

ORIGINAL PAPER

IMMUNOLOGICAL PARAMETERS AND CORTISOL LEVELS IN CHILDREN WITH ATOPIC DERMATITIS

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ABSTRACT

Introduction. The role of individual cytokines in the development of various clinical forms of atopic dermatitis (AD) and the effect of cortisol on this process in children have not been sufficiently studied.

Objective. To study the nature of cellular and humoral immunity disorders and the content of cytokines and cortisol in children with AD.

Methods. The study involved 168 children, aged 1 to 18 years, with AD, in whom their immune status was determined. CD3, CD4, CD8 CD16 CD19 were determined by flow cytometry, the concentration of IgA, IgM, IgG, IgE, IL-2, IL-4, IL-6, IL-10, INF- γ , and cortisol by the ELISA method. The Excel program, Statistica 6.0 and the on-line SISA calculator were used. Average values are given in the form ($M \pm m$), where M is the average value of the indicator, m is the standard error of the mean; n – the volume of the analyzed group.

Results. The total number of leukocytes in children with AD did not exceed the norm, but it was likely to increase in case of a severe course. In all age groups

RÉSUMÉ

Paramètres immunologiques et niveaux du cortisol chez les enfants avec dermatite atopique

Introduction. La contribution des cytokines individuelles dans la formation de différentes formes cliniques de la dermatite atopique (AD), les effets du cortisol sur ce processus chez les enfants ont été insuffisamment étudiés.

Le but de l'étude. L'investigation des violations de l'immunité cellulaire et humorale et le contenu de cytokines et de cortisol chez les enfants atteints de la MA.

Méthodes. 168 enfants âgés de 1 à 18 ans atteints de BP ont été examinés. La détermination de CD3, CD4, CD8 CD16 CD19 a été réalisée par la cytofluorométrie d'écoulement, la concentration des IgA, IgM, IgG, IgE, l'IL-2, IL-4, IL-6, IL-10, INF- γ , Cortisol ELISA.

Le programme Excel, Statistica 6.0 et le calculateur SISA en ligne ont été utilisés. Les valeurs moyennes présentées sous la forme de ($M \pm m$), où M – le taux moyen, m – erreur standard de la moyenne; n – le volume du groupe analysé.

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and at varying severity of AD, there was a tendency to lymphocytosis, with severe course of the disease – to neutrophilia ($p < 0.05$), an increase in the concentration of CD19 and CD16 ($p < 0.05$). In severe forms of AD, higher IL-4, IL-6, IL-10 and lower IL-2 and INF- γ were reported. Proliferation to dysimmunoglobulinemia was revealed: reduction of serum IgA concentration, increase of serum IgE and IgG concentrations. Concentration of cortisol decreased in boys with AD and increased in girls.

Conclusion. In the immunopathogenesis of AD there is an imbalance between individual subpopulations of T- and B-lymphocytes, changes in the differentiation of T-lymphocytes and their profile of cytokine secretion, dysimmunoglobulinemia, signs of adrenal cortex dysfunction.

Keywords: atopic dermatitis, T- and B-lymphocytes, immunoglobulins, cortisol.

Abbreviations: AD – atopic dermatitis, subpopulation of T- and B-lymphocytes – CD₃, CD₄, CD₈, CD₁₆, CD₁₉, IRI – immunoregulatory index (CD₄/CD₈), IgA, IgM, IgG, IgE – immunoglobulins, IL-2, IL-4, IL-6, IL-10 – interleukins, INF- γ – interferon-gamma.

INTRODUCTION

The importance of atopic dermatitis (AD) is due to:

- the epidemiological aspect. The AD incidence in children ranges between 10% and 20% and continues to grow steadily^{1,3}. Persistent atopic dermatitis is found in 50% of adult patients diagnosed at school age⁴;
- the psychosocial aspect: AD, as a chronic disease, significantly impairs the quality of life of the patient and his family, leads to disorders in the emotional sphere and social maladaptation⁵;
- the scientific and practical medical aspects: the pathogenesis of AD can not be considered to be finally elucidated, the current methods of treatment do not ensure full recovery;
- the economic aspect: treatment per a patient with mild, moderate or severe AD costs 330, 818 and 1255 Australian dollars per year respectively and this is higher than the cost of treating children with insulin-dependent diabetes mellitus and asthma⁶.

