

CYTOKINES PROFILE (IL-4, IL-13) OF CHILDREN WITH SEVERE FORMS OF ATOPIC DERMATITIS VERSUS OTHER GRADES OF SEVERITY

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Atopic dermatitis (AD), or atopic eczema, is chronic recurrent inflammatory skin disease that often manifests in the first year of life and affects up to 12-20% of children [6, 9]. It's prevalence is steadily increasing worldwide. Development of skin inflammatory process in patients with AD is caused by a complex interactions of genetic mechanisms, environmental factors, infectious agents, skin barrier defects and immune mechanisms [3].

Atopy is a genetic predisposition to develop allergic symptoms caused by environmental antigens (allergens). Numerous studies indicate that two of the key cytokines are interleukin-4 (IL-4) and IL-13 [1, 3]. They are potent mediators of the induction and effector phases of the Th-2 immune response. IL-4 is a differentiation factor that polarizes CD4 + cells to the Th2 phenotype, a growth factor for B cells, an inducer of IgE isotope class transition, as well as an activator of eosinophil chemotaxis [5].

Early studies strongly suggested that AD is also linked to Innate lymphoid cells2 (ILC2) dysregulation. ILC2 express pro-allergic mediators IL-4, IL-5, IL-9, IL-13 [1, 16]. It was proved that in comparison to CD4+, ILC2 are capable of producing IL-5 and IL-13 cytokines per-cell in case of allergic inflammation against house dust-mite independently. IL-4, produced by ILC2, induces mast cell responsiveness to degranulation and inhibits Tregs induction, it decreases tolerance that may influence on food allergy (FA) development. Simultaneously, ILC2 interact with CD4+ Th2 cells to induce FA by IL-13 production [12]. So, a deeper study of ILC2 inhibiting pathway may promote searching of new target therapy opportunities [11].

As it has been shown, IL-4 has several functions in acute inflammation period of AD. Increased expression of IL-4 in the epidermis contributes to the activity of inflammation, which leads to manifestation of itching and intradermal edema. IL-4 can induce gene suppression (such as filaggrin, loricrin), that are responsible for skin barrier function [6, 14]. In addition, IL-4 stimulates normal keratinocytes, it leads to increase of serine proteases activity, which promotes skin desquamation and increases transepidermal water loss (TEWL) [7].

IL-4 and IL-13 have 20-25% sequence homology and effector function. IL-13 affects synthesis of desmosomes proteins, increases the infiltration of skin by inflammatory cells, promotes skin desquamation and increases TEWL [4, 7]. In chronic course of AD IL-13 is thought to be responsible for such symptoms as itching [8]. In animal models, increased expression of IL-13 in the skin induced itching, increased IgE levels, and eosinophil infiltration [19].

Previously, IL-4 was thought to be one of the major cytokine involved in the development of AD because of its IgE synthesis regulation. Recently, however, scientists have suggested that IL-13 is considered to be more significant in type 2 inflammation than previously has been thought [13]. Researchers have assumed that IL-13 promotes inflammation in the periphery, and IL-4 has a central effect. Up to date studies have shown a significant data of increased cytokine IL-13 expression in AD patients (in affected skin areas) and normal IL-13 level in healthy people, however, there were no significant changes in IL-4 levels [1]. Elevated levels of IL-13 were correlated with the severity of AD due to the SCORAD scale [2, 9]. These assumptions are still being discussed by scientists, since the role of IL-4 and IL -13 in the development of AD is confirmed by rather high efficiency of monoclonal antibodies (against IL -4, IL-13) treatment of this

disease [5, 10]. Despite the fact that treatment in early stages of development of AD can stanch the patient's condition in adulthood, early onset of AD is still a subject of debates. Changes of skin barrier structure and characteristics of the immune response in early onset are likely to differ from adults. Hitokazu Esaki et al. in their study review distorting of Th-2 in patients with early-onset of AD, which restricts research of targeted therapy [15].

