Research Article

Mojtaba Doulatpanah, Meltem Kocamanoğlu*, Eser Yıldırım Sözmen, Gökçen Kartal Öztürk, Esen Demir, Figen Gülen and Yasemin Akçay

Nasal fluid sample as a reliable matrix for determination of cytokine levels in childhood asthma

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Abstract

Objectives: Childhood asthma is a chronic disease with high incidence worldwide. As a lifelong disease, asthma has episodes. Inflammation continues to occur in the clinical remission of asthma. It can be difficult to diagnose childhood asthma, especially in clinical remission. We hypothesized that some cytokines secreted to nasal fluid from the airway during inflammation might help diagnose clinical remission of asthma. Moreover, sampling nasal fluid is an easy and non-invasive procedure, so it may be a preferable sampling method.

Methods: We measured levels of some interleukins (ILs), which are IL-4, IL-5, IL-6, IL-12p70, IL-13, IL-33, granulocyte-macrophage colony-stimulating factor (GM-CSF), periostin and thymic stromal lymphopoietin (TSLP) by Luminex magnetic bead-based immunoassay in nasal fluid and in serum of asthmatic children in clinical remission.

Results: We found that IL-5, IL-6, IL-33, and periostin had elevated levels in nasal fluid. IL-5 and IL-33 had increased levels in the nasal fluid of the patients with immunoglobulin E (IgE) high and low phenotypes. While the nasal fluid TSLP levels were positively correlated with most of the increased serum cytokine levels of non-allergic asthmatic children, the nasal fluid GM-CSF levels were positively correlated with most of the increased serum cytokine levels of the allergic asthmatic children.

Conclusions: IL-5, IL-6, IL-33, and periostin had elevated levels in the nasal fluid of the patients in clinical remission. The nasal fluid GM-CSF levels of the allergic patients and nasal fluid TSLP levels of the non-allergic patients had a positive correlation with most of the serum cytokine levels. Thus, our results showed that nasal fluid might be a preferable biological sample to diagnose asthma in children.

Keywords: biomarker; childhood asthma; cytokines; immune response; remission

Introduction

Asthma is the most common chronic childhood respiratory disease all over the world [1]. This disease is characterized by reversible airway obstruction and remodeling. The development risk of asthma is related to genetic and environmental factors [2], and it includes asthma episodes. Remission of childhood asthma is experienced in more than half of asthmatic children [3]. While clinical remission is characterized by normal lung function, absence of symptoms, use of no medication, at least one year of no attack of asthma, complete remission of asthma has no bronchial hyperresponsiveness [4]. Several studies show that it is necessary to evaluate lower airway inflammation and bronchial hyperresponsiveness via bronchoalveolar lavage (BAL) [5].

It has been complicated to describe asthma due to the development of diagnosis and treatment strategies for this disease with recent approaches [6]. Asthma is mostly associated with T-helper type 2 (Th2) inflammatory responses [7]. Clinical observations showed that type 2 asthma is usually early-onset allergic asthma, late-onset eosinophilic asthma, or exercise-induced asthma [8], and the patients are allergic

***Corresponding author: Meltem Kocamanoğlu**, Department of Medical Biochemistry, Faculty of Medicine, Ege University, Bornova, İzmir, Türkiye, Phone: +(90) 542 261 2999, E-mail: meltemkcmnoglu@gmail.com. https://orcid.org/0000-0002-1018-6182

Mojtaba Doulatpanah, Eser Yıldırım Sözmen and Yasemin Akçay, Department of Medical Biochemistry, Faculty of Medicine, Ege University, Bornova, İzmir, Türkiye. https://orcid.org/0000-0001-7447-9112 (M. Doulatpanah). https://orcid.org/0000-0002-6383-6724 (E.Y. Sözmen). https://orcid.org/0000-0002-0497-9346 (Y. Akçay)

Gökçen Kartal Öztürk, Esen Demir and Figen Gülen, Department of Paediatric Allergy and Clinical Immunology, Faculty of Medicine, Ege University, Bornova, İzmir, Türkiye. https://orcid.org/0000-0002-0793-9710 (G.K. Öztürk). https://orcid.org/0000-0003-2736-8924 (E. Demir). https://orcid.org/0000-0002-5431-3913 (F. Gülen)

to common aeroallergens, have high blood eosinophilia, increased serum periostin levels, and high exhaled nitric oxide [9].

For patients who have allergic asthma, it is necessary to measure Ig levels, especially IgE, to determine the allergens that trigger asthma attacks [10]. Inflammatory responses in asthma have a local basis, so serum IL levels have poor diagnosis efficiency [11]. It may be helpful to investigate interleukins in nasal fluid in terms of their biomarker potential because of their direct connection to secretions of airway epithelial cells.

Thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 are produced by epithelial and some haematopoietic cells and contribute to the promotion of Th2 cell development and the initiation of asthma pathogenesis [12]. Stimulated overproduction of type 2 cytokines such as IL-4, IL-5, IL-6, and IL-13 by these regulators results in hypersensitivity to aeroallergens, activation of airway epithelial cells, chemoattraction of mast cells, eosinophils, and basophils, remodeling of epithelium and subepithelial matrix [8]. IL-5 contributes migration of eosinophils in the bloodstream to the affected tissues in eosinophilic inflammation, and allergen exposure induces IL-5 production by Th2 cells in asthma [13]. IL-13 has an important role in the recombination of B cells to IgE production [14]. Moreover, IL-13 mediated activation of airway epithelial cells is related to upregulated gene expression of periostin in the subepithelial region of the airway [15]. IL-4, IL-5, and IL-13 are closely related to each other; therefore, they reach high amounts in blood serum to respond to several antigens [16]; in many chronic diseases, IL-6 is a proinflammatory cytokine as well as in asthma, and IL-6 has increased levels in serum and sputum [17]. IL-12p70 decreases the severity of asthma by inhibiting the development and proliferation of Th2 cells [18]. IL-33 acts as an alarmin to stimulate several immune responses when endothelial or epithelial tissue has damage [19]. GM-CSF secretion is not detectable in blood circulation except in response to endotoxins, GM-CSF has increased concentrations at local sites in tissue inflammations, and it is required for the accumulation of eosinophils in airways and lung tissue during allergic inflammation in asthma [20].

Our aim was to determine L-4, IL-5, IL-6, IL-12p70, IL-13, IL-33, GM-CSF, periostin, and TSLP cytokine levels in the nasal fluid of childhood asthma patients in clinical remission. Because sampling nasal fluid is an easy, non-invasive, non-traumatic sample collection method from children, and local inflammation response occurs via cytokines secreted by airway epithelial cells in asthma. For this purpose, we divided the patient group into two different subgroups: Allergic and non-allergic, serum IgE>100 IU/mL and serum IgE≤100 IU/mL, and we evaluated the serum and nasal fluid levels of the cytokines in terms of their biomarker potential.

Materials and methods

This is a prospective study involving asthma and healthy cohort groups at Ege University Children's Hospital Department of Paediatric Allergy and Pediatric Pulmonology. Serum and nasal cytokine levels were compared in the patient and the control groups. This study was approved by the Ethics Committee of Ege University Faculty of Medicine on 8 May 2017 with approval No 17–4/11, and written informed consent was obtained from the subjects and their parents.

Twenty asthmatic children were between 7 and 18 years old and in clinical remission, and 20 healthy subjects were included. Subjects with any underlying disease and upper respiratory tract infection in the last three months, who live with smoker parents, use any type of medication, have severe asthma, and cannot acceptably perform spirometry were excluded from the study.

Asthma was diagnosed according to The Global Initiative for Asthma [21]. The patients with asthma who do not have exacerbations and symptoms of asthma and who do not use any medications, also who do not have airflow obstruction in one year were assumed to be in clinical remission. The pulmonary function test was performed by spirometry (Flowhandy ZAN 100, Oberthulba, Germany) by measuring forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), and forced expiratory flow during the middle half of FVC (FEF25-75) in accordance with the standards of American Thoracic Society [22]. Spirometry parameters at the last visit of the patients were recorded.

The patients were divided into two subgroups in this study. While the first subgroup was divided into allergic and non-allergic patients according to the blood allergy test results, the second group was divided into patients with serum IgE>100 IU/mL and serum IgE≤100 IU/mL.

Blood allergy test parameters were as follows

Dermatophagoides pteronyssinus, *Dermatophagoidesfarinae*, *Alternaria alternata*, tree, grass, mold, weed panels, olive tree, eucalyptus tree, white egg-cow milk-whiting-wheat flour-peanut-soy, milk, Gum arabick, Tragacanth, Carob, Guar gum, orange-apple-peach, strawberry, tomato, spinach, cabbage, red pepper.

The sample collection procedure for nasal fluid was previously defined [23]. 300 µL saline solution was added to collected nasal fluid samples, and they were centrifuged for 5 min at 3000×*g*. 2 mL of blood samples were collected into blood tubes with EDTA from subjects' veins centrifuged for 10 min at 5000×*g*, and the plasmas were obtained. After that, the nasal fluid and the plasma samples were divided into two separate plastic tubes and stored at −80 °C until the analyses were performed.

