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The Levels of Serum Biomarkers in Stable Asthma Patients with Comorbidities

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ABSTRACT

The effects of comorbidities on systemic inflammation markers in stable asthmatics and the consequences of such effects have not been well evaluated. We aimed to evaluate the effect of comorbidities on clinical manifestations and systemic inflammation in asthmatic patients under control.

The study group consisted of asthmatic patients who applied to our pulmonology outpatient clinic and volunteered to participate. 120 clinically stable asthma patients (71 females and 49 males) and 35 healthy controls (19 females and 16 males) with similar age, gender, and body mass index distributions were admitted to the study. The levels of osteopontin, interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 13 (IL-13), eosinophilic cationic protein, adiponectin, and high-sensitivity C-reactive protein of the individuals were evaluated using commercial ELISA kits by taking venous blood samples.

Of 120 asthmatic subjects, 47 (39, 2%) had comorbidities and allergic rhinitis (15%) coexisted most frequently. Other comorbidities associated with asthma were gastroesophageal reflux, sinusitis, hypertension, diabetes, gastritis, and peptic ulcus respectively. There was no physician-diagnosed comorbidity in the control group. The levels of IL-6 and IL-8 were found higher in asthma group with comorbidities when compared to those with no comorbidities (p were 0.032 and 0.046, respectively).

Comorbidities interfere with the diagnosis and treatment of asthma, besides affecting the disease control. Our findings suggest the possibility of the impact of comorbidities on systemic inflammation markers, especially IL-6 and IL-8. To evaluate the impact of comorbidities on asthma control and systemic markers, further studies are needed.

Keywords: Asthma; Biomarkers; Comorbidities; Serum interleukin 6; Serum interleukin 8

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INTRODUCTION

Asthma is a disease characterized by chronic airway inflammation resulting from the effect of environmental factors on an existing genetic background.[1-3] Despite regular anti-inflammatory treatment, optimum control can be obtained in only 30-70% of patients.[4,5] It is a disease with heterogeneous characteristics in terms of diagnosis, treatment response, and prognosis. Personal factors such as age and gender, environmental exposures such as airborne irritants and conditions such as the patient's asthma phenotype may be responsible for this heterogeneity.[6] Another factor that makes the diagnosis and control of asthma difficult is accompanying diseases (comorbidities).[7]

Severity of both asthma and the accompanying comorbid disease may increase and asthma control may be disturbed in the presence of comorbidity.[7,8] The term comorbidity is defined as a medical condition that develops independent from and accompanies the primary disease. They may be associated with or independent from asthma, but may affect the diagnosis, treatment, and follow-up of the disease.[7] Rhinosinusitis, gastroesophageal reflux (GER), obstructive sleep apnea syndrome (OSAS), chronic infections and psychological diseases are the conditions most commonly seen in patients with asthma. [6,8-10] The Canadian Community Health Survey showed the presence of chronic comorbidity in 59% of asthma patients when all age groups were taken into consideration.[9]

Eosinophils had been considered for a long time to play a key role in the development of airway inflammation in chronic asthma. However, it was shown that 50% of patients are non-eosinophilic and non-allergic patients.[11,12] Asthma can be basically divided into two main subgroups in terms of phenotypic characteristics: the subtype with predominant Th-2 weighted eosinophilic inflammation and the subtype with interleukin 8-mediated neutrophilic inflammation together with bronchial epithelial cells. Increased levels of biomarkers of eosinophilic origin eosinophilic cationic protein (ECP), Immunoglobulin E (IgE), eotaxin 1, interleukin 15 (IL-5), interleukin 13 (IL-13), osteopontin (OPN), periostin and 8-isoprostane were shown for the first group whereas the second group is mainly characterized by increased levels of proinflammatory cytokines such as, interleukin 6 (IL-6), interleukin 8 (IL-8), tumor necrosis factor - alpha (TNF-alpha), and of systemic inflammation markers such as high-sensitivity C-reactive protein (hs-CRP). [10,13-16] In asthmatic inflammation, the bronchial epithelium was shown to have important effects such as releasing proteinases which lead to accumulation of other inflammatory cells in the inflammation site.[13]