The aim was to study serum levels of cytokines IL-4 and IL-13 and its' possible relation to immunophenotypes of T-lymphocytes and levels of total serum IgA, IgM, IgG in children with atopic dermatitis.

Materials and methods. The study involved 85 patients aged 3 months to 3 years with a verified diagnosis of AD. The study was conducted in Department of Faculty Pediatrics, Zaporizhzhia state medical university and Municipal non-profit enterprise "Children City Hospital № 5" of Zaporizhzhia City Council, Ukraine. 21 children without history of atopy were examined as a control group. Children with AD were divided into 3 groups (I, II, III) due to the SCORAD severity scale. Then additionally children with severe manifestations of dermatitis were divided into 2 subgroups (IIIa, IIIb). Children of the IIIb subgroup had total decrease of total IgA, IgM, IgG levels ($< 6,0$), low levels of CD3+, CD 56+ T-lymphocytes in comparison to the reference ranges. The study was conducted after obtaining informed consent that complies with generally accepted ethical standards. Interview questionnaires were used to study the anamnesis. AD was diagnosed according to the Hanifin and Rajke criteria, the severity of AD was measured by the SCORAD index. All patients were prescribed standard treatment per protocol which included emollients, steroid ointment, eliminative diet if needed. Blood samples were collected twice (at exacerbation and remission period) in vacutaner (EDTA) and immediately centrifuged (4°C for 3.000×30 min). ELISA method was used for the detection of levels of IL-4, IL-13 (Human IL-13 ELISA Kit and Human IL-4 Hight Sensitivity ELISA Kit, Thermo Fisher Scientific, Austria). Immunological parameters were analysed automatically by flow cytometry («Synevo» laboratory). Statistical processing of the results was carried out using the Statistica 13.0 official software package.

Results. No significant difference was found in immunological parameters (C3, C4-2, CD3+, CD19-, CD4+, CD8-, CD4-, CD8+, CD3-, CD56-, CD3-, CD19+, CD14, CD45 T-lymphocytes, C1Cs) due to reference levels, except total IgA, IgM, IgG levels, CD3+, CD 56+ T-lymphocytes. Children with decreased levels of these indicators had severe grade of the course of AD and were assigned to group IIIb.

Study of IL-13 cytokine level revealed a significant difference between children of I, II, IIIa groups and control group (table 1).

Table 1.

IL-13 cytokine levels in study groups, Me (Q25; Q75).

Marker, units	Group of patients	
	I, II, IIIa (n=70)	Control (n=21)
IL-13, pg/mL	19,95* (10,58; 35,64)	4,77 (1,58; 15,87)

*significance (Mann-Whitney criteria) $p < 0,001$.

It was found that level of IL-13 correlated with SCORAD scale severity of AD ($r = +0,73$; $p < 0,05$). The highest levels of IL-13 were detected in serum of patients with normal levels of CD3+, CD 56+ T-lymphocytes and total IgA, IgM, IgG levels and the highest severity of AD symptoms. The same time children with severe AD but low levels of CD3+, CD 56+ T-lymphocytes and total IgA, IgM, IgG levels had the lowest levels of serum IL-13 in compare with mild to moderate forms (table 2).

Table 2.

IL-13 cytokine levels depending on AD severity, Me (Q25; Q75).

