Open Access Article

STATE OF IMMUNE STATUS AND CYTOKINE PROFILE IN CHILDREN WITH CYSTIC FIBROSIS

Furkat Mukhitdinovich Shamsiev
Head of the Department of Pulmonology of the Republican Specialized Scientific and Practical Medical Center of Pediatrics, Tashkent, Uzbekistan

Xikmatbek Pirnazarovich Atajanov
Assistant of the Department of Pediatrics and Neonatology of the Urgench branch of the Tashkent Medical Academy, Urgench, Uzbekistan

Rano Anvarbekovna Musajanova
Senior researcher Department of Pulmonology of the Republican Specialized Scientific and Practical Medical Center of Pediatrics, Tashkent, Uzbekistan

Durumboy Musaevich Yakubov
Assistant of the Department of Propedeutics of Childhood Diseases of the Urgench branch of the Tashkent Medical Academy, Urgench, Uzbekistan

Bakhtiyor Gofurovich Bekchanov
Ph.D., Associate Professor, Head Department of Propedeutics of Childhood Diseases Urgench branch of the Tashkent Medical Academy, Urgench, Uzbekistan

ABSTRACT

Progressive lung pathology in children with cystic fibrosis is the cause of mortality in patients, despite the active use of various drugs that prolong their life, including mucolytic, antimicrobial therapy, and the emergence of pathogenetic targeted therapy.

The aim of the study was to study the immune status and profile of cytokines - IL-1β and IL-8 in cystic fibrosis in children. The data obtained allow us to conclude that significant changes in the immune system are determined in children with mixed form cystic fibrosis.

The study included 65 children with mixed cystic fibrosis aged 0 to 14 years. As a comparison group, 25 children with a recurrent course of obstructive bronchitis were examined. The control group consisted of 20 apparently healthy children of the same age.

In the group of children receiving basic therapy, the immunological parameters did not recover, within a year, in some cases, even decreased from the initial value. Studies have shown that through cytokine network failures and immune disorders, cystic fibrosis contributes to the aggravation of

Received: September 18, 2021 / Revised: October 13, 2021 / Accepted: October 31, 2021 / Published: November 30, 2021

About the authors：Furkat Mukhitdinovich Shamsiev

Corresponding author- *Email:

immunodeficiency and, possibly, the development or aggravation of the course of complications, which dictates the need for immunocorrective therapy.

KEY WORDS: children, cystic fibrosis, immunity, cytokines.

抽象的

尽管积极使用各种药物来延长他们的生命，包括粘液溶解剂、抗菌治疗和致病靶向治疗的出现，但囊性纤维化儿童的进行性肺病理学是导致患者死亡的原因。

该研究的目的是研究儿童囊性纤维化中细胞因子 - IL-1β **和** IL-8 **的免疫状**态和特征。获得的数据使我们能够得出结论，混合型囊性纤维化儿童的免疫系统发生了显着变化。

该研究包括 65 **名** 0 **至** 14 岁的混合性囊性纤维化儿童。作为对照组，对 25 **名反复**发作阻塞性支气管炎的儿童进行了检查。对照组由20**名明**显健康的同龄儿童组成。

在接受基础治疗的儿童组中，免疫学参**数在一年内没有恢复，在某些情况下，甚至从初始**值下降。研究表明，通过细胞因子网络故障和免疫紊乱，囊性纤维化会导致免疫缺陷的恶化，并可能导致并发症的发展或恶化，这就需要免疫矫正治疗。

关键词：儿童，囊性纤维化，免疫，细胞因子。

INTRODUCTION

Bronchopulmonary pathology occupies a leading place in the structure of morbidity in children of all age groups [7]. Progressive lung pathology in children with cystic fibrosis is the cause of mortality in patients, despite the active use of various drugs that prolong their life, including mucolytic, antimicrobial therapy, and the emergence of pathogenetic targeted therapy [1,5]. Cystic fibrosis developing as a result of the production of secretions of increased viscosity by exocrine glands causes secondary changes mainly in the bronchopulmonary, digestive, and reproductive systems. However, the determining factor in the clinical picture of CF is the chronic bronchopulmonary process [3].

