

Cytokines Levels in Children Affected by Atopic and Nonatopic Eczema

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Abstract: Patients with atopic eczema present higher serum levels of total IgE and different pattern of cytokines, compared to nonatopic eczematous patients. This study aimed to evaluate serum levels of IL-2, IL-4, IL-5, IL-10, IFN γ , TNF α in eczematous children in relation to atopic and nonatopic form. We enrolled 45 eczematous children and 12 controls. Total IgE and specific IgE against principal food and inhalant allergens were evaluated by UniCap1000 and levels of cytokines by Cytometric Bead Array System. The geometric means of levels of IL-4 were higher in eczematous patients than controls ($P=0.036$). Serum levels of IL-10 and IFN γ were higher in nonatopic eczematous children than in atopic eczematous patients and controls ($P=0.004$, $P=0.003$). Our data confirm that cytokines may play an important role in the pathogenesis of eczema: nonatopic eczema seems to be characterized by increased levels of IL-10 and IFN γ compared to atopic form.

Keywords: Atopic eczema, childhood, cytokines, IL-10, IFN γ , nonatopic eczema.

INTRODUCTION

Eczema is a chronic inflammatory skin disorder characterized by pruritic chronic lesions. Two subsets of eczema have been described: 70-80 % of eczematous patients present atopic form, with elevated serum IgE and allergic sensitization, whereas 20-30% of patients exhibit nonatopic form, with normal serum IgE levels and lack allergen-specific sensitisation.

Some immunological differences between atopic eczema and the nonatopic type have been observed in the cytokine pattern of peripheral blood and in the skin lesions, also by phenotyping characterization of epidermal dendritic cells, but few data are available [1].

Th2 cells play a key role in pathogenesis of eczema, this notion is supported by the presence of blood eosinophilia and enhanced serum IgE levels in the majority of patients affected, but recent studies suggests that both Th1 and Th2 cells may play roles in the pathogenesis of eczema. Novak *et al.* [1] show patients with atopic eczema have increased serum levels of IL-4, IL-5, while IL-13 seem to prevail in nonatopic form. Instead, Akdis *et al.* [2] show nonatopic form, compared with atopic form express significantly lower levels of IL-4 and IL-13 in their skin and peripheral blood. Shimada *et al.* [3] investigated serum levels of Th2 and Th1 chemokine pattern in patients affected by atopic eczema compared with patients with contact dermatitis and normal controls; serum chemokine levels were examined in 55 patients (22 females, 33 males, mean age: 23 years). They demonstrated increased serum levels of Th2 but also Th1 chemokines in patients affected by atopic eczema compared

with normal control subjects, suggesting that both Th1 and Th2 chemokines may be related to the development of this condition. Katsunuma *et al.* [4] investigated the relationship between Th1/Th2 serum cytokines and severity of eczema. In particular they assessed the transcription and production of IFN γ and IL-5 in peripheral blood mononuclear cells of 29 subjects: 17 patients affected by severe eczema (mean age: 15 years), 4 mild eczema (mean age: 25.8 years), 8 healthy nonatopic controls (mean age: 27.4 years). The study evidenced decreased levels of IFN γ in severe atopic eczema, suggesting that decreased production of this chemokine may account for the abnormal immunopathogenesis of severe, intractable eczema.

Data from skin biopsies obtained from uninvolved skin of eczematous patients indicate that acute skin lesions contain significantly more cells producing IL-4, IL-5 and IL-13, but not IFN γ compared with chronic lesions [5]. In chronic lesions cells expressing IL-4 and IL-13 were significantly decreased, while cells expressing IL-5 and IFN γ were increased. Antunez *et al.* [6] evaluated serum cytokines levels in patients with acute and chronic eczematous lesions; the study showed an increased percentage of T cells secreting IL-13, IL-4 in peripheral blood of patients with acute lesions there, increased serum levels of TNF α in those with chronic lesions. Cutaneous T cells of patients with nonatopic form produce similar amounts of IL-5 and IFN γ , but less of IL-4 and IL-13 than cells of atopic patients. This distinct cytokine pattern might be both cause and effect of the lower IgE levels found in patients with nonatopic eczema. Many studies [7, 8] have showed an increased expression of IL-10 in skin lesions of patients with nonatopic eczema, moreover Shin *et al.* [9] showed a correlation between increased serum levels of IL-10 and decreased total IgE among eczematous patients, suggesting a probably association between this type of eczema and increased levels of IL-10. A recent study by Jacobi [10] demonstrated that targeted therapeutic approaches, like

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inhibition of TNF α cytokine that can be detected in high concentrations in skin and serum of patients with eczema maybe a novel therapeutic option, suggesting a role of this chemokine in pathogenesis of eczema.