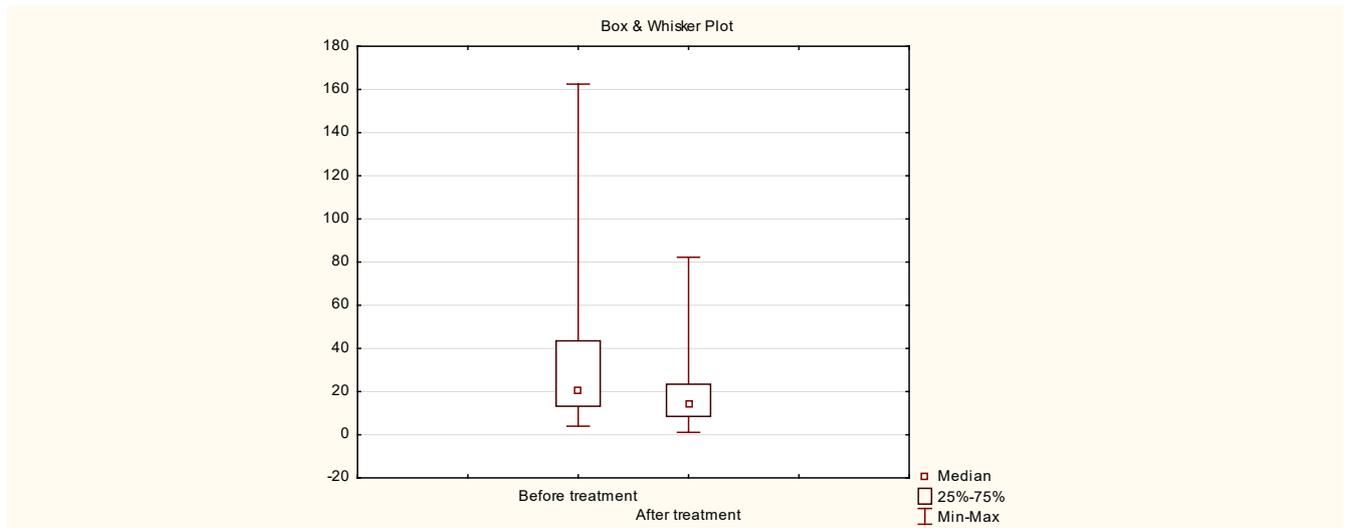
Marker, units	Subgroups in the main group of patients (n=85)			
	I (n=23)	II (n=28)	IIIa (n=19)	IIIb (n=15)
IL-13, pg/mL, before treatment	8,58 (5,29; 12,66)	20,60 (13,88; 26,56)	74,36* (29,84;148,28)	1,1 (0,42;1,96)
IL-13, pg/mL, after treatment	5,74 (3,42;10,52)	13,76 (8,58;18,88)	23,75 (14,08;35,34)	4,62 (2,20;6,50)

*- significance of the difference between groups I, II, IIIa (Kruskal-Wallis criteria) $p < 0,001$.

As it can be seen on fig. 1, treatment of AD resulted in significant decrease of IL-13 serum levels in all groups of patients except IIIb.

Figure 1.

Evaluation of the ratio of cytokine levels of IL-13 before and after treatment in groups I, II, IIIa.