Evidence of systemic inflammation that develops because of the overflow of mediators that are released due to chronic airway inflammation into the blood in asthma has been increasing during the recent years. It has been associated with diabetes, CVD, systemic rheumatic diseases and psychiatric disorders that accompany asthma.[17] Biomarkers that allow detection of inflammation in blood are currently used only in the research and clinical studies but may be employed in everyday practice in the future for diagnosis, detection of attacks and evaluation of treatment response.[13] Meanwhile, eosinophilia, total IgE, and specific IgE are commonly used in daily practice for diagnosis and evaluation of treatment response. Biomarkers could play a role in establishing targeted therapies with respect to disease phenotype and are still being studied.[6]

Serum ECP level may be used as an indicator of both the number and the activity of eosinophils. That is why it may be a better inflammatory marker compared to eosinophil numbers. Serum level of ECP may increase due to intensive exposure to allergens or provocation and may decrease due to termination of allergen exposure or regular use of inhaled steroids. [13,18] It has been reported that the blood levels of ECP may be elevated before the clinical symptoms develop.[18] Measurement of serum ECP level may be used to assess the severity of asthma, for the treatment and the evaluation of response to the treatment.[18]

Osteopontin and adiponectin, which are among novel biomarkers, are also investigated for assessment of airway inflammation. OPN plays a role in the development of diseases associated with a Th1 immune response by increasing production of interferon gamma and interleukin 12 and reducing interleukin 10 (IL-10). In asthma, OPN is released from airway epithelial cells and from inflammatory cells around the bronchi. Since it has been detected at high levels in serum in other IgE-mediated allergic diseases such as allergic conjunctivitis and allergic rhinitis during the recent years, OPN has been considered to be also involved in

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Th2-associated diseases.[19]

Adiponectin is an insulin-sensitive hormone that is released into the bloodstream mainly from fat cells and, to a lesser extent, from airway epithelium. It is important that it inhibits the effects of proinflammatory cytokines such as TNF-alpha and IL-6 and also increases the release of anti-inflammatory cytokines (IL-10 and IL-1 receptor antagonist).[20] Therefore, it may be used as a biomarker that reflects inflammation in asthma.

The effects of comorbidities on systemic inflammation markers and the consequences of such effects have not been well evaluated so far. It has been suggested that the presence of comorbidities may be responsible for asthma and inflammation may be responsible for its clinical progression.[10] The present study was aimed at evaluating the effect of existing comorbidities on systemic inflammation in asthmatic subjects and then contributing to the existing literature.

MATERIAL AND METHOD

Patient Characteristics

The study population consisted of 120 stable asthma outpatients. All the patients, between 18 and 65 years of age, were on inhaled steroids, none of them used systemic steroids in last six months, and none had recent respiratory infection. 35 age group and body mass index matched volunteers who did not have any health problem were included as control group. Patients who had clinical conditions that may lead to systemic inflammation such as malignancies, severe trauma or recent surgical intervention and conditions such as connective tissue disorders requiring the use of steroids were excluded from the study.

A survey including demographic data, medical history and information about asthma were applied by the investigating physicians to all patients. Physician-diagnosed medical conditions already known by the subjects were recorded as comorbidities. In line with the current literature, the presence of a history of rhinitis, gastroesophageal reflux, sinusitis, hypertension, gastric ulcer/gastritis, diabetes, thyroid disease, atherosclerotic vascular disease/ hyperlipidemia, anxiety disorder/depression, and stroke were queried. Although the presence of obesity was assessed by body mass index (BMI), it's not considered as comorbidity. The presence of allergy was questioned but it's not considered as comorbidity. It was evaluated by the results of priorly performed skin prick tests or specific IgE tests in blood.