The aim of our study was to evaluate serum levels of IL-2, IL-4, IL-5, IL-10, IFN γ , TNF α in patients affected by eczema and their relation to atopic and nonatopic form.

MATERIALS AND METHODOLOGY

We studied 45 children (mean age: 2,5 years) affected by eczema who consecutively referred to our Centre. The diagnosis was made on the basis of Hanifin and Rajka's criteria; the evaluation of the severity of the disease was made using the SCORAD index [11]. Patients with a SCORAD < 25 were considered with mild eczema, 25-50 moderate eczema and > 50 severe eczema.

Total IgE and specific IgE antibodies against principal food (egg, cow's milk, wheat, peanut, fish) and inhalant allergens (grass pollen, *D. Pteronyssinus*, *D. Farinae*, cat, dog) were evaluated by UniCap 1000 System (Phadia, Sweden). We have considered sensitized patients with specific IgE levels >0.35 Ku/L. Atopic eczema was characterized by the presence of sIgE against food or inhalant allergens or by elevated serum total IgE levels, according to the WAO nomenclature [12].

Of the 45 children with eczema consecutively enrolled, 10 (22%) were affected by nonatopic eczema, 35 (78%) were affected by atopic eczema. Twelve healthy, non allergic children were designed as the control group. There were no differences in the age and clinical severity between the two groups of eczematous patients.

Serum levels of IL-2, IL-4, IL-5, IL-10, IFN γ , TNF α were examined by Cytometric Bead Array System that involve flow cytometry (CBA; Human Inflammation Kit, B&D™), which provides the opportunity to perform multiplex cytokine assay in a single analysis. This technique combines flow cytometry with traditional sandwich enzyme-linked immunosorbent assay (ELISA), as each bead is analogous to one coated well in an ELISA. The CBA technique uses different light intensities to label the desired molecule and the flow cytometer is then used to analyse the light intensity that reflects the concentration of each cytokine.

Cytokines levels in serum of patients with eczema were compared with cytokines levels examined in the serum of control group.

Statistical Methods

The Statistical Package for Social Science (SPSS/PC; SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The Student's t-test was used to decide the significance of the difference between the groups. Data were analyzed by looking for linear correlations between SCORAD and serum levels of cytokines and total IgE. Results were considered as statistically significant for $P \leq 0.05$.

Ethical Consideration

This research was conducted in accordance with the principles of the Declaration of Helsinki. Written informed con-

sent was obtained from parents before the children were included in the study.

RESULTS

Ten patients (22%) were affected by mild eczema, 33 (73%) were affected by moderate eczema and 2 (5%) by severe eczema. No difference in clinical severity between children affected by atopic and nonatopic eczema was found (Table 1). Fourteen patients with atopic eczema presented sIgE against foods and 23 were sensitized to inhalant allergens. The number of children with atopic eczema (n=35) with specific IgE level >0.35 kU/L against principal food and inhalant allergens are described in Table 2. The geometric means (GM) of serum cytokines levels in patients with eczema were higher than those found in normal controls; in particular serum levels of IL-4 were significantly higher in patients with eczema) than controls (2.67 pg/ml vs 0.23 pg/ml; $P=0.036$). Serum levels of IL-10 and IFN γ in children with nonatopic eczema were significantly higher ($P=0.004$, $P=0.003$) than those found in children affected by atopic eczema and controls (Table 3, Fig. (1)).

A statistically significant correlation was found between SCORAD index and IL-5 ($R=0.3$; $P=0.016$; Fig. (2)). A statistically significant correlation was found between total IgE and IL-5 ($R=0.46$; $P=0.01$).

No association was found between levels of sIgE against principal foods and inhalants and cytokines levels.