The research over the last decade has identified genetic, environmental and immunological pathogenetic factors of AD⁷⁻¹². The factors involved in AD development are shown in the figure 1¹². More and more attention is paid to the immune system condition while searching after fundamental pathogens

Résultats. Le nombre total de globules blancs chez les enfants atteints de la MA ne dépasse pas la norme, mais a augmenté de manière significative en cas d'une évolution sévère. Dans tous les groupes d'âge et à différents degrés de gravité de la MA on a observé une tendance à la lymphocytose, une maladie grave la neutrophilie ($r < 0,05$), des concentrations croissantes de CD19 et CD16 ($r < 0,05$). Dans les formes sévères de l'AD on a rapporté des niveaux élevés des IL-4, IL-6, IL-10 et diminués des IL-2 et INF- γ . Une prolifération avec tendance à la dysimmunoglobulinémie a été soulignée: la réduction de l'IgA dans le sérum, les concentrations sériques d'IgE et d'IgG augmentent. La concentration de cortisol chez les garçons avec hypertension artérielle abaissée, chez les filles avec hypertension artérielle sévère – a augmenté.

Conclusion. Dans l'immunopathogenèse de l'AD il y a un déséquilibre entre les différentes sous-populations de lymphocytes T et B, des changements dans la différenciation des lymphocytes T et leur profil de sécrétion de cytokines, des signes de dysimmunoglobulinémie, de dysfonctionnement du cortex surrénal.

Mots-clés: dermatite atopique, lymphocytes T et B, immunoglobulines, cortisol.

mechanisms and improvement of prevention and treatment options¹³. According to modern concepts of AD pathogenesis, an important place in the functioning of the immune system in this pathology is given to the indices of the cellular level of immunity – T cells with helper activity, which produce cytokines with a multi-directional action. The most important value in the regulation of the immune response in AD is given to the cytokines. The changes in the cytokines system reflect an imbalance between different populations of the immune system. The immune response mediated by type 2 T-helper cells (type 2 immunity) predominates in the skin inflammation¹⁴.

It has been proved that the difference in the immune response by atopic and nonatopic types is determined by the function of T-cell populations. The memory cell population stimulated by the antigen can direct the CD4 T-cell response of the body to the Th1 or Th2 pathway.

THE OBJECTIVE was to study the nature of cellular and humoral immunity and the content of cytokines and cortisol in children with atopic dermatitis.

MATERIAL AND METHODS

The study of the immune status involved 168 AD children aged between 1 and 18 years, who were

treated in Chernivtsi Regional Clinical Hospital between 2012 – 2017. The average age of the patients with AD was 7.4 ± 0.6 years. The study included 108 boys and 60 girls. The inclusion criteria were: age of patients from 1 year to 18 years; residence in Chernivtsi region; confirmed diagnosis of AD. The exclusion criteria were: age of children under 1 year; combined allergic and another pathology with inflammatory genesis. The control group consisted of 30 practically healthy children. The criteria for including children in the control group were: the absence of an allergic disease, non-burdened hereditary history of atopy; the absence of chronic diseases; absence of infectious diseases within 3 months before the survey; compliance of the full blood and urine count with the age norm.

AD in children was diagnosed according to diagnostic criteria of Hanifin and Rajka (1980)⁶. Children were divided into groups, according to the severity of the clinical course of the disease (mild, moderate, severe). The severity of AD was determined using the SCORAD-TIS (Scoring of Atopic Dermatitis – The Item Severity) dermatologic index. Only objective findings were evaluated determining the index by the formula: $SCORAD-TIS = A / 5 + 7B / 2$, where: A – prevalence of skin lesions, B – intensity of clinical manifestations. 6 signs of the intensity of lesions in the form of erythema, edema/papules, mycosis/crust, exacerbation, lichenification, dryness on a scale from 0 to 3 points were taken into account: 0 – absence, 1 – weakly expressed, 2 – moderately pronounced, 3 – severe. The prevalence was evaluated according to the Wallace rule of nines, where the area of the palmar surface of the hand was taken as a unit.

