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IL-5, IL-8 and MMP -9 levels in exhaled breath condensate of atopic and nonatopic asthmatic children

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Summary
Rationale: Asthma is a heterogeneous disease, and a great majority of pediatric patients with asthma demonstrate atopic characteristics and develop a Th2 type cytokine response. Nonatopic asthma, on the other hand, is seen more rarely.
Methods: In this study, levels of IL-5, IL-8 and MMP-9 were measured in exhaled breath condensate (EBC) of the subjects to demonstrate the extent of tissue damage as well as eosinophilic and neutrophilic inflammation in children with atopic and nonatopic asthma. A total of 37 children with atopic asthma and 37 children with nonatopic asthma were enrolled in the study. Patients who exhibited protease positive aeroallergen (House dust mite, mould mix, olea, grass mix) sensitivity in allergen skin prick test were included in the atopic asthma group. To evaluate the EBC, the fluid content of the breath was collected by having the patients exhale into an EBC device, after which the IL-5, IL-8 and MMP-9 levels were assayed using the ELISA method.
Results: The atopic asthmatics exhibited significantly higher IL-5 levels in their EBC samples than the nonatopic asthmatics (0.271 [0.198–0.489] pg/ml and 0.198 [0.125–0.344] pg/ml, respectively, $p = 0.04$), while no significant differences were observed in the levels of IL-8 and MMP-9 in the EBC samples of the atopic and nonatopic asthmatics.
Conclusions: IL-5 levels, as a marker of eosinophilic inflammation, were demonstrated to be higher in the children with atopic asthma when compared to those with nonatopic asthma in EBC. The fact that no significant difference was apparent in the IL-8 levels between the groups

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suggests that it is the severity of the disease rather than the atopic state that plays an important role in IL-8 levels. Since no difference was recorded between the groups in terms of MMP-9 levels, lung damage in asthma sufferers seems to develop independent of atopia.
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Introduction

Asthma is a chronic inflammatory disease with various different etiologies [1]. Most cases of pediatric asthma are atopic and are identified from positive SPT results, the presence of IgE antibodies, hyperreactivity of the airways and infiltration of the bronchial mucosa by eosinophils and Th2 lymphocytes. On the other hand, nonatopic asthma with negative SPT results which is less common among children and is not accompanied by allergic disease findings or a family history of atopy [2].

Airway inflammation in asthma cases is a multicellular process that involves primarily eosinophilic infiltration, but also netrophils, CD4 (+) T lymphocytes and mast cells [3]. While IL-3, GM-CSF and Eotaxin 1-3 are important in the early transformation of easinophils in the bone marrow from CD34 + progenitor cells, IL-5, as the most important eosinopoetin, is known to be involved in maturation, increased lifecycle and gathering of eosinophils in the airways [3,4]. Pathological studies performed on patients with asthma suggest that eosinophilic inflammation is not characteristic for all patients, as a neutrophilic inflammation in which IL-8, independent of the atopy, is probably effective either direct or indirectly to varying degrees, predominantly in the airways of asthma patients [5—7].

Proteolytic enzymes play an important role in the tissue remodelling and repair of airway walls [8]. MMP-9 is an endopeptidase that originates from various inflammatory cells, such as bronchial epithelial cells, neutrophils, mast cells, eosinophils and macrophages, but especially from neutrophils [8—10]. MMP-9 is a marker of the damage that inflammation creates in the epithelial mesenchymal trophic unit, and is found to be increased in patients with asthma compared with healthy controls [8].

Numerous invasive techniques such as bronchoscopy, bronchoalveolar lavage (BAL) and airway biopsies are available to assess local inflammation, remodelling, biomarkers in asthma. However, the number of noninvasive methods for identifying and assessing asthma is far more limited. The EBC method has been extensively used to identify respiratory tract diseases in children, and the method's reliability has been demonstrated in previous studies [11,12].

In this study, to allow a comparison of eosinophilic and neutrophilic inflammation, the IL-5, IL-8 and MMP-9 levels of children with atopic and nonatopic asthma were measured using EBC, which is a noninvasive method. The intention in this regard was to assess the level of tissue damage and to evaluate the results through a comparison with clinical findings.

