FLU IN CHILDREN: CLINICAL, LABORATORY INDICATORS AND CYTOKINE PROFILE PARAMETERS

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ABSTRACT

Introduction. *Respiratory diseases occupy a leading place in the structure of pathology of childhood. The proportion of influenza and acute respiratory viral infections among all infectious diseases is 90 %. The literature describes gender differences in the immune response to respiratory infections in children, but there is a gap in the description of the cytokine profile in children with influenza depending on gender and age.*

The aim of the study. *To analyze clinical and laboratory parameters as well as cytokine profile parameters in children with influenza.*

Materials and methods. *A single-stage descriptive study was conducted with the participation of 50 children from 1 to 11 years of age with a diagnosis of influenza who were on inpatient treatment at the Irkutsk Regional Infectious Diseases Clinical Hospital from December 2018 to January 2019. The clinical and laboratory features of the course of influenza in children and the duration of treatment were determined. The concentration of cytokines interleukin (IL) 1β, IL-4, IL-6, IL-8, tumor necrosis factor alpha (TNF-α), interferon alpha and gamma (INF-α, INF-γ) in blood plasma was determined by enzyme-linked immunosorbent assay (ELISA) using diagnostic test systems manufactured by Vector-Best (Novosibirsk, Russian Federation) on the analyzer Multiscan EX (Thermo Electron, Germany). The control group consisted of practically healthy children without signs of acute respiratory viral infection (n = 50; mean age – 5.3 ± 2.6 years).*

Results. *When comