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Relationship of Fatty Acid Binding Protein 4 with Asthma and Severity of Asthma

Semra DEMIR[1], Derya UNAL[1], Derya SONMEZ[2], Ozlem OZDEMIR[3], Berrin BERCIK INAL[2]

[1]Department of Internal Medicine, Division of Immunology and Allergic Diseases, Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey
[2]Clinic of Biochemistry, Istanbul Training and Research Hospital, Istanbul, Turkey
[3]Clinic of Adult Allergy and Immunology, Istanbul Training and Research Hospital, Istanbul, Turkey

Corresponding Author: Semra Demir ✉ ertansemra@yahoo.com

ABSTRACT

Objective: To investigate the relation of circulating Fatty acid binding protein 4 (FABP4) molecule with asthma, severity of asthma, and eosinophil and neutrophil counts in the peripheral blood as well as to evaluate the potential risk factors including demographic and clinical features for severe asthma.

Materials and Methods: Sixty-seven patients older than 18 years of age with asthma and 20 healthy controls were included in the study. Patients were grouped as those with severe (n=34) and non-severe (n=33) asthma. Additional analysis was performed depending on the peripheral eosinophil count.

Results: The mean age of the patients was 42.37 ± 11.26 years and 77.6% of them were female. In severe asthmatic patients, the mean age and the presence of house dust mite allergy and familial history of asthma were higher, and the actual asthma control test (ACT) score was lower than in the non-severe asthma group ($p<0.001$, $p=0.005$, $p=0.001$, $p<0.001$). Neutrophil counts and serum total IgE levels were higher in patients with severe asthma than those with non-severe asthma ($p<0.001$, $p=0.02$). According to multivariate analysis, age and familial history of asthma were associated with severe asthma ($p=0.003$, $p=0.008$). FABP4 levels were not different between the three groups and there was no correlation between serum FABP4 levels and eosinophil counts; however, a positive correlation was observed between FABP4 levels and neutrophil counts ($p>0.05$; $r=0.106$ $p>0.05$; $r=0.4$, $p=0.001$).

Conclusion: Circulating FABP4 levels were not related to asthma, severity of asthma, or peripheral eosinophil count but may reflect neutrophilic asthma.

Keywords: Asthma, allergic asthma, severity of asthma, fatty acid binding protein 4, biomarker

INTRODUCTION

Asthma is a heterogeneous chronic inflammatory respiratory disease with variations in presentation, disease progression, and response to treatment, influencing all the ages (1). Asthma influences almost 300 million subjects all over the world and increases the burden of treatment cost and loss of work for both the patients and the community (2). The majority of the asthma patients can be treated successfully with the usual treatment modalities. However, 5%–10% of the patients are severely affected and need further treatment options (3,4).

Since asthma is a heterogeneous disorder, personalized medicine as a novel approach in its treatment has become important (5). Moreover, the detection of biomarkers makes understanding the mechanisms of the disease possible, further aiding in the determination of correct treatment modalities and prediction of the response to treatment (6). The Th2 cytokines involving interleukins 3, 4, 5, 9 and 13 are well known to contribute to allergic inflammation in the airways, leading to increased production of immunoglobulin E (IgE) and activation and recruitment of mast cells, basophils, and eosinophils (7,8). However, there are still unmet needs including lack

ORCID Semra Demir / 0000-0003-3449-5868, Derya Unal / 0000-0001-9741-5939, Derya Sonmez / 0000-0002-9098-4140, Ozlem Ozdemir / 0000-0001-8136-3994, Berrin Bercik Inal / 0000-0002-9098-4140

of knowledge on biomarkers to describe the different types of asthma and plan an effective treatment.

Fatty acid binding protein 4 (FABP4) is a member of a family of intracellular lipid binding proteins, which transports the hydrophobic ligands through cellular compartments and plays a role in leukotriene metabolism (9-11). Its roles in the pathophysiology of metabolic and cardiovascular diseases as well as in some biological processes including inflammation, angiogenesis, and tumorigenesis are known (9,12-15). However, there are few animal studies that have investigated the role of FABP4 in asthma.

In murine models, it was observed that FABP4 played a role in allergic airway inflammation by leading to the migration and adhesion of eosinophils (16,17). Additionally, epithelial barrier dysfunction caused by FABP4 via Forkhead BoxM1 activation was shown in another mouse study (18).