The cellular immunity values (CD_3 , CD_4 , CD_8 , CD_{16} , CD_{19}) were determined using the flow cytometry method. (Epics XL-MCL, «BeckmanCoulter», USA). The following rates were taken as normal: $CD_3 = 61.0-85.0\%$, $CD_4 = 35.0-55.0\%$, $CD_8 = 19.0-35.0\%$, $CD_{16} = 12.0-17.0\%$, $CD_{19} = 7.0-17.0\%$. The immunoregulatory index was calculated (IRI): CD_4/CD_8 . The value 1.5-2.1 was considered to be normal. The concentration of the serological IgA, IgM, IgG was determined by the method of competitive immunoenzyme analysis (ELISA), while IgE was measured using the double-antibody method of ELISA. The following values were considered to be normal: IgA = 1.25-2.5 g/L, IgG = 7.5-18.0 g/L, IgE: < 6 months = 12 IU/mL, 6-12 months = 30 IU/mL, 1-3 years = 45 IU/mL, 4-6 years = 70 IU/mL, 7-9 years = 90 IU/mL, 10-15 years = 120 IU/mL, > 15 years = 130 IU/mL. The concentration of IL-2, IL-4, IL-6, IL-10 and INF- γ in serum was determined by the solid-phase double-antibody method of ELISA. The following values were considered to be normal: IL-6 = 0-10 ng/L, IL-10 =

0-31 ng/L, INF-1, IL-6 = 0-10 ng/L, IL-6 = 0-10 ng/ γ = 0-10 ng/L. The levels of cortisol in the serum were determined by the method of solid-phase double-antibody method of ELISA (norm: 155-660 nM/L).

All studies were conducted after the informed consent was signed by the children (aged over 6 years) and their parents. The work follows the ethical principles of the people who act as subjects of the study taking into account the main provisions of the ICH GCR and the Helsinki Declaration of the World Medical Association for Biomedical Research, where a person acts as their object (World Medical Association Declaration Helsinki 1964, 2000, 2008), The Council of Europe Convention on Human Rights and Biomedicine (2007)

Statistical processing of the findings was carried out using standard algorithms of variation statistics, a computer program Excel (Microsoft Office, USA), Statistica 6.0 was used for calculations as well as on-line calculator SISA (Simple Interactive Statistical Analysis) using correlation and parametric analysis. The average values are presented in the form of ($M \pm m$), where M – average value, m – standard error of the average; n – number of people in the experimental group.

RESULTS AND DISCUSSION

The results of the examination of the cellular level of immunity in children with AD were evaluated and compared, depending on the severity of the clinical course of the disease. The total number of leukocytes in children with AD did not exceed the norm, but it was likely to increase in case of severe course, and the rates were higher than in the control group. In all age groups and with different degrees of severity of AD it was observed the tendency to lymphocytosis, with severe disease – tendency to neutrophilia ($p < 0,05$). The subpopulation composition of peripheral blood lymphocytes, according to the differentiation clusters, had certain features depending on the severity of the disease. There was a statistically significant increase in the concentration of CD_{19} and CD_{16} ($p < 0,05$). At the same time, more expressed changes and deviations in these indicators were also observed in the more severe course of AD. In severe form of AD, higher IL-4, IL-6, IL- and lower IL-2 and INF- γ have been registered. Children with AD have a tendency to dysimmunoglobulinemia: decreased serum of IgA concentration, and increased serum of IgE and IgG concentrations. Concentration of cortisol in boys with AD is lower, in girls with AD – higher.

The first type of immune response is observed in non-atopic patients, and the second one – in patients