Material and methods

Study population

The study was carried out in the Department of Pediatric Allergy and Immunology in the Celal Bayar University Medical Faculty. A total of 74 pediatric patients with asthma (37 atopic and 37 nonatopic) were enrolled in the study consecutively. The Global Initiative for Asthma (GINA) criteria were used for diagnosing asthma [13], while exclusion criteria included the presence of other diseases that may affect the inflammatory markers of the EBC method (such as primary ciliary dyskinesia and cystic fibrosis); the use of systemic or inhaled antiinflammatory therapies within the last three months; a history of asthma exacerbation within the last three months; and respiratory infections within the last month.

Children who exhibited at least one protease positive aeroallergen (Houst dust mite, mould mix, olea, grass mix) [14] sensitivity in the allergen skin prick test were included in the atopic asthma group, while those with negative allergen skin prick test results were included in the nonatopic asthma group. The ages of the children in the nonatopic group were similar to those in the atopic group.

Study design and ethics committee approval

This case control study was approved by the Ethics Committee of Celal Bayar University (Decision No: 03-2011/72). The study was carried out in accordance with the Declaration of Helsinki, and written informed consent was obtained from each child and parent.

Data collection

The patients' age, sex, number of exacerbations, days of hospitalization, and bronchodilator and systemic corticosteroid use within the last year were recorded at the time enrolment. The pulmonary function test results were also recorded.

Blood samples were collected in order to assess serum IgE levels and peripheral blood eosinophil count on the same day. In addition, the patients exhaled breath condensate was obtained on the day of enrolment, with samples maintained at −80° C until the time of measurement of IL-5, IL-8 and MMP-9.

Total eosinophil count

Eosinophil counts were recorded using a complete blood count device (Beckman Coulter LH 780 Analyzer, California, United States).

Total IgE measurement

Venous blood samples of 2 cc were collected in standard biochemical tubes, and the samples were studied as part of the routine assessment of each patient using the chemiluminescent immunometric assay method (Immulight 2000, NJ, United States) in a biochemistry laboratory.

Pulmonary function test

Spirometric evaluations were carried out using Spirobank G (Rome, Italy). The tests were conducted at room temperature while the patients were in a sitting position with their nasal airways closed using a clip [15].

Assessment of asthma severity

The pediatric allergy specialist who examined the patients evaluated asthma severity with full consideration of both daytime and nighttime symptoms, the number of exacerbations, and FEV_1 and PEF values, as follows. Based on these evaluations, the patients were classified as having either intermittent asthma, mild persistent asthma, moderate persistent asthma or severe persistent asthma [13].

Allergen skin prick test

Inhalant allergen sensitivity was assessed with an allergen skin prick test using the most common aeroallergens (Allergpharma©, Reinberg Germany) and in accordance with EAACI recommendations [16]. The intake of short acting antihistamines by the patients was stopped at least five days in advance of testing, while the intake of long-acting antihistamines were stopped 10 days in advance.

The skin tests were carried out by qualified technicians. The allergy test kit consisted of eight different aeroallergens Dermatophagoides pteronyssinus, Dermatophagoides farinae, Mould mix I (Alterneria tenius, Botrytis cinerea, Cladosporium herbarun, Curvularia lunata, Fusarium moniliforme, Helminthosporium halodes), Dog epithelium, Weed mix (Artemisia vulgaris, Urticia dioica, Toraxacum vulgare, Plantago Lanceolata), Grass mix (Holcus lanatus, Dactylis glomerata, Lolium perenne, Pheleum pratense, Poa pratensis, Festuca pratensis), Plantago lanceolata, Olea europeae along with positive and negative controls. A histamine solution in 10 mg of distilled water was used as the positive control, while a phyglycerol buffered physiological saline solution was used as the negative control. The skin tests were carried out on the volar surface of the forearm using prick lancets. The skin reactions triggered by the application of each allergen was compared with the reactions triggered by the positive and negative controls. Indurations with a diameter equal to or greater than 3 mm were considered as positive reactions.

