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ORIGINAL ARTICLE

RESPIRATORY MICROBIOME AND ITS RELATIONSHIP WITH INFLAMMATORY MARKERS

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ABSTRACT
The aim: This study aims to investigate and analyze the microbiome of the nasopharyngeal zone in acute respiratory infections (ARI) and their relationship with inflammatory markers.
Materials and methods: Examination of 112 children (10-14 years old) with acute respiratory infections (ARI) of the upper respiratory tract was carried out. The control group consisted of 25 healthy children identical in age and examination parameters.
Results: When analyzing the microflora of the nasopharynx of patients, 29,0% of strains were gram-positive bacteria and 71,0% were gram-negative bacteria (*Escherichia coli* representing 37,0%). Biochemical examination of the biomaterial revealed the presence of sucrase (n=69), maltase (n=87), lactorepoxidase (n-89) and alcohols - sorbitol (hexahydric alcohol, n=102), mannitol (hexahydric alcohol, n=84), xylitol (pentahydric alcohol, n=86). Regarding the markers of inflammatory response, the following dynamics was noted: increase in the level of IgM (3,91 ± 1,79 g/l, p<0,01) by 2,2 times, elevation of Ig G level by 10 times (145, 91 ± 53,04 g/l, p< 0,01), slightly higher than the reference values IgE level. In addition, increased IL-1, IL-4, IL-6, γ-IFN, TNF-α, Neopterin levels were detected. The level of Thyroid stimulating hormone (TSH) was significantly different compared to the control group (0,62 ± 0,57 vs. 1,98 ± 0,30 mIU/ml, p< 0,01), but within the reference values.
Conclusions: Predominance of Gram-negative bacteria in the nasopharyngeal microflora of patients along with elevated inflammatory markers and lactoperoxydase enzyme predominance was detected in the study.

KEY WORDS: acute respiratory infections, nasopharyngeal microflora, inflammatory markers, correlation, children

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INTRODUCTION
Pathogenetic features of acute respiratory infections (ARI) are related to the clinical presentation caused by the respiratory microbiome of the upper respiratory tract. ARI, mainly of the upper respiratory tract, are especially common in preschool-age children [1].

In healthy children, acute inflammation of the mucous membrane and lymphoid structures of the oropharynx is usually a self-limiting disease, except for episodes caused by GAS. Due to an insufficiently developed immune system, children primarily suffer from ARI and are prone to the development of complications, including bronchitis, pneumonia, sinusitis, otitis. Each year, up to 12 cases of ARI can occur in a child, and the frequency of complications reaches 30% and leads to cases where the use of antibiotics is considered [2,3].

Colonization of the nasopharyngeal zone is the first stage in the development of pathology. The next stage of primary colonization is the transmission of infection in the environment. Nasopharyngeal carriage of microorganisms can play a leading role in the development and spread of respiratory infections, and the so-called «healthy» carriage under the influence of various pathological influences can transform into an infectious process [4].

Active reproduction of microorganisms occurs during acute respiratory viral infections. Under the influence of infectious factors and other factors that suppress immunity, the bacterial process develops [5,6].

THE AIM
To Investigate and analyze the microbiome of the nasopharyngeal zone in ARI and their relationship with inflammatory markers

MATERIALS AND METHODS
Examination of 112 sick children (10-14 years old) with acute respiratory infections (ARI) of the upper respi-

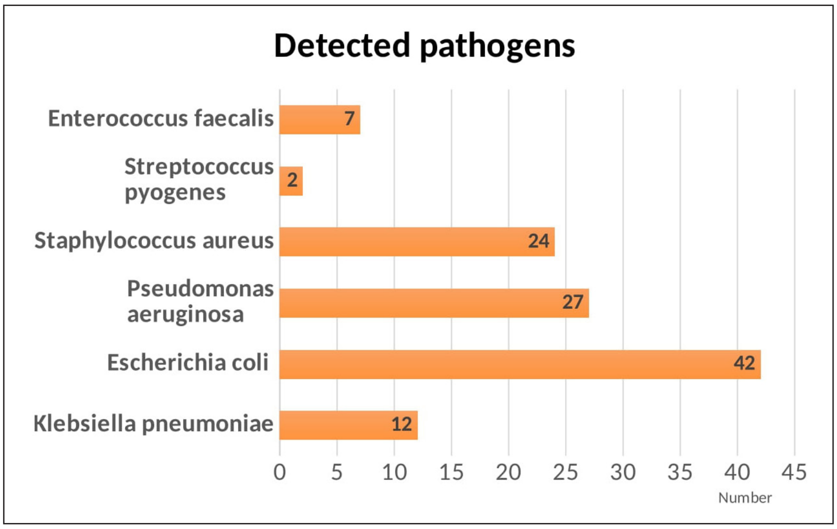

Fig. 1. Characteristics of detected pathogens in children (absolute values)

