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Original paper

THE SERUM EOSINOPHIL CATIONIC PROTEIN CONCENTRATION IN PATIENTS WITH BRONCHIAL ASTHMA AND ITS CORRELATION WITH SEVERITY AND EXACERBATION OF THE DISEASE

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Summary: Activated eosinophilic leukocyte in asthma secrete numerous mediators, among which is ECP as well. The object of our study was to measure the serum ECP concentrations in 46 asthmatic patients with exacerabating and stable asthma, and to correlate the serum ECP concentrations with severity and exacerbation of the disease. Geometric mean of ECP in serum (Gecp) in our group of patients was 7.5 mcg/l, while it was 3.05 mcg/l in the 15 healthy subjects (controls). Highly significant correlation of serum ECP concentrations with the activity of the disease (R=0.897) and the severity of clinical picture (R=0.79) was found. The patients with stable asthma had significant correlation of ECP and the severity of disease (R=0.6). The patients with exacerbating asthma have significantly higher serum ECP concentrations than the patients with stable asthma. Serum ECP concentrations in patients with exacerbating asthma correlate with the severity of the disease.

Key words: asthma, eosinophil cationic protein, exacerbation of asthma

Introduction

One of the crucial effector cells of chronic inflammation in asthma is the activated eosinophilic leukocyte, whose role is accomplished through the secretion of wide spectrum of preformed (ECP, MBP, EPO, EDN, etc) and newly synthesized mediators (1). Following the release from specific granules, ECP undergoes structural changes, what is verified by studies of monoclonal antibodies (EG2) that can make difference between the secreted form and the one kept in the specific granules. That is why the secreted form of ECP is considered marker of eosinophilic activity (2 4). ECP can be measured in body fluids and quantitated in serum as well, which is an accessible medium in clinical practice. Results from previous clinical evaluation of serum ECP in chronic asthma suggested that serum ECP is a sensitive marker of inflammatory airflow obstruction and that elevated levels denote patients at risk from inflammatory exacerbations, independent of atopic status, indicating the need to intensify treatment with corticosteroids (5).

The object of our study was to measure the serum ECP concentrations of asthmatic patients in exacerbation and stable forms; to correlate the serum ECP concentrations with the severity of clinical picture in exacerbating asthmatic patients; to correlate the serum ECP concentrations with the severity of clinical picture in stable asthmatic patients; and to correlate the serum ECP concentrations in asthmatic with exacerbating and stable forms of the disease.

Material and Methods

The prospective, randomized study included 46 patients with asthma (33 females and 13 males) who were examined at the Institute of Allergology and Clinical Immunology, aged from 18 to 72 years, mean-age 42.15 years.The control group consisted of 15

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healthy subjects, aged from 18 to 70 years, mean-age 41.14 years, with no history of allergy, asthma, allergic rhinitis and atopic dermatitis. The diagnosis of asthma was based on the guidelines for diagnosis and treatment of asthma of the international expert group from the National Health Institute and National Institute for Heart, Lung and Blood Institute WHO, Jan 1995 (6). Lung function (measured by the apparatus Autospir Discom-14 Chest Corporation Tokyo, Japan), physical examination and blood sampling for ECP was performed in all patients. We have devided our patients: 1) according to status of disease activity into the following groups: exacerbation and stable; and the criteria were: symptoms and signs of the disease, and the degree of airway obstruction measured by FEV1 at the time of testing; 2) according to severity of disease into the groups: mild, moderate and severe asthma; based on criteria: symptoms and signs of the disease, drug application and testing of functional airway status (FEV1). Our group comprised 29 (63%) patients with asthma exacerbation and 17 (37%) patients with stable disease. Mild asthma in phase of exacerbation was evidenced in 5 patients, moderate asthma in phase of exacerbation was recorded in 9 patients, while severe asthma in phase of exacerbation was found in 15 patients. In group of the patients with stable asthma mild asthma was evidenced in 7 patients, moderate in 6 and severe in 4 patients.