Collection and evaluation of exhaled breath condensates

For the measurement of Exhaled Breath Condensates, the fluid volume of the breath was collected by having the patient exhale into an Exhaled Breath Condensate device (Ecoscreen; Jaeger, Hoechberg, Germany) for 10 min without a nose clip. The EBC tests were carried out in line with ERS/ATS recommendations [17] after the patients had rinsed their mouths following a 2-h fasting period, and before any asthma treatment was initiated. The accumulated liquid portion of the collected air was placed into 130 μL Eppendorf tubes made from translucent polypropylene, and stored at −80 °C until the IL-5, IL-8 and MMP-9 measurement.

IL-5 levels in exhaled breath condensate

Serum IL-5 levels were tested through the ELISa method using a commercial kit (R&D Systems, Quantikine ELISA, IL-5, Abingdon, United Kingdom). The correlation coefficient of the kit in the study was determined to be 3.2 percent, 2.2 percent and 4.2 percent in concentrations of 28.1 pg/ml, 89.3 pg/ml and 170 pg/ml, respectively. The correlation coefficient of the kit between the studies was determined to be 3.8 percent, 4.5 percent and 5 percent in concentrations of 31.2 pg/ml, 92.6 pg/ml and 174 pg/ml, respectively.

IL-8 levels in exhaled breath condensate

Serum IL- 8 levels were tested through the ELISa method using a commercial kit (R&D Systems, Quantikine ELISA, IL-5, Abingdon, United Kingdom). The correlation coefficient of the kit in the study was determined to be 4.6 percent, 4.4 percent and 4.7 percent in concentrations of 115 pg/ml, 386 pg/ml and 802 pg/ml, respectively. The correlation coefficient of the kit between the studies was determined to be 8.1 percent, 6.8 percent and 5.2 percent in concentrations of 132 pg/ml, 410 pg/ml and 817 pg/ml, respectively.

MMP-9 levels in exhaled breath condensate

Serum MMP-9 levels were tested through the ELISA method using a commercial kit (R&D Systems, Quantikine ELISA, MMP-9, Abingdon, United Kingdom). The correlation coefficient of the kit in the study was determined to be 2 percent, 1.9 percent and 2.9 percent in concentrations of 0.833 ng/ml, 2.04 ng/ml and 11 ng/ml, respectively. The correlation coefficient of the kit between the studies was determined to be 7.9 percent, 7.8 percent and 6.9 percent in concentrations of 0.972 ng/ml, 2.35 ng/ml and 12.2 ng/ml, respectively.

Statistical analysis

The statistical analysis was performed using the SPSS 15.0 software program (Chicago, IL), with p-values of <0.05 being considered statistically significant. The frequencies of asthma severity were compared between the groups using Pearson's chi-squared test.

Mann—Whitney U Test was employed to compare disease severity parameters, as well as the cytokine and mediator levels between the atopic and nonatopic asthma groups. The IL-5, IL-8 and MMP-9 levels in EBC lower than the lower

Table 1 Sociodemographic characteristics of the children enrolled in the study.

	Atopic asthma group n = (37)	Nonatopic asthma group n = (37)
Age[a]	9.0 (7.0–12.0)	9.0 (7.0–12.0)
Gender[b]		
Male	48.6 (18)	40.5 (15)
Female	51.4 (19)	59.5 (22)

[a] Expressed as median (interquartile range).
[b] (Percent) number.

Table 2 Distribution of patients in the study according to severity of asthma[a].

Severity of asthma[b]	Atopic asthma group n = (37)	Nonatopic asthma group n = (37)	P[a]
Intermittent	21.6 (8)	10.8 (4)	0.28
Mild persistent	67.6 (25)	62.2 (23)	
Intermediate persistent	10.8 (4)	24.3 (9)	
Severe persistent	0 (0)	2.7 (1)	

[a] Pearson Chi-square.
[b] (Percent) number.

limit of the measurement kit were recorded as the lowest detection limit value of the kit.

