Characteristics of subpopulation composition of peripheral blood lymphocytes in children with different forms of congenital ichthyosis

SCO — краткое сообщение

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Характеристика субпопуляционного состава лимфоцитов периферической крови у детей с различными формами врожденного ихтиоза

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Congenital ichthyosis (CI) is a large heterogeneous group of rare genetic skin diseases with immune disorders and malformation of keratinization of the skin [1]. Defective differentiation of keratinocytes and abnormal formation of the epidermal barrier are observed in these diseases [1, 2]. The resulting dysfunction of the skin barrier causes an increase in transepidermal water loss (TEWL-value of the skin barrier function) and inflammation [3, 4]. Impaired keratinization of the skin regardless of CI form is clinically characterized by dryness, peeling, hyperkeratosis and erythema [1, 2]. Studies in recent years have shown that clinical manifestations of CI and progression of the disease is also caused by uncontrolled activation of immune system cells and inflammatory mediators in the skin [3, 5, 6]. Regarding patients with CI, it was found that a strong activation of cytokines in the IL-17 and TNFα families is closely associated with the severity of the disease and change in TEWL value [7, 8].

CI covers the spectrum of syndromic and non-syndromic dermatosis with a different genetic basis [1,

6]. Non-syndromic ichthyoses include the most described and common phenotype — ichthyosis vulgaris (IV), caused by *FLG* gene mutation (encoding filaggrin). The most prevalent rare non-syndromic CI forms are lamellar ichthyosis (LI, *TGM1* gene mutation, encoding transglutaminase 1 and others) and congenital ichthyosiform erythroderma (CIE; multiple genes), known collectively as autosomal recessive congenital ichthyosis as well as keratinopathic ichthyosis (KPI) with mutations in *KRT1, KRT2* and *KRT10* genes (genes, encoding keratin proteins) [1, 9, 10]. Syndromic forms of CI are characterized by the monogenic type of inheritance, low prevalence and include Netherton syndrome (NS), which is characterized by a large number (> 80) of *SPINK5* gene mutations and specific clinical manifestations [1, 7].

The relevance of the study of the immune system role in developing chronic inflammation with ichthyosis is driven by a search for efficient targeted methods of treating children with various forms of CI. Nowadays, there is still a huge need for safer and more efficient treatment methods of ichthyosis in children [1, 5, 11]. Complex blood immunophenotyping in more patients with ichthyosis will help characterize the immune profile of different disease forms and choose targeted therapy in children with CI.

STUDY OBJECTIVE: to determine value characteristics of major and small lymphocyte populations in children with various forms of congenital ichthyosis.

MATERIALS AND METHODS. 96 children were screened with non-syndromic (n = 65) and syndromic (n = 31) forms of congenital ichthyosis, including Netherton syndrome (NS — group 1, n = 20), X-linked ichthyosis (group 2, n = 11), lamellar ichthyosis (LI — group 3, n = 17), ichthyosiform erythroderma (CIE — group 4, n = 16), keratinopathic ichthyosis (KPI — group 5, n = 15) and ichthyosis vulgaris (IV — group 6, n = 14). The age of the screened children was from 2 months to 18 years. The examination was carried out on the basis of the laboratory of experimental immunology and virology, screening and treatment of patients — in the Department of Dermatology and Allergology of FSAI NMRC for Children's Health" of the Ministry of Health of the Russian Federation. Diagnosis and subtype of CI were made based on the results of molecular genetic studies with NGS.

The study is approved by the local ethics committee (Protocol № 6 of 17.06.2021). Written informed consent was obtained from the children's parents during examination.

All the patients had immunophenotyping of peripheral blood lymphocytes by flow cytometry on «Novocyte» cytofluorimeter (ACEA Biosciences, USA), using monoclonal antibodies (Beckman Coulter, USA). The method of step-by-step gating in $CD45^+$ region determined the composition of: T-lymphocytes ($CD3^+$), T-helpers ($CD3^+CD4^+$), cytotoxic T-lymphocytes ($CD3^+CD8^+$), B- lymphocytes ($CD3^-CD19^+$), NK-cells ($CD^3{-}CD16^+CD56^+$), regulatory T-cells ($CD4^+CD25^{high}CD127^{low}$ — Treg), activated T-helpers ($CD4^+CD25^+CD127^{high}$ — Thact), Th17-lymphocytes ($CD3^+CD4^+CD161^+$ — Th17) and Th2- lymphocytes ($CD3^+CD4^+CD294^+$ — Th2).