The objectives of this study were to investigate the relation of circulating FABP4 molecule with asthma, severity of asthma, and eosinophil and neutrophil counts in the peripheral blood as well as to evaluate the potential risk factors including demographic and clinical features for severe asthma.

MATERIALS and METHODS

Sixty-seven patients older than 18 years of age with asthma and 20 healthy controls without any known comorbidity or allergic diseases were included in the study. The diagnosis of asthma was made depending on the presence of characteristic respiratory symptoms and the confirmed variable expiratory airflow obstruction (2, 19). The asthma patients who were smokers, obese, had comorbidities including metabolic and cardiovascular diseases, had malignancies which could lead to increase in FABP4 levels, or in whom reversibility (at least 200 ml and 12% increase in forced expiratory volume (FEV1) of the baseline value after inhaling short-acting beta-blockers) was not detected, in addition the patients who had diseases causing a decrease or increase in peripheral granulocytes including infections or hematological diseases were excluded from the study (2). In patients who had elevated total IgE levels, the other reasons leading to total IgE increase such as parasitic diseases, allergic bronchopulmonary aspergillosis, and hyper IgE syndrome were excluded.

Asthma severity was determined according to the Global Initiative for Asthma (GINA) criteria, and patients were grouped as severe or non-severe asthma (2). Severe asthma was defined as asthma that needed high-dose inhaled corticosteroid (ICS) and at least a second controller medication such as long-acting beta-agonist (LABA), systemic corticosteroids, montelukast, or theophylline to control the symptoms or uncontrolled asthma under this treatment. Asthma not meeting the above criteria was defined as non-severe asthma (2,20).

Demographic, clinical and laboratory features including serum FABP4 and total IgE levels, eosinophil and neutrophil counts, and fractional exhaled nitric oxide (FeNO) were measured, as well as performing an asthma control test (ACT) including 5 items with 4-week recall of symptoms and daily functioning, and respiratory function tests (RFT) depending on the severity. Additional analysis was performed according to the peripheral eosinophil counts of the patients. Patients were further grouped into those who did or did not have eosinophil counts ≥150/μl and those who did or did not have counts ≥300/μl (21,22).

FeNO and serum FABP4 levels were measured in healthy controls as well. Blood samples were collected and serum samples were frozen at −80°C. The FABP4 levels were measured using a Human FABP4 Elisa kit (Assay Max, USA, catalogue number: EF2702-1) by the sandwich enzyme immunoassay method according to the manufacturer's instructions. FeNO levels were monitored by Bedfont's breath analyser (Bedfont® Technical Instruments, Harrietsham, England).

Atopy was determined as per the skin prick test performed with common inhaled allergens or the serum level of specific IgEs.

Written informed consent was obtained from all the participants, and the study for collecting the participants' blood samples to measure serum FABP4 levels and monitor FeNO as well as share their demographic and clinical data was approved by the ethics committee of Istanbul Training and Research Hospital (Tarih: 23/02/2018, No:1188).

Statistical Analysis

SPSS Software version 21.0 was used for statistical analysis. The categorical and continuous variables were presented as percentages, mean and standard deviation (SD), or median. The features of the patient groups were

compared by student's t-test or Mann–Whitney test and Chi-square test; the continuous values were compared by the Pearson's or Spearman's correlation tests; and the variables of the three groups were compared with Kruskal–Wallis or analysis of variance tests, depending on the distribution of the data. Bonferroni or Tamhane tests were used for post hoc analyses. For the related factors obtained from univariate analysis, multivariate analysis was performed by binary logistic regression analysis. A p-value lower than 0.05 was accepted as statistically significant.