Quantities of IL-4, IL-5, IL-6, IL-12p70, IL-13, IL-33, GM-CSF, TSLP, and periostin were analysed with custom design multiple panels (Luminex®, LXSAHM, RnDSystems, MN, USA). These panels were designed for the cytokines of this study. A 96-well plate with magnetic beads (MagPlex, RnDSystems, MN, USA) coated with antibodies. 50 µL of the nasal fluid and the plasma samples, and the reagents that were prepared according to the manufacturer's instructions were added into the wells with three replicates. After antibody-cytokine interactions for

90 min, the plates were read (Luminex® INTELLIFLEX, RnDSytems, MN, USA) with a set at 7,000 and 17,000 doublet discriminator gates. Total protein measurement was carried out by Bicinchoninic Acid Assay (23,225, Thermo Scientific, IL, USA). The calibration curves were achieved using current concentrations (0.1, 0.2, 0.4, 0.8, 1.2, 1.6, 2.0 µg/mL) of bovine serum albumin.

Statistical analysis was performed using Statistical Package for the Social Sciences Statistics Package V.21.0 (IBM Corp., NY, USA). A two-sided power analysis with an α value (default)=0.05 of the variables was performed to assess the size of the samples is adequate to evaluate the results. While the χ^2 test was used to compare categorical variables between groups, the Kolmogorov–Smirnov test was used to evaluate the normal distribution assumption for numerical variables. For normally distributed variables, the difference between the two groups was examined by independent samples t-test, and for non-normally distributed variables, the Mann–Whitney U-test was used. Following the Kruskal Wallis test, a post-hoc test (LSD) was performed in the three groups -the control group and the two patient groups with different IgE levels-to evaluate the differences in the variables. Statistically, a $p<0.05$ value was considered significant.

Results

The study included 20 subjects with asthma in clinical remission (8 females, 12 males) and 20 healthy subjects (11 females, 9 males) of similar age. The clinical characteristics of the groups are shown in Table 1.

Table 1: General and spirometric data of the subjects.

	Healthy group	Asthma group in clinical remission	p-Value
n	20	20	–
Male gender, n (%)	9 (45)	12 (60)	0.352
Age, years (range)	9.1 (7–12)	8.6 (7–11)	0.291
Serum IgE>100 IU/mL, n (%)	0 (0)	10 (50)	–
Allergy positive, n (%)	0 (0)	10 (50)	–
Serum eosinophil, cells/µL ± SD	230 ± 162	231 ± 112	0.878
FVC, % ± SD	–	82.80 ± 14.08	–
FEV1, % ± SD	–	91.70 ± 14.18	–
FEV1/FVC, % ± SD	–	109.20 ± 6.24	–
PEF, % ± SD	–	80.30 ± 12.14	–
MEF75, % ± SD	–	85.85 ± 15.76	–
MEF50, % ± SD	–	91.60 ± 21.32	–
MEF25, % ± SD	–	94.30 ± 29.34	–
MEF2575, % ± SD	–	95.55 ± 23.57	–

Data are represented in mean with standard deviations (mean ± SD) FVC, forced vital capacity; FEV1, first forced expiratory volume; FEV1/FVC, forced expiratory volume/forced vital capacity; PEF, peak expiratory flow; MEF75, mid-expiratory flow at 75 % of FVC; MEF50, maximal expiratory flow at 50 % of vital flow capacity; MEF25, maximal expiratory flow at 25 % of forced vital capacity; MEF2575, forced medium expiratory flow.

Blood allergy tests "were performed, and mixed allergens, olive tree, weeds, *Dermatophagoides pteronyssinus*, trees, *A. alternata*, and eucalyptus tree were detected alone or together some of the patients, and 10 of the patients were sensitive to at least one of them. The other 10 patients were not sensitive to any type of allergens. 10 of the patients had high levels of serum IgE (>100 IU/mL), and five of these patients were sensitive to some type of allergens; serum eosinophil levels of the patients were in the reference interval out of the four patients who have higher levels than the interval. Spirometry tests were in the normal range.

The cytokine levels in the serum and the nasal fluid for both groups are shown in Table 2. IL-5 (p=0.015), IL-12p70 (p=0.024), IL-13 (p=0.020), and TSLP (p=0.030) serum levels of the patient group were found to be significantly higher than the control group. In the patient group, IL-5 (p=0.001), IL-6 (p=0.022), IL-33 (p=0.006), and periostin (p=0.000) nasal fluid levels were significantly higher than the control group.

Nasal IL-33 and IL-5 levels of patients with serum IgE≤100 IU/mL and IgE>100 IU/mL were significantly higher than the control group. Nasal IL-13 levels of patients with blood IgE>100 IU/mL were significantly higher than patients with serum IgE≤100 IU/mL (Figure 1).

