

SERUM THYMUS AND ACTIVATION-REGULATED CHEMOKINE LEVELS IN CHILDREN WITH ATOPIC DERMATITIS

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ABSTRACT

Introduction. It is known that atopic dermatitis (AD) is characterized by infiltration of Th2 cells into affected skin. Infiltration of inflammatory cells in the tissue is mediated by specific chemokines. An important chemokine is Thymus and Activation-Regulated Chemokine (TARC). The purpose of the study is to investigate TARC levels in blood serum of children of different ages with different AD severity degrees. **Methods.** The scrutiny of immune status was conducted in 168 children with AD aged from 1 to 18 years. The inclusion criteria in the study were: age of patients from 1 to 18 years; residence in Chernivtsi region; confirmed AD. The severity of AD was set by the SCORAD Index. Serum IgE and TARC levels were determined.

Results. Often, AD has occurred in young children, particularly from 2 to 6 years of age. The most prevalent in children with AD is food sensitibilisation, which is found in 89.9% of cases. With age, the level of allergenic IgE in the blood of patients with AD and sensitibilisation increased. The average indicator in the group of children with AD was 1322.8 ± 114.6 pg / ml, while in the group of healthy children – 399.8 ± 56.4 pg / ml. TARC levels decreased gradually with the age of children and correlated with activity and severity of AD ($r = 0.58$ and 0.77 , accordingly, $p < 0.001$). The highest TARC levels are registered in patients with severe AD.

Conclusions. Biomarkers, such as TARC, can be used in pediatric practice to accurately assess the grade of invisible subclinical disorders and the severity of AD.

Keywords: atopic dermatitis, serum thymus and activation-regulated chemokine (TARC), children

Abbreviations

AD – atopic dermatitis

TARC – Thymus and Activation-Regulated Chemokine

SCORAD Index – Severity scoring of atopic dermatitis

DC – dendritic cell

IL – interleukin

LC – Langerhans cell

LDH – lactate dehydrogenase

Th – T-helper cell

TLR – Toll-like receptor

TNF – tumor necrosis factor

INTRODUCTION

In recent years, the researcher's concern in atopic dermatitis (AD) has significantly increased, due to the rapid prevalence elevation of the disease in different regions of the world [1]. AD usually presents during early infancy and childhood, but it can persist or start in adulthood. The lifetime prevalence of AD is 10-

20% in children and 1-3% in adults [2]. The research over the last decade has revealed the genetic, ecological and immunological pathogenetic factors of AD [3-5]. It has been shown that Levels of serum lactate dehydrogenase (LDH) [7] and total immunoglobulin (Ig) E [8-9] and peripheral eosinophil counts [10] are well known to be correlated with AD severity. In the search for fundamental pathogenic mechanisms and

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the options improvements for prevention and treatment, more and more attention is paid to the state of the immune system [11]. It is known that AD is characterized by infiltration of Th2 cells into affected skin. Infiltration of inflammatory cells in the tissue is mediated by specific chemokines. An important chemokine is Thymus- and Activation-Regulated Chemokine (TARC), described in 1996 [12]. The TARC receptor is CCR4, which is expressed mainly on the skin, skin lymphocyte antigen-positive (CLA+) T-cells [13]. In vitro studies have shown that TARC causes selective lymphocyte migration of the phenotype Th2 [14]. Infiltration of effector / memorizing CD4 + T-cells of Th2 phenotype in the skin or respiratory tract plays an important role in the pathogenesis of allergic diseases, such as AD [15]. Chemokine TARC belongs to the CC subfamily, whose members are synthesized in thymus and also produced by dendritic cells, endothelial cells, keratinocytes, bronchial epithelium cells and fibroblasts [16-18]. The TARC protein is a ligand for CC-chemokine receptors types 4 (CCR4) and 8 (CCR8) and it facilitates to replenishing the cell population that synthesizes these receptors, for example, Th2 cells [19-21]. Chemokine TARC and CCR4 receptor play an important role in the pathogenesis of AD, which is considered to be the 7b2-dominant disease. Recently, [21] it had been shown, that TARC levels in blood serum have been elevated in patients with AD. In addition, they had shown that TARC levels correlate with the severity of AD [22]. The results of recent studies [23-26] suggest, that chemokine TARC plays an important role in the pathogenesis of AD. Abnormally high TARC levels are considered to reflect the

presence of vicious spirals of positive feedback (Fig. 1), [26].

