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Biomarkers of Inflammation in Peripheral Blood during Treatment with Inhaled Corticosteroids in Patients with Asthma

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Abstract

BACKGROUND: The main attribute of asthma is inflammation, which leads to airway remodelling, bronchial hyper-reactivity and reversible or partly reversible airway obstruction. According to GINA, asthma is a chronic inflammatory disorder of the airways in which many cells play a role, in particular, mast cells, eosinophils (Eo), and T lymphocytes. Many cells and mediators take part in creating the asthmatic inflammatory reaction, but eosinophils play a central role.

AIM: The aim of this study was to show the values of biological inflammatory markers (IL-5, Eo, and ECP) during treatment with ICS by non-invasive methods.

MATERIAL AND METHODS: This study includes 30 patients of the Pulmonology and Allergology Clinic, Skopje, with confirmed asthma, treated with ICS. In all of the patients, we followed Eo count, ECP and IL-5 in peripheral blood at the beginning of the study, after 2 and six months treatment. Following the parameters during treatment with ICS we registered changes in all of the tested parameters.

RESULTS: Our results show high level of the tested biomarkers at the beginning of the study, IL-5 in all patients (100%), Eosinophils in 80%, and ECP in 90% of the asthmatics. Following the parameters during treatment with ICS we registered changes in all of the tested parameters.

CONCLUSIONS: Our conclusion is that the ICS objectively suppress the inflammatory reaction in asthma and the biologic markers (IL-5, Eo and ECP), which we have followed, can measure the accomplished effect. They could be used in everyday practice, not only as diagnostic parameters but also as valid therapeutic guides in the treatment of asthma.

Introduction

Asthma is one of the most common chronic diseases all over the world, resulting from a state of persistent sub-acute inflammation of the airways. Asthma is a chronic disease defined by three features: inflammation, which leads to airway remodelling, bronchial hyper-reactivity and reversible or partly reversible airway obstruction. It is known that the basis of the pathogenesis of asthma lays chronic inflammation. According to GINA, asthma is a chronic inflammatory disorder of the airways in which many cells play a role, in particular, mast cells, eosinophils (Eo), and T lymphocytes (GINA) [1]. Many cells and mediators take part in creating the asthmatic inflammatory reaction, but eosinophils play a central role.

The immune response is stimulated by T lymphocytes by the release of Th2 type cytokines, such as IL-3, IL-4, IL-5, and GM-CSF. IL-5 is the primary interleukin involved in the production, differentiation, maturation, and activation of Eo. It takes an important place in the allergic inflammation by promoting the chemotaxis, activation and survival of Eo and their apoptosis. Il-5 increases the number and activity of Eo. Their number in the bone marrow, peripheral blood and tracheobronchial mucosa increases. Eosinophils are very potent cells. They can generate more than 28 newly synthesised cytokines, chemokines and growth factors, which are capable of modulating the immune response [2]. They contain four basic proteins which degranulate during the asthmatic reaction: major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil peroxidase (EPO) and the eosinophil neurotoxin (EDN), which damage the bronchial mucosa and increase the airway hyper-reactivity [3-5]. With the degranulation of Eo, other mediators are released as

well, such as LTC_4, LTD_4, LTE_4, and PAF, which influence the contractility of airway smooth muscle and the permeability of the vessels. Eo are also responsible for the local control of the asthmatic reaction because they release active biological substances which inactivate mediators released by mast cells [3, 6].

Many studies show the correlation between the severity of asthma and the number and activity of Eo [7, 8].

Up to this date, the most efficient anti-inflammatory medications in the treatment of bronchial asthma are inhaled corticosteroids [9, 10]. Although they were introduced in the treatment of asthma more than 40 years ago; they still are the first line treatment for most patients with asthma [11, 12]. Corticosteroids affect almost all cells which take part in the inflammatory process in asthma. Laitinen [13] shows a decrease in the total cell count in the mucosa of asthmatics treated with BDP for three months.

Corticosteroids cause a decrease of the Eo count [14] and consecutive improvement of asthma symptoms. They inhibit the synthesis of IL-4 and IL-5, which influence Eo survival and decrease the release of cellular mediators and basic protein molecules [15, 16]. Inhaled corticosteroids decrease the release of GM-CSF, which leads to a decrease in the number of low-density Eo, meaning inhibition of cytokine production. They also influence the apoptosis of Eo. Inhaled corticosteroids inhibit the late asthmatic reaction within hours of the first application, while continuous treatment for at least 4-6 weeks is necessary for suppression of the early asthmatic reaction. We have followed the anti-inflammatory response to ICS by following the serum levels of IL-5 and ECP and the total Eo count in peripheral blood.

The aim of this study was to show the values of biological inflammatory markers (IL-5, Eo, and ECP) during treatment with ICS by non-invasive methods.

Material and Methods

The study was performed at the Pulmonology and Allergy Clinic, The Institute for Biochemistry and Institute for Immunology, at the Medical Faculty in Skopje. We included 30 patients with severe asthma, 22 (73.33%) female and 8 (26.66%) male, age 18-65 years. The average age was 35.30 ± 9.65 years. The patients were treated with spray Beclomethasone dipropionate a 250 μg in doses from 1000 to 2000 μg/per day.

