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### **Središnja medicinska knjižnica**

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**TITLE:**

Serum eosinophil cationic protein (ECP) in children with atopic dermatitis

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## ABSTRACT

**Background:** Eosinophil cationic protein (ECP) is a cytotoxic agent secreted by activated eosinophils during allergic and inflammatory processes. The aim of the study was to determine ECP level, absolute and relative eosinophil count and IgE antibodies in children with atopic dermatitis (AD), compare them to the levels in non-atopic children, and to assess correlation of these laboratory parameters with clinical severity of AD. **Methods:** The prospective study encompassed 70 children. There were 49 children with AD aged 3 to 36 months and the control group comprised 21 children, with negative personal and family history for atopic diseases. Detailed history, serum ECP levels (UniCAP FEIA), relative and absolute eosinophil counts and total serum IgE antibodies were determined in both groups. In AD children the skin involvement was measured by SCORAD index. **Results:** The calculated SCORAD index was between 16 and 83. IgE antibodies, relative and absolute eosinophil counts showed a much wider range of values and a statistically higher median ( $p < 0.001$ ) in AD patients compared to the control group. These laboratory parameters did not correlate with the severity of AD. The serum ECP median level, in children with AD, was  $16.2 \mu\text{g/L}$  (range 3.01-65.30) compared to  $5.92 \mu\text{g/L}$  (range 2.76-21.90) in the control group. The correlation of total SCORAD index and serum ECP levels was negative, weak ( $r = -0.065$ ) and statistically not significant ( $p > 0.05$ ). The same was found for the correlation of serum ECP and intensity of skin changes ( $r = -0.095$ ) and serum ECP and subjective symptoms ( $r = -0.045$ ). The correlation was positive, but weak and statistically not significant for serum ECP and extent of skin lesions ( $r = 0.079$ ,  $p > 0.05$ ). **Conclusion:** Elevated levels of ECP, relative and absolute eosinophil counts, as well as IgE antibodies were determined in the patients with AD. Since these laboratory findings did not correlate with the severity of AD, they can be considered only as additional methods in the evaluation of patients with AD.

## INTRODUCTION

Atopic dermatitis (AD) is a chronic, recurring, allergic inflammatory skin disease caused by genetic predisposition (atopic constitution) and characterized by typical clinical presentation. This disorder, primarily occurring in infants and children, is the most common disease seen in pediatric dermatology practice with the prevalence reaching 15% in this age group (1,2). The development of AD is dependent on multiple genes but environmental factors influence the expression of the disease (3,4).

The pathogenesis of the disease is still unclear. In the past, considerable debate was led whether AD is primarily an allergen-induced disease or simply an inflammatory skin disorder (5). Recent data strongly support that AD is an allergic disorder associated with asthma and allergic rhinitis (6).

Serum levels of IgE antibodies are elevated in 80-85% patients with AD. Disturbance of skin function, reflected by increased skin sensitivity, dryness and infection, appear to be more important than allergy in inducement and maintain of skin inflammation in some patients (6).

Eosinophilia is a common finding in patients with atopy. The investigations in the last few decades have clearly pointed out that eosinophils, owing to their functional capabilities and toxic proteins, represent active proinflammatory cells that cause different symptoms in allergic diseases (7-9). Eosinophils are activated through various cytokines and inflammatory mediators, which lead to invasion of eosinophils to the site of inflammation (10,11). At the site of inflammation eosinophils secrete leukotriens, prostaglandins, bradikinin, various cytokines, enzymes as well as different toxic proteins from their granules (12). Eosinophil cationic protein (ECP), secreted by eosinophils, is a cytotoxic agent that plays an important role in the propagation of allergic inflammation and has an immunomodulatory capacity (13).

There are various means of eosinophil monitoring and determination of their involvement in diseases: eosinophil count in blood and tissue, identification of activated eosinophils in blood (hypodense eosinophils), identification of activated eosinophils in tissue with monoclonal antibody staining-EG2 antibody (14) and eosinophil cationic protein (ECP) in different body fluids.