The role of cytokines in the development and maintenance of a chronic inflammatory process, including in cystic fibrosis, is known. Cytokines, being the initial link in the activation of the immune response, determine the effectiveness and type of immunological response to infectious and non-infectious agents, are directly involved in the development and regulation of inflammatory and immune responses. According to some authors, the chronic inflammatory process in cystic fibrosis is maintained due to the imbalance of pro- and anti-inflammatory cytokines, including IL-4, IL-8, IFNγ, and their determination can be considered as markers of inflammation. The reason for the high concentrations of IL-8 in the airways may be a primary defect in the cystic fibrosis protein, the transmembrane conductance regulator. TNF-α and IL-6 contribute to the chronicity of inflammation by increasing the adhesion of the main effector cells of chronic inflammation - macrophages, as well as by activating platelets

and inducing the synthesis of reactive oxygen species [6,8].

Progressive lung pathology in children with cystic fibrosis is the cause of mortality in patients, despite the active use of various drugs that prolong their life, including mucolytic, antimicrobial therapy, and the emergence of pathogenetic targeted therapy [4].

Some issues of clinical diagnosis of cystic fibrosis require further study, there are many unresolved problems related to the diagnosis and prognosis of the outcome of the disease.

Purpose of the study: To study the immune status and profile of cytokines - IL-1β and IL-8 in cystic fibrosis in children.

MATERIALS AND METHODS

The study included 65 children with mixed cystic fibrosis aged 0 to 14 years. As a comparison group, 25 children with a recurrent course of obstructive bronchitis were examined. The control group consisted of 20 apparently healthy children of the same age.

The diagnosis of cystic fibrosis was verified on the basis of complex clinical and instrumental studies: on the basis of complaints, a carefully collected history, clinical symptoms, a positive result of neonatal screening for immunoreactive trypsin, by collecting and analyzing sweat, mandatory X-ray confirmation. The analysis of the general condition of the body was also carried out according to the consultations of related specialists. The diagnosis of cystic fibrosis was confirmed by a sweat test on a US MACRODUCT VESCOR-3700 machine.

The nosological diagnosis in the observed children with cystic fibrosis was formed in accordance with the "International Statistical Classification of Diseases and Related Health Problems" X revision (ICD-X) [2].

During the survey, we used a research questionnaire developed by us. It includes passport data, data of the ante-, intra- and postnatal period, anthropometric data at birth and at admission, the state of organs and systems during an objective examination.

In the process of a comprehensive clinical and laboratory examination of children general clinical, instrumental, immunological and statistical research methods were used.

Sweat samples were studied by studying the conductivity of chlorine ions in the patient's sweat using sweat analyzers "Macroduct" - Sweat-Chek, Vescor (USA) 3700 - SYS.

Immunological studies. Study of the immune system: CD3 + -, CD4 + -, CD8 + -, CD16 + -, CD20 + - lymphocytes, phagocytic activity of neutrophils was determined using anti-lymphocytic antibodies produced by the Research Institute of Immunology of the Russian Federation (Moscow).

The concentration of cytokines - IL-1β and IL-8 in blood serum was determined by enzyme immunoassay on a semiautomatic IF analyzer "Multiskan FC" (Finland) in the laboratory of biochemistry of the Republican Specialized Scientific and Practical Medical Center of Pediatrics of the Republic of Uzbekistan.

Statistical processing of the obtained results was carried out by a program developed in the Microsoft Office Excel-2012 package. The

methods of variation statistics were used with the calculation of arithmetic mean values (M), their standard errors (m) and significant differences according to the Fisher-Student test.

RESULTS AND DISCUSSION

The results of studies of the immune status of the examined patients made it possible to establish a different severity of an immune deficiency state with signs of tension in the humoral link of immunity and a multidirectional nature of immunological changes. In patients with cystic fibrosis in the phase of exacerbation of the disease, the following deviations were revealed: a significant decrease in the relative number of CD3 + - lymphocytes by 1.9 times, in relation to the control group (33.1± 1.1% with 65.2 ± 6.4% in children of the control group, P <0.001) and 1.4 times in relation to the comparison group (45.1-2.2% in children with recurrent obstructive bronchitis, P <0.001). In turn, in children with a recurrent course of obstructive bronchitis, there is a significant decrease in the relative number of CD3 + - lymphocytes by 1.4 times, in relation to the control group (P <0.001). When studying the number of CD4 + lymphocytes, their significant decrease was revealed by 1.5 times in children with cystic fibrosis in relation to the control group (25.8 ± 0.5%, while 39.1 ± 2.1% in children of the control group, P <0.001) and 1.0 times in relation to the comparison group (at 30.3-2.9% in children with recurrent obstructive bronchitis, P <0.01).

