

THE VALUE OF THE MICROBIAL FLORA OF THE NASAL AND OROPHARYNGEAL MUCOSA IN FORMATION OF CLINICAL AND IMMUNOLOGICAL FEATURES OF INFECTIOUS MONONUCLEOSIS IN CHILDREN

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Abstract

Objective. The article presents the results of studies determining significance of the microbial flora of the nasal mucosa and oropharynx in the formation of the clinical course and immune response in children with infectious mononucleosis (IM). **Materials and methods.** The study involved 93 children aged three to nine years, with mononucleosis. In 32 children (group 1), *Streptococcus pyogenes* at concentrations of 10^{-5} and higher was isolated during bacteriological examination of the mucosa of the nasopharynx and oropharynx. 30 (group 2) – 10^{-4} degrees or less. In 31 (group 3), *Staphylococcus aureus*, *Spirochetes buccalis*, *E. Coli* and other bacteria, except streptococcus, were shown in smears from the mucous membrane of the nasopharynx. The immune status of patients was assessed by indicators of levels of lymphocytes CD3⁺, CD4⁺, CD8⁺, CD22⁺ and the content of interleukins 1 β , 4, TNF- α . **Results.** The acute period of the mononucleosis in children of group 1 was characterized by more severe symptoms of intoxication, more severe morphological changes in the tissues of the tonsils, lymph nodes, liver and spleen. Also a significant decrease in the relative amount of CD3⁺, CD4⁺, CD8⁺ was observed compared with the indicators of children of the second and third groups. The increase in blood CD22⁺ content was more significant in children of the first group. The content of pro-inflammatory IL-1 β and TNF- α in patients of all groups was significantly higher than in healthy children. The IL-4 increased in children of the second and third groups. In the period of early convalescence in children of the second and third groups, the relative content of CD3⁺, CD4⁺, CD8⁺ cells approached the corresponding indices of the control group. This was not observed in children of the first group. CD22⁺ levels in all observation groups decreased by the convalescence period, but remained high compared with the control group. In children of the studied groups, by the period of reconvalescence, a decrease in the levels of IL-1 β , TNF- α was noted, more significant in children of the second and third groups. At the same time, in children of the first group, the level of pro-inflammatory interleukins by the period of reconvalescence remained at high numbers. The content of IL-4 was a significant difference in the indicators of its content in comparison with the digital characteristics of healthy ones in children of the second and third groups. **Conclusion.** An analysis of the results of the study found that the presence of streptococcus in its high concentration on the mucosa of the nasopharynx of children with mononucleosis already contributes to the formation of cellular immunosuppression and a pronounced reaction of pro-inflammatory interleukins at the initial stage of the disease, which, in general, leads to aggravation of the clinical manifestations of the disease and, in our opinion, may be a causative factor of a possible unfavorable course of the disease.

Keywords: Children, infectious mononucleosis, Epstein-Barr virus, microbial flora, immunity.

Introduction

Diseases of herpes virus etiology, including infectious mononucleosis (IM), in which Epstein-

Barr virus (EBV) (herpes type 4 virus) is a causative agent, are currently a widespread pathology among the child population, both in Ukraine and abroad [1]. According to the WHO, more than 5 million children die every year from these diseases and their consequences in the world [2].

The results of laboratory studies of recent years convincingly prove infection with Epstein-Barr virus in almost 98% of people living on the

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Earth [3]. However, EBV diseases with the presence of appropriate symptoms are recorded much less frequently [4]. It is believed that in children under the age of 2 years, the disease can occur in the clinical form resembling acute respiratory viral infections, which is apparently due to their initial contact with the virus and, possibly, a feature of the immune response [5]. At the same time, in older people, a typical clinical picture of infectious mononucleosis develops [6].

The clinical picture of IM is characterized by fever, tonsilopharyngitis, lymphadenopathy, hepato- and splenomegaly, specific changes in the peripheral blood parameters [7].

Scientists argue that the clinical picture of the disease and the complications that can develop with IM depend on the microbial flora of the nasal and oropharynx mucosa of the patients [8, 9]. However, there are very few works devoted to the study of this problem in the literature, and their results are very contradictory [10].

Some authors prove that tonsillitis in IM has a viral and bacterial origin, while the role of the microbial flora is dominant [11, 12]. Others indicate the leading role of the virus in formation of pathological changes in the lymphoid formations of the nose and oropharynx of patients, and the participation of pathogenic microorganisms in the occurrence of tonsillitis is considered secondary [13, 14].

