Levels of Serum Acute-Phase Proteins in Patients with Asthma

ASTIMLI HASTALARDA SERUM AKUT FAZ PROTEİN DÜZEYLERİ

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Abstract

Objective: Bronchial asthma is an inflammatory disease which high numbers of cytokines play an important role in the pathogenesis of the disease. Production of the acute-phase proteins (APP) is under the control of several cytokines. The aim of this study was to investigate serum APP levels in patients with stable asthma.

Material and Methods: Forty asthma patients and thirty apparently healthy controls attended to this study. All the patients and controls were undertaken to history, physical examination, chest radiogram, blood count, routine biochemical investigation, skin prick test and pulmonary function tests. Asthma was diagnosed according to the American Thoracic Society Statement. Venous blood samples were obtained and used for C-reactive protein (CRP), ceruloplasmin, transferrin, haptoglobin and α1-acid glycoprotein (AAGP) detection. APP levels were determined by immunoturbidometrical methods.

Results: CRP levels were higher in the patients group than the controls (p= 0.007). While AAGP (p= 0.006) and ceruloplasmin (p= 0.000) levels were higher, transferrin (p= 0.000) and haptoglobin (p= 0.009) levels were lower in the patients than the controls. APP levels were not affected from smoking, FEV1 levels, steroid use, allergic rhinitis, atopy and eosinophili in the patient group.

Conclusion: All of the acute-phase protein levels, which were measured in this study, were observed significantly different in the asthmatic patients in comparison to the controls. In order to determine whether these APP are useful for the demonstration and follow-up of the inflammation in asthma patients, patients who have different stages of the disease and other certain inflammation markers should be performed in future studies.

Key Words: Asthma, acute-phase proteins

Özet

Amaç: Günümüzde bakteriyel enfeksiyon, inflamasyon, kanser ve plevral hastalıklar gibi birçok hastalıkta akut faz proteinleri (AFP)'nin düzeyleri ve biyolojik kullanımları ile ilgili pek çok çalışma sunulmaktadır. Bronşiyal astım, patogenezinde çok sayıda sitokinin rol aldığı inflamatuar bir hastalıktır ve AFP'nin üretimi de birçok sitokinin kontrolü altındadır. Bu çalışmada, stabil dönemdeki astımlı hastalarda serum AFP'nin düzeyini araştırmayı amaçladık.

Gereç ve Yöntemler: Kırk stabil astımlı hasta ve 30 sağlıklı kontrol çalışmaya alındı. Tüm hastalar ve kontrollerden detaylı anamnez alındıktan sonra, fizik muayene AC grafisi, tam kan sayımı, rutin biyokimyasal incelemeler, cilt testleri ve solunum fonksiyon testleri yapıldı. Astım tanısı Amerikan Toraks Derneği kriterlerine gore koyuldu. Çalışmaya katılanlardan venöz kan örnekleri alındı, ve serumlar C-reaktif protein (CRP), seruloplazmin, transferrin, haptoglobin ve α1-asit glikoprotein (AAGP) tayini için kullanıldı. AFP düzeyleri immunotürbidometrik yöntemlerle ölçüldü.

Bulgular: CRP düzeyi hastalarda kontrollerden anlamlı derecede yüksekti (p= 0.007). Hastalarda AAGP (p= 0.006) ve seruloplazmin (p= 0.000) düzeyleri anlamlı yüksek, transferrin (p= 0.000) ve haptoglobin (p= 0.009) düzeyleri anlamlı düşük bulundu.

Sonuç: Astımlı hastalar kontrollerle karşılaştırıldığında bu AFP düzeylerinin kontrollerden farklı olduğu görülmüştür. AFP'nin astımdaki inflamasyonu gösterme ve izlemede faydalı olup olmadığını saptamak için, hastalığın farklı evrelerindeki hastalarda ve kesin inflamasyon göstergelerinin de değerlendirildiği ileri çalışmalara gereksinim vardır.

Anahtar Kelimeler: Astım, akut faz proteinleri

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Bronchial asthma is an inflammatory disease which high numbers of cytokines play an important role in the pathogenesis of the disease. Biopsies and bronchoalveolar lavage fluid investigations in bronchial mucosa have shown

increased numbers of eosinophils, mast cells, macrophages and lymphocytes. Lymphocytes and the other inflammatuar cells contribute to development of inflammation by secreting interleukin (IL) 1, 4, 5, 6, 10 and 13, interferon γ, tumor necrosis factor-alpha.[1,2] Eosinophil derived proteins such as eosinophilic cationic protein, major basic protein and epithelial derived neurotoxin are important markers indicating eosinophilic inflammation. Histamine and tryptase are the products of mast cell/basophil activation. These markers are detected in sputum, broncoalveolar lavage, serum and urine, and increased in asthmatics. Since asthma has been recognized as a chronic inflammatory airway disease, inflammatory markers may be useful tools to show the degree of airway inflammation. In addition to these markers, nitric oxide (NO) in exhaled air and chemokines such as eotaxin, leukotrien E4, matrix metalloproteinase are inflammatory markers to indicate the quality and quantity of asthmatic airway inflammation.[3]