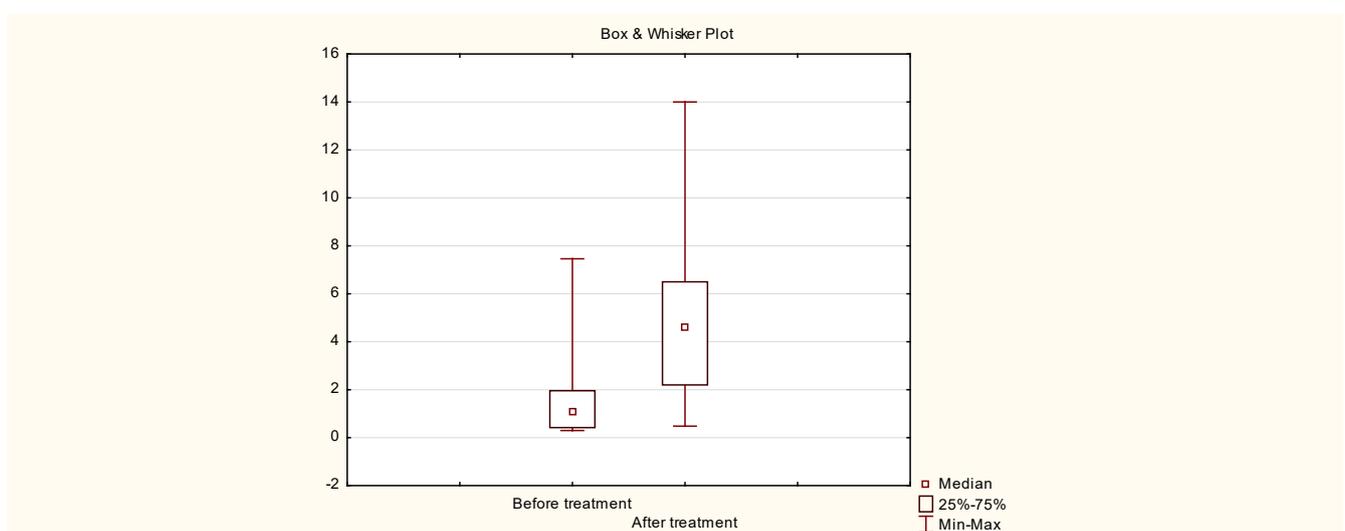


*- significance (Wilcoxon criteria) $p < 0,001$.

As results showed, children with severe AD and low CD3+, CD 56+ T-lymphocytes and total IgA, IgM, IgG had significant increase of IL-13 levels after treatment, which remained still lower than IL-13 levels in other groups and was approximately the same as in control ($p < 0,05$) (fig.2).

Figure 2.

Evaluation of the ratio of cytokine IL-13 levels before and after treatment in group IIIb.



* - significance (Wilcoxon criteria) $p = 0,002$.

As it was expected, level of IL-4 was significantly higher in children with AD versus to the control group (table 3).

Table 3.

IL-4 cytokine levels in study groups, Me (Q25; Q75).

Marker, units	Group of patients	
	I, II, IIIa (n=70)	Control (n=21)
IL-14, pg/mL	0,3 (0,08; 0,5)*	0,1 (0,02; 0,38)

*- significance (Mann-Whitney criteria) $p < 0,02$.

The results showed that IL-4 levels were significantly different in children with different grades of severity of AD. But patients with severe forms had levels as control group (table 4).

Table 4.

IL-4 cytokine levels depending on AD severity, Me (Q25; Q75).

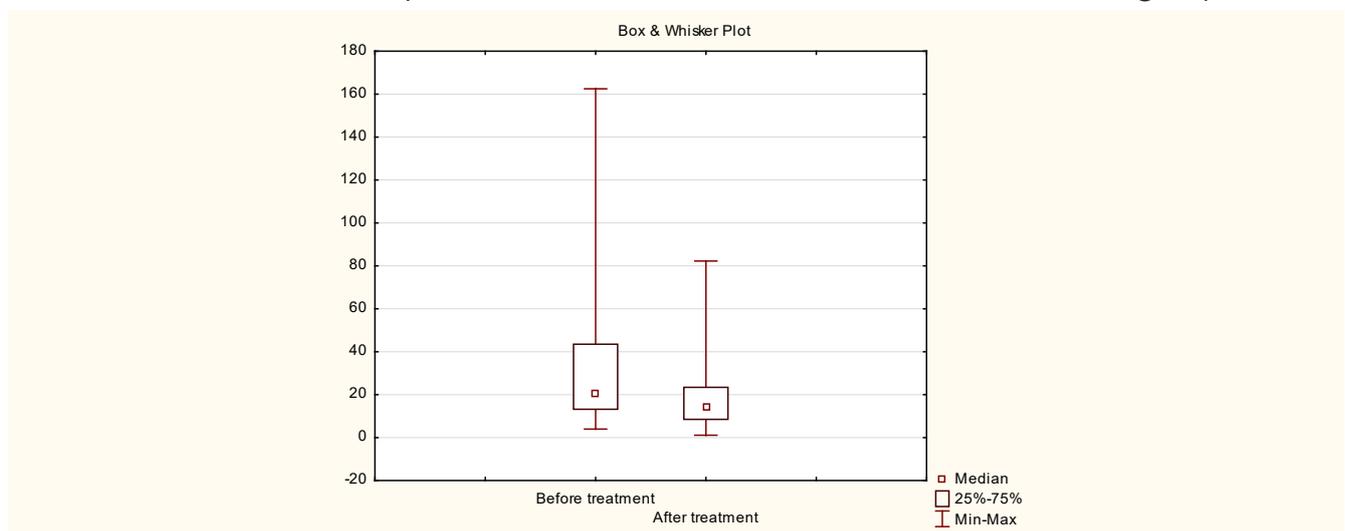
Marker, units	Subgroups in the main group of patients (n=85)			
	I (n=23)	II (n=28)	IIIa (n=19)	IIIb (n=15)
IL-4, pg/mL, before treatment	0,26 (0,04; 0,42)	0,42* (0,12; 0,56)	0,14 (0,08; 0,49)	0,12 (0,04; 0,048)
IL-4, pg/mL, after treatment	0,08 (0,02; 0,34)	0,12 (0,02; 0,32)	0,08 (0,04; 0,1)	0,42 (0,1; 0,46)