RESULTS

The mean age of the patients was 42.37 ± 11.26 years and 77.6% of them were female. The mean ages at the onset of symptoms and at diagnosis were 27.67 ± 9.37 years and 30.07 ± 9.9 years, respectively. Eleven patients who were newly diagnosed were not under any treatment for asthma, and there were no newly diagnosed patients in the group of severe asthma. A total of 50.7% of the patients were under omalizumab treatment, and the mean dose of omalizumab was 630.9 ± 381.5 mg/month. Five patients experienced drug hypersensitivity reactions caused by nonsteroidal anti-inflammatory drugs (n=4) and parenteral iron replacement (n=1). Comparison of the demographic and clinical features of the patients depending on the severity is given in Table I. Regarding gender, presence of concomitant diseases, eosinophil count, and FABP4 levels, there were no differences between the severe and non-severe asthma groups. In severe asthmatic patients, the mean age and the presence of house dust/mite allergy and familial history of asthma were higher and the actual ACT score was lower than in the non-severe asthma group (p<0.001, p=0.005, p=0.001 and p<0.001). Moreover, the mean FEV1 values of the patient groups were similar; however, the mean FEV1/forced vital capacity (FVC) value was higher in the non-severe asthma group (p=0.001) (Table I). There was no significant difference between asthmatic patients regarding eosinophil counts (p > 0.05); however, the neutrophil counts and serum total IgE levels were higher in severe asthma patients than the non-severe ones (p<0.001, p=0.02) (Figure 1). According to multivariate analysis of the related factors obtained from univariate analysis, age and familial history of asthma were associated with severe asthma (p=0.003, p=0.008) (Table II).

FABP4 levels were not different between the three groups (Figure 2), and although there was no correlation between serum FABP4 levels and eosinophil counts in the peripheral blood, a positive correlation was observed between FAB4 levels and peripheral blood neutrophil counts (Figure 3) (p>0.05; r=0.106, p>0.05; r=0.4, p=0.001).

FeNO levels were lower in the healthy controls than both the severe and non-severe asthma groups (p=0.007, p=0.01) and were higher in severe asthma than the non-severe asthma group (p=0.013; p=0.02; p<0.001) (Figure 2). FeNO levels were positively correlated with eosinophil counts in the peripheral blood, while not correlated with the neutrophil counts (r=0.347, p=0.004; r=0.168, p>0.05) (Figure 3).

On further analysis between patients who did or did not have eosinophil counts more than 150 µl (n=21; n=46), and those who did or did not have eosinophil counts more

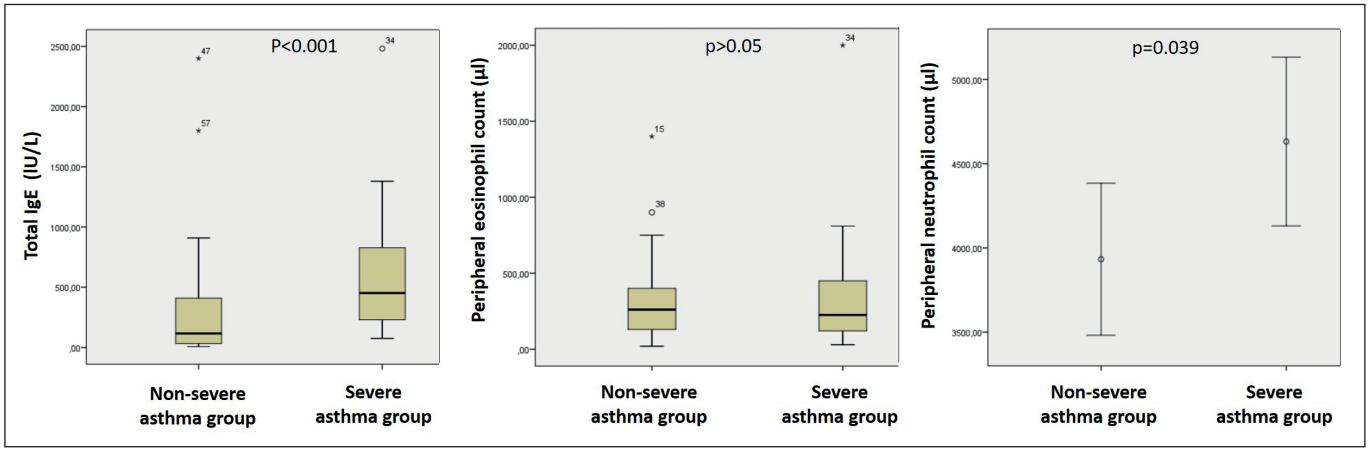

Figure 1. Comparison of eosinophil and neutrophil counts in peripheral blood and serum total IgE levels between severe and non-severe asthmatic groups.