Pulmonary function tests were performed according to the ATS/ERS standards using Jaeger MasterScope PC. Every measurement was repeated three times, and the best values were selected. Forced expiratory volüme in one second (FEV1), Forced vital capacity (FVC), and FEV1/FVC were used as spirometric parameters. In the reversibility test, an increase of at least 200 mL and 12% compared to baseline in FEV1 and/or FVC and an increase of 15% in PEF 15 minutes after inhalation of salbutamol at 200 µg (2 puffs) measured dose were considered positive. [6]

The Asthma Control Test (ACT) is a 5-item questionnaire that queries daytime and nocturnal asthma symptoms, use of rescue medications, and the effect of asthma on daily functioning. In this study, its validated Turkish form was used. For ACT scoring, scores of 25 points were considered as complete control, scores of 20-24 points were considered as good control and scores lower than 20 were considered as uncontrolled.[6]

The study was approved by the Ethics Committee of Adnan Menderes University, School of Medicine (N.: BAP-TPF 14014).

Patient Samples

IL-6, IL-8, hs-CRP, ECP, IL-13, osteopontin, and adiponectin were studied as biomarkers in the sera of asthma patients. Written informed consent was obtained from all the participants before the collection of blood specimens. The blood samples collected from the patients and the control group were centrifuged, the sera were separated, and stored in capped Eppendorf tubes at -80ºC at the "Research Laboratory of Adnan Menderes University Faculty of Medicine Department of Biochemistry". After completion of procedures for all individuals included in the study, serum biomarker levels were measured using commercial ELISA kits for adiponectin, osteopontin, IL-6, and IL-8 (Bender MedSystems GmbH, Campus Vienna Biocenter 2, 1030 Vienna, Austria). The evaluation of serum hs-CRP and ECP was measured by SunRed human ELISA kits (SunRed Biological Technology, No.128 Lane 628, Jufengyuan Road Baoshan, District, Shanghai, China). Serum IL-13 was evaluated by human ELISA kits (Human IL-13 ELISA kit; RayBiotech, 3607 Parkway Lane, Suite 100, Norcross, Georgia).

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Statistical Methods

For the statistical analysis of the data, SPSS Statistics, version 16.0 (SPSS Inc., Chicago, Ill., USA) was used. Descriptive statistics of categorical measures (gender, educational status, group, smoking history, respiratory symptoms, and presence of comorbidity) were expressed as frequency (percentage). Chi-square test was used for comparisons of categorical measures. Kolmogorov-Smirnov test was used to examine whether quantitative variables (age, height, weight, BMI.) show normal distribution. The descriptive statistical results were expressed as a mean ±standard deviation for quantitative variables showing normal distribution, and as median (25^{th}-75^{th} percentile) for quantitative variables not showing normal distribution. Independent samples t-test was applied to examine whether quantitative variables showing normal distribution are different between the groups, and Mann-Whitney U analysis was applied to examine whether quantitative variables not showing normal distribution are different between the groups. The statistical significance level was set at $p<0.05$ for all tests.

RESULTS

Our study group consisted of 155 subjects in total, 120 of whom were asthmatics, and 35 were controls. Their mean age was 38.14±11.21 (min. 18–max. 63) years. The asthma group included 71 female (59.2%) and 49 male (40.8%) subjects. The gender distribution in the control group was as 19 female (54.3%) and 16 male (45.7%) volunteers. There were no differences between asthma patients and controls in terms of mean age, body mass index (BMI) and gender distribution (Table 1). The rate of smoking history was 14% in the asthma group. None of the volunteers included in the control group had a smoking history. The comparisons according to BMI and age between the groups were not found statistically different. No smoking history, comorbidities and respiratory symptoms were reported in the controls.

The subjects have been followed-up for asthma for 7.06±5.34 (2-24) years and have been receiving regular inhaler steroid therapy for 4.24±3.74 years on average. The Asthma Control Test revealed complete control in 63 (52.5%) and good control in others. Asthma-associated symptoms, especially cough, dyspnea, chest tightness, wheezing, were reported in 63 (52.5%) patients despite therapy. 18.2% of the patients had dyspnea, 52.9% had cough, 5% had wheezing, and 1.7% had chest tightness.