*- significance between groups I, II (Kruskal-Wallis criteria) $p < 0,001$.

Fig. 3 presents data on the dynamic changes of IL-4 levels after treatment. IL-4 levels decreased significantly in groups I, II, IIIa. Interestingly that no significant changes of IL-4 levels were detected after treatment in subgroup IIIb ($p=0,1$) (table 4).

Figure 3.

Evaluation of the ratio of cytokine levels of IL-4 before and after treatment in groups I, II, IIIa.



*- significance (Wilcoxon criteria) $p < 0,001$.

As it was found, the higher was severity of AD, correlated with SCORAD, the higher was level of IL-13 ($r=+0,73$; $p < 0,05$). But children with low CD3+, CD 56+ T- lymphocytes and decreased serum total IgA, IgM, IgG levels and severe course of AD had IL-13 levels comparable with control group. This finding indicated possible role of low CD3+, CD 56+ T- lymphocytes and B-lymphocytes in IL-13 production. Or may be result of low IL-13 level. The same data were published by Metwally S.S. et al, 2004, who reported higher expression of IL-13 mRNA in patients with severe forms of AD [18]. Study of animal models demonstrated that IL-13 deficient mice had elevated IL-5 skin mRNA levels

[17]. In this regard it is possible to suppose that patients in our study subgroup IIIb are thought to have other mechanisms of AD symptoms, which differs from others. This suspicion was supported by our results of IL-4 levels study. It was found that IL-4 level in subgroup IIIa and IIIb did not differ from control group. This corresponds to the previously suggested relation of severe forms of AD with mechanisms of allergy dependent on other cytokines (IL-5). The underlying arrangement of AD in children remains unclear, but studies display that levels of IL-13 and IL-4 can depend on activation of ILC2 or elevated IL-5 skin mRNA levels. Prospective studies of the characteristics of the immune response in early onset of AD may prompt to take a fresh look at targeted therapy in this age group.

Conclusion. Levels of IL-4, IL-13 were controversial depending on grades of severity. Children with severe course of AD with normal levels of total had serum total IgA, IgM, IgG, CD3+, CD 56+ T- lymphocytes had higher levels of IL-13 and lower levels of IL-4 in comparison with mild to moderate forms ($p < 0,05$). Children with severe AD and decreased levels of total IgA, IgM, IgG, CD3+, CD 56+ T- lymphocytes had lower levels of IL-13, IL-4 than children with mild to moderate forms of AD and control group ($p < 0,05$).

Conflict of interest. The authors declare that there is no conflict of interest regarding the publication of this article.

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