Nasal fluid GM-CSF levels of the allergic patients had a positive correlation with serum cytokine levels out of the periostin. Nasal fluid TSLP levels of the non-allergic patients had a positive correlation with serum cytokine levels out of the IL-6 and periostin (Supplementary Figure).

Discussion

Serum levels of IL-5, IL-6, IL-12p70, IL-13, and TSLP and nasal fluid levels of IL-5, IL-6, IL-33, and periostin of the patient group were found to be significantly higher than the control group in this study (Table 2). Besides, IL-5 and IL-33 levels in the nasal fluid of the patient groups with IgE≤100 IU/mL and IgE>100 IU/mL were significantly higher than in the control group, also IL-13 levels in nasal fluid from the patients with IgE>100 IU/mL were significantly higher than the IgE≤100 IU/mL group. There was no significant difference in blood eosinophil counts between the patients and the control group. Furthermore, while nasal fluid GM-CSF levels of the allergic patients had a positive correlation with serum cytokine levels out of periostin levels, nasal fluid TSLP levels of the non-allergic group had a strong positive correlation with serum cytokine levels out of IL-6 and periostin in this study.

Characteristic symptoms and limitation in airflow at spirometry are the diagnostic criteria of childhood asthma. However, spirometric values can be in the normal range in

Table 2: Serum and nasal fluid cytokine levels of the subjects.

Cytokines	Serum levels							Nasal fluid levels						p-Value
	Healthy group (n=20)			Asthma group in clinical remission (n=20)			p-Value	Healthy group (n=20)			Asthma group in clinical remission (n=20)			
	Median	Q1	Q3	Median	Q1	Q3		Median	Q1	Q3	Median	Q1	Q3	
IL-4, pg/μL	1.020	0.440	3.320	1.175	0.590	2.780	p=0.169	7.040	3.718	18.273	11.050	5.420	23.870	p=0.361
IL-5, pg/μL	1.040	0.870	1.168	1.040	1.040	1.740	p=0.015[a]	0.870	0.870	1.040	1.125	1.040	1.898	p=0.010[a]
IL-6, pg/μL	0.615	0.440	1.215	0.870	0.640	1.338	p=0.093	1.800	1.295	8.705	9.220	5.708	13.873	p=0.022[a]
IL-12p70, pg/μL	18.16	14.06	34.31	24.31	17.68	65.78	p=0.024[a]	20.13	17.20	30.00	21.17	18.39	28.58	p=0.577
IL-13, pg/μL	76.42	62.94	107.79	103.59	76.42	195.56	p=0.020[a]	263.76	147.77	359.42	261.45	117.14	440.64	p=0.988
IL-33, pg/μL	5.67	1.50	20.92	3.05	2.26	10.14	p=0.988	13.55	7.63	32.87	113.52	17.94	181.60	p=0.006[a]
GM-SCF, pg/μL	0.700	0.455	1.093	0.780	0.515	0.940	p=0.150	2.120	1.850	2.270	1.980	1.850	2.560	p=0.828
TSLP, pg/μL	0.430	0.340	0.990	0.645	0.430	0.843	p=0.030[a]	0.545	0.390	1.350	0.515	0.400	2.113	p=0.613
Periostin, pg/μL	128.2	109.9	142.2	127.3	115.6	144.0	p=0.718	926.1	390.5	1,325.5	2,358.0	1,448.0	8,974.0	p=0.000[a]

Data are represented in median and interquartile ranges. The variables were non-normally distributed because of this result; p-values were presented with a non-parametric Mann-Whitney U test. IL, interleukine; GM-CSF, gronulocyte-macrophage colony-stimulating factor; TSLP, thymic stromal lymphopoietin. [a]p<0.05 value considered statistically significant.