The frequency of symptoms was reported as 42.3% in female subjects with asthma and 67.3% in male subjects, the rates being significantly higher in males (Chi-square: 6,349; df:1, $p<0.012$). No significant differences in terms of tested inflammatory parameters were found between the asthmatics subjects who report symptoms and those who do not.

Table 1. Demographic characteristics and respiratory symptoms of the asthma patients and controls

	Asthma (n=120)	Control (n=35)	*p* value
Age* (years)	38.8±11.93	35.86±7.97	0.093
Gender distribution(F/M)			
Female (n, %)	71 (59.2)	19 (54.3)	0.749
Male (n, %)	49 (40.8)	16 (45.7)	
Body mass index (kg/m^2)	25.52 (23.04-28.28)	25.61 (23.14-27.18)	0.620
Presence of smoking history			
No (n, %)	103 (85.8)	34 (100)	**0.025**
Yes (n, %)	17 (14.2)	0 (0)	
Presence of comorbidities			
No (n, %)	73 (60.8)	35 (100)	**<0.001**
Yes (n, %)	47 (39.2)	0 (0)	
Presence of respiratory symptoms			
No (n, %)	57 (47.5)	35(100)	**<0.001**
Yes (n, %)	63 (52.5)	0 (%0)	

*The descriptive statistical results were expressed as a mean±standard deviation for quantitative variables showing normal distribution and T-test was used for comparisons. Chi-square test was used for comparisons of categorical measures. The statistical significance level was set at $p<0.05$.

Higher levels of biomarkers associated with systemic inflammation were found in the asthma patients compared to the controls (Table 2). However, a statistically significant difference was found only in IL-8 levels. Although no significant difference in serum levels of osteopontin was found between the groups, the median level of osteopontin was higher in the controls (Table 2).

While the investigated comorbidities were found in 47 (39.2%) asthmatic subjects, none of the individuals in the control group had physician-diagnosed comorbidities. The distribution of the comorbidities in our asthma group is shown in Table 3.

The age of asthmatic subjects with comorbidities was found higher compared to those without comorbidities (p<0,015). The proportion of women was found higher in the group of asthmatics with comorbidity (Chi-square:7,209, df:1, p<0,01), but there was no difference in other comparisons such as the duration of asthma, duration of inhaled steroid use, asthma control scores, having an allergy and BMI, between the groups according to the presence of comorbidity. Allergy has been reported in 20 (%42,6) of the asthmatics with comorbidity and 24 (%32,9) of those without comorbidity (p=0,379). Pulmonary function tests were not different between the groups with and without comorbidities. No difference was found between the groups with and without comorbidities in terms of symptom frequency and smoking history.

Comparative evaluations based on the presence of comorbidities revealed higher IL-6 and IL-8 levels in asthmatics with comorbidities, with the difference between the groups being significant (Table 4).

Table 2. Comparison between the data of the patient and control groups in terms of the tested inflammatory parameters

	Asthma n=120	**Control n=35**	***p* value**
Interleukin 6 (pg/ml)	11.8 (5.9-19.15)	9.9 (5.5-14.3)	0.458
Interleukin 8 (pg/ml)	241.15 (116.9-419.7)	131.1(68.3-208.3)	**<0.001**
Interleukin 13 (pg/ml)	17.3(10.85-26.8)	13.5(7.2-28.8)	0.292
Eosinophilic cationic protein (ng/ml)	7.05(5.02-12.65)	6.10(5.0-11.20)	0.559
High-sensitivity C-reactive protein (mg/L)	4.95(2.93-12.88)	5(3-11.6)	0.876
Adiponectin (ng/ml)	15.9(9.3-25.75)	15.3(7.5-23.8)	0.303
Osteopontin (ng/ml)	7.56(2.75-12.92)	9.36(3.96-13.56)	0.426

Mann-Whitney U analysis was applied to compare the differences between the groups. The results were showing as median (25^{th}-75^{th} percentile). The statistical significance level was set at p<0.05 for all tests.