The purpose of the study is to investigate TARC levels in blood serum of children of different ages with different AD severity degrees.

METHODS

The scrutiny of immune status was conducted in 168 children with AD, aged 1 to 18 years, who were treated at Chernivtsi Oblast Children's Clinical Hospital during 2012-2017 years. The average age of AD patients was 7.4 ± 0.6 years, among them: 108 boys and 60 girls. The inclusion criteria in the study were: age of patients from 1 to 18 years; residence in Chernivtsi region; confirmed AD. The exclusion criteria were: age of children under 1 year; combined allergic and other pathology of inflammatory genesis, use of corticosteroids and antihistamines during the last two weeks before the study. The control group consisted of 60 practically healthy children. The criteria for inclusion of children in the control group were: the absence of an allergic disease, not-burdened hereditary history of atopy; the absence of chronic and infectious diseases for 3 months before the survey; compliance with the normal parameters of the general analysis of blood and urine in the age.

Diagnosis of AD in children was exposed according to the diagnostic criteria of J. M. Hanifin and G. Rajka [27]. Children were divided into groups according to the severity of the clinical course of the disease (mild, moderate, severe). The severity of AD was set by the SCORAD Index [28]. The intensity of clinical

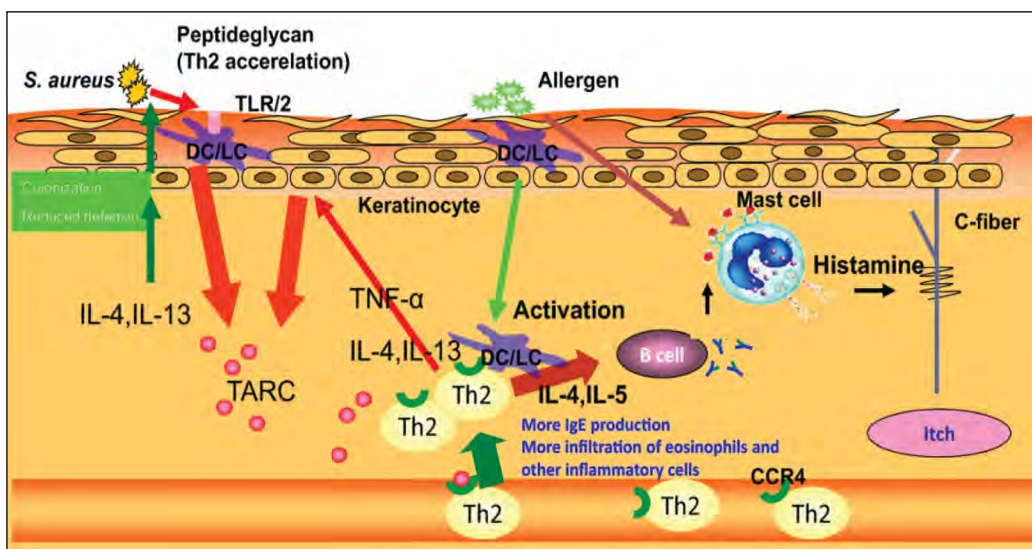


FIGURE 1. Speculated mechanism underlying the abnormally high serum thymus and activation-regulated chemokine (TARC) levels characterized by vicious inflammatory spirals of positive feedback. DC, dendritic cell; IL, interleukin; LC, Langerhans cell; LDH, lactate dehydrogenase; Th, T-helper cell; TLR, Toll-like receptor; TNF, tumor necrosis factor [26]

manifestations was scaled from 0 to 3 points: 0 – absent, 1 – weak, 2 – moderately expressed, 3 – acutely expressed. Prevalence was evaluated according to the rule of “nine”, where the area of the palmar surface of the wrist is taken as unit. The SCORAD Index formula is: $A/5 + 7B/2 + C$. In this formula, “A” is defined as the extent (0-100), “B” is defined as the intensity (0-18) and “C” is defined as the subjective symptoms (0-20). The maximal score of the SCORAD Index is 103. Children were divided into groups according to the severity of the clinical course of the disease (light – up to 20 points, moderate – 20-40 points, severe – more than 40 points). Medical intervention included a collection of 3-5 ml of blood from the elbow vein by the single use system BD Vacutainer Safety-Lok (Bectar Dickinson and Company, USA). The IgE level was determined by an indirect immunofluorescence method using an auto-analyzer, and the test was considered positive at $IgE > 0.35kE/l$.