Elevated ECP levels have been measured in serum, bronchoalveolar and nasal secretions in patients with allergic diseases such as bronchial asthma and allergic rhinitis (15,16). Significantly elevated levels of ECP have been found in patients with AD (17-27). It seems likely that elevated levels reflect the state of eosinophil activation in the skin so ECP may represent a laboratory parameter for identification of pathologic inflammatory processes and degree of eosinophil involvement in the skin of AD patients.

The aim of this study was to determine levels of ECP, absolute and relative eosinophil count and IgE antibodies in children with AD, compare them to the levels in non-atopic children and to assess correlation of these laboratory data with clinical severity of AD.

## **MATERIALS AND METHODS**

### *Patients*

Seventy children divided in two groups were included in the study. The first group consisted of 49 children, aged 3-36 months, with the diagnosis of AD according to the criteria established by Hanifin and Rajka (28). There were 17 girls and 32 boys. The patients were recruited from the Outpatient Clinic or were hospitalized at the Department of Dermatovenerology, Zagreb University Hospital Center, Croatia, from February to October 2000. The children were otherwise healthy, had no symptoms that could be related to any other allergic illness, and their only current

treatment were skin moisturizers. They were not previously treated by ultraviolet therapy, systemic corticosteroids or immunosuppressive medications.

The control group comprised 21 children, of the same age, with negative personal and family history for atopic diseases. They were selected, between patients who were monitored and considered healthy after treatment of urinary or respiratory infection at the Outpatient Pediatric Clinic of Zagreb University Hospital Center, Croatia.

### *Methods*

Detailed history, relative and absolute eosinophil counts, total serum IgE antibodies and serum ECP levels were determined in both groups. Serum ECP value was determined by UniCAP-ECP Fluoroimmunoassay-Pharmacia (29). Blood samples for serum ECP were collected, handled and prepared according to the instructions of the manufacturer (29). The blood was collected by venipuncture using Becton Dickinson 2,5 ml Vacutainer hemogard SST<sup>®</sup> tubes for serum separation. After collection the tubes were gently inverted 5 times then left for 90 minutes at room temperature (20-24<sup>0</sup>C) for clotting. Centrifugation at 1300xg for 10 minutes was performed, the serum was transferred to a new tube and stored at -20<sup>0</sup>C until analysed. The absolute number of eosinophils was calculated from the total number of leukocytes and percentage of eosinophils determined by counting of 400 leukocytes. Total IgE antibody values were determined by UniCAP 100 method-Pharmacia CAP System IgE FEIA (30).

In AD children, the extent of the disease and severity of symptoms was calculated by SCORAD index (31).

### *Statistics*

Data analysis was carried out by release 6.12, the SAS computer program. The Mann-Whitney U test was used to determine differences between the study and

control group. Relationship between different parameters was determined by Spearman coefficient correlation. The results of statistical analysis with  $p < 0.05$  were considered statistically significant.

### *Results*

Out of 49 children with AD, 26 (53.1%) had a positive family history for atopy. The mean age of AD onset was 3.0 months.

SCORAD index values were between 16 and 83. The majority of our patients (35 patients) had SCORAD index between 31 and 65 (Figure 1) therefore the clinical picture was moderately severe.

IgE antibody values ranged between 2.0 and 124.0 kIJ/L in healthy children, and between 2.6 and 5,000.0 kIJ/L in patients with AD. The median value of IgE in AD patients was 98.3 kIJ/L, which was significantly higher ( $p < 0.001$ ) compared to the median of healthy controls (10.0 kIJ/L).

Healthy controls had values of relative eosinophil numbers from 0 to 11% compared to 0 to 34% in AD children. Median for this laboratory parameter was 2% in healthy children and 7 % in children with AD.