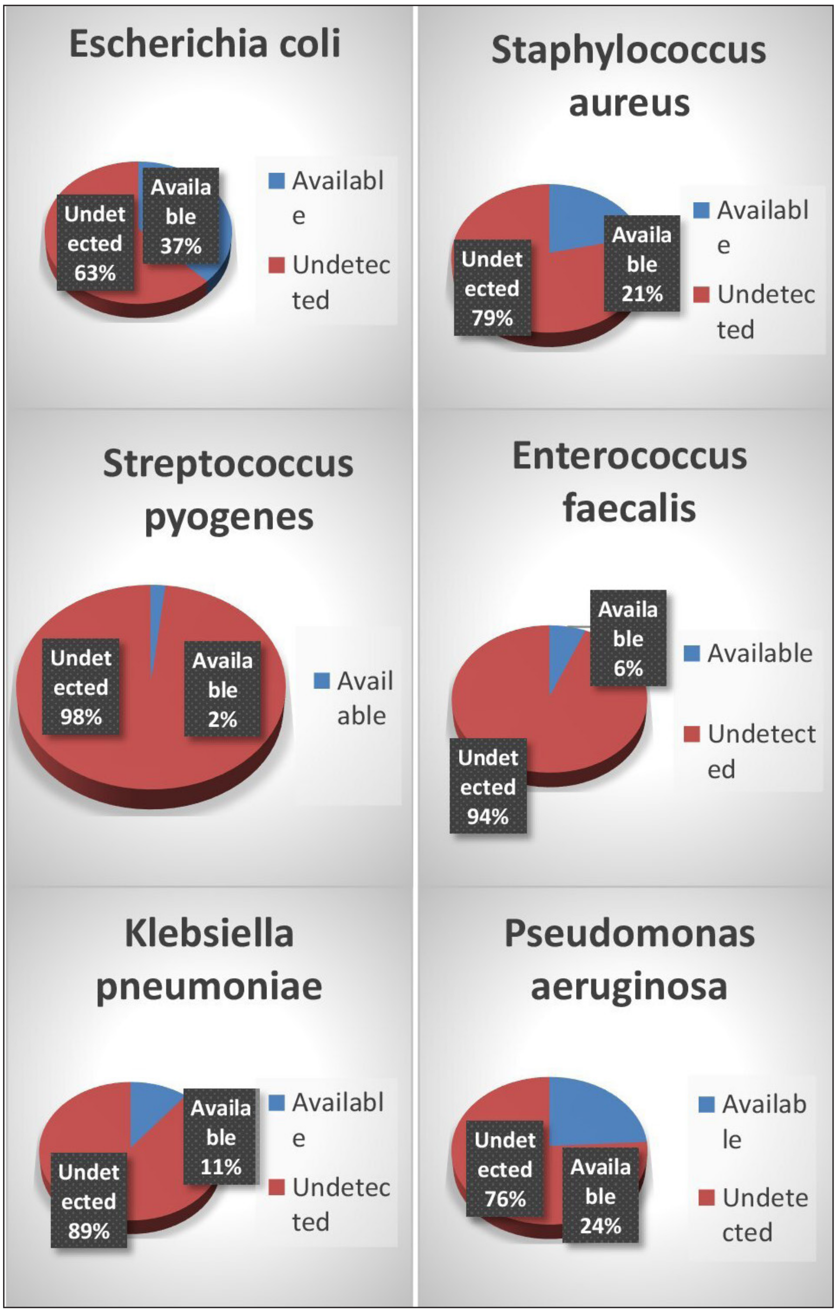

Fig. 2. The structure of representatives of the microflora of the nasopharynx in children with ARI: A) Percentage of *Escherichia coli* strains in the microflora of nasopharynx in children with ARI; B) Percentage of *Staphylococcus aureus* strains in the microflora of nasopharynx in children with ARI; C) Percentage of *Streptococcus pyogenes* strains in the microflora of nasopharynx in children with ARI; D) Percentage of *Enterococcus faecalis* strains in the microflora of nasopharynx in children with ARI; E) Percentage of *Klebsiella pneumoniae* strains in the microflora of nasopharynx in children with ARI; F) Percentage of *Pseudomonas aeruginosa* strains in the microflora of nasopharynx in children with ARI

