic inflammation of the epithelium alters the modulation of cytokines and reduces the expression of AMPs [22]. This disrupts the epithelial barriers and promotes bacterial/viral invasion of the respiratory tract. The process of goblet cell metaplasia in chronically inflamed epithelium leads to changes in the secretion and rheology of mucus, which in turn contributes to the disruption of mucociliary clearance and the growth of pathogens. The fact that children at high risk of developing asthma and children with established asthma may have an increased risk of respiratory viral infections is also noted by Hamid Ahanchian *et al.* [23].

Many scientists point to a link between the microbiome and human health. According to research by Qian Liu, *Staphylococcus epidermidis* works in tandem with the host's immune system, eliminating pathogenic microflora and promoting the expression of antimicrobial peptides of nasal epithelial keratinocytes. Thus, a change in the microbiome can affect the development of allergic diseases. Studies by Dimitri-Pinheiro *et al.* show that *Prevotella buccalis* and *Gardnerella vaginalis* were more

common in the nasal microbiome of patients with asthma [24]. This may cause a decrease in AMP expression in patients with allergic diseases.

It is also essential that our study identified at least 2 potentially modifiable factors, which, if being addressed, could theoretically mount the content of hCAP-18/LL-37 and HβD-2 in the upper airways secretions in paediatric patients with respiratory allergy; namely, passive smoke exposure and breastfeeding duration in infancy. Elimination of the former and prolongation of the latter could serve as a basis for a targeted preventive interventions in children with atopic predisposition. Significant impact of maternal smoking on the mucosal innate immunity function in the upper airways was also reported by Bhat *et al.* [6].

CONCLUSIONS

Asthma and allergic rhinitis are associated with an altered mucosal innate immune response in the upper airways. In children with AR and BA, a significant decrease was found in the concentrations of antimicrobial peptides in the upper respiratory tract secretions as compared to healthy controls.

Higher severity of allergic disease, poor symptom control, atopic burden in family history, and passive smoking are associated with significantly higher odds for the decline in the mucosal AMP concentrations in the upper airways. In contrast, good symptom control, adherence to the basic treatment, and longer breastfeeding duration appear to affect the levels of AMPs positively and could be used as modifiable protective factors for targeted interventions in atopic children.