Table I: Demographic and clinical features of the patients depending on the severity of asthma

	Non-severe asthma n=33 (49.3%)	Severe asthma n=34 (50.7%)	p
Female	27 (51.9)	25 (48.1)	>0.05
Newly diagnosed asthma	11 (33.3)	0	<0.001
Current treatment			
None	10 (30.3)	0	<0.001
SABA when needed	14 (42.4)	19 (55.8)	>0.05
Montelukast (±antihistamine)	24 (72.7)	29 (85.3)	>0.05
ICS	8 (24.2)	5 (14.7)	>0.05
ICS + LABA	13 (31)	29 (69.0)	<0.001
Omalizumab	0	34 (100)	<0.001
Theophylline	0	3 (8.8)	>0.05
Inhaler anticholinergic	0	5 (14.5)	0.029
SABA	0	3 (8.8)	>0.05
Immunotherapy	2 (6.1)	0	>0.05
Concomitant diseases			
Chronic rhinosinusitis	29 (48.3)	31 (51.7)	>0.05
Nasal polyposis	1 (3)	0	>0.05
Conjunctivitis	8 (24.2)	4 (11.8)	>0.05
Atopic dermatitis	0	2 (5.9)	>0.05
Chronic urticaria	2 (6.1)	3 (8.8)	>0.05
Drug allergy	4 (12.1)	1 (2.9)	>0.05
Presence of atopy	27 (81.8)	34 (100)	0.011
Mite	26 (78.8)	34 (100)	0.005
Pollen	16 (48.5)	17 (50)	>0.05
Mould	3 (9.1)	5 (14.7)	>0.05
Family history			
Asthma	5 (15.2)	18 (52.9)	0.001
Hay fever	13 (39.4)	13 (38.2)	>0.05
	Mean ± SD	**Mean ± SD**	
Age (year)	36.42 ± 10.7	48.15 ± 8.5	<0.001
Age of onset of asthma-related symptoms (year)	28.33 ± 10.26	27.03 ± 8.6	>0.05
The age of asthma diagnosis (year)	31.03 ± 10.11	29.03 ± 9.8	>0.05
Asthma control test (actual)	24.33 ± 1.49	20.03 ± 5.7	<0.001
Reversibility (at diagnosis/ ml)	257.87 ± 72.74	246 ± 46.5	>0.05
FEV1/FVC (actual)	94.24 ± 11.83	80.02 ± 2.27	0.001
FEV1 (ml) (actual)	2.77 ± 0.68	2.63 ± 0.63	>0.05

than 300 μl (n=25; n=42), the serum FABP4 levels were similar (p>0.05). Moreover, the FeNO measures were similar in patients who did or did not have eosinophil counts ≥150 μl (p>0.05), but were higher in the patients who had peripheral eosinophils count more than 300 μl than those who had less than 300 μl (p=0.003) (Table III).

DISCUSSION

The main outcome of the present study, which is the first human study in this subject and investigated the levels of circulating FABP4, a potential biomarker for severe asthma, was that FABP4 was not related to asthma, severity of asthma, or peripheral eosinophil counts, but was associated with neutrophil counts in the blood.

FABP4 has been shown to be an important mediator in inflammatory processes mediated by the T cells, dendritic cells, and macrophages (9,12,13,15,23,24). Animal studies have demonstrated that FABP4 contributes to the development of airway inflammation by the recruitment and activation of eosinophils, leading to an increase in Th2 cytokines and airway hyperresponsiveness (16,25,26). The present study was the first human study that evaluated the effect of the serum FABP4 level in asthma depending on the severity and peripheral eosinophil count, and we found that there was no relationship between the FABP4 levels and asthma, severity of asthma, and peripheral eosinophil count; however, the circulating FABP4 levels correlated with peripheral blood neutrophil counts, which were higher in the severe asthma group than the non-severe one, although the significance of this effect disappeared in multivariate analysis. Hence, we may assume that

Table II: Multivariate analysis of the features related to severe asthma.