The number of CD8 + lymphocytes in children with cystic fibrosis was increased by 1.3 times in comparison with the indicators of practically healthy children. There was a significant increase in the relative number of CD8 + lymphocytes in children with cystic fibrosis (23.9± 0.9% with 19.5 ± 1.9% in children of the control group, P <0.05), and a significant increase in 1.5 times in relation to the comparison group (15.9 ± 1.4%, P <0.01). At the same time, there was a significant 1.7-fold increase in the relative number of CD16 + lymphocytes, compared with children of the control group - and amounted to 25.8± 0.9%, while 15.4 ± 1.9% in practically healthy children; P <0.01).

We also determined a significant decrease in the phagocytic activity of neutrophils in children with cystic fibrosis and recurrent obstructive bronchitis. In children with cystic fibrosis, it was 33.9 ± 1.0%, which was 1.7 times less than in the control group (58.5 ± 2.3%, P <0.001). In the group of children with cystic fibrosis, there was a tendency to increase in CD20 + - lymphocytes by 1.5 times (26.9 ± 0.5%, while 18.2 ± 1.7% in children of the control group, P <0.01). The children from the comparison group also showed a significant increase in CD20 + - lymphocytes, by 1.6 times and amounted to 29.5 ± 0.8% (P <0.01).

The activation of the humoral link of immunity is evidenced by an increased concentration of immunoglobulins IgG, IgA, IgM. In particular, in children with cystic fibrosis, there was a significant increase in the IgG content up to 1234.2 ± 33.9 mg /%, which is 1.4 times higher than in the control group (890 ± 61.4 mg /%, P <0.001) and 1.3 times higher than in the comparison group (950.7 ± 30.0 mg /%, P <0.01). The IgA content in children with cystic fibrosis was increased by 1.1 times in comparison with the indicators of practically healthy children. There was a significant increase in the IgA content in children with cystic fibrosis (131.3 ± 5.2 mg /% at 112.3 ± 4.1 mg /% in children of the control group, P <0.01) and a

significant increase in 1.3 times in relation to the comparison group (99.2 ± 5.0 mg /%, P <0.001). At the same time, there was a significant increase of 1.2 times in the IgM content, compared with children of the control group - and amounted to 130.51 ± 3.49 mg /%, while 109.5 ± 2.24 mg /% in practically healthy children; P <0.001). The IgM content in children with cystic fibrosis in relation to the comparison group was 1.1 times higher, this indicator in children with recurrent obstructive bronchitis was 117.0 ± 2.9 mg /%, (P <0.01).

Our studies of the cytokite status and analysis of the level of IL-1β in the blood serum of patients with cystic fibrosis allowed us to obtain the following results (Table 1).

Table 1.

Comparative analysis of cytokines in the examined children, (M ± m)

Indicators	healthy children (n = 20) (I)	Recurrent obstructive Bronchitis (n=25) (II)	Children with cystic fibrosis (n=65) (III)	P	P1
IL-1β (pg / ml)	29,7±3,5	75,1±4,5	101,0±4,8	<0,001	<0,01
IL–8 (pg / ml)	21,4±3,6	80,0±3,5	59,3±2,4	<0,001	<0,01

Note: P - reliability of differences in indicators between groups I and II of patients;

P1 - reliability of differences in indicators between II and III groups of patients.

It was found that in children with cystic fibrosis in the phase of exacerbation of the disease, the level of IL-1β increased 3.4 times compared with the data of practically healthy children and averaged 101.0--4.8 pg / ml (P <0.001), according to in relation to the comparison group increased by 1.3 times (75.0 ± 4.5 pg / ml, P <0.01). The IL-8 level significantly increased 2.9 times compared with the data of children in the control group, which averaged 59.3□2.4 pg / ml (P <0.001), but in relation to the comparison group, this indicator was significantly lower 1.3 times (P <0.01).