It is now well established that the response to inflammatory stimuli is associated with a variety of changes in the concentration of plasma proteins referred to as acute-phase proteins (APP).[4,5] C-reactive protein (CRP), AGPP, α1-antitrypsin, haptoglobin, ceruloplasmin, transferrin, fibrinogen and serum amyloid A protein are important APP and all of them are mainly produced in the liver.[4] The production of the APP is under the control of several cytokines.[4-6] Although the cytokines and markers mentioned above are more predictable tools to indicate the inflammation in asthma, their detections are limited in clinical chemistry laboratories. Their detections are difficult and expensive to use in routine laboratories. However, most of the APP can measured in clinical chemistry laboratories. Therefore, we aimed to determine the effect of chronic airway inflammation on the serum levels of CRP, alpha 1 acid glycoprotein (AAGP), ceruloplasmin, haptoglobin and transferrin in the patients with asthma.

Material and Methods

Forty consecutive patients with diagnosed asthma on the basis of clinical history of asthma and pulmonary function tests were studied. All the patients were admitted to the outpatient clinic of the chest disease department of the University Hospital, Mersin. Thirty apparently healthy individuals were selected as the control group from hospital staff. All the patients and the controls were undertaken to extensive history, physical examination, chest radiogram, complete blood count, routine biochemical investigation, skin prick test and pulmonary function tests. Asthma was diagnosed according to the American Thoracic Society (ATS) Statement.[7] All the patients had both clinical history of asthma and positive reversibility test. All the asthmatic subjects were clinically stable who had never experienced exacerbation of symptoms, away from systemic steroid usage and surgical treatment, trauma and had no signs suggestive of respiratory infection for at least 3 month before the study. The patients were classified as intermittent mild asthma (n= 14), persistent mild asthma (n=13) and persistent moderate asthma (n= 11), persitent severe asthma (n= 2) according to Global Strategy for Asthma Management and Prevention.[8]

Pulmonary function tests were performed by using Vmax 22 D Sensormedix (California, USA). Atopy was evaluated by skin prick test applying 13 standard aeroallergen (Stallergenes SA, Pasteur, France). The allergenes used together with histamine hydrochloride (10 mg/mL) and glycerol diluent controls were provided by Stallergenes S.A., France, at concentrations of 1/10 IR (indice dé reactive) for Dermatophagoides farinea, D. Pteronyssinus and of 1/20 IR for grasses, cereals, trees, weeds and W/V (weight/volume) Helianthus annuus.

Sixteen patients were using inhaled steroids (400 μg/day Budesonide, Turbohaler). Venous blood was collected into sterile glass tubes before the skin prick test and allowed to clot for 1-2 h at room temperature. Following centrifugation, sera were collected and used for CRP, ceruloplasmin, transferrin, haptoglobin and AAGP. All the APP were determined by immunoturbidometrical methods (Cobas Integra 700, Roche Diagnostics,

Table 1. Clinical characteristics of the patient and control groups.

	Patients (n= 40)	Controls (n= 30)	p	Tests statistics
Age (year)	38.95 ±2.06	37.57 ±0.69	0.25	t= 0.56
Sex (M/F)	10/30	15/15	0.04	Fisher's Exact
Smoking +/- (%)	9/31 (22.5%)	8/22 (26.7%)	0.78	Fisher's Exact
FEV1 (expected %)	82.35 ±2.41	91.43 ±1.89	0.02	t= -2.81

n= Number of patients
Significance was defined as p< 0.05. Data were presented as mean ±SEM

Table 2. APP levels in the patient and control groups.

Test	Reference range	Patients (n= 40)	Controls (n= 30)	p	Test statistics
*CRP (+) n (%)		21 (52.5%)	0 (0%)	0.001	z= 4.74
CRP (mg/L)	0-5	5.77 ±0.80	1.28 ±0.17	0.007	t= 2.785
Transferrin (g/L)	2-3.6	2.63 ±0.05	3.19 ±0.01	0.000	t= -4.773
Ceruloplasmin(g/L)	0.16-0.53	0.28 ±0.01	0.16 ± 0.008	0.000	U= 80.50
Haptoglobin (g/L)	0.3-2	0.83 ±0.05	1.13 ±0.1	0.009	t= -2.670
AAGP (g/L)	0.5-1.2	1.05 ±0.02	0.88 ±0.05	0.003	t= 3.065

Data was expressed as mean ±SEM. Significance was defined as p< 0.05
CRP: C-reactive protein, AAGP: Alpha 1 acide glycoprotein
**CRP (+):* CRP levels higher than 5 mg/L (over normal reference range).

Mannheim, Germany).