Features	Severe Asthma	
	OR (95% CI)	p
Age	1.19 (1.06–1.34)	0.003
Asthma history in family	0.06 (0.08–0.47)	0.008
Presence of atopy	0.01 (0.01–13.4)	>0.05
Presence of house dust mite allergy	0.01 (0.01–12.8)	>0.05
Total IgE level	1.001 (0.99–1.003)	>0.05
Neutrophil counts in peripheral blood	1.0 (0.99–1.001)	>0.05
FeNO	1.05 (0.97–1.14)	>0.05

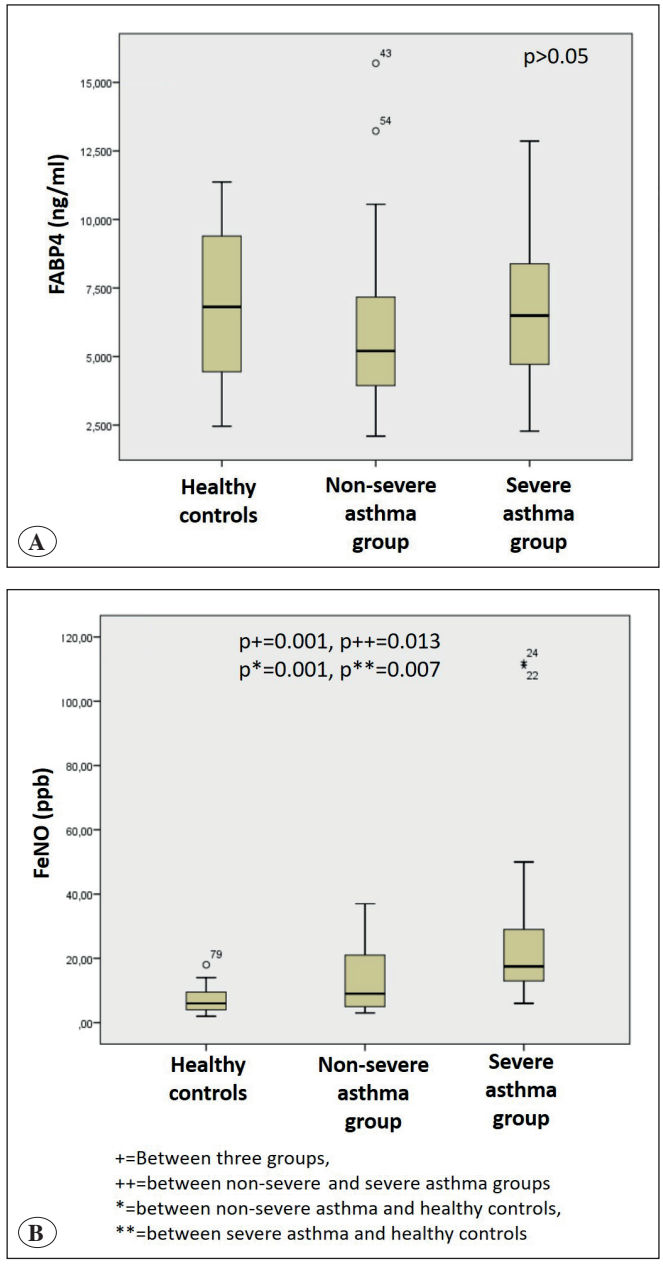

Figure 2. The comparison of the FABP4 and FeNO levels between three groups. Serum FABP4 levels were not different between the three groups **(A)**. The FeNO values were higher in severe asthmatic patients than the non-severe asthma group and in non-severe asthmatic patients than in healthy controls **(B)**.

Table III: FABP4 and FeNO measures according to eosinophils counts.

	Eosinophils		p	Eosinophils		p
	<150 µl	≥150 µl		<300 µl	≥300 µl	
FABP4 (ng/ml) *(median)*	4.718	6.149	>0.05	5.657	6.483	>0.05
FeNO (ppb) *(median)*	12	15	>0.05	12.5	22	0.003