Results

Sociodemographic characteristics

Median (interquartile range) ages of the 37 atopic asthmatic children and the 37 nonatopic children were 9.0 (7.0–12.0) and 9.0 (7.0–12.0) respectively (p = 0.96).

Males represented 48.6 percent of the atopic asthma patients, and 40.5 percent of the nonatopic asthma patients (Table 1).

Clinical characteristics of the asthma group

Asthma severity did not differ significantly between the atopic and nonatopic asthma groups (Table 2).

Median (interquartile range) ages of initial asthma diagnosis in the atopic and nonatopic asthma groups were 5.0 (2.5–7.0) and 4.0 (2.0–8.0), respectively (p = 0.7) (Table 3).

The number of asthma exacerbations and the number of emergency visits within the last year were higher in the nonatopic group when compared to the atopic group (p = 0.001 and p = 0.003, respectively).

No statistically significant difference was observed between the atopic and nonatopic asthma patients with respect to the number of hospitalization days within the last year (p = 0.06).

While bronchodilator drugs had been used in the nonatopic group for an average of 5 (0–10) days within the last year, they had been used for an average of 0 (0–0) days in the atopic group (p = 0.001).

No statistically significant difference was observed between the atopic and nonatopic asthma patients with respect to their systemic corticosteroid requirement over the last year (p = 0.97) (Table 3).

Table 3 Distribution of patients enrolled in the study according to asthma status[a].

	Atopic asthma group n = (37)	Nonatopic asthma group n = (37)	P[b]
Age of diagnosis of asthma	5.0 (2.5–7.0)	4.0 (2.0–8.0)	0.7
Bronchodilator treatment[c]	0 (0–0)	5 (0–10)	0.001
Systemic corticosteroid treatment[d]	0 (0–0)	0 (0–0)	0.97
Number of exacerbations[e]	1 (0–2)	2 (1–4)	0.001
Presentation to emergency service[f]	0 (0–0)	0 (0–1)	0.003
Duration of hospitalization[g]	1 (0–2)	2 (1–3)	0.06

[a] Expressed as median (interquartile range).
[b] Manney Whitney U test.
[c] Total number of days in the last year with bronchodilator use.
[d] Total number of days in the last year with systemic corticosteroid use.
[e] Total number of days in the last year with asthma exacerbations.
[f] Total number of days in the last year with presentation to emergency services.
[g] Total number of days in hospital in the last year.

Table 4 Median and interquartile ranges of EBC IL-5, IL-8, MMP-9 levels, serum immunoglobulin E levels and eosinophil counts of the atopic and nonatopic asthmatic patients group included in the study[a].

	Atopic asthma group N = (37)	Nonatopic asthma group N = (37)	P[b]
IL-5 pg/mL	0.271 (0.198—0.489)	0.198 (0.125—0.344)	0.04
IL-8 pg/mL	0.437 (0.004—1.736)	0.004 (0.004—1.303)	0.47
MMP-9 ng/mL	0.592 (0.329—1.907)	0.592 (0.417—0.618)	0.35
Immunoglobulin E (IU/mL)	198.0 (95.9—341.0)	52.0 (28.0—127.0)	0.001
Eosinophil number/mm^3	400 (200—500)	190 (100—200)	0.006

[a] Expressed as median (interquartile range).
[b] Mann Whitney U test.

Pulmonary function tests

The results of the Pulmonary Function Test were not significantly different between the asthma groups and the control group.

Serum immunoglobulin-E (IgE) and eosinophil

The atopic asthma patients exhibited significantly higher IgE levels in comparison to the nonatopic asthmatic patients median (interquartile range) 198.0 [95.9—341.0] IU/ml and 52.0 [28.0—127.0] IU/ml, respectively; p = 0.001) (Table 4).

The eosinophil counts were significantly higher in the atopic asthma groups comparison to the nonatopic asthmatic group median (interquartile range) (400 [200—500]/mm^3 and 190 [100—200]/mm^3, respectively) (Table 4).