Since the study involves children of different age, to assess changes in major and small lymphocyte populations, deviations of individual indicators from the level of the age norm were calculated by the formula:

$X_n = (X_{min} - X) / 0{,}01 \times (X_{max} - X_{min})$, где

X_n — the value of the individual indicator, standardized on the age norm; X — the value of the studied indicator; X_{max} — the upper limit of the age norm; X_{min}— the lower limit of the age norm. The range of the age norm was accepted as 100 %.

Statistical processing of the data obtained was performed using Statistica 10.0 program. Descriptive statistics of quantitative trait is presented in the format: median (lower and upper quartiles) — Me ($Q_{0,25}$– $Q_{0,75}$). The non-parametric Mann-Whitney test was used to evaluate significance of differences between groups. Differences were considered statistically significant at $p < 0{,}05$.

RESULTS AND DISCUSSION

The first stage of the study included analysis of the percentage of major and small lymphocyte populations in children with syndromic and non-syndromic forms of congenital ichthyosis. Regardless of CI form, the children had an increase in the concentration of activated T-helpers relative to the values of the age norm. Analysis revealed a significant increase in the

relative composition of Th17-lymphocytes in the group of children with syndromic forms of the disease as to rates in the group with non-syndromic ones: for the relative composition of Th17 (% from LF) deviation from the norm was 84,3 (30–203) % versus 29,0 (−2,3–77) %, p = 0,005; for the relative composition of Th17 (% from CD4) — 111,8 (22,4–221) % versus 18,2 (−12–80) %, p = 0,001.

Composition analysis of major lymphocyte populations in children with Netherton syndrome, X-linked ichthyosis, lamellar ichthyosis, ichthyosiform erythroderma, keratinopathic ichthyosis and ichthyosis vulgaris showed that the relative and absolute composition of T-lymphocytes, T-helpers, NK-cells, B-lymphocytes was mainly within the age reference values, however, there were statistically significant differences and a large range between the forms of the disease. Patients with Netherton syndrome experienced a reduction in the percentage of cytotoxic T-lymphocytes by 12,5 % (−21–42,6) below the level of the age norm. A similar decrease in the relative composition of $CD8^+$ T-cells was detected in children with lamellar ichthyosis: −10,8 (−27,1–20,7) %. The group of children withc X-linked ichthyosis showed a decline in the relative and absolute composition of B-lymphocytes ($p < 0,05$), and the group with KPI — a decrease in the composition of B-lymphocytes below the age norm. Children with vulgar ichthyosis are characterized by a significant reduction in the composition of NK-cells relative to the age norm and indicators of children with lamellar ichthyosis (p = 0,025) and keratinopathic ichthyosis (p = 0,002).

The greatest changes in the lymphocyte composition were identified in the analysis of small populations of $CD4^+$ T-cells in the peripheral blood in children with different forms of CI. composition content of activated T-helpers by 1,8–3,9 times in regard to the values of the age norm. As for regulatory T-cells, the largest increase in the composition of this population was shown in children with Netherton syndrome and was 126 (46–200) %—for the relative value (% CD4) and 155 (−5–349) % — for the absolute one (cells/ μL). The relative composition of Treg was within normal range in the other groups of children with CI, while the absolute composition of the population was increased in the groups with LI, CIE, KPI и IV. It is worth noting that there was a large spread of Treg content in all forms of congenital ichthyosis.

The content analysis of Th17-lymphocytes in children with different forms of CI showed that the increased population was observed in children with Netherton syndrome: for the relative composition (% CD4) by 112 (65–209) %; for the absolute one (cells/ μL) by 168 (43–342) %. An increase in the absolute number of Th17 was also found in the group of children with ichthyosiform erythroderma and was 190 (76–311) % in regard to the age norm. A rise in the composition of Th2- lymphocytes was revealed with NS, CIE and KPI. The highest growth in the composition of Th2-lymphocytes relative to the values of the age norm was detected in the group of patients with keratinopathic ichthyosis at 200 (131–393) % for the relative composition and at 399 (172–571) % for the absolute one.

Thus, the study has allowed to determine characteristic deviations of the values of major and small lymphocyte populations for different forms of congenital ichthyosis in children. A significant increase in the composition of activated T-helpers and Th17-lymphocytes was shown for pediatric patients with Netherton syndrome, ichthyosiform erythroderma and lamellar ichthyosis, and a rise in the composition of regulatory T-cells — for children with Netherton syndrome, keratinopathic ichthyosis, ichthyosiform erythroderma and ichthyosis vulgaris.

CONCLUSION

The obtained data on the state of cellular immunity in children with different forms of congenital ichthyosis expands the understanding of the disease immunopathogenesis and may serve as a basis for choosing targeted biological therapy and thus allows to improve patients' condition and to predict the course of the disease.

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