Absolute eosinophil count in AD children showed values from 0.01 to  $3.69 \times 10^9/L$  compared to 0.02 to  $1.27 \times 10^9/L$ . The median values were 0.70 (AD children) and 0.09 (healthy controls).

The mean value, of serum ECP in AD patients, was 16.20  $\mu g/L$  and significantly higher ( $p < 0.001$ ) compared to 5.92  $\mu g/L$  in healthy controls. As found for IgE and eosinophil numbers the values varied greatly in AD patients (3.01-65.30  $\mu g/L$ ) compared to healthy controls (2.76 –21.90  $\mu g/L$ ).

Correlation between clinical (SCORAD) and laboratory parameters (ECP, eosinophils, IgE) in children with AD are shown in Tables 1-3.



The correlation of serum ECP levels and total SCORAD index (Table 1) was negative, weak ( $r=-0.065$ ) and statistically not significant. The same was found for the correlation of serum ECP and intensity of skin lesions ( $r=-0.095$ ) as well as the correlation between serum ECP and subjective symptoms ( $r=-0.045$ ). The correlation between ECP and extent of skin lesions was positive, weak ( $r=0.079$ ) and statistically not significant.

The relationship of serum ECP with relative and absolute eosinophil counts (Table 2) showed medium strong, positive ( $r=0.533$ ;  $r=0.459$ ) and statistically highly significant correlation. The correlation between ECP and IgE (Table 2) antibodies was positive, weak ( $r=0.038$ ) and statistically not significant.

Negative, weak and statistically nonsignificant correlation was determined between SCORAD index and relative as well as absolute eosinophil counts ( $r=-0.059$ ;  $r=-0.104$ ). The correlation of SCORAD index and IgE antibodies was positive, weak ( $r=0.150$ ) and statistically nonsignificant (Table 3).

## **DISCUSSION**

Atopic dermatitis is a chronic-relapsing disease characterized by exacerbations for which the cause is usually not evident. For clinical evaluation of momentary disease activity SCORAD index is widely used because of its simplicity and reliability. It combines numerous symptoms of the disease. The intensity of skin symptoms accounts for 60% of the total score while subjective symptoms and extent of the disease account for 20% each (31).

Some laboratory parameters are frequently elevated in patients with AD so their correlation with SCORAD index can be assumed.

Although our results confirm data from the literature according to which patients with AD have elevated levels of eosinophils (7-9), IgE antibodies (6) and

ECP (17-27), the correlations between any of these laboratory parameters and SCORAD index were not determined.

Comparison of our correlation results with those from other authors is hard because of the differences in patients' age and/or severity of their disease. Some studies, in which severity of the disease was measured by the SCORAD method, showed positive and statistically significant correlation (25,27), others (26) gained results as we did - negative, weak and statistically not significant correlation. Positive, medium strong and statistically significant correlations were determined in studies that used COSTA (20), SIS (21) and a modified method (24) for clinical evaluation of the disease.

Lack of correlation between ECP and SCORAD can be explained by several reasons. SCORAD index is widely used for clinical evaluation of disease activity in patients with AD because of its simplicity and reliability. It reflects momentary disease state and does not give information about the severity of the disease in general. Subjective symptoms such as pruritus and sleep loss that account for 20% of the total SCORAD index are hard to evaluate in children.

The nature of data and their statistical interpretation should also be taken with certain caution. In our patients the SCORAD index ranged from 16 to 83. The large majority (45 patients) had moderate to severe clinical picture with SCORAD index over 30. Our results are probably influenced by low percentage of patients with high and low SCORAD index values. Comparing a quantitative parameter like ECP with a semi quantitative one, like SCORAD index, straight correlation is rarely observed.

The serum level of ECP is considered to express "activation" status of peripheral blood eosinophils but mechanisms of the release, however, are still not completely understood. Results of some investigations (33) conclude that the serum level of ECP represents the level found in vivo, plus additional protein released in vitro from

peripheral blood eosinophils during the coagulation period. Other investigators concluded that serum ECP level measurements are unrelated to the coagulation process (34).