The difficulties in establishing the role of certain microorganisms in the defeat of the oropharynx are also due to the fact that healthy people have an extremely diverse microbial flora in the oral cavity and tonsils [15].

Staphylococcus epidermidis, *Streptococcus pyogenes*, *Streptococcus pneumoniae* and other *Streptococcus*, *Enterococcus*, *Lactobacillus*, *Actinomyces*, *Neisseria*, *Actinomyces*, *Clostridium*, *Pseudomonas*, *Staphylococcus aureus* are most often detected in the oral cavity and nasopharynx. *Streptococcus* dominates of all these bacteria and makes up 30–60% of the entire microflora. [16].

Owing to the recent studies, it was found that in 60–80% of healthy children with mucous membranes of the nasopharynx and oropharynx, *Streptococcus* is shown, which has a high pathogenic potential and can cause the development of a disease [17].

However, many scientists claim that the presence of *Streptococcus* is not in all concentrations pathogenic [18].

It is known that the clinical picture and outcome of any disease depends on the timeliness and adequacy of the immune responses of the human body. This is especially observed with

herpesvirus pathology, including mononucleosis, a disease that is considered a disease of the immune system [19]. Active proliferation of EBV in all lymphoproliferative organs leads to their structural changes, which is reflected in the immune response (cellular and humoral) [20].

At the same time, in the available literature there are no works considering the effect of the microbial flora of the nasopharynx on formation of the immune response of patients, and hence the clinical picture of the disease, its course and outcomes. In our opinion, studies in this direction will improve prediction of the course of IM in children, outcomes and more reasonably outline ways to increase the effectiveness of treatment of patients.

2. Purpose, subjects and methods:

2.1. The purpose of the study was to determine the significance of the microbial flora of the nasal and oropharyngeal mucosa in the formation of the clinical course and immune response of children with infectious mononucleosis.

2.2. Subjects & Methods

The study involved 93 children aged three to nine years with mononucleosis of moderate severity who were treated at the Regional Children's Infectious Clinical Hospital in Kharkiv, Ukraine. The diagnosis of IM was verified by positive results of the investigation for the disease markers by ELISA (anti-EBV IgM and IgG) and PCR (detection of EBV DNA in the blood). In 32 children (group 1), *Streptococcus pyogenes* at concentrations of 10^{-5} and higher was isolated during bacteriological examination of the mucosa of the nasopharynx and oropharynx and in 30 children (2nd group) – in 10^{-4} degrees or less. In 31 children (group 3) *Staphylococcus aureus*, *Spirochetes buccalis*, *E. Coli* and other bacteria, except streptococcus, were shown in smears from the mucous membrane of the nasopharynx. The immune status of patients was assessed by indicators of levels of populations and subpopulations of peripheral blood lymphocytes, which were determined by indirect immunofluorescence using monoclonal antibodies to surface antigens of lymphocytes CD3⁺, CD4⁺, CD8⁺, CD22⁺ as well as the content in their blood of interleukins 1 β , 4, TNF- α . The studies were carried out in the acute period (1–2 days of illness) and in the period of early convalescence (8–13 days). As a comparison, we took the corresponding indicators of 30 healthy children of the same age and gender.

Statistic processing of the results was carried out using computer programs Excel and Statistica 6.0. The reliability of the difference in values was

revealed using Student's test and Fisher's method. Differences were considered significant at a significance level of $P < 0.05$.

Conflict of interests

The authors of the article declare no conflict of interest.

3. Results & Discussion

When comparing the clinical and laboratory parameters of the children of the compared groups, it was found that the children, whose *Streptococcus pyogenes* was isolated on the rhinopharyngeal mucosa at concentrations of 10^{-5} and higher, the clinical picture was characterized in the onset of the disease by a higher temperature reaction of the body, more severe morphological changes in the tissues of the tonsils, significantly more pronounced increase in submandibular and cervical lymph nodes, liver and spleen. In the blood of children of group 1, higher numbers of the relative content of neutrophils and low lymphomonocytes were determined (Table 1).

nasal and oropharyngeal mucosa in the acute period of the disease, a significant decrease in the number of CD3+, CD4+ CD8+ was observed compared with the indicators of children of groups 2 and 3 ($P_2, P_3 < 0.05$). The increase in blood CD22+ content was more significant in children of group 1 ($P_1 < 0.05, P_2 < 0.05, P_3 < 0.05$).

Some authors argue that violation of the cellular-humoral reactivity of the body with a tendency to suppress cell-mediated mechanisms and enhance the humoral mechanisms of the immune response affects the clinical and biochemical manifestations of the disease and leads to its long-term undulating course. [21].