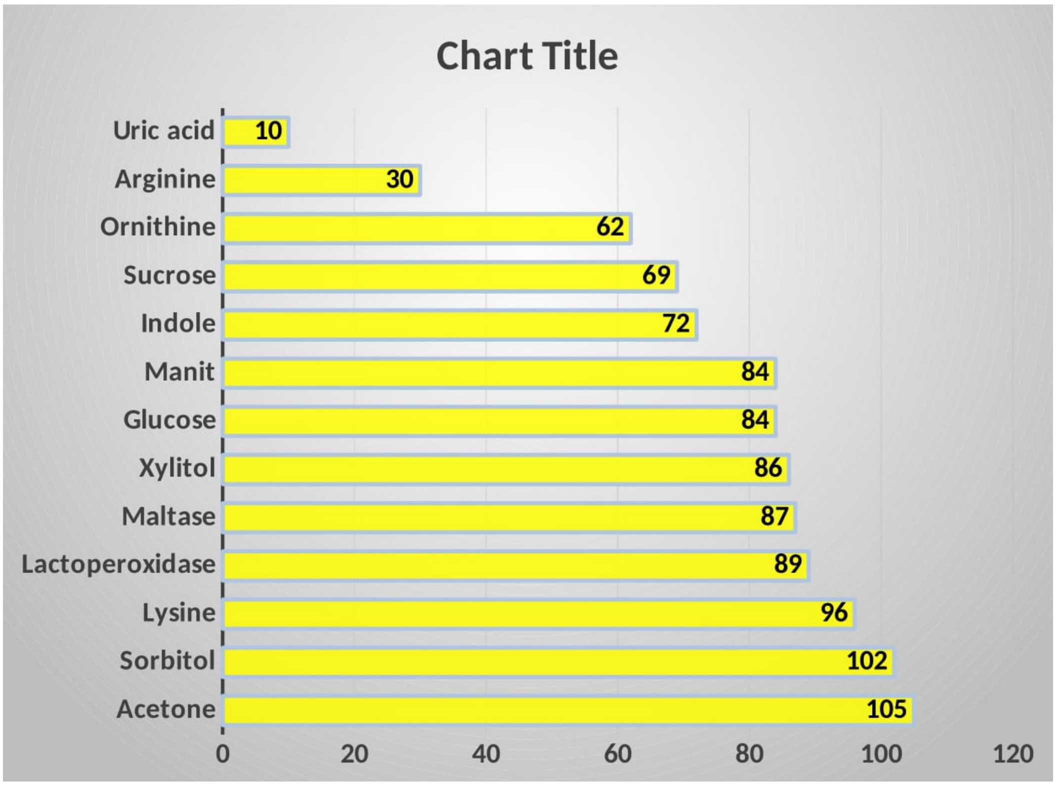

Fig. 3. Biochemical components of the biomaterial in children with ARI

ratory tract who were being treated at the Uzhgorod Clinical City Hospital was carried out. The control group consisted of 25 healthy children identical in age and examination parameters.

A study of the immunological and hormonal status was conducted to identify the levels of markers of the inflammatory response. Microbiological research included the selection of pathogens of the pathological process, identification by morphological, cultural and biochemical properties based on the analysis of nasopharyngeal washes and the establishment of correlational relationships between them.

RESULTS

When analyzing the microflora of the nasopharynx of patients with acute upper respiratory tract infections, 114 strains of opportunistic microorganisms were isolated, of which 33 strains (29,0%) were gram-positive bacteria (*Streptococcus pyogenes, Staphylococcus aureus, Enterococcus faecalis*) and 81 strains (71,0%) were gram-negative bacteria (*Klebsiela pneumoninae, Pseudomonas aeruginosa, Escherichia coli*) (fig. 1, 2).

According to the research data, there is a predominance of 81 strains of gram-negative bacteria (71.0%), in particular, *Klebsiela pneumoninae* (11%), *Pseudomonas aeruginosa* (24%), *Escherichia coli* (37.0%) and 33 gram-positive bacteria (29.0%) - *Streptococcus pyogenes* (2.0%), *Staphylococcus aureus* (21.0%), *Enterococcus faecalis* (6.0%). According to groups, the leading pathogens were *Escherichia coli* (37.0%) and *Staphylococcus aureus* (21.0%). The composition of the microflora of the nasopharynx of healthy children depends on many factors, both on the age category and on the hormonal status and pathology [4].