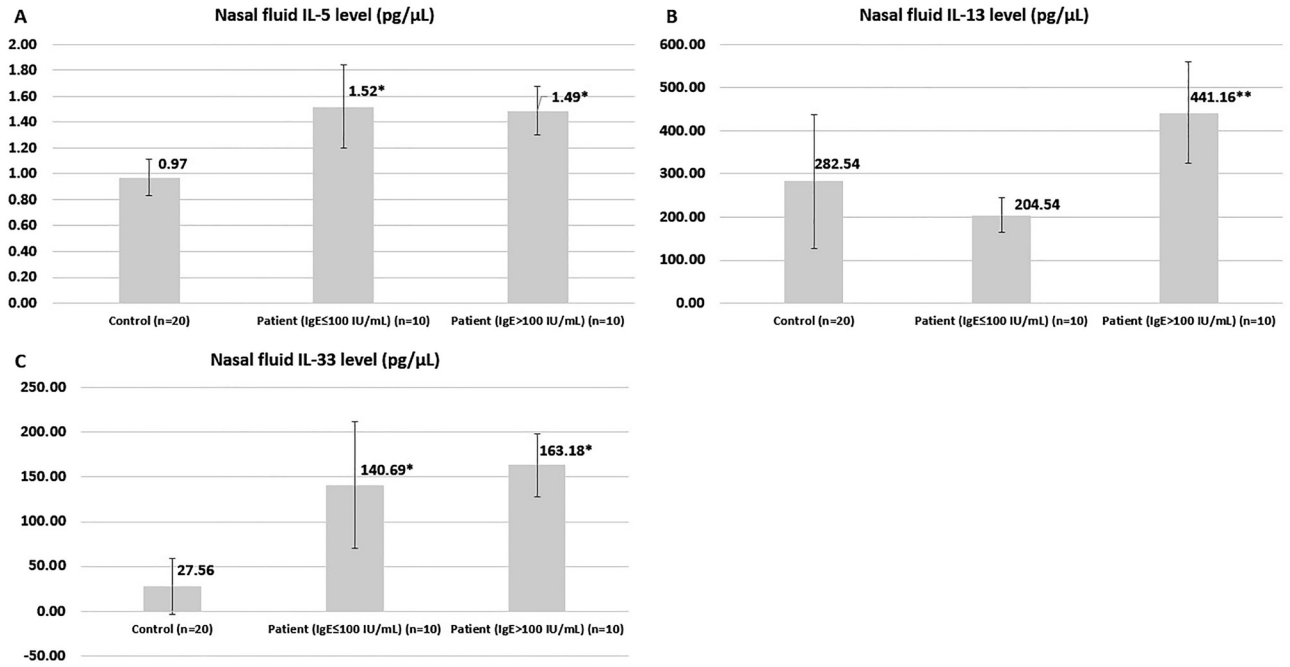

Figure 1: Nasal fluid levels of IL-5 (A), IL-13 (B), and IL-33 (C). IgE, immunoglobulin E; IL, interleukin. *Statistically significant compared with the control group, $p<0.05$. **Statistically significant compared with the IgE≤100 patient group, $p<0.05$.

some cases. Also, running spirometry tests under age 6 in clinical practice is impossible. Because of these reasons, there are some difficulties in diagnosing asthma in children. Moreover, there is no serological biomarker for diagnosis or follow-up of asthma, and novel biomarkers of asthma in all patients and especially in children, have been investigated for long years. Because of these reasons, it is important to discover new biomarkers with a non-invasive sampling method for diagnosis and follow-up of childhood asthma. In other words, asthma diagnosis and follow-up are performed by anamnesis of the patient and spirometry tests. A biomarker or a panel of biomarkers is not clinically available for the diagnosis of asthma [24]. There are several cytokines that we evaluated for their biomarker potential in nasal fluid for clinical remission in this study. It has been chosen nasal fluid samples for two main reasons; firstly, nasal fluid samples can be easily collected from patients, and secondly, nasal fluid may show a local inflammatory response from epithelial cells of the airway due to its direct link to the airway.

We performed spirometry tests on the patient group to evaluate their pulmonary function. The results were in the normal range, supporting their clinical remission.

There is no consensus about serum IgE levels in healthy or asthmatic children in the literature. A study that included 786 allergic asthmatic children in Turkey showed that the mean value of serum IgE levels was 153 IU/mL [25]; the other research with 179 asthma patients and 93 healthy subjects in Turkey showed that the mean value of serum IgE levels was 257.57 IU/mL in asthmatic children and 68.26 IU/mL in healthy children [26]. Because half of the children in the patient group had allergic asthma, and the patient group was in clinical remission in our study, we determined the cut-off value of total serum IgE level as 100 IU/mL assumptionally. There were 10 patients with IgE>100 IU/mL, and five of them were also allergic patients. It was controversial that the five patients of the IgE>100 IU/mL group were not allergic because IgE levels increase in allergic diseases [27].

Serum IL-5 and IL-13 but not serum IL-4 levels of the patients were significantly higher than the control group in this study. IL-4, IL-5, and IL-13 are highly relevant to each other because they are essential cytokines in the Th2 response in asthma [8]. IL-5 is associated with an eosinophilic pathway of the immune response [13], IL-13 has an important role in increased smooth muscle contractility associated with asthma to contribute to the secretion of inflammatory mediators from bronchial tissue [28]. Production of IL-5 and IL-13 from mast cells is induced by TSLP [29], so elevated levels of serum IL-5 and IL-13 of the asthmatic subjects from this study might be induced by increased levels of TSLP. We found that IL-5 nasal fluid levels of the patients were significantly higher than the control group, but we did not find significantly higher levels for IL-4 and IL-13 in nasal fluid. Low levels of IL-4 and IL-13 may be

caused by low blood eosinophil levels, production of these cytokines may not have been increased in clinical remission, or a low amount of these cytokines may have been secreted to the nasal fluid.