ECP was measured in blood samples obtained by cubital vein puncture using Vacutainer SST (Becton Dickinson, France), without EDTA addition. The sample was left to coagulate at room temperature between 60 and 120 minutes, and subsequently spun at 1300g for 10 min to separate the serum. These are the instructions for extracting eosinophil cationic protein (ECP) issued by the manufacturer (Pharmacia Upjohn, Uppsala, Sweeden). The serum was stored at 20 °C, before the ECP analysis was performed by the Pharmacia UniCAP fluoroenzyme immunoassay (FEIA) ECP system according to the manufacturer instructions. The detection limit of the assay was 2.0 μg/L.

Statistical analysis

Statistical analyses were performed using Basic Statistic software (EPI INFO ver 6.0). Statistical differences were calculated with the non-parametric Mann-Whitney test. Correlations between the different parameters were sought by calculating the Spearman' s rank correlation coefficient (R).

Results

Serum concentration of the eosinophilic cation protein (ECP) is expressed in mcg/l. Lower detection border of serum ECP was 2 mcg/l (the method according to Pharmacia UniCAP FEIA). Geometric mean of ECP value (Gecp) in 100 healthy individuals was 4.4 μg/L (manufacturer's standardization). Our control group composed of 15 individuals free of history of atopy, asthma, allergic rhinitis and atopic dermatitis had the geometric mean of serum ECP values of 3.056 mcg/l, which was consistent with the manufacturer's reference values.

Geometric mean of ECP serum concentrations in our asthma patients (46) was 7.5 mcg/l. *Table I* presents patients based on condition of their disease (exacerbation/stable) and severity of the clinical picture (mild, moderate, severe asthma).

Table I Geometric mean of serum eosinophil cationic protein in asthmatic patients in relation to the severity and state of activity of the disease

State of Asthma	Severity of Asthma	No of Subjects	Geometric mean (μg/L)
Exacerbated	Mild	5	8.6
Exacerbated	Moderate	9	6.6
Exacerbated	Severe	15	10.4
Exacerbated Total		29	8.7
Stable	Mild	7	6.6
Stable	Moderate	6	5.9
Stable	Severe	4	4.8
Stable Total		17	5.9
Total		46	7.5

It is intriguing that the lowest geometric mean of ECP in patients with exacerbating asthma was found in the group with moderate asthma (Gecp=6.6), and in patients with stable form of asthma, the lowest Gecp was reported in patients with severe asthma (Gecp= 4.8) (*Table I*). Serum ECP correlation coefficient between the groups with exacerbation and stable disease was R = 0.8977, indicating highly significant correlation of serum ECP values and status of asthma, meaning that the patients with asthma exacerbation had significantly higher ECP concentrations in comparison to those with stable asthma.

As for the patients with asthma in phase of exacerbation, correlation coefficient of clinical picture severity and serum ECP concentration was R= 0.638, indicating highly significant correlation of ECP concentration in exacerbating asthma with severity of the disease, meaning that the patients with severe exacerbating asthma had higher ECP concentrations in comparison to those with mild asthma in phase of exacerbation.

In patients with stable asthma, correlation coefficient of clinical picture severity and serum ECP con-

centration was R = 0.6, indicating significant correlation of ECP concentration in stable state with clinical picture severity, meaning that the patients with stable mild disease had lower ECP concentrations in comparison to those with severe stable asthma.

In asthmatic patients with a stable form of disease there was a significant correlation of serum ECP concentration and the severity of clinical picture.