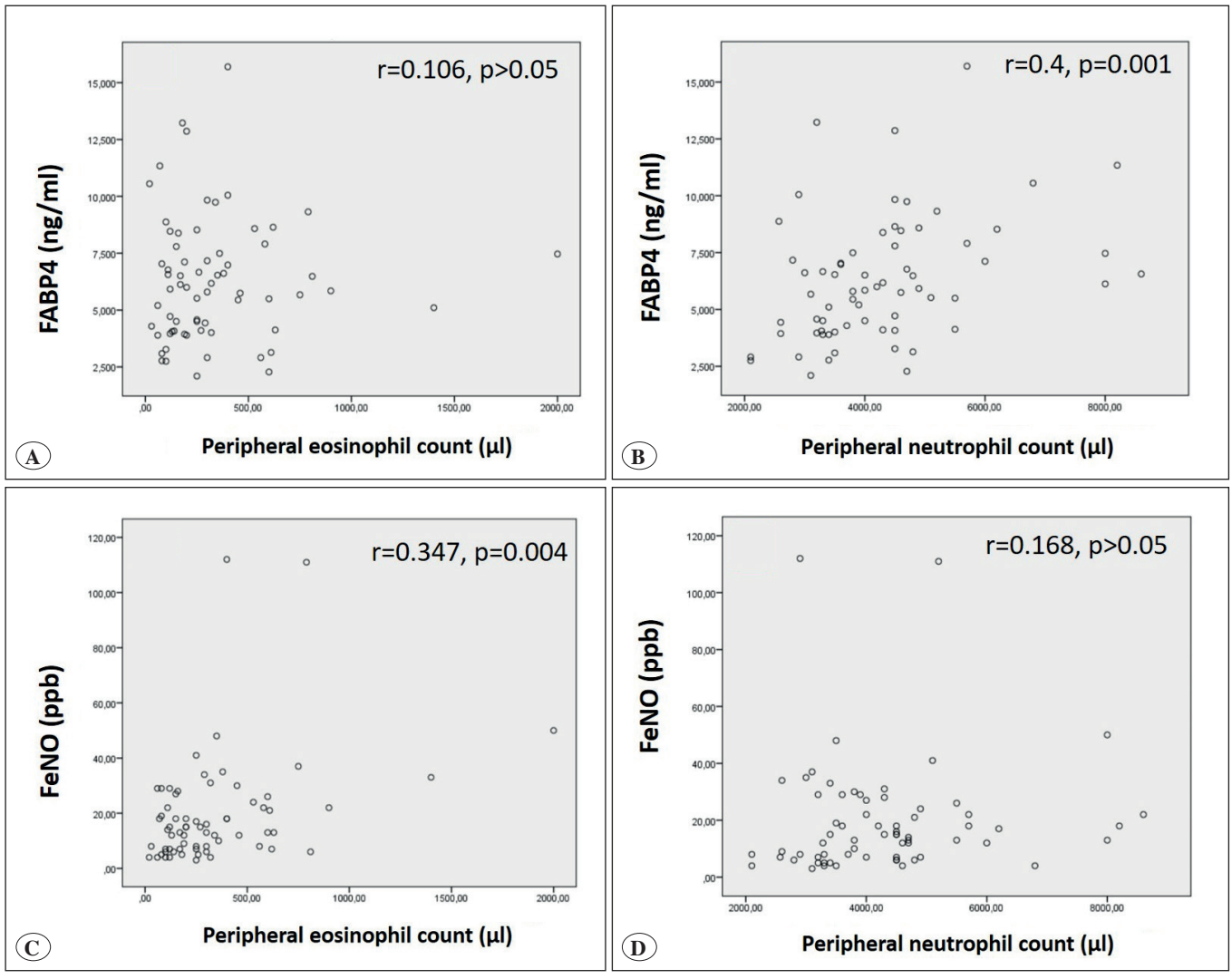

Figure 3. There was no correlation between serum FAB4 levels and peripheral blood eosinophil count **(A)** but the serum FABP4 levels were correlated with peripheral blood neutrophil count **(B)**. FeNO measures were positively correlated with eosinophil counts **(C)**, but were not correlated with neutrophil counts **(D)** in the peripheral blood.

circulating FABP4 levels might reflect neutrophilic asthma that needs to be confirmed by further studies. In animal studies, the relationship of FABP4 and peripheral neutrophil counts were not investigated; however, they showed that the neutrophil counts were decreased in the bronchoalveolar lavage fluid of allergen-challenged FABP4-deficient mice (16,26). On the other hand, it has already been shown that FABP4 has an important role in metabolic inflammation, and a murine model has demonstrated a relationship between macrophage FABP4 and neutrophil recruitment, which can explain this correlation between FABP4 levels and neutrophil counts (27,28). Combining the previous data obtained from animal studies on asthma and our main results, which showed that the serum FABP4 levels were not related to asthma, severity of asthma, and higher peripheral eosinophil count, it could be said that FABP4 acts locally in the lung, and should be investigated locally such as in bronchoalveolar lavage (BAL) or tissue specimens to confirm this.