Because IL-5 enhances IgE production with cytokines IL-4 and IL-13 [27] and it has a key role in Th2 immune response [13] as well, IL-5 levels in nasal fluid for both the patients with IgE≤100 IU/mL and IgE>100 IU/mL may be significantly higher than the control group. Elevated IL-13 nasal fluid levels in the patient group with IgE>100 IU/mL support its role in mucus production, airway remodeling, and bronchial hyperresponsiveness [13]. IgE levels are over-increased by IL-4 secretion from airway mucosa during the pathogenesis of asthma [30]. However, this study did not show any significant difference between the control and the patient group in terms of serum or nasal fluid IL-4 levels. Interestingly, IL-4 levels do not have a significant elevation even in the patient group with IgE>100 IU/mL in our study because IL-4 directly promotes higher IgE levels [13, 31].

Our data showed that nasal IL-6 levels of the patients were significantly higher than the control group. Proinflammatory cytokine IL-6 is secreted from inflamed tissue [17]. Therefore, IL-6 levels in the nasal fluid of the patient group may have been significantly higher than IL-6 levels of the control group. Manise et al. showed that IL-6 levels increased in the airway with IgE high phenotype as well [32]. Controversially, there was no correlation between nasal fluid IL-6 levels and serum IgE levels in our study.

Although we have no data on IL-17 and IL-23 levels in this study, it might be speculated that increased serum IL-12p70 levels of the patients and serum IL-12p70 levels of the non-allergic patient group may be an indicator for mixed granulocytic non-type 2 non-eosinophilic asthma rather than type 2 non-allergic asthma [33]. Nasal fluid levels of IL-12 in the patient group have not significantly increased, this discrepancy might be explained by the fact that it's secreted by T cells, not by airway epithelial cells [34].

IL-33 in nuclei and cytoplasm of nasal mucosa epithelium is overexpressed when the Th2 immune response occurs, and overexpressed IL-33 may induce the production of IL-4, IL-5, and IL-13 [35]. IL-33 acts as an alarmin to stimulate several immune responses when endothelial or epithelial tissue is damaged [19]. Therefore, nasal fluid levels of IL-33 in the patients might have increased but not serum levels. IL-33 has a modulator role for all the immune responses of human organisms, specifically in asthma. Due to its significant role in allergic inflammation [35], we observed increased levels of IL-33 in the patients independent of IgE levels (Figure 1C).

Since TSLP induces eosinophilia in the airway and blood and enhances the production of proinflammatory cytokines [36], we found significantly elevated serum levels of TSLP in the patients. Interestingly, we did not find a significant increase of TSLP in the nasal fluid of the patients. Because TSLP has an important role in Th2 responses of mucosal barriers [12], a non-significant increase of TSLP in the nasal fluid was unexpected, however, TSLP may have been increased at only the early stage of inflammation because of its proinflammatory effect, or it may be a result of lower nasal fluid levels of TSLP in clinical remission. Because TSLP is a molecule that is produced by epithelial and haematopoietic cells [37], nasal fluid TSLP levels in non-allergic patients might have a positive correlation with serum cytokine levels out of IL-6 and periostin.

Nasal fluid levels of periostin increased in the patients because of the overexpressed levels of it by inflammation in airway epithelial cells [15]. Since airway epithelial cells, not circulating cells are responsible for the production of periostin, our results showed that serum periostin levels of the patient group were not significantly higher than the control group. Unexpectedly we did not find a correlation between serum or nasal fluid levels of IL-13 and periostin, probably because our patients were in clinical remission when we collected their samples. It is known that the periostin gene of airway epithelium cells is overexpressed by IL-13 activation in the active inflammatory state [15]. Serum IL-13 and nasal fluid periostin levels may have a positive correlation in only exacerbated asthma.

In this research, we did not observe a significant difference ($p>0.05$) in blood eosinophil counts between the patient and the control groups, the blood eosinophil levels were 231 ± 112 cells/μL and 230 ± 162 cells/μL, respectively. The decision limit of circulating eosinophil levels to identify the severity of asthma is not clear in the literature, the levels changed from 150 cells/μL to 757 cells/μL according to several studies. For instance, Gupta A. et al. reported that blood eosinophil levels which are higher than 150 cells/μL represents eosinophilic childhood asthma [38]; results of another study showed that if blood eosinophil counts per μL are higher than 436, it is accepted that the child patient has childhood asthma [39]. Another study indicated that moderate to severe child asthma patients have 757 cells/μL circulating eosinophils, while healthy subjects have 282 cells/μL of circulating eosinophils [40], all these results can be interpreted that childhood patients had moderate or severe asthma in these studies; as it was shown that their blood eosinophil counts were similar to those of the healthy subjects, patients in our study were in clinical remission.