Table 3. Patient-reported comorbidities in the asthma group

Comorbidities*	**n**	**%**
Rhinitis	18	15
Reflux	15	12.5
Sinusitis	13	10.8
Hypertension	8	6.7
Gastric ulcer/gastritis	3	2.5
Diabetes	3	2.5
Thyroid disease	2	1.7
Others ASHD/Hyperlipidemia, Anxiety disorder/Depression, Stroke, Sleep apnea syndrome)	0	0

* Possible to have multiple comorbidities in the same patient

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Table 4. Comparison of various cytokine and inflammatory parameters of the patients with asthma according to the presence of comorbidities

	Presence of comorbidity n=47	Absence of comorbidity n=73	*p* value
Asthma duration (years)	5 (3-10)	3 (0-6)	0,252
Asthma test score	23 (21-25)	25 (22-25)	0.114
BMI (kg/m^2)	26.26 (23.73-30.11)	25.31 (23.04-28.03)	0,317
Interleukin 6 (pg/mL)	13.9(7.7-22.0)	9.7(4.9-16.5)	0.032
Interleukin 8 (pg/mL)	262.6(142.6-439.7)	205.4(104.0-362.6)	0.046
Interleukin 13 (pg/mL)	17,3 (10,4-24,3)	17,3 (11,45-31,85)	0,556
Eosinophilic cationic protein(ng/ml)	8,00 (5.1-13.3)	6,10(4.9-11.43)	0,259
High-sensitivity C-reactive protein (mg/L)	6.8 (3.9-12.9)	4 (2.6-12.5)	0.055
Adiponectin (ng/mL)	18.8(9.3-26.2)	15.5(9.45-25.55)	0.805
Osteopontin (ng/mL)	8.68(4.64-14.76)	7.08(1.88-11.9)	0.100

Mann-Whitney U analysis was applied to compare the differences between the groups. The results were showing as median(25th-75th percentile). The statistical significance level was set at p<0.05 for all tests.

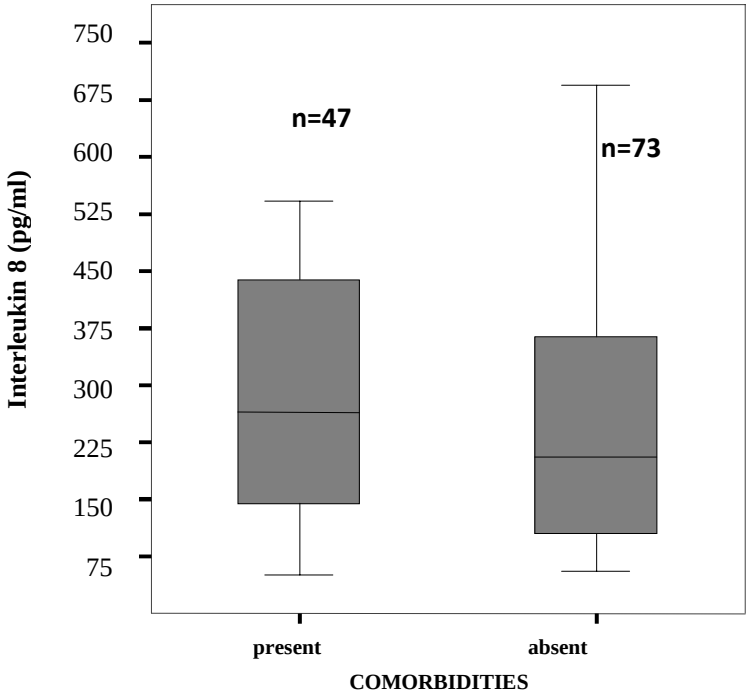

Figure 1. The comparison of serum interleukin 8 levels between the asthmatic subjects with and without comorbidities