Measurement of FeNO is a simple method, and helps diagnose and manage asthma and it can also specify the patients with Th2/Type 2 inflammation in airways and help identify the response to steroid treatment (29,30). In the light of these data, we measured the FeNO levels and observed that FeNO levels were higher in severe asthma

than non-severe asthma patients indicating a higher inflammatory burden in the severe asthma group than the non-severe asthma group in univariate analysis; however, in multivariate analysis, we did not observe this effect in accordance with the literature and the association of asthma severity and FeNO levels was not well confirmed (31).

Furthermore, FeNO levels were correlated with peripheral blood eosinophil counts and were higher in the patients in whom the counts were more than 300/μl than the patients in whom the counts were less than 300/μl, but similar in patients who did or did not have eosinophil counts more than 150/μl. Although peripheral eosinophil counts do not indicate eosinophilic asthma as we mentioned above, it can be speculated that an FeNO increase in patients with more than 300/μl of peripheral blood eosinophil counts may reflect eosinophilic asthma, in contrast to Katz et al. who showed that blood eosinophil count of at least 150/μl was helpful to identify eosinophilic asthma (21).

In the current study, we also investigated the potential risk factors including demographic and clinical features for the development of severe asthma. We found that the patients with severe asthma were older than the ones with non-severe asthma, in accordance with previous studies (20, 32-34). In addition to the well-known association between mortality and increasing age, it can be said that older patients with asthma need more attention and close follow-up (35).

Another result of the current study was that the frequency of asthma history in the family was higher in severe asthmatic patients than the non-severe ones. The estimated inheritance of asthma ranges from 48% to 79%, since it is associated with various genetic abnormalities, and some genetic defects related to severe asthma have been defined that could explain our result (36,37). Nwaru et al. very recently reported that a history of asthma in the family increased the risk of developing asthma without showing any genetic defects, as in the results of our study; however, they did not investigate the relationship to severity (38). Although *de novo* mutations can develop, family history may reflect the presence of genetic defects and may increase the risk of severity in asthma.

Moreover, we detected that severe asthma was associated with the presence of atopy, house dust/mite allergy, and higher total IgE levels in univariate analysis, but these associations could not be shown in multivariate analysis. Previous studies have shown that childhood asthma strongly was associated with atopy while in adult-onset asthma most of the patients were non-allergic and 34% of the patients were atopic (39, 40).

Although the current study is very valuable as the first human study evaluating the FABP4 level in asthma, it has an important limitation as the local impact of FABP4 in the airways was not investigated.

In conclusion, we suggest that circulating FABP4 levels might reflect neutrophilic asthma and play a role in metabolic inflammation like in the animal models. Although serum FABP4 levels were not related to asthma, severity of asthma, and peripheral blood eosinophil count, it could be said that FABP4 acts locally in the lung and should be investigated locally such as in BAL or tissue specimens for confirmation.

The main outcome of the present study, which is the first human study on this subject, investigating the circulating levels of FABP4, a potential biomarker for severe asthma, was that FABP4 was not related to asthma, severity of asthma, or peripheral eosinophil counts, whereas it was associated with neutrophil counts in the blood.

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Istanbul Training and Research Hospital

Conflict of Interest

We declare that we have no conflict of interest.

Authorship Contributions

Concept: **Semra Demir, Derya Unal, Derya Sonmez, Ozlem Ozdemir, Berrin Bercik Inal,** Design: **Semra Demir, Derya Unal, Derya Sonmez, Ozlem Ozdemir,** Data collection or processing: **Semra Demir, Derya Unal, Derya Sonmez, Ozlem Ozdemir,** Analysis or Interpretation: **Semra Demir, Derya Sonmez, Ozlem Ozdemir, Berrin Bercik Inal,** Literature search: **Semra Demir, Derya Sonmez, Ozlem Ozdemir,** Writing: **Semra Demir, Derya Unal, Derya Sonmez, Berrin Bercik Inal,** Approval: **Semra Demir, Derya Unal, Derya Sonmez, Ozlem Ozdemir, Berrin Bercik Inal.**

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