IL-5, IL-8 and MMP-9 levels in exhaled breath condensate

IL-5 levels were significantly higher in the atopic asthma group when compared to the nonatopic asthma group median (interquartile range) (0.271 [0.198—0.489] pg/ml and 0.198 [0.125—0.344] pg/ml, respectively; p = 0.04) (Fig. 1) (Table 4). No statistically significant difference was observed between the atopic and nonatopic asthma patients with respect to their IL-8 and MMP-9 levels median (interquartile range) (0.437 [0.004—1.736] pg/ml and 0.004 [0.004—1.303] pg/ml for IL-8, respectively; 0.592 [0.329—1.907] ng/ml and 0.592 [0.417—0.618] ng/ml for MMP-9, respectively. For IL-8 and MMP-9, p = 0.47 and p = 0.35, respectively) (Table 4).

Discussion

In this study, the IL-5 levels in exhaled breath condensed, as a marker of eosinophilic inflammation, in children with atopic asthma were found to be higher than in children with nonatopic asthma; however, no significant differences were found between IL-8 levels, which is a marker of neutrophilic inflammation, or in MMP-9 levels, which is a known cause of tissue damage.

Phenotypes of asthma may demonstrate clinical differences due to differences in pathophysiological pathways, and airway inflammation and airway remodelling are characteristic features of the disease, occurring even if the severity of asthma is clinically mild.

In developed countries, atopy is observed in up to 40 percent of children and young adults, although only one-third of these individuals develop asthma. In addition, sensitization to local aeroallergen in the lower airways has a more significant role and effect than systemic allergy in the pathogenesis of asthma [18—20]. In concordance with our observation concerning the higher IgE levels among asthmatics in comparison to the nonatopic controls, previous studies have also demonstrated higher total serum IgE levels among asthma patients [21,22].

Nonatopic asthma patients generally exhibit more severe disease symptoms and require earlier corticosteroid treatment [23,24]. Furthermore, asthma exacerbations in nonatopic patients are more commonly triggered by irritants, infections, gastroesophageal reflux disease, stress and exercise [24]. In the present study, patients with nonatopic asthma were found to have more frequent exacerbations, use more rescue drugs and present more often to the emergency services.

Walker et al. were the first to report the differences between atopic and nonatopic asthma in immunopathology

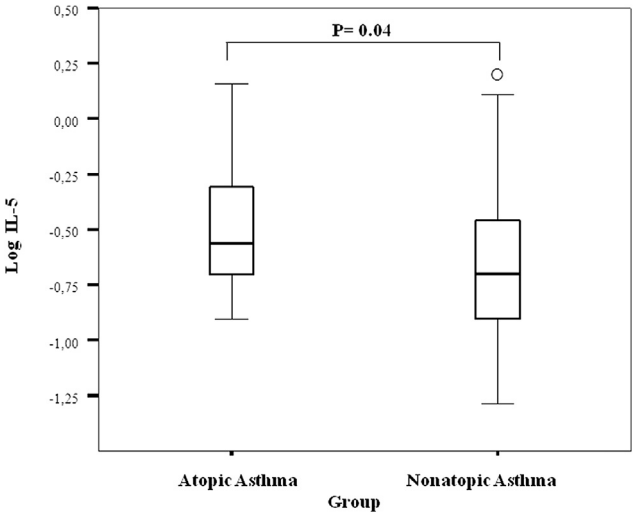

Figure 1 Logaritmic boxplot scala of IL-5 levels in atopic and nonatopic asthma groups.

[25]. To date, a relatively low number of studies have been carried out on the differences between atopic and nonatopic asthma that have garnered controversial results [1,26–33].

Damage to the airway epithelium was demonstrated to be significantly higher in patients with atopic asthma when compared to those with nonatopic asthma in a study in a study of airway epithelium biopsy samples. The inflammation pattern was shown to consist primarily of eosinophils, mast cells and T-lymphocytes in patients with atopic asthma, and of neutrophils and mast cells in patients with nonatopic asthma [29]. In another study in which transmission electron microscopy was used, diffuse epithelial damage at the ultrastructural level was reported to be present in both asthma groups, while inflammation patterns were found to be similar to the previous study [30].