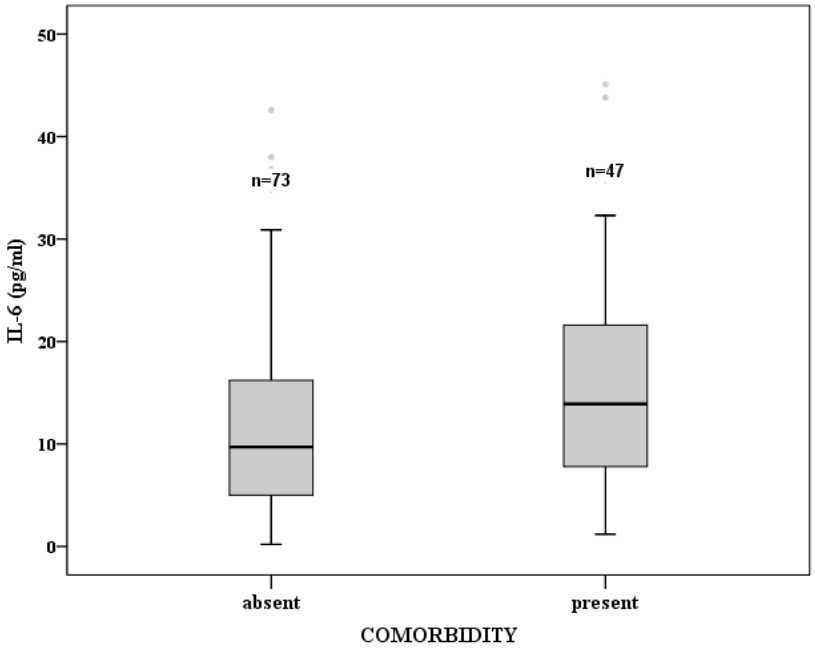

Figure 2. The comparison of serum interleukin 6 levels between the asthmatic subjects with and without comorbidities

Although the median levels of hs-CRP, ECP, IL-13, adiponectin and osteopontin were found higher in the asthmatic subjects with comorbidities, there was no significant difference between the groups. No difference was found between the groups with and without comorbidities in terms of other inflammatory parameters investigated.

In asthma patients, low-level negative correlations were found to be significant between hs-CRP and age and between hs-CRP and BMI (r:-0,195 *p*:0,032; r:-0,210 *p*:0,021, respectively). Low-level significant positive correlations was found between hs-CRP and the presence of comorbidity (r:0,149 *p*:0,048). Low-level significant positive correlations were found between IL-6 and symptom score (r:0,154 *p*:0,041) and between IL-6 and the presence of comorbidities (r:0,166 *p*:0,027), and low-level significant positive correlations were found between IL-8 and age (r:0,216 *p*:0,018) and between IL-8 and the presence of comorbidities (r:0,150 *p*:0,046). No significant correlations were present between the asthma control test score and disease duration and inflammatory markers. In the group of asthmatics under control and receiving regular inhaler steroids, low level significant negative correlation was found between ECP and age and ECP and BMI (r:-0.193, *p*:0.035; r:-0.229, *p*:0.012, respectively).

The comparative plot of the mean group IL-8 levels between the asthma patients with and without comorbidities is shown in Figure 1. The comparison of the mean of IL-6 levels between the groups is shown in Figure 2.

DISCUSSION

In the present study which evaluated the frequency of comorbidity and their effect on the inflammatory parameters in stable asthmatics, comorbidities were detected in 47 (39.2%) of asthma patients. In terms of frequency rates, rhinitis was the most common with 18 (15%) patients, gastroesophageal reflux was the second most common with 15 (12.5%) patients, and sinusitis was the third most common with 13 (10.8%) patients. Hypertension was seen in 8 (6.7%), diabetes in 3 (2.5%) and gastritis and ulcer in 3 (2.5%) patients. Comorbidities such as cerebrovascular disease, anxiety disorder, and sleep apnea syndrome were not reported by our cases. Allergic rhinitis and gastroesophageal reflux were also reported as the most common comorbidities of asthma in the study conducted by Cazzola et al.[21] In an epidemiologic study based on patient-reported information, arthritis, rhinitis,

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depression, and obesity were found to be the most common comorbidities of asthma in Korea.[22]