Table 1. SCORAD Index [11] in 45 Children (Mean Age: 2.5 Years Old) with Atopic (n=35; 78%) and Nonatopic Eczema (n=10; 22%). Patients with a SCORAD Index < 25 were Considered with Mild Eczema, 25-50 Moderate Eczema and > 50 Severe Eczema

SCORAD INDEX	All Children (n=45)	Nonatopic Eczema (n=10)	Atopic Eczema (n=35)
Mild (<25)	10 (22%)	2 (20%)	8 (23%)
Moderate (25-50)	33 (73%)	8 (80%)	25 (72%)
Severe (>50)	2 (5%)	0 (0%)	2 (5%)

DISCUSSION

Research during the last decade has established that several abnormalities in soluble factors, cellular characteristics, and other mediators in the blood are characteristic of the complex pathogenesis of eczema. Atopy is understood to be an imbalance between the Th2 and Th1 type immune responses, with the cutaneous inflammatory response in eczema being at least partly mediated by Th2 cytokines [1, 13, 14]. Our data showed all patients affected by eczema present serum levels of IL-4, IL-5, IL-2, IL-10 higher than normal controls, confirming that Th2 cells play an important role in the pathogenesis of this form. Katsunuma *et al.* [4] assessed the transcription and production of IFN γ and IL-5 in peripheral blood mononuclear cells of patients affected by severe eczema. They showed an opposite relation between serum levels of IFN γ and severity of lesions suggesting that production of this cytokine may account for the abnormal immunopathogenesis of severe, intractable eczema. No correla-

Table 2. Number of Children with Atopic Eczema (n=35) with Specific IgE Level >0.35 kU/L Against Principal Food and Inhalant Allergens Tested

Allergen	Number of Children with Specific IgE Level >0.35 kU/L
Food Allergen	
Egg	9
Milk	8
Peanut	2
Fish	1
Wheat	1
Inhalant Allergen	
<i>P. Pratense</i>	12
Cat Dander	10
<i>C. Dactylon</i>	9
<i>D. Pteronyssinus</i>	9
<i>D. Farinae</i>	9

Table 3. SCORAD Index, Total IgE Levels (Geometric Mean) and Serum Cytokines Levels (Geometric Mean) in 45 Children with Eczema (35 Cases of Atopic Eczema, 10 Nonatopic Eczema) and 12 Controls

	Nonatopic Eczema (10 cases)	Atopic Eczema (35 cases)	Control Group (12 Cases)
SCORAD	32.3	33	-
IgE (kU/L)	31.2	408	25
IFN γ (pg/ml)	2.52 * †	0.55 *	0.58 †
TNF α (pg/ml)	5.22	1.33	2.97
IL-10 (pg/ml)	7.42 § ‡	2.53 §	1.28 ‡
IL-5 (pg/ml)	1.67	1.73	0.22
IL-4 (pg/ml)	2.13	0.54	0.23
IL-2 (pg/ml)	1.32	0.41	0.25

*P=0.004; †P=0.004; §P=0.004; ‡P=0.003.

tion was found between the levels of IL-5 and severity of eczema. Our data, in contrast, show a statistically significant correlation between SCORAD index and IL-5 (R= 0.3, P=0.016), and between serum levels of IL-5 and total IgE; no correlation between severity of eczema and other interleukines considered. Moreover a significant correlation was found between IL-2 and total IgE levels, confirming that Th1 mechanism seems to take part in pathogenesis of eczema [15].

Our study show serum levels of IL-10, TNF α , IFN γ and IL-4 in patients with nonatopic eczema higher than those found in patients affected by atopic eczema, in particular serum levels of IL-10, and IFN γ in patients with nonatopic eczema seem to be significantly higher than those found in patients affected by atopic eczema and controls (P= 0.004),

in according to Novak and Bieber [1] that showed elevated serum IL-4 and IL-13 levels in this form suggesting the predominance of an immune response of Th2 type in this variant, but in contrast to Akdis *et al.* [2].

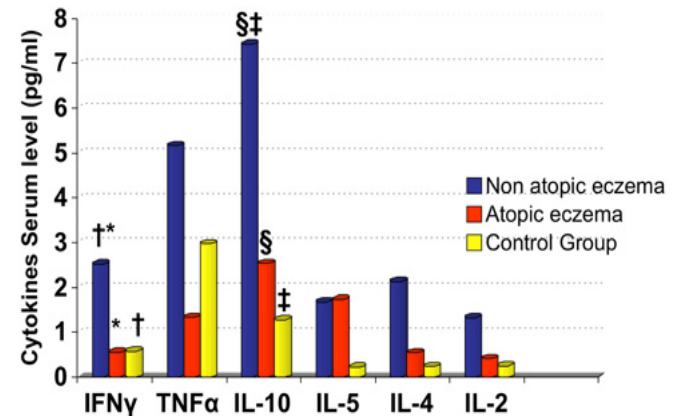


Fig. (1). Serum cytokines levels (geometric mean) in 45 children with eczema (35 cases of atopic eczema, 10 nonatopic eczema) and 12 controls. *P= 0.004; †P= 0.004; §P= 0.004; ‡P= 0.003.