At the same time, other studies have revealed an increase in the activity of the cellular component of the immune response in children with IM in the acute period of the disease. In our opinion, the immune response in mononucleosis depends on many factors, including the patient's age, activity of the process, viral load, the initial

Table 1

Clinical and laboratory characteristics of the acute period of mononucleosis in children of the compared groups

Clinical and laboratory manifestations of the disease	Compared groups (M±m)		
	Group 1 (n=32)	Group 2 (n=30)	Group 3 (n=31)
1. Temperature of the body, °C	39.2±0.21 ^{1,2}	38±0.31	37.7±0.27
2. Tonsillitis:			
- catarrhal (%)	11.28±1.91 ^{1,2}	29.56±2.03	33.32±1.43
- purulent (quinsy) (%)	88.72±2.04 ^{1,2}	71.44±1.87	67.67±0.09
3. Lymph node size centimeters (cm)			
- submandibular	2.5±0.13 ^{1,2}	1.5±0.32	1.5±0.12
- cervical	1.5±0.31 ^{1,2}	1.2±0.24	0.9±0.32
4. Enlargement of the spleen (cm)	3.3±0.26 ^{1,2}	2.1±0.24	2.0±0.13
5. Enlargement of the liver (cm)	2.1±0.24 ^{1,2}	1.03±0.17	1.03±0.36
6. Analysis of the blood:			
- count of the neutrophils (%)	62.08±1.62 ^{1,2}	41.33±1.97	42.26±1.13
- count of the lymphocytes (%)	34.03±1.22 ^{1,2}	49.17±2.03	50.13±1.17

Note:

P1 – probability of the characteristic of group 1 relative to group 2;

P2 – probability of the characteristic of group 1 relative to group 3;

P3 – probability sign between groups 2 and 3.

In the children of group 1, the course of the disease was longer and amounted to 17.56 ± 1.56 days, group 2 – 13.24 ± 1.37 and in the children of group 3 – 10.24 ± 1.54 days.

The differences in the severity of clinical manifestations and the results of paraclinical examination which was given, as well as taking into account the importance of immune factors in this, we conducted studies to determine the immune status of children in all groups (Table 2).

It should be noted that in children with a high degree of insemination of streptococcus of the

background of the patient and the presence of comorbidities, etc., and needs further investigation [22].

However, these studies concerned children with mononucleosis in the form of mono-infection without taking into account the presence of coccal flora on the mucous membranes of the nasopharynx and its amount, which may affect the immune response of children.

All children have characteristic signs of activation of anti-infection protection in the acute period of mononucleosis. The content of pro-

Table 2*Indicators of the immune status of patients in the acute period of mononucleosis (M ± m)*

Indicator	Group 1 (n=32)	Group 2 (n=30)	Group 3 (n=31)	Controls (n=30)
CD 3 ⁺ , %	47.16±0.74 ^{1,2,3}	57.30±0.52 ¹	58.10±0.72 ¹	60.60±1.20
CD 4 ⁺ , %	30.57±0.42 ^{1,2,3}	33.86±0.34 ¹	34.02±0.22 ¹	36.30±0.75
CD 8 ⁺ , %	19.37±0.34 ^{1,2,3}	23.40±0.39 ¹	23.60±0.12 ¹	25.70±0.68
CD 22 ⁺ , %	35.60±0.25 ^{1,2,3}	32.56±0.42 ¹	32.63±0.28 ¹	17.30±0.79
Interleukins: pg / ml				
IL-1β	18.66±1.19 ^{1,2,3}	9.80±0.91 ¹	7.90±0.84 ¹	5.32±1.73
IL-4	3.66±0.24 ^{1,2,3}	5.40±0.30 ¹	5.10±0.21 ¹	2.06±0.94
TNF-α	11.20±1.43 ^{1,2,3}	6.90±1.12 ¹	6.30±1.08 ¹	2.69±1.67

Note here and further:

P1 – reliability of the difference in the digital values of indicators of healthy and sick children;

P2 – probability of the characteristic of group 1 relative to group 2;

P3 – probability of the characteristic of group 1 relative to group 3;

P4 – probability sign between groups 2 and 3.

inflammatory IL-1β in blood serum in the onset of the disease in patients of all groups was significantly higher than in healthy children (P1<0.05). In children with additional infection of the nasal and oropharynx mucosa with streptococcus with a high degree of seeding, the concentration of IL-1β was 18.66 ± 1.19 pg/ml and was significantly higher than the corresponding indicators of the second group - 9.8 ± 0.91 pg/ml (P2<0.05) and the third – 7.90 ± 0.84 pg/ml (P3<0.05).