In each subject the following investigations were undertaken at the beginning of the study, after two months and at the end of the sixth month of continuous treatment:

- Interleukins: (IL-5) in peripheral blood, by the standard methodology of the Institute of Biochemistry (ELISA System with appropriate antibodies for the IL-5 was used. Referent values: 0 pg/ml for IL-5).
- Total Eo counts and ECP in peripheral blood. (Pharmacia CAP System for determination of serum ECP was used. Referent values: Eo = 120-240 x 10^6/l, ECP < 11.3 mg/l).

The data were statistically analysed by "STATISTICA for Windows", version 4.5, from 1993. The changes of the evaluated parameters were tested with ANOVA. Values for $p < 0.05$ were considered statistically significant.

Results

At the beginning of the study in all 30 (100 percent) of the patients the serum level of IL-5 was increased (44.94 ± 28.87 pg/ml). After two months of treatment, the values decreased to 37.44 pg/ml. During the 6-months follow-up the average value of IL-5 decreased from 44.94 ± 28.87 to 30.44 ± 18.05 pg/ml ($p = 0.000044$), but still remained high above the referent values (0 pg/ml).

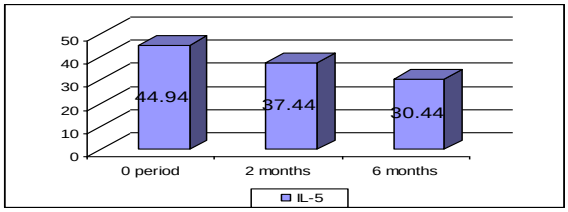

Figure 1: Serum level of the IL-5 during six months treatment

In 24 (80%) of the subjects, we found high levels of peripheral blood Eo at the beginning of the study. The total Eo count was found to be from 150 to 390 x 10^6/l; average value was 292.66 ± 67.05 x 10^6/l. After two months, this number decreased to 270 ± 59.71 x 10^6/l and at six months it was 224.33 ± 59.17 x 10^6/l.

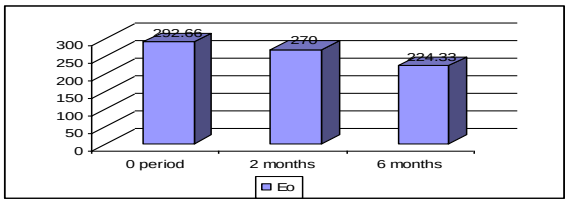

Figure 2: Total Eo count in peripheral blood during six months treatment

In 27 (90%) of the subjects we found increased levels of serum ECP, before treatment.

Values ranged from 4.9 to 42.1 mg/l, average value was 22.92 ± 10.08 mg/l. At two months these values decreased to 19.36 ± 8.59 mg/l. After six months of treatment, the ECP values in peripheral blood were from 7 to 30.4 mg/l. Average value decreased from 22.92 ± 10.08 to 14.47 ± 6.22 mg/l.

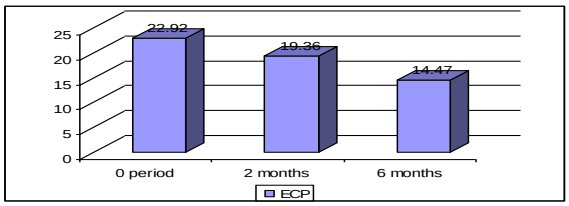

Figure 3: Level of the ECP during six months treatment

Discussion

Our results are similar to those found in the literature [17, 18]. The positive therapeutic effect of the inhaled corticosteroids in asthma is promoted by induction of apoptotic cellular death of Eo. This mechanism can explain the clinical efficiency of corticosteroids in the control of asthma symptoms and the prevention of exacerbations. Corticosteroids markedly decrease the Eo count as well as their mediators, by reducing the production of Eo in the bone marrow and by their influence on apoptosis.

The new studies suggest that the efficiency of ICS in the reduction of airway hyperreactivity and asthma symptoms depends on the increased number of Eo in the airways before beginning of treatment [19]. A group of Japanese authors used serum ECP and the total Eo count as leading markers for step down of treatment with ICS. They recommend that the serum levels of ECP can be a very sensitive marker for determination of the exact time for stepping down the treatment [20].

The anti-inflammatory effect of corticosteroids on Eo is mostly accomplished through reduction of the number of IL-5 producing T-cells [21]. Corticosteroids inhibit the production of cytokines [22]. A group of Swedish authors refer about decrement of the expression of IL-5 receptor on Eo during treatment with ICS [23]. Numerous studies confirm the reduction of Th_2 cytokines influenced by aortic therapy [24-27].

In our study, we found a decrease in the serum levels of *IL-5* during treatment with ICS, which is consistent with the data from the literature.

The aim of every form of anti-asthmatic treatment is to prevent and suppress the inflammation of the airways. Still, objective parameters are necessary to evaluate the effects of the chosen treatment. The markers that we have evaluated correlate significantly with the severity of inflammation and the effects of the applied therapy. The follow-up of these markers in peripheral blood (although less sensitive than in bronchoalveolar lavage of bronchial mucosa) has an advantage of being non-invasive and therefore repeatable as many times as necessary. The possibility to determine and follow markers of inflammation should be a stimulus for each doctor to measure the effects of prescribed treatment or to choose the effective therapeutic measures.

The results of our study show that the ICS objectively suppress the inflammatory reaction in asthma and the biologic markers (IL-5, Eo and ECP), which we have followed, can measure the accomplished effect. They could be used in everyday practice, not only as diagnostic parameters but also as valid therapeutic guides in the treatment of asthma

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