Thus, the data obtained allow us to conclude that significant changes in the immune system are determined in children with mixed cystic fibrosis. In the group of children receiving basic therapy, the immunological parameters did not recover, within a year, in some cases, even decreased from the initial value. Studies have shown that through cytokine network failures and immune disorders, cystic fibrosis contributes to the aggravation of immunodeficiency and, possibly, the development or aggravation of the course of complications, which dictates the need for immunocorrective therapy.

CONCLUSION

1. In children with cystic fibrosis: an imbalance of the cellular link of immunity due to a decrease in CD3 + -, CD4 + - lymphocytes and an increase in CD8 + -, CD16 + -, CD20 + lymphocytes, a decrease in the phagocytic activity of neutrophils in comparison with children of the control group.

2. In case of cystic fibrosis in children in the phase of exacerbation of the disease, overproduction of the cytokine profile (IL-1β by 3.4 times, IL-8 by 2.9 times) is determined, which is a diagnostic marker of the inflammatory response under hypoxic conditions.

REFERENCES

1. Amelina E.L., Kashirskaya N.Yu., Kondratyeva E.I. and etc.,ed. Register of patients with cystic fibrosis in the Russian Federation. 2018 year. M .: Medpraktika-M; 2020.- 68s.

2. Geppe N.A., Rozinova N.N., Volkov I.K., Mizernitsky Yu.L. Working classification of the main clinical forms of bronchopulmonary diseases in children // Difficult patient. -2019. - Volume 7. -No. 1-2. -S.35-40.

3. Golubtsova O.I., Dianova T.I., Krasnov M.V., Safonov D.V., Krasovsky S.A. Modern possibilities of diagnosing lesions of the bronchopulmonary system in cystic fibrosis in children in Chuvashia // Practical Pulmonology. - 2015.-№3.-P.54-61.

4. Goryainova AV, Kashirskaya N.Yu., Shumilov PV, Polikarpova SV, Semykin S.Yu .. General recommendations for the diagnosis and treatment of various forms of pulmonary aspergillosis in patients with cystic fibrosis // Pediatrics. 2018; 97 (5): 118-130.

5. Goryainova A.V., Shumilov P.V., Semykin S.Yu., Zobkova G.Yu., Donnikov A.E. Clinical and genetic features of connective tissue dysplasia syndrome in cystic fibrosis in children // Russian pediatric journal. - 2018; 21 (4) .- S.203-207.

6. Ivanova ON Cystic fibrosis in children // Successes of modern science. -2017.-№7.-C. 180-183.

7. Ilchenko S.I., Mishina N.V., Fialkovskaya A.A., Zhukova L.A. Features of the microbial landscape of the upper respiratory tract in children with microaspiration syndrome against the background of lesions of the central nervous system // Clinical Pediatrics.-2018.- Volume 13, No. 8.-P. 749-753.

8. Klimov L.Ya., Kondratyeva EI, Ilyenkova NA, et al., Features of innate immunity against the background of chronic respiratory tract infection in children with cystic fibrosis // Pediatrics. Consilium Medicum.-2019; 1: 59-66.

9. Farrell, Philip M. "The prevalence of cystic fibrosis in the European Union." *Journal of Cystic Fibrosis* 7.5 (2008): 450-453.

10. Brennan, A. L., Geddes, D. M., Gyi, K. M., & Baker, E. H. (2004). Clinical importance of cystic fibrosis-related diabetes. *Journal of cystic fibrosis*, *3*(4), 209-222.

11. Cohen, Taylor Sitarik, and Alice Prince. "Cystic fibrosis: a mucosal immunodeficiency syndrome." *Nature medicine* 18.4 (2012): 509-519.

12. van Heeckeren, A. M., Sutton, M. T., Fletcher, D. R., Hodges, C. A., Caplan, A. I., & Bonfield, T. L. (2021). Enhancing Cystic Fibrosis Immune Regulation. *Frontiers in pharmacology*, *12*, 269.

13. Ratner, D., & Mueller, C. (2012). Immune responses in cystic fibrosis: are they intrinsically defective?. *American journal of respiratory cell and molecular biology*, *46*(6), 715-722.

14. Bruscia, Emanuela M., and Tracey L. Bonfield. "Innate and adaptive immunity in cystic fibrosis." *Clinics in chest medicine* 37.1 (2016): 17-29.