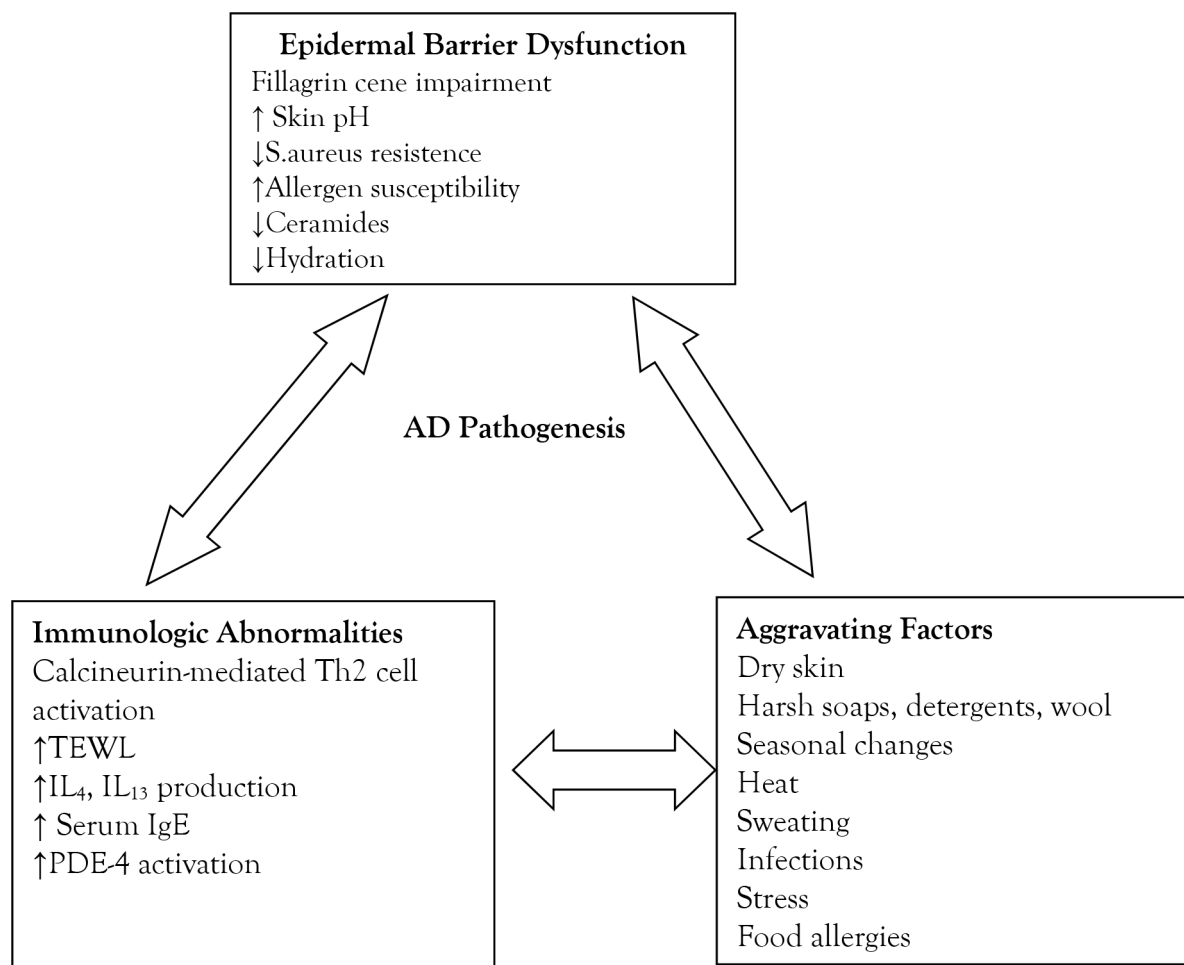


Figure 1. Complex pathophysiology of AD¹³. AD, atopic dermatitis; PDE-4, phosphodiesterase-4; IgE, immunoglobulin E; IL – interleukin; TEWL, transepidermal water loss; Th2, T helper 2.

with the presence of atopy. Th1 type cells synthesize interleukin -2 (IL-2), γ -interferon (IFN- γ), tumor necrosis factor- α (TNF - α) and others – these cells and cytokines cause delayed-type reactions. Th2 type cells produce interleukins 4, 5, 10, 13 (IL-4, IL-5, IL-10, IL-13), which are responsible for the development of atopic reactivity. Cytokines which are synthesized by

Th2 cells are in dynamic opposition to Th1 cells and vice versa.

Endocrine disorders are one of the leading factors which explain the course of AD from pathogenesis position¹⁵. It is proved that low hormones concentrations make the immune mechanisms active, and high hormones concentrations suppress it. However,

Table 1. Subpopulation composition of lymphocytes in children with AD, depending on the severity of the clinical course and in the control group (M \pm m)

The severity of the clinical course	L, $\cdot 10^9/l$	CD ₃ , %	CD ₄ , %	CD ₈ , %	CD ₁₆ , %	CD ₁₉ , %	IRI, CD ₄ /CD ₈
Mild (n=89)	6.78 \pm 0.27*	69.95 \pm 0.11*	48.18 \pm 0.09*	22,89 \pm 0,07*	14.79 \pm 0.08*	15.21 \pm 0.11*	2.10 \pm 0.10*
Moderate (n=60)	6.79 \pm 0.23*	71.98 \pm 0.14*	53.02 \pm 0.11*	19,01 \pm 0,17*	16.77 \pm 0.08*	18.04 \pm 0.07*	2.78 \pm 0.15*
Severe (n=19)	7.24 \pm 0.51*	73.11 \pm 0.15*	54.97 \pm 0.15*	15,84 \pm 0,11*	17.97 \pm 0.12*	19.71 \pm 0.13*	3.47 \pm 0.21*
The control group (n=30)	5.41 \pm 0.13	68.40 \pm 0.08	45.03 \pm 0.17	27,98 \pm 0,19	13.17 \pm 0.06	11.92 \pm 0.11	1.61 \pm 0.22

Note. *p – the reliability of the difference between the values in children with AD and in control groups (<0,05).

the contribution of individual cytokines to the formation of various clinical forms of AD and the impact of cortisol on this process in children are not sufficiently studied.

The results of the examination of cellular immunity in children with AD, depending on the severity of the clinical course of the disease were evaluated and compared (Table 1).

Analyzing the parameters of cellular immunity, it was found that the total number of leukocytes in children with AD did not exceed the reference value, but it was increased in the case of severe course, and the values were higher than in the control group. There was a tendency to lymphocytosis, and with severe course of the disease a tendency to neutrophilia ($p < 0,05$) in all age groups and with different degrees of AD severity. The subpopulation composition of peripheral blood lymphocytes according to the differentiation clusters had certain features, depending on the severity of the disease. For instance, all patients

with AD, in comparison with the control group, had a reliable decrease in the absolute concentration of the general population of CD_3 , at the same time, CD_4 growth was likely to increase with a possible decrease of CD_8 , moreover this ratio was more pronounced in case of severe course of the disease ($p < 0,05$). As a result of the imbalance between CD_4 and CD_8 lymphocytes, IRI increased in AD, and its growth occurred in relation to the increase of the severity of the clinical course of the disease ($p < 0,05$). There was a statistically significant increase in the concentration of CD_{19} and CD_{16} ($p < 0,05$). At the same time, more pronounced changes and deviations in these values were also observed in the severe course of AD.

The findings of cytokine status in children with AD were different from the control group as well (Table 2).

The analysis of average interleukin levels in children with AD showed a statistic reliable decrease in IL-2, with a possible increase in IL-4, IL-6, IL-10

Table 2. The concentration of serum cytokines and interferon-gamma in children with AD, depending on the severity of the clinical course (M ± m)

The severity of the clinical course of AD	IL-2, ng/L	IL-4, ng/L	IL-6, ng/L	IL-10, ng/L	INF-γ, ng/L
Mild (n=89)	3.68±0.04*	1.35±0.02*	5.19±0.07*	7.39±0.06*	6.09±0.08*
Moderate (n=60)	3.11±0.01*	2.19±0.04*	9.04±0.13*	9.91±0.23*	3.81±0.05*
Severe (n=19)	2.23±0.08*	3.69±0.11*	17.19±0.53*	17.02±0.51*	2.45±0.18*
The control group (n=30)	5.81±0.09	0.44±0.01	2.13±0.08	5.41±0.10	8.09±0.10

Note. *p – the reliability of the difference between the values in children with AD and those in control groups (<0,05).