GM-CSF is secreted by tissues with inflammation, and its concentration increases with Th2 inflammation response [20]; therefore, nasal fluid GM-CSF levels of the allergic

patients may have a positive correlation with serum cytokine levels out of the serum periostin levels in this study.

In conclusion, IL-5, IL-6, IL-33, and periostin had elevated levels in the nasal fluid of asthmatic children in clinical remission. Nasal fluid GM-CSF levels of the allergic asthma group had a positive correlation with serum cytokine levels out of the serum periostin, and nasal fluid TSLP levels of the non-allergic patients had a strong positive correlation with serum cytokine levels out of IL-6 and periostin. Our results showed that nasal fluid might be a preferable biological sample to diagnose asthma in asthmatic children. However, it is necessary to investigate and compare nasal fluid levels of these cytokines, IL-17 and IL-23, in different stages and subtypes of asthma together in further studies.

Research ethics: The study was approved by the Ethics Committee of Ege University Faculty of Medicine on 8 May 2017 with approval No 17–4/11.
Informed consent: Informed consent was obtained from all individuals included and their parents in this study.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
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References

1. Leong KP, Huston DP. Understanding the pathogenesis of allergic asthma using mouse models. Ann Allergy Asthma Immunol 2001;87: 96–110.
2. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, et al. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med 2010;363:1211–21.
3. Sears MR. Predicting asthma outcomes. J Allergy Clin Immunol 2015; 136:829–36.
4. Menzies-Gow A, Bafadhel M, Busse WW, Casale TB, Kocks JWH, Pavord ID, et al. An expert consensus framework for asthma remission as a treatment goal. J Allergy Clin Immunol 2020;145:757–65.
5. Van den Toorn LM, Overbeek SE, Prins JB, Hoogsteden HC, De Jongste JC. Asthma remission: does it exist? Curr Opin Pulm Med 2003;9:15–20.
6. Taylor SL, Leong LEX, Choo JM, Wesseling S, Yang IA, Upham JW, et al. Inflammatory phenotypes in patients with severe asthma are associated with distinct airway microbiology. J Allergy Clin Immunol 2018;141:94–103.
7. Fajt ML, Wenzel SE. Asthma phenotypes and the use of biologic medications in asthma and allergic disease: the next steps toward personalized care. J Allergy Clin Immunol 2015;135:299–310.
8. Fahy JV. Type 2 inflammation in asthma-present in most, absent in many. Nat Rev Immunol 2015;15:57–65.
9. Castro M, Corren J, Pavord ID, Maspero J, Wenzel S, Rabe KF, et al. Dupilumab efficacy and safety in moderate-to-severe uncontrolled asthma. N Engl J Med 2018;378:2486–96.
10. Rajakulasingam K, Till S, Ying S, Humbert M, Barkans J, Sullivan M, et al. Increased expression of high-affinity IgE (FcεRI) receptor-α chain mRNA and protein-bearing eosinophils in human allergen-induced atopic asthma. Am J Respir Crit Care Med 1998;158:233–40.
11. Dimitrova D, Youroukova V, Ivanova-Todorova E, Tumangelova-Yuzeir K, Velikova T. Serum levels of IL-5, IL-6, IL-8, IL-13 and IL-17A in pre-defined groups of adult patients with moderate and severe bronchial asthma. Respir Med 2019;154:144–54.
12. Paul WE, Zhu J. How are Th2-type immune responses initiated and amplified? Nat Rev Immunol 2010;10:225–35.
13. Lambrecht BN, Hammad H, Fahy JV. The cytokines of asthma. Immunity 2019;50:975–91.
14. Parulekar AD, Diamant Z, Hanania NA. Role of T2 inflammation biomarkers in severe asthma. Curr Opin Pulm Med 2016;22:59–68.
15. Woodruff PG, Boushey HA, Dolganov GM, Barker CS, Yang YH, Donnelly S, et al. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. Proc Natl Acad Sci USA 2007;104:15858–63.
16. Ho IC, Miaw SC. Regulation of IL-4 expression in immunity and diseases. Adv Exp Med Biol 2016;941:31–77.
17. Neveu WA, Allard JL, Raymond DM, Bourassa LM, Burns SM, Bunn JY, et al. Elevation of IL-6 in the allergic asthmatic airway is independent of inflammation but associates with loss of central airway function. Respir Res 2010;11:28–37.
18. Durrant DM, Metzger DW. Emerging roles of T helper subsets in the pathogenesis of asthma. Immunol Invest 2010;39:526–49.
19. Cayrol C, Girard JP. IL-33: an alarmin cytokine with crucial roles in innate immunity, inflammation, and allergy. Curr Opin Immunol 2014; 31:31–7.
20. Hamilton JA, Anderson GP. GM-CSF biology. Growth Factors 2004;22: 225–31.
21. Horak F, Doberer D, Eber E, Horak E, Pohl W, Riedler J, et al. Diagnosis and management of asthma – statement on the 2015 GINA guidelines. Wien Klin Wochenschr 2016;128:541–54.
22. Graham BL, Steenbruggen I, Miller M, Barjaktarevic IZ, Cooper BG, Hall GL, et al. Standardization of spirometry 2019 update an official American Thoracic Society and European Respiratory Society technical statement. Am J Respir Crit Care Med 2019;200:70–88.
23. Ruocco L, Fattori B, Romanelli A, Martelloni M, Casani A, Samolewska M, et al. A new collection method for the evaluation of nasal mucus proteins. Clin Exp Allergy 1998;28:881–8.
24. Danvers L, Lo DKH, Gaillard EA. The role of objective tests to support a diagnosis of asthma in children. Paediatr Respir Rev 2020;33:52–7.
25. Kılıç M, Taşkın E. Alerjik astımlı çocukların klinik özelliklerinin ve risk faktörlerinin değerlendirilmesi. Firat Med J 2015;20:199–205.
26. Tülübaş F, Gürel A, Donma MM, Nalbantoğlu B, Topçu B, Mut ZD. Evaluation of total IgE, CRP, and blood count parameters in children with asthma and allergic rhinitis. Dicle Med J 2013;40:57–61.
27. Poulsen LK, Hummelshoj L. Triggers of IgE class switching and allergy development. Ann Med 2007;39:440–56.
28. Bara I, Ozier A, Tunon De Lara JM, Marthan R, Berger P. Pathophysiology of bronchial smooth muscle remodelling in asthma. Eur Respir J 2010;36:1174–84.
29. Allakhverdi Z, Comeau MR, Jessup HK, Yoon BP, Brewer A, Chartier S, et al. Thymic stromal lymphopoietin is released by human epithelial