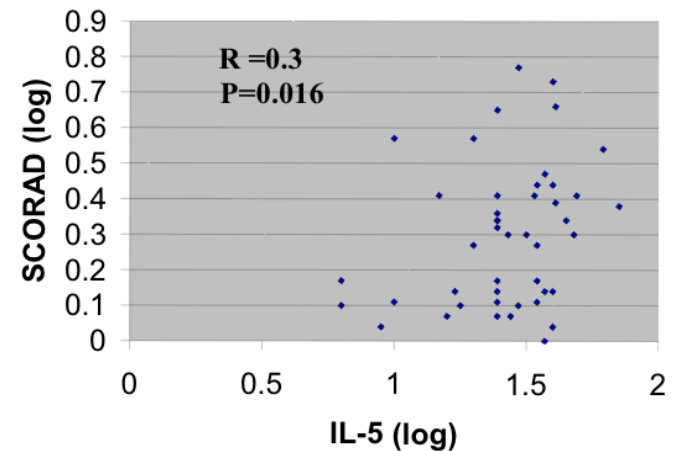


Fig. (2). Correlation between SCORAD index (log) and IL-5 (log) serum level in 45 children (mean age: 2.5 years old) with atopic (n=35; 78%) and nonatopic eczema (n=10; 22%).

Leung *et al.* [7] showed an increased expression of IL-10 in skin lesions of patients with nonatopic eczema compared with patients affected by psoriasis and controls, suggesting a probably association between nonatopic form of eczema and serum levels of IL-10. Shin *et al.* [9] scrutinized the single nucleotide polymorphisms in the IL-10 gene in serum of 334 patients (mean age: 28.9 years) affected by eczema. Subjects were categorized into three groups: low IgE group whose serum total IgE concentration was < 165 IU/ml, intermediate group whose serum total IgE concentration was between 165 and 1500 UI/ml, and high IgE group whose serum total IgE concentration was higher than 1500 UI/ml. Logistic regression analyses were performed with single polymorphisms and aptotypes to determinate their association with level of serum total IgE. The study demonstrated that the inhibition of innate immunity by increased IL-10 production might be associated with decreased total serum IgE levels among eczematous patients, suggesting an inverse association between IL-10 production and total serum IgE levels.

Howell *et al.* [16] observed significantly increased levels of IL-10 in skin lesions of patients affected by nonatopic eczema; using primary keratinocytes they found that the deficiency in anti-microbial peptide (AMP) expression is an acquired defect and showed that neutralizing antibodies to IL-10 augmented the production of TNF α and IFN γ by peripheral blood mononuclear cell from eczematous patients, suggesting that increased levels of IL-10 may contribute to the AMP deficiency by reducing cytokines that induce AMP. According to Leung *et al.* [7] our data show increased serum IL-10 levels in patients with eczema, in particular serum levels were significantly higher in nonatopic form ($P=0.004$) than atopic form and controls ($P=0.003$). No significant association was found between total serum IgE levels and IL-10. In contrast to Howell *et al.* [16] our data demonstrate that serum IFN γ levels in patients with nonatopic eczema were significantly higher than those found in patients affected by atopic eczema and controls, suggesting a probable association between IL-10, IFN γ levels and nonatopic form.

Our results show increased serum levels of TNF α in eczematous patient, in particular higher levels in nonatopic form; the importance of this cytokine has been investigated by Jacobi *et al.* [10]. In a recent study they demonstrated that TNF α can be detected in high concentrations in skin and serum of patients with eczema. They suggest that targeted therapeutic approaches, like inhibition of TNF α cytokine, that may be a novel therapeutic option in patients affected by severe eczema, suggesting the importance of this chemokine in pathogenesis of eczema, but few data are available.

We conclude that immunological differences can be detected in peripheral blood of patients affected by atopic and nonatopic eczema, in particular Th2 cells seem to detect a key role in the pathogenesis of atopic form, while nonatopic form seem to be characterized by Th1/Th2 mechanisms. Nevertheless further studies are necessary to clear the complex pathogenesis of nonatopic eczema.

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