During the biochemical examination of the biomaterial, the following components were observed (fig. 3)

The design of biomaterial research included the detection of a group of enzymes such as sucrase (n=69), maltase (n=87) and lactorepoxidase (n-89). Lactoperoxidase, which is a heme-containing glycoprotein, was shown to have the highest level in our research. Its function is to use H_2O_2 for the synthesis of hypothiocyanate (OSCN–), which has the ability to suppress the replication of bacteria, fungi, viruses, parasites, neutralization of intestinal pathogens in infants. The enzyme maltase (α-glucosidase) splits maltose disaccharide to form glucose. Sucrase breaks down both sucrose and maltose [4]. The obtained level of glucose in children (n=84) indicates the functionality of the system of destructuring and formation of monosaccharides. Amino acids Lysine (n-96), ornithine (n=62), arginine (n=30) were also found in the biomaterial.

The presence of alcohols - sorbitol (hexahydric alcohol, n=102), mannitol (hexahydric alcohol, n=84), xylitol (pentahydric alcohol, n=86) indicates the possibility of detoxification by the organism. Factors of the intoxication plan were also found: acetone (n=105), which indicates excessive replication of bacteria in the oral cavity. Iden-

Table I. Immunogram of children with ARI

Laboratory indicators	Main group (n = 112) M ± m	Control group (n = 25) M ± m	Statistical significance (p)
Ig M (0,31-1,79, g/l)	3,91 ± 1,79	1,05 ± 0,09	< 0,01
Ig G (6,98-15,49, g/l)	145, 91 ± 53,04	10,39 ± 0,79	< 0,01
Ig E (till 120 IU/ml)	144,68 ± 61,09	41,71 ± 3,18	< 0,01

Table II. Markers of the inflammatory response of children with ARI

Laboratory indicators	Main group (n = 112) M ± m	Control group (n = 25) M ± m	Statistical significance (p)
IL-1 (0-11, pg/ml)	30,37 ± 28,19	2,08 ± 0,49	< 0,01
IL-2 (0-10, pg/ml)	9,43 ± 5,80	0,41 ± 0,05	< 0,01
IL-4 (< 0,5 ng/ml)	5,98 ± 3,19	0,37 ± 0,14	< 0,01
IL-6 (0-10, pg/ml)	16,99 ± 6,89	3,29 ± 0,66	< 0,01
IL-10 (0-20, pg/ml)	15,55 ± 11,88	2,13 ± 0,31	< 0,01
γ-IFN (< 15, pg/ml)	54,16 ± 7,35	5,65 ± 0,85	< 0,01
TNF-α (< 6, pg/ml)	156,70 ± 20,70	3,43 ± 0,47	< 0,01
Neopterin (< 10, nmol/l)	90,42 ± 54,33	6,34 ± 1,14	< 0,01
Cortisol (110-692, nmol/l)	540,32 ± 132,17	251,46 ± 118,82	< 0,01

tification of indole in 72 cases indicates effects in the regulation of various aspects of bacterial physiology and the level of virulence. Tryptophan is an indole derivative and precursor of the neurotransmitter serotonin and can cause vomiting and angiospasm in the patient [5].

Respiratory diseases of the upper respiratory tract (URI), like all inflammatory diseases, are accompanied by an adequate response of the child's organism. We investigated the inflammatory response of the child's organism by studying the following markers (Table I).

As we can see from Table I, there is an increase in the level of IgM (3,91 ± 1,79 g/l, p<0,01) by 2,2 times, which indicates an increase in their synthesis after the pathogen enters the body for bactericidal activation of human blood serum.

Let's consider the next stage of the humoral link of immunogenesis - the synthesis of IgG molecules. According to our data, Ig G level is increased 10 times (145, 91 ± 53,04 g/l, p< 0,01). According to scientific data, immunoglobulins of class G (IgG) are the main class of antibodies and are synthesized in response to a secondary infectious factor. In the process of implementation and formation of the immune response, the synthesis of IgM is switched to IgG. We observe the predominance of IgG synthesis.

The level of IgE is slightly higher than the reference values, but 3,5 times higher than the level in children of the control group (144,68 ± 61,09 versus 41,71 ± 3,18 IU/ml, p<0,01). According to literature data, it is believed that the main role of IgE is the protection of mucous membranes due to the induction of a local inflammatory reaction. This causes an inflammatory reaction [6].