In pediatric asthma, data on the airway cytokine profile is limited, since it is difficult to obtain airway secretions in this group of patients, and most of our knowledge in this regard comes from studies of adult patients [34]. As such, this study provides new data, given its focus on the pediatric age group; however this factor also prevents a comparison of the results with previous studies, alongside the methodological differences.

The identification of specific, noninvasive markers and methods that permit early diagnosis, monitoring and follow-up for lung disease is an important goal in pulmonology [35], and the collection of EBC is one such method. Recent advances in technology have allowed the EBC collection method to be used as a noninvasive research tool, and analyses of inflammatory substances within the EBC is a promising approach, in that it can be applied effectively even to young children and requires little cooperation from the patients [36]. The diagnostic utility of EBC has been reported and discussed in a joint report published by the American Thoracic Society and the European Respiratory Society (ATS/ERS) [17]. EBC provides information on substances in the epithelial surface fluids, which in turn enables the assessment of airway inflammations or other conditions [36,37]. To date, various biomolecules from the alveolar membrane (such as surface active proteins) and from the central and peripheral airways (such as cytokines and prostaglandins) have been identified within EBC samples; however, the specific effects of these biomolecules on the airways has not yet been fully elucidated. The EBC contains no cells, and mutations in exhaled DNA can be successfully studied from EBC samples [37]. The EBC is particularly important in providing information on local pathogenetic mechanisms, and it is for these reasons that we chose to assess IL-5, IL-8 and MMP-9 in the asthmatic children participating to this study using the EBC method.

Eosinophils have long been considered as markers of asthma [38], and a high number of eosinophils was also recorded in the peripheral blood of patients with atopic asthma in the present study [38]. However, eosinophilic infiltration in the airways creates the characteristic feature of the disease rather than systemic eosinophilia. Furthermore, eosinophils are present not only in the walls of the airways, but also in the sputum and BAL of patients with uncontrolled asthma [3].

Amin et al., in their study in which they compared the biopsy samples of patients with atopic and nonatopic asthma, demonstrated that the level of IL-5 positive cells was higher in the atopic asthma group when compared to the nonatopic asthma group [29]. Furthermore, IL-5 levels and eosinophilia in the induced sputum were found to be significantly higher in pediatric patients with atopic asthma compared to the patients with nonatopic asthma and the control group in a more recent study [40]. Similar to these studies, the IL-5 levels in the EBC samples from children with atopic asthma in the present study were found to be higher when compared to the children with nonatopic asthma. In another study, IL-5 was demonstrated to be released spontaneously in high amounts from BAL and PBMC cells in both atopic and nonatopic cases of asthma; however the IL-5 release in the atopic group was increased when compared to the nonatopic group when stimulated with household dust mites [27]. Although the results of that study demonstrated that IL-5 levels are different in atopic and nonatopic asthma patients in the presence of allergen stimulus, the different results of EBC and IL-5 in the two groups in our study may point to a difference at the baseline level in the two groups. Furthermore, the allergens to which the cases displayed a sensitivity and the season in which the samples were obtained may also affect the results.

The amount of IL-5 secretion from PBMC cells as a result of allergen stimulation has been similar in the atopic and nonatopic patients in some studies [1,41,42], in contrast with the findings of the present study. This discrepancy in results may be based on the fact that an in vitro method was used in those studies, and that the local allergic response of the cells in bronchial mucosa is different from the systemic response.

Since asthma is a heterogeneous disease with different clinical types, differences in inflammation patterns may also be apparent [43].

No increase was identified in the number of neutrophils in the airway secretions of patients with asthma of mild and intermediate severity: however they were found to be increased in the airway secretions of patients with severe asthma [6,43]. IL-8 plays a major role in neutrophilic inflammation in the lungs [8,44].