Statistical Analysis

The differences between the groups were analyzed by Student t-test and Fisher's Exact test using SPSS 9.0.5© (SPSS Inc, 1989-1999) package program except and p-values < 0.05 were accepted as significant. Non-Gaussian distribution groups were analyzed by Mann-Whitney U test for serum ceruloplasmine. The difference between the groups was analyzed with z approximation test for two independent proportions of CRP.

Results

Clinical characteristics of the patient and the control groups were given in the Table 1.

Serum AAGP and ceruloplasmin levels were significantly higher (p= 0.006, p= 0.000 respectively); and transferrin and haptoglobin were significantly lower than the controls (p= 0.000, p= 0.009 respectively). CRP levels were higher in the patient group than the control group and this difference was statistically significant (p= 0.007). All of the APP levels, except CRP were found to be in reference range. Levels of the APP were given in the Table 2.

The patients were divided into 2 groups according to their expected forced expiratory volume at first second (FEV1) values. 16 patients have FEV1 values above 80% and 24 patients have FEV1 values below 80%. Mean APP levels did not show significantly difference between **the two groups.** In addition presence of peripheral eosinophili (>3%), atopy, allergic rhinitis and steroid usage did not affect the APP levels in serum. Similarly, the APP levels did not affected from to be smoker or non-smoker.

Discussion

The presence of inflammatory cells such as eosinophils, mast cells, macrophages and lymphocytes has been observed in different specimens of asthmatic patients.[2] These inflammatory cells secrete several kinds of cytokines.[1,2] Therefore, asthma and allergic inflammation include wide cytokine network. This cytokines and their complex relationship have important role in inflammation and immune

regulation.[1]

Acute-phase reaction is a general response to inflammation and probably triggered by interleukins, released from the site of injury or inflammation.[4,5,10,11] IL-2, IL-6 and TNF-α are important mediators in the synthesis of APP by liver.

CRP is an important and sensitive APP and has been used to monitor progression and remission of many diseases.[4] Recent studies suggested that CRP levels play important role in the development of atherosclerosis, cyctic fibrosis, bronchiectasis, postoperative complication monitoring, exacerbation of chronic obstructive pulmonary disease e.g.[12] Increased CRP levels were also detected in plasma and several biological fluids in malignant diseases.[5]

In this study, all of the APP levels, except CRP, were found to be in reference range. CRP levels were detected to be increased in the asthmatic patients. The results suggest a possible role for CRP in the pathogenesis of asthma and CRP measurement may use for follow up the inflammation in asthmatic patients. Further studies should be performed in the different stages of the disease such as mild, moderate and severe stable period and attack.

Although exact role of ceruloplasmin is not known, antioxidant effect of it's has been reported.[4,13] The presence of oxidant/antioxidant imbalance has been proposed to play important role in the pathogenesis of asthma.[14-16] Increased ceruloplasmin levels were demonstrated in asthma patients by Vural *et al*.[17] We also found increased ceruloplasmin levels in the patient group in comparison to the control group. However, the serum levels of it were in reference range in the two groups.

It has been shown that AAGP is an important immunomodulator in response to pulmonary inflammatory process. AAGP has been proposed as a protective agent against to inflammation-mediated tissue damage.[18] Crestani *et al*. have shown that hyperplastic alveolar type 2 cells (AT2) have positive immunoreactivity for AAGP staining while normal AT2 cells lacking for immunoreactivity.[19] AAGP gene expression has also been observed on AT2 cells in case of lung inflammation.[19,20] While increased plasma AAGP levels were observed in patients with interstitial lung disease, which is often associated with chronic inflammation, plasma AAGP levels did not show any elevation in asthmatic patients.[20] But, in that study, increased branching of AAGP was observed in plasma and alveolar lavage fluid of asthmatics. In addition, this phenomenon has also correlated with factors related to chronic airway inflammation in the form of an increased number of eosinophils in blood and airways, decreased lung function and increased bronchial hyperreactivity.[20] In our study, increased levels of AAGP were observed in the patient group. This increase was independent from smoking, inhale steroid usage, values of FEV1 and the presence of atopy and allergic rhinitis.

In our study, serum haptoglobin and transferrin levels were significantly lower in the patients than the controls. As for the other APP, they were similar in following groups: Smoker vs. nonsmoker, allergic vs. nonallergic patients, patients with low vs. high FEV1, and patients receiving steroid treatment vs. those not receiving. A slight inverse relationship was demonstrated between serum haptoglobin levels and airway obstruction.[21] In that study, subjects who exhibited bronchial hyperresponsiveness to methacholine had haptoglobin levels higher than those who did not. In addition, haptoglobin level was unreleated to IgE level and skin prick tests.[21]

In conclusion, while transferrin and haptoglobin levels decreased in serum, increased CRP, ceruloplasmin and AAGP levels were observed in the asthmatic patients in comparison to the controls. But, only CRP levels were over of the reference range. In order to determine whether CRP is useful for the follow-up of the inflammation in asthma patients, it should be performed in different stages of disease. In addition, whether there was any relationship with

other certain inflammation markers, eg. nitric oxide should also shown in future studies.

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