In the vast majority of cases, cytokines are close-acting mediators of local interactions between cells in foci of certain processes in tissues, even pairs of cells. Depending on the known parameters of irradiation, the effects of cytokines are classified into autocrine effects (on the cell itself that secreted cytokines) and paracrine effects (on cells located nearby). There are also endocrine or remote effects, they are also called systemic,

Table III. Hormonal status of children with ARI

Laboratory indicators	Main group (n = 112) M ± m	Control group (n = 25) M ± m	Statistical significance (p)
Free Triiodothyronine (1,2 - 2,8, nmol/l)	1,38 ± 0,57	1,32 ± 0,12	0,60
Free thyroxine (12,5 - 21,0, pmol/l)	14,59 ± 3,03	13,72 ± 0,51	0,15
Thyroid stimulating hormone (0,4 - 4,0, mIU/ml)	0,62 ± 0,57	1,98 ± 0,30	< 0,01
Thyroid peroxidase antibody (< 0,9, IU/ml)	7,26 ± 4,71	5,52 ± 0,21	0,07

Table IV. Correlation relationships of nasopharyngeal microflora and inflammatory response markers

Laboratory indicators		Correlation coefficient (r)	Statistical significance (p)
Escherichia coli	Free T4	0,19	0,05
	TNF-α	0,20	0,04
Staphylococcus aureus	Lactoperoxidase	-0,20	0,04
	Acetone	-0,21	0,03
Pseudomonas aeruginosa	Free T3	0,20	0,04
	Free T4	-0,28	0,003
	TNF-α	-0,20	0,04
Streptococcus pyogenes	γ-IFN	0,32	0,001
Klebsiella pneumoniae	Free T4	0,20	0,04
Enterococcus faecalis	Cortisole	0,26	0,007

since in this case the cytokine reaches the target cell, circulating in the blood. But endocrine effects were found only for four cytokines (TNF-α, IL-1, IL-6, M-CSF) and not in healthy organisms, only in severe systemic pathology such as septic shock [6,7].

According to the data of Table II, there is significant increase in the level of the following cytokines with a significant predominance in comparison with the data of the control group of children: IL-1 (30,37 ± 28,19 vs. 2,08 ± 0,49 pg/ml, p< 0,01), IL-2 (9,43 ± 5,80 vs. 0,41 ± 0.05 pg/ml, p< 0,01), IL-4 (5,98 ± 3,19 vs. 0,37 ± 0,14ng/ml, p< 0,01), IL-6 (16,99 ± 6,89 vs. 3,29 ± 0,66 pg/ml, p< 0,01), IL-10 (15,55 ± 11,88 vs. 2,13 ± 0,31 pg/ml, p< 0,01), γ-IFN (54,16 ± 7,35 vs. 5,65 ± 0,85pg/ml, p< 0,01), TNF-α (156,70 ± 20,70 vs. 3,43 ± 0,47pg/ml, p< 0,01), Neopterin (90,42 ± 54,33 vs. 6,34 ± 1,14nmol/l, p< 0,01), Cortisol (540,32 ± 132,17 vs. 2 51,46 ± 118,82 nmol/l, p< 0,01).

The level of IL-1 increased 2,7 times, IL-4 11,8 times, IL-6 1,7 times, γ-IFN – 3,6 times, TNF-α – 26 times, Neopterin – 9 times. IL-2 and Cortisol levels are within reference values.

The formation and biological activity of cytokines are interconnected and mutually regulated in response to a stimulus. They form the so-called cytokine cascade, which corresponds to the inflammatory response of the child's body and, as a result, the clinical presentation is formed. Regulators of natural resistance - interferons α and β, interleukins 1, 6 and 12, TNF-α, chemokines (IL 8, MCP-1, RANTES, etc.) are the main activators and regulators of nonspecific reactions of the body to protect it from colonization by carriers of foreign genetic information [6].

The investigated parameters of the hormonal status of children with ARI are considered in Table III.

According to the data in Table III, changes are observed only in the Thyroid peroxidase antibody (TPO) indicator (7,26 ± 4,71 IU/ml), but they are unreliable in comparison with the data of the control group p=0,07. Indicators of Free triiodothyronine (T3) and Free thyroxine (T4) do not show differences with the control group (0,60 and 0,15 respectively). The level of Thyroid stimulating hormone (TSH) is significantly different compared to the control group (0,62 ± 0,57 vs. 1,98 ± 0,30 mIU/ml, p< 0,01), but within the reference values. Changes in thyroid status correspond to scientific research on their involvement in the inflammatory process [8].