Comorbidities can lead to confusion in diagnosis of asthma, as well as assessing treatment response, even changing the disease phenotype.[6,8,] Asthma patient with comorbidities may be more difficult to control and sometimes requires more intense treatment. Pérez De Llano et al found that asthma control was impaired in more than 90% of asthma patients with comorbidities.[23] Control of asthma becomes even more difficult if there are more than one comorbidity. The frequency rate of 3 or more comorbidities was found 23.2% in patients with controlled asthma while it was 44% in patients with uncontrolled asthma.[23] In such cases, inflammatory parameters increased due to uncontrolled asthma may pose a risk for new comorbid conditions. In a recently published meta-analysis, a positive correlation was found between respiratory, cardiovascular and metabolic diseases and asthma.[24] The meta-analysis revealed that with asthma, respiratory comorbidities increase by 5.60 fold, cardiovascular comorbidities by 1.90 fold, cerebrovascular comorbidities by 1.44 fold, hypertension by 1.66 fold, diabetes by 1.25 fold, metabolic and endocrine comorbidities by 1.60 fold, and psychiatric and neurological comorbidities by 1.62 fold .[24] Disease control is more difficult in asthmatics with comorbidities, and this may increase the risk of attack in asthma.[8,17,25,26]

The ratio of chronic comorbidity accompanied by asthma was found in 59% of patients with asthma when all age groups were taken into consideration in a Canadian study.[9] In the above-mentioned study, the rate of detection of at least one major comorbidity in asthmatic subjects aged 55 years or over was reported as 83%.[9] In a population-based study from Australia showed a rate of 31,1% chronic comorbidity in asthmatics.[27] The rates of comorbidities found in our study were lower than Canadian study but closer to the results of the Australian study. We excluded the patients who had attacks within the last 6 months based on the inclusion criteria, which might lead to false negative results. Moreover, since we excluded patients with clinical conditions that can lead to a systemic inflammatory response such as connective tissue disorder, we might have excluded patients with significant comorbidities.

While asthma is associated with chronic inflammation in the respiratory tract, studies also revealed the systemic effects of local inflammation.[10,17,28] In fact, there are studies related to the use of airway inflammatory markers in the evaluation of treatment response in asthma.[13] Although there were no differences between groups in terms of anthropometric parameters such as BMI, Ali Hasan et al demonstrated significantly increased IL-8 levels in asthma patients compared to healthy volunteers.[29] In our study, IL-8 levels were found higher than healthy volunteers and the difference was found significant between the groups. IL-6 levels were also higher than controls, but the difference was not significant. When we classified the asthma patients according to the presence of comorbidities, both IL-8 and IL-6 levels were found to be statistically significantly different in the group with comorbidities. hs-CRP and osteopontin levels were also higher in the group with comorbidities; however, the difference between the groups was not significant. IL-8 showed a significant and positive correlation with increased age and presence of comorbidities, but this correlation was poor. Comorbidity burden may partly be responsible for higher level of IL-8 in asthmatics with comorbidities, since all patients were under control with regular inhaled steroids.

In the cross-sectional study with asthmatics, Takamura and colleagues demonstrated that the levels of hsCRP were increased in steroid-naive subjects but not in those on inhaled steroids.[30] Serum levels of ECP and hs-CRP were found higher in steroid-naive asthma patients compared to the healthy controls in a study by Sileem and colleagues in which it was also found that the serum levels of ECP were decreased two months after the treatment instituted, together with functional and clinical improvement.[18] In the present study, serum ECP levels of asthmatics tend to be higher than controls although it was not statistically different. This indifference may indicate the good response of our asthmatic patients to regular inhaled steroid therapy, which is supported by the similar ECP levels of asthma groups when compared for the presence of chronic comorbidities.