Discussion

Nowadays, serum ECP concentration is adopted as the indicator of eosinophilic activity (7). The object of our study was to explore the influence of severity and exacerbation of the disease on serum ECP concentration in asthmatic patients. Geometric mean of serum ECP concentration in our studied group (7.5 μg/L) was slightly lower than Gecp=9.6 μg/L found by Roquet et al. in the patients with suspected mild asthma (8). Lower Gecp in our studied group was probably the result of the heterogeneity of the given group related to the severity and exacerbation of the disease. Geometric mean of ECP in our studied group (3.056 μg/L) was compatible with the control values (4.4 μg/L) of the producer of the serum ECP testing commercial kit, as well as with the reference data (5, 9). Ahlstedt et al. (10) reported that Gecp=3 5 μg/L in healthy persons, while somewhat higher values were found in children: 6.5 μg/L (11). Highly significant correlation of serum ECP concentration with the activity of disease (exacerbated/stable form) means that the patients with exacerbating asthma have significantly higher ECP concentrations in relation to patients with stable asthma. The same conclusion was drawn by numerous authors (9, 10, 12, 13, 14), and they also have found that the initial ECP concentration was higher in patients having more severe attack. In our group of patients with exacerbating asthma, highly significant correlation of ECP concentration and the disease severity was found (R=0.638), and these data were consistent with findings of other authors (12). The patients with stable asthma also have the poorest but still significant correlation with the severity of the disease, meaning that the patients with severe stable asthma have higher ECP concentrations in comparison to patients with mild stable asthma, but such difference, although significant, is not big. Similar results were reported by Oymar et al. (15). Given that serum ECP concentration is the indicator of eosinophilic leukocyte activity (7), it is reasonable that the significant activation of eosinophilic leukocytes occurred in the group of patients on the basis of exacerbation, and that the severity of the disease was less significant, all the more so the usage of corticosteroids is more intensive in the severe forms of the disease. Our finding of highly significant correlation of serum ECP concentration and the disease severity is compatible with numerous statements in literature (12, 16, 17). Vanto and Koskinen (18) found poor correlation of serum ECP concentrations and severity of the disease in children (R=0.21). There are some authors who failed to find a significant correlation of ECP concentration and severity of the disease (19). The severity of asthma, however, could not be determined with certainty on the basis of ECP concentration in serum, because we found lower geometric mean of ECP in the group with moderate asthma than in the group with mild condition. Apparently, the severity of asthma does not reflect directly the intensity of airway inflammation. It means that patients with severe stable asthma may have lower ECP concentrations than the patients with mild exacerbating asthma. This is congruent with the published data (20, 21), and the similar results were obtained by Bousquet et al.: in patients with the most severe asthma score 4, geometric mean was lower in comparison to the group with score 3. Such result was explained by natural process of chronic inflammation, during which the mediators of inflammation abate in the subsequent stage of disease, giving place to reparative process (22). On the basis of the results obtained throughout this study, it could be concluded that the patients with exacerbating asthma have significantly higher serum ECP concentrations in relation to patients with stable asthma. Serum ECP concentration in patients with exacerbating asthma is proportional to the severity of the disease. It is necessary to keep on with further investigations on the role of ECP and other eosinophilic mediators in chronic inflammation of asthma.

KONCENTRACIJA EOZINOFILNOG KATJONSKOG PROTEINA KOD BOLESNIKA SA BRONHIJALNOM ASTMOM I NJEGOVA KORELACIJA S TEŽINOM I EGZACERBACIJOM BOLESTI

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Kratak sadržaj: Aktivisani eozinofilni leukocit u astmi sekretuje brojne medijatore među kojima i ECP. Cilj ove studije je bio da se izmeri serumska koncentracija ECP kod 46 astmatičara u stabilnom stanju i pogoršanju i korelira koncentraciju ECP kod bolesnika u pogoršanju bolesti sa težinom kliničke slike. Za bolesnike dobijena je geometrijska sredina ECP (Gecp) 7.5 μg/L, dok je kod 15 zdravih kontrola iznosila 3.05 μg/L. Nađeno je da postoji statistički visoko značajna korelacija koncentracije serumskog ECP sa aktivnošću bolesti (R=0,897) i težinom kliničke slike (R=0,79). Bolesnici sa stabilnom astmom imali su značajnu korelaciju koncentracije serumskog ECP sa težinom bolesti (R=0,6). Bolesnici sa pogoršanjem astme imali su značajno više koncentracije serumskog ECP od bolesnica sa stabilnom astmom. Koncentracije serumskog ECP kod bolesnika s pogoršanjem astme bile su proporcionalne težini bolesti.

Ključne reči: astma, eozinofilni katjonski protein, egzacerbacija astme

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