IL-8 levels were found to be similar in the EBC samples of the healthy children, patients with atopic asthma and with viral wheezing and in another study performed in preschool children, BALF [45] samples and PBMC cytokine response in response to allergen application in different asthma groups [12,41].

IL-8 levels were found to be higher in the cytokine measurements in induced sputum in children when compared to the control group, although no difference was reported between the atopic and nonatopic asthma groups [40]. Confirming the findings of previous studies, in our study the IL-8 levels in the EBC samples in children with atopic and nonatopic asthma showed little difference between the asthma groups. In contrast, in a study in which bronchial biopsies from adult atopic and nonatopic asthma patients were compared, IL-8 positive cells were found to be more prominent in the nonatopic group [29]. The reason for the discrepancies in the results of the two studies could be the pediatric nature of our study, methodological

differences and the analysis of milder asthmatic patients in our study group.

The severity of the disease constitutes the most important factor in terms of neutrophilic inflammation in asthma, and a significant correlation was identified between the neutrophil number in BAL and the IL-8 level in a study of children with asthma and persistent wheezing [12]. Similarly, the neutrophil numbers in BAL and IL-8 levels were demonstrated to be significantly increased in cases with non-infectious status asthmaticus when compared to patients with stable asthma [5]. We evaluated the association of IL-8 level which is a marker of neutrophilic inflammation and atopia, no evaluation was made that took into account disease severity.

Disease severity may be considered to have greater influence on IL-8 levels, as was proven in previous reports, since most of our patients with atopic and nonatopic asthma suffered from intermittent, mild and intermediate persistent asthma. For this reason, no significant differences could be identified between the groups in terms of neutrophilic inflammation and IL-8 levels being effective on the neutrophilic inflammation.

Airway remodelling is characteristic of asthma, and data obtained from adult patients with asthma resulted in the identification of physiological differences in both bronchial epithelial cells and smooth airway muscle cells, which suggest that airway remodelling takes place in inflammatory cells rather than in these cells [9]. MMP-9 originates in various inflammatory cells, such as bronchial epithelial cells, mast cells, eosinophils and macrophages, and especially neutrophils [8,10]. MMP-9 may increase in the blood, sputum and BAL fluid of patients during asthmatic exacerbations [38,39], and MMP-9 expression is seen to be induced in airways following an allergen loading [1,9,46].

MMP-9 levels have been demonstrated to increase BAL in preschool wheezers when compared to healthy controls; however, the difference disappeared when the wheezers were divided into those with atopic and nonatopic asthma [47]. Similarly, MMP-9 levels were demonstrated to be higher in the induced sputum of patients with atopic asthma when compared to the patients with nonatopic asthma, but with no statistical significance [48]. Similarly in our study, the MMP-9 levels in the EBC samples obtained from patients with atopic and nonatopic asthma were found to be similar, which demonstrates that the level of airway damage is not significantly different in atopic and nonatopic asthma patients.

It has been shown in our study that the level of IL-5, one of the markers of eosinophilic inflammation, is higher in EBC of children with atopic asthma than in those with nonatopic asthma. Since there is no significant difference in IL-8 levels between the two groups, the severity of the disease is suggested as playing a more important role on the level of IL-8 rather than atopic state itself. The fact that there is no difference between the levels of MMP-9 between the two groups supports the development of lung damage independent of atopy in asthma. Accordingly, MMP-9 can be used for the early diagnosis of tissue damage and re-modelling, and the use of EBC may be encouraged as an appropriate approach when faced with such patients and treatments.

Conflict of interest statement

All authors of this manuscript have no conflict of interests to declare.

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Hasan Yuksel and Ozge Yilmaz were involved in the formulation of the study hypothesis and the conception and development of the study design, while Ahmet Turkeli and Ozge Yilmaz wrote the study manuscript. Revisions to the manuscript were carried out by Hasan Yuksel.

Ahmet Turkeli, Esra Toprak Kanik and Metehan Kizilkaya carried out the data collection for the study, while Fatma Taneli, Ceyhun Gozukara and Gonul Dinc Horasa were involved in the analysis and interpretation of the study data.

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