Elevated infectious respiratory morbidity and protracted course of upper respiratory tract infections could be additional predictors of altered mucosal immunity function in asthmatic children, and further investigations are required to clarify the potential mutual impact of allergic disease course, innate immunity function, and patterns of infectious respiratory morbidity in this cohort of patients.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 2017; 390: 1211-1259.
2. Meltzer EO, Bukstein DA. The economic impact of allergic rhinitis and current guidelines for treatment. Ann Allergy Asthma Immunol 2011; 106 (2 Suppl): S12-S16.
3. Gibson GJ, Loddenkemper R, Lundbäck B, Sibille Y. Respiratory health and disease in Europe: the new European Lung White Book. Eur Respir J 2013; 42: 559-563.
4. Mahlapuu M, Håkansson J, Ringstad L, Björn C. Antimicrobial Peptides: An Emerging Category of Therapeutic Agents. Front Cell Infect Microbiol 2016; 6: 194.
5. Xia X, Cheng L, Zhang S, et al. The role of natural antimicrobial peptides during infection and chronic inflammation. Antonie Van Leeuwenhoek 2018; 111: 5-26.
6. Bhat TA, Kalathil SG, Bogner PN, et al. Secondhand Smoke Induces Inflammation and Impairs Immunity to Respiratory Infections. J Immunol 2018; 200: 2927-2940.
7. de Groot EP, Nijkamp A, Duiverman EJ, Brand PL. Allergic rhinitis is associated with poor asthma control in children with asthma. Thorax 2012; 67: 582-587.
8. Bogefors J, Kvarnhammar AM, Millrud CR, et al. LEAP-2, LL-37 and RNase7 in tonsillar tissue: downregulated expression in seasonal allergic rhinitis. Pathog Dis 2014; 72: 55-60.
9. Zhang LJ, Gallo RL. Antimicrobial peptides. Curr Biol 2016; 26: R14-R19.
10. Chessa C, Bodet C, Jousselin C, et al. Antiviral and Immunomodulatory Properties of Antimicrobial Peptides Produced by Human Keratinocytes. Front Microbiol 2020; 11: 1155.
11. Olvera DPR, Gutiérrez CC. Multifunctional activity of the β-defensin-2 during respiratory infections, immune response activation and immunomodulation, Rajeev K. Tyagi and Prakash S. Bisen, IntechOpen. Available at: https://www.intechopen.com/books/immune-response-activation-and-immunomodulation/multifunctional-activity-of-the-defensin-2-during-respiratory-infections
12. Amirkhanov NV, Tikunova NV, Pyshnyi DV. Synthetic antimicrobial peptides: I. Antimicrobial activity of amphiphilic and nonamphiphilic cationic peptides. Russ J Bioorg Chem 2018; 44: 492–503.
13. Dale BA, Tao R, Kimball JR, et al. Oral antimicrobial peptides and biological control of caries. BMC Oral Health 2006; 6: S13.
14. Abaturov AE, Kryuchko TA, Lezhenko GA, Zavgorodnyaya NY. Antimikrobnye peptidy i proteiny respiratornogo trakta, diagnosticheskaya znachimost i terapevticheskie vozmozhnosti. Planeta-Print, Kharkov 2018.
15. Currie SM, Gwyer Findlay E, McFarlane AJ, et al. Cathelicidins have direct antiviral activity against respiratory syncytial virus in vitro and protective function in vivo in mice and humans. J Immunol 2016; 196: 2699-2710.
16. Davidopoulou S, Diza E, Menexes G, Kalfas S. Salivary concentration of the antimicrobial peptide LL-37 in children. Arch Oral Biol 2012; 57: 865-869.
17. Milani M. Approaching atopic dermatitis treatment differently: from skin barrier preservation to allergen-specific immunotherapy. Immunotherapy 2012; 4: 561-564.
18. Zuberbier T, Lötvall J, Simoens S, et al. Economic burden of inadequate management of allergic diseases in the European Union: a GA(2) LEN review. Allergy 2014; 69: 1275-1279.
19. Pałgan K, Tykwińska M, Bartuzi Z. Udział peptydów antydrobnoustrojowych w patogenezie astmy oskrzelowej [Antimicrobial peptides in asthma pathogenesis]. Postepy Hig Med Dosw (Online) 2015; 69: 10-13.
20. Persson LJ, Aanerud M, Hardie JA, et al. Antimicrobial peptide levels are linked to airway inflammation, bacterial colonisation and exacerbations in chronic obstructive pulmonary disease. Eur Respir J 2017; 49: 1601328.
21. Marcinkiewicz M, Majewski S. The role of antimicrobial peptides in chronic inflammatory skin diseases. Postepy Dermatol Alergol 2016; 33: 6-12.
22. van der Does AM, Amatngalim GD, Keijser B, et al. Contribution of Host Defence Proteins and Peptides to Host-Microbiota Interactions in Chronic Inflammatory Lung Diseases. Vaccines (Basel) 2018; 6: 49.

23. Ahanchian H, Jones CM, Chen YS, Sly PD. Respiratory viral infections in children with asthma: do they matter and can we prevent them? BMC Pediatr 2012; 12: 147.
24. Dimitri-Pinheiro S, Soares R, Barata P. The Microbiome of the Nose-Friend or Foe? Allergy Rhinol (Providence) 2020; 11: 2152656720911605.
25. Bousqet J, Hellings PW, Agache I, et al. ARIA 2016: Care pathways implementing emerging technologies for predictive medicine in rhinitis and asthma across the life cycle. Clin Transl Allergy 2016; 6: 47.