The blood serum levels of IL-13, which has a significant role in asthma pathogenesis, was found higher in patients with acute allergic asthma[31], although the studies of stable asthmatics have controversial results. Joseph J. et al found higher levels of IL-13 in mild and moderate asthmatics compared to normal controls, regardless of atopic condition.[32] No difference

was found in serum IL-13 levels in both the patients receiving regularly inhaled glucocorticoids and those receiving only an inhaled beta-2 agonist.[32]

Davoodi P et al found no significant difference in the median levels of serum IFN-gamma and IL-13 between asthmatic and healthy subjects in a case-control study. They suggested that IFN gamma and IL-13 exhibit a neutral role in the inflammatory process of asthma.[33] Although no significant difference in serum levels of IL-13 was found between the asthmatics and healthy controls, the median level of IL-13 was higher in the asthmatic group in the present study. In sub-analyses of the asthmatic group according to the presence of comorbidity, there was no difference between the groups, probably since the inflammation was under control by use of regular inhaled steroids and leukotriene receptor antagonist.

Zhao et al compared groups with different severity levels of asthma and controls in terms of OPN levels. They found higher OPN levels in the asthma patients when compared to the controls and demonstrated that these high levels were independent of disease severity.[34] In our study, osteopontin levels were not found different between the asthma patient group and the controls. However the levels of OPN were found relatively high similar to the results of other investigated parameters. When the subjects with or without comorbidities were compared, a slight increase was seen in OPN levels in the asthma group with comorbidities but no statistically significant difference was found based on the results of our study.

Liu et al studied OPN levels in 58 allergic rhinitis patients with and without asthma comorbidity and 24 controls.[35] The study reported significantly increased serum OPN, ECP, and IL-5 in allergic rhinitis patients compared to the controls. Clinical severity of allergic rhinitis was correlated with serum OPN levels. Comparisons between the subjects with and without asthma revealed a statistically significant difference with higher serum OPN in asthma in association with allergic rhinitis. The investigators commented that if high serum OPN levels are found in patients with allergic rhinitis, activation of systemic response that induces eosinophil migration and activation may contribute to asthma comorbidity.[35] In the analyses that we performed to test the results found by Liu et al., OPN and hs-CRP levels were higher in the subjects with rhinosinusitis in our study group; however, the difference was not statistically significant. Additionally, no significant correlations were present between serum OPN level and asthma disease duration, age, body mass index, asthma control test score, symptom score, and comorbidities.

There are studies suggesting the protective role of adiponectin in the development of asthma, especially in the female gender. In the study conducted by Sood et al high serum adiponectin levels are associated with active disease in male asthma subjects (p<0.05).[36] Higher serum adiponectin levels were statistically significantly correlated with the more frequent presence of respiratory symptoms, a higher number and more extensive use of asthma drugs. Additionally, body mass index was only correlated with unfavorable clinical outcomes in female subjects with asthma.[36] In our study, adiponectin was relatively higher in cases with asthma when compared with the age group, BMI, and gender-matched control group; however, the difference was not statistically significant. In subjects with BMI>30 kg/m^2, adiponectin levels were low, which was consistent with the literature. Comparisons among the asthma patients according to the presence of comorbidities did not reveal a statistically significant difference between asthma patients with and without comorbidities regarding the adiponectin level. Indeed, the comparison between genders demonstrated higher adiponectin levels in female subjects, which was consistent with the literature, and the difference between the groups was statistically significant (p=0.001). Additionally, the female asthma patients with high adiponectin levels had statistically significantly lower symptom frequency than the males (42.3% and 67.3% respectively; p<0,012).

Limitations of the Study

Although our results suggest that comorbidities may have effects, albeit partial, on systemic inflammation in asthma patients, these results have poor strength. In the present study, this situation could not be clearly demonstrated in our subject group most of whom had completely or partially controlled asthma. Regular anti inflammatory therapy of our subjects might have effected the plasma levels of inflammatory biomarkers investigated. Because of the stringent criteria we applied in subject recruitment, it is possible that uncontrolled asthma patients were excluded from the study; with this respect, there may have been an involuntary bias in subject recruitment. In a study group including uncontrolled patients, the frequency of

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comorbidities would possibly be greater, which must be evaluated by further studies.

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