Table 3. Concentration of serum immunoglobulins in children with AD, depending on the severity of the clinical course and in the control group (M ± m)

The severity of the clinical course of AD	IgA, g/L	IgE, IU/mL	IgG, g/L
Mild (n=89)	1.53±0.03*	305.000±7.24*	10.24±0.05*
Moderate (n=60)	1.05±0.02*	481.24±11.51*	11.79±0.08*
Severe (n=19)	0.74±0.03*	801.57±38.91*	13.19±0.23*
The control group (n=40)	2.21±0.03	26.10±1.96	8.33±0.12

Note. *p – the reliability of the difference between the values in children with AD and in control groups (<0,05).

Table 4. The level of cortisol in children, depending on the AD severity

Hormone	Moderate form (M±m)		Severe form (M±m)		Healthy children	
	Boys	Girls	Boys	Girls	Boys	Girls
Cortisol nM/l	372.8±58.7	345.5±20.0	148.0±9.8*	406.7±27.3*	407.5 (CI:155-660)	407.5 (CI:155-660)

Note. *p – the reliability of the difference between the values in children with moderate and severe AD (<0,05); CI – confidence interval.

compared with the control group ($p < 0.05$), which was more pronounced in a severe course of the disease. Investigation of levels of concentration of $INF-\gamma$ showed its reliable decrease in children with AD compared with the control group ($p < 0.05$).

Children with AD have a tendency to dysimmunoglobulinemia compared with the control group. There was a possible decrease in the serum IgA concentration, which was especially expressed during a severe course of the disease, and in the same time there was an increase in the serum IgE and IgG concentration in children with AD ($p < 0.05$), which interdependent on the severity of the clinical course of the disease (Table 3).

Studying the hormonal profile, significant differences in the values depending on gender have been obtained. The concentration of cortisol in boys with severe AD was significantly lower than in children with moderate AD ($p < 0.001$, Table 4). At the same time, the level of cortisol in girls with moderate severity is somewhat lower compared to those in healthy girls, while in severe cases it is higher both compared to healthy girls and compared to AD boys. The more severe course of atopic dermatitis is likely to be accompanied by a situation of chronic stress, which causes girls to have a higher level of stress-limiting hormones, specially cortisol.¹⁷⁻²⁰

Deviation of the cellular level of immunity with the development of imbalance between individual subpopulations of lymphocytes can be found in children with AD. The course of allergic dermatitis is accompanied by a decrease in the total number of T-lymphocytes, with an increase in the number of T-lymphocyte helper cells and a decrease of the number of T-lymphocyte suppressors, and a corresponding increase in the immunoregulatory index, as well as an increase in the number of B-lymphocytes and T-lymphocytes of natural killers.²¹⁻²⁵

The study of immunopathogenesis of AD showed that the process of allergic inflammation involves a number of immune cells, whose interaction is regulated by a cascade of cytokines. In children with AD the cytokine system was activated. The analysis of the values of humoral immunity in children with AD showed a pronounced dysimmunoglobulinemia, with a decrease in serum IgA concentration, an increase in serum IgE and IgG concentrations. IgA is contained in the blood serum and synthesized on the skin and mucous membranes. It belongs to non-specific factors of immunity protection. IgE and IgG are contained in the blood serum and easily penetrate into tissues through the vascular wall. They belong to specific factors of immunity protection. Thus, the children with AD had a decrease in the factors of specific and nonspecific protection, which significantly increases with increasing severity of allergic dermatosis. The

severe course of AD leads to a pronounced inhibition of the IgA synthesis, which creates preconditions for supporting the pathophysiological mechanisms of sensitization, the penetration of allergens through the skin and mucous membranes and increases the risk of developing infectious complications. At the same time, the synthesis of IgG is stimulated in response to the re-exposure of antigens, the pathophysiological mechanisms of allergic inflammation trigger and maintain IgE.

CONCLUSIONS

In the AD immunopathogenesis, the deficiency of cellular immunity manifests at all levels – quantitative (imbalance between some subpopulations of T- and B-lymphocytes) and functional (changes in differentiation of T-lymphocytes and their profile of their cytokine secretion – decrease in the concentration of IL-2 and growth of IL-4, IL-6, IL-10). Changes in humoral immunity are accompanied by a decrease in the concentration of IgA, and an increase in IgE and IgG in the blood serum. Signs of dysfunction of adrenal cortex were found in children with moderate and severe AD. The intensity of changes in the values is interdependent on the severity of the clinical course of AD.

Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article“

„The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study“

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