It is important that recommendations for blood sample collection, handling and preparation are strictly followed. In our study recommendations of the manufacturer were strictly followed and the blood was left for coagulation for 90 minutes at room temperature before centrifugation.

ECP is generally regarded as protein specific for eosinophils. It has been documented that ECP, or protein immunologically cross-reactive with ECP, is present in neutrophilic leukocytes as well (35). Therefore, increased ECP serum levels can partly be the result of neutrophil degranulation, and influenced by skin infection often observed in patients with AD.

Clinical scoring of disease activity in patients with AD still remains the most effective and relevant method for determination of the severity and course of the disease as well as response to various treatments. Based on our results we can conclude that eosinophils and proteins from their granules, such as eosinophil cationic protein, have a role in the pathogenesis of AD. Therefore these laboratory data can give additional information on pathogenesis and inflammatory mechanisms involved (36). More investigations of correlation between SCORAD index and ECP are needed. They should include a greater number of patients with mild and severe clinical picture. If correlation exists the determination of ECP level will be justified as an additional, laboratory method in the evaluation, follow up and treatment of patients with AD.

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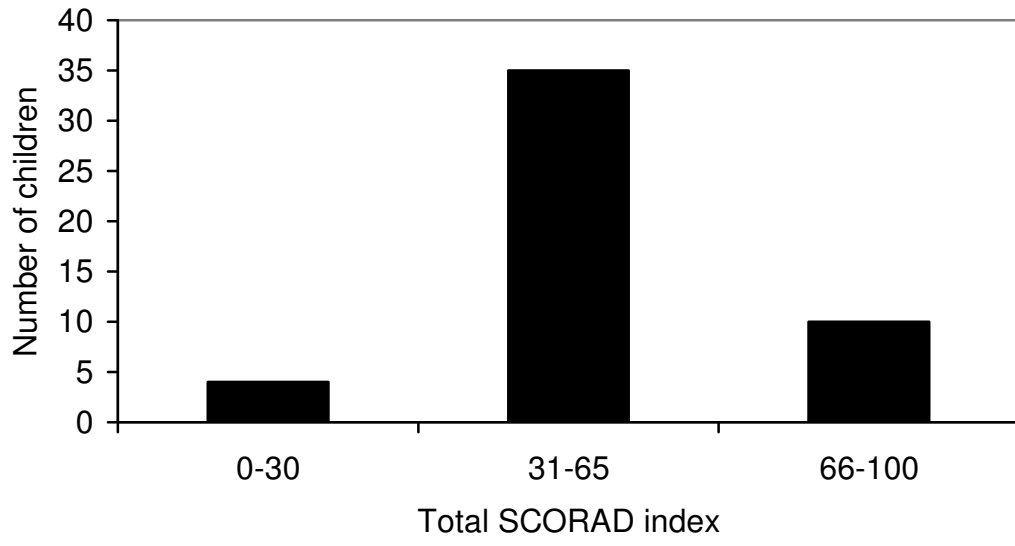




Table 1. Correlation coefficients for SCORAD index parameters and serum ECP level in children with AD

SCORAD index parameter	E C P level	
	r	p
Total SCORAD index	-0.065	0.655
Extent of skin lesions	0.079	0.586
Intensity of skin lesions	-0.095	0.515
Subjective symptoms	-0.045	0.758

Table 2. Correlation coefficients for laboratory parameters and serum ECP level in children with AD

Laboratory parameter	E C P level	
	r	p
Eosinophils (%)	0.533	0.000
Absolute eosinophil count	0.459	0.001
IgE	0.038	0.804

Table 3. Correlation coefficients for laboratory parameters and total SCORAD index in children with AD

Laboratory parameter	Total SCORAD index	
	r	p
Eosinophils (%)	-0.059	0.684
Absolute eosinophil count	-0.104	0.475
IgE	0.150	0.330