TARC measurements in serum were performed by a plate (Becton Dickinson Franklin Lakes, NJ), coated with mouse monoclonal antibodies against human TARC (MAB364, R & D Systems, Abingdon, UK). The optical densities were measured at 450 nm using Bio-Rad microplate reader (Biorad Laboratories, Inc., Hercules, CA). A recombinant human TARC (364-DN, R & D Systems) was used as a standard. The concentration was calculated according to the standard curve, obtained by the curve selection program. The minimum TARC level determined was 20 pg/ml. Indicators were considered normal: Infant 1-2 years: < 998 pg/ml, Child 2-18 years: <743 pg/ml [29]. The statistical processing of the study results were carried out by standard algorithms of variation statistics, for calculations were used the Excel software (Microsoft Office, USA), Statistica 6.0 and the on-line SISA calculator (Simple Interactive Statistical Analysis), using correlation and parametric analysis. Average values were given as $(M \pm m)$, where «M» is the average value of the indicator, «m» is the standard error of the average; «n» – the volume of the analyzed group. Data was analyzed using the Mann-Whitney U-criterion. Correlation coefficients were determined using Spirman’s rank correlation test. All comparisons were 2-sided. The values $p < 0.05$ were considered statistically significant.

RESULTS

Often, AD has occurred in young children, particularly from 2 to 6 years of age. According to the results of the study, the most prevalent in children with AD is food sensibilisation, which is found in 89.9% of cases. Almost half of the patients were sensitive to home

dust and bacterial allergens, one third – to the medicinal ones; and about one quarter – to the pillow feathers, epidermal and pollen allergens, and in 48.2% of cases – in combination with each other (Fig. 2).

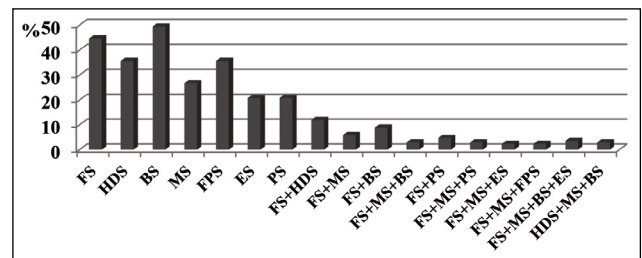


FIGURE 2. FS – food sensibilisation, HDS – home dust sensibilisation, BS – bacterial sensibilisation, MS – medical sensibilisation, FPS – feather pillow sensibilisation, ES – epidermal sensibilisation, PS – pollen sensibilisation

With age, the level of allergenic IgE in the blood of patients with AD and sensibilisation increased (Table 1).

TABLE 1. Age dynamics of IgE parameters in the blood of examined children

Indicator	Age (years)			
	1-6	7-10	11-14	15-18
IgE, kO/l	80.6±19.1	106.6±23.1	234.9±22.8*	312.5±33.9**,**

Note: *the indicator difference in groups of 7-10 years and 11-14 probable, **the indicator difference in groups of 11-14 years old and 15-18 probable, $p < 0.05$.

Most children under observation had a light or moderate severity of AD. The distribution of children is presented in Table 2.

TABLE 2. Distribution of children with AD according to age and severity of AD

Age (years)	Severity degree of AD (Number)			Total
	light	moderate	severe	
1	4	2	2	8
2	5	3	2	10
3	8	5	4	17
4	8	5	3	16
5	6	6	3	15
6	7	3	2	12
7	5	6	0	11
8	4	3	3	10
9	4	3	2	9
10	5	3	2	10
10	3	4	3	10
11	4	2	2	8
12	3	3	1	7
13	4	1	1	6
14	3	2	0	5
16	2	2	0	4
17	3	2	0	5
18	4	1	0	5
Total	80	56	30	168

TARC levels in serum were increased in all AD patients with a statistically significant ($p < 0.001$) compared to healthy children (Table 3). Thus, the average indicator in the group of children with AD was 1322.8 ± 114.6 pg/ml, whereas in the group of healthy children – 399.8 ± 56.4 pg/ml. The levels of TARC decreased gradually with the age of children. The highest TARC levels were found in children with AD of the first year of life (from 1,356 to 1,848 pg/ml, with an average value of $1,655 \pm 125.6$ pg/ml).