The acute period of mononucleosis is accompanied by a significant increase in the blood level of patients with TNF-α compared with its content in healthy children (P1<0.05), while the level of pro-inflammatory TNF-α in patients of the first group was significantly higher than similar indicators in children of the second and third groups (P2<0.50; P3<0.05).

When studying the levels of IL-4 in the blood serum of children of the studied groups, an increase in its content was revealed in comparison with healthy children, however, a significant difference in its content was determined only in the indicators of children of the second, third and

control groups (P2<0.05, P3<0.05). And although in patients of the first group there was an increase in the level of IL-4 in the blood, it was less significant than in children of the second and third groups to healthy children (P1<0.05).

In the period of early convalescence in children of groups 2 and 3, the relative content of CD3⁺, CD4⁺, CD8⁺ cells approached the corresponding indices of the controls (P2,3≥0.05), which indicated a tendency to normalize the cellular immunity of patients. This was not revealed in children which were seeding streptococcus with a high degree on the mucosa of the nasal and oropharynx. in children of group 1, the content of CD3⁺, CD4⁺, CD8⁺ increased in the period of IM convalescence compared with the acute period, but was significantly lower (P1<0.05) compared with the control group. As in the acute period, in the period of IM convalescence in children of group 1, signs of a cellular immune response deficiency were found, which must be taken into account in the dynamics of correction of the therapy (Table 3).

CD22⁺ levels in all observation groups decreased by the convalescence period, but

Table 3

Indicators of the immune status of patients in the early convalescence period of mononucleosis (M±m)

Indicator	Group 1 (n=32)	Group 2 (n=30)	Group 3 (n=31)	Controls (n=30)
CD 3 ⁺ , %	47.16±0.74 ^{1,2,3}	57.30±0.52 ¹	58.10±0.72 ¹	60.60±1.20
CD 4 ⁺ , %	30.57±0.42 ^{1,2,3}	33.86±0.34 ¹	34.02±0.22 ¹	36.30±0.75
CD 8 ⁺ , %	19.37±0.34 ^{1,2,3}	23.40±0.39 ¹	23.60±0.12 ¹	25.70±0.68
CD 22 ⁺ , %	35.60±0.25 ^{1,2,3}	32.56±0.42 ¹	32.63±0.28 ¹	17.30±0.79
Interleukins: pg / ml				
IL-1β	18.66±1.19 ^{1,2,3}	9.80±0.91 ¹	7.90±0.84 ¹	5.32±1.73
IL-4	3.66±0.24 ^{1,2,3}	5.40±0.30 ¹	5.10±0.21 ¹	2.06±0.94
TNF-α	11.20±1.43 ^{1,2,3}	6.90±1.12 ¹	6.30±1.08 ¹	2.69±1.67

remained high compared with the controls ($P1 < 0.05$).

In children of the studied groups, by the period of convalescence, a decrease in the levels of IL-1 β , TNF- α was noted, which was more significant in children of groups 2 and 3, in which there was no mathematical difference in the levels of these interleukins in blood compared with healthy children ($P_{2,3} > 0.05$).

At the same time, in children of group 1, the level of pro-inflammatory interleukins by the period of convalescence remained at high numbers ($P1 < 0.05$).

The content of anti-inflammatory interleukin-4 in the blood of children by the period of their recovery exceeded these indicators of the acute period. However, in the convalescence period only in children of groups 2 and 3 there was a significant difference in IL-4 in comparison with the digital characteristics of healthy ones ($P_{2,3} < 0.05$).

Conclusions

1. Microorganisms that are present on the mucous membrane of the nasal and oropharynx

have a different effect on the formation of the immune response of children with mononucleosis. This explains the differences in the severity of clinical symptoms and the duration of the disease.

2. Streptococcus is the most aggressive microbial structure that negatively affects the immune response of patients with mononucleosis. Moreover, the degree of the indicated effect is proportional to the level of streptococcus contamination of the nasopharyngeal mucosa.

3. The most significant deviations from the norm of the indicators of the immune response of patients with mononucleosis are observed in children in whom streptococcus 10-5 and higher is sown on the mucous membrane of the nasal and oropharynx. In our opinion, the immunosuppressive state is a factor in the prolongation of the disease.

4. The differences that were identified as a result of the study can serve as an additional criterion for predicting the course of mononucleosis and the choice of therapy, which will reduce adverse outcomes and improve treatment.

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