cells in response to microbes, trauma, or inflammation and potently activates mast cells. J Exp Med 2007;204:253–8.
30. Kabesch M, Schedel M, Carr D, Woitsch B, Fritzsch C, Weiland SK, et al. IL-4/IL-13 pathway genetics strongly influence serum IgE levels and childhood asthma. J Allergy Clin Immunol 2006;117:269–74.
31. Pelaia C, Paoletti G, Puggioni F, Racca F, Peleia G, Canonica GW, et al. Interleukin-5 in the pathophysiology of severe asthma. Front Physiol 2019;10:1514.
32. Manise M, Holtappels G, Van Crombruggen K, Schleich F, Bachert C, Louis R. Sputum IgE, and cytokines in asthma: relationship with sputum cellular profile. PLoS One 2013;8:e58388.
33. Agache I, Akdis CA. Endotypes of allergic diseases and asthma: an important step in building blocks for the future of precision medicine. Allergol Int 2016;65:243–52.
34. Zhang YL, Luan B, Wang XF, Qiao JY, Song L, Lei RR, et al. Peripheral blood MDSCs, IL-10 and IL-12 in children with asthma and their importance in asthma development. PLoS One 2013;8:e63775.
35. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity 2005;23:479–90.
36. Zhou B, Comeau MR, De Smedt T, Denny Liggitt H, Dahl ME, Lewis DB, et al. Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. Nat Immunol 2005;6:1047–53.
37. Wang W, Li Y, Lv Z, Chen Y, Li Y, Huang K, et al. Bronchial allergen challenge of patients with atopic asthma triggers an alarmin (IL-33, TSLP, and IL-25) response in the airway's epithelium and submucosa. J Immunol 2018;201:2221–31.
38. Gupta A, Ikeda M, Geng B, Azmi J, Price RG, Bradford ES, et al. Long-term safety and pharmacodynamics of mepolizumab in children with severe asthma with an eosinophilic phenotype. J Allergy Clin Immunol 2019;144:1336–42.
39. Ross KR, Gupta R, DeBoer MD, Zein J, Phillips BR, Mauger D, et al. Severe asthma during childhood and adolescence: a longitudinal study. J Allergy Clin Immunol 2020;145:140–6.
40. Covar RA, Spahn JD, Martin RJ, Silkoff PE, Sundstrom DA, Murphy J, et al. Safety and application of induced sputum analysis in childhood asthma. J Allergy Clin Immunol 2004;114:575–82.

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