TARC levels in serum correlated with activity and severity of AD ($r = 0.58$ and 0.77 , respectively, $p < 0.001$). The highest TARC levels were recorded in patients with severe AD (Table 4, Figure 3). In children with AD and sensibilisation to certain factors, TARC levels were higher than those in children with AD, but without definite sensibilisation.

TABLE 3. TARC levels (pg/ml) in blood serum of children with AD

Age (years)	Children with AD (n=168)	Healthy children (n=60)
1	1,655±125.6*	655±25.6
2	1,497±134.6*	643.8±12.9
3	1,346.2±112.5*	605.5±32.6
4	1,309.5±105.1*	554.3±21.7
5	1,295.7±123.7*	505.8±30.4
6	1,264.1±99.3*	493.2±12.6
7	1,256.8±125.8*	455.5±26.3
8	1,250.9±95.6*	412.9±25.7
9	1,207.7±102.6*	399.8±19.6
10	1,200.9±98.9*	387.2±25.3
10	1,189.4±112.6*	379.9±17.6
11	1,178.6±121.2*	378.8±19.1
12	1,189.3±93.6*	370.0±19.6
13	1,099.4±89.8*	367.4±25.6
14	1,110.7±101.1*	359.8±18.7
16	989.3±85.6*	345.6±12.9
17	967.9±97.7*	308.8±21.1
18	912.8±76.6*	299.4±16.6

Note: * $p < 0.01$.

TABLE 4. TARC levels (pg / ml) in blood serum of children with AD

Index	Severity degree of AD		
	light (n=80)	moderate(n=56)	severe (n=30)
TARC, pg/ml	956.6±76.1*	1,366.9±67.1*	1,646±54.6**

Note: * $p < 0.01$ ** – $p < 0.001$.

DISCUSSIONS

The results of our and other [30-31] studies indicate a dynamic increase in the prevalence of AD. In our studies, we confirmed the recommendations [32] to grade the severity of AD, while agreeing that the



FIGURE 3. A 6-months-old boy with severe atopic dermatitis. His serum thymus and activation-regulated chemokine level were 1684 pg/ml

best tool for assessing clinical signs of AD is the SCORAD scale. Other authors recommend using for children with eczema paediatric version- ISS and also Patient-Oriented Eczema Measure (POEM), Patient-Oriented SCORing Atopic Dermatitis (PO-SCORAD), Self-Administered Eczema Area and Severity Index (SA-EASI) and adapted SA-EASI, which are currently the most appropriate instruments and therefore have the potential to be recommended as core symptom instrument in future clinical trials. These findings will be utilized for the development of a core outcome set for atopic eczema [33-34]. Attention is paid to assessing the quality of sleep in patients with AD [35]. In recent decades, a large number of studies have been published to investigate the connection between AD severity and various biomarkers, the TARC level in particular [36-37]. This indicator is important for assessing the severity of AD because of the diversity of clinical manifestations and the complexity of their assessment in childhood. Even in a healthy population, TARC levels in serum are higher in children than in adults, according to the studies [38]. We conclude that, if the initial skin lesions are localized, high TARC levels in serum indicate a strong disease activity and this may lead to immediate eczema development and increased Ig E levels with multiple sensibilisation to different factors. The levels of TARC in cord blood serum can predict the development of AD in childhood [39]. However, the issue of defining TARC as an absolute biomarker of AD is currently under discussion. The feature of elevated TARC level in serum is not specific to AD, because its levels change with other skin lesions [40-42]. In our opinion, TARC levels can be used as a marker of predicting the development and severity of AD. Persistent high levels of serum-like TARC may indicate inadequate treatment, but more clinical studies are required.

CONCLUSIONS

Nowadays, for successful treatment of atopic dermatitis a certain and objective assessment of clinical manifestations is required. TARC can be considered as a useful clinical biomarker that can be used in pediatric practice to accurately assess invisible subclinical

disorders, assess the severity of atopic dermatitis and to predict its course. Further studies of TARC levels in tissues and peripheral blood in patients with atopic dermatitis may help to explain the role that this chemokine plays in the pathogenesis of the disease and pave the way for further therapeutic tactics.

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