On the basis of the data obtained from the study of inflammatory markers and indicators of the nasopharyngeal microflora in children with ARI, a correlation analysis of relationships was carried out (Table IV).

According to Table IV, correlations between the frequency of detection of microorganisms and indicators of the inflammatory response of the child's organism are the following. The values of *Escherichia coli* infection have reliable positive relationships with Free T4 (r =0,19, p=0,05), TNF-α (r =0,20, p=0,04). The value of *Staphylococcus aureus* has a negative correlation with the levels of the *enzyme Lactoperoxidase* (r =-0,20, p=0,04) and Acetone (r =-0,21, p=0,03). The microorganism Pseudomonas aeruginosa presents negative correlations with Free T4 (r =-0,28, p=0,003), TNF-α (r =-0,20, p=0,04) and positive with Free T3 (r =0,20, p=0,04). The microorganism *Streptococcus pyogenes* has the highest correlations in our study with γ-IFN (r = 0,32, p = 0,001). Positive correlations of Klebsiella pneumoniae with Free T4 (r =0,20, p=0,04), *Enterococcus faecalis* with Cortisol (r =0,26, p=0,007) are observed.

DISCUSSION

Given the high prevalence of acute respiratory diseases in childhood, there is a need for new developments to identify pathogenetic factors for the treatment and prevention of this pathological process. The respiratory microbiome plays a leading role in diseases[1,2]. Deviations of the microflora and carriage causes the clinical presentation of acute respiratory diseases. Disturbances of the balance and the presence of pathogenic microorganisms cause an inflammatory reaction in the mucous membranes of the respiratory tract. Changes in the enzymatic, detoxification and characterological features of the microbial landscape present the inflammatory response of the child's organism with the presence of increased levels of their markers, in particular Il-1,4,6, γ-IFN, TNF-α [4,5].. Correlation relationships with thyroid hormones, TNF-α in the detection of *Escherichia coli* are observed. *Staphylococcus aureus* causes a violation of enzymatic properties in the oral cavity, in particular Lactoperoxidase and signs of intoxication of the organism. Respiratory pathology is the most common problem in clinical pediatrics and its relevance in childhood is related to the prevalence, predicted severe course and complications and requires further treatment and solving the etiopathogenetic plan for understanding the disease and its prevention.

CONCLUSIONS

1. 114 strains of opportunistic microorganisms were identified, of which 33 strains (29,0%) were Gram-positive bacteria (*Streptococcus pyogenes, Staphylococcus aureus, Enterococcus faecalis*) and 81 strains (71,0%) were Gram-negative bacteria (*Klebsiela pneumoninae, Pseudomonas aeruginosa, Escherichia coli*). The leading pathogens were *Escherichia coli* (37,0%) and *Staphylococcus aureus* (21,0%).
2. The biomaterial included the detection of enzyme groups such as sucrase (n=69), maltase (n=87), lactorepoxidase (n-89). Lactoperoxidase showed the highest level in our research.
3. Sucrase breaks down both sucrose and maltose. The obtained level of glucose in the analysis of children (n=84) testifies to the functionality of the system of destructuring and formation of monosaccharides. The presence of alcohols - sorbitol (hexahydric alcohol, n=102), mannitol (hexahydric alcohol, n=84), xylitol (pentahydric alcohol, n=86) indicates the possibility of detoxification of the child's body. Factors of the intoxication plan were also found: acetone (n=105), which indicates excessive replication of bacteria in the oral cavity. Identification of indole in 72 cases indicates effects in the regulation of various aspects of bacterial physiology and the level of virulence. Tryptophan is an indole derivative and precursor of the neurotransmitter serotonin and may cause vomiting and angiospasm in the patient.
4. The level of IL-1 is increased 2,7 times, IL-4 11,8 times, IL-6 1,7 times, γ-IFN – 3,6 times, TNF-α – 26 times, Neopterin – 9 times. IL-2 and Cortisol levels are within reference values.
5. According to the data of the immunogram, changes are observed only in the indicator of Thyroid peroxidase antibody (TPO) levels (7,26 ± 4,71 IU/ml), but they are unreliable in comparison with the data of the control group p=0,07.
6. The most frequent detection of *Escherichia coli* microorganisms has a reliable positive relationship with Free T4 (r = 0,19, p = 0,05), TNF-α (r = 0,20, p = 0,04) and *Staphylococcus aureus*, which has a negative correlation with Lactoperoxidase enzyme (r =-0,20, p=0,04) and Acetone levels (r =-0,21, p=0,03).

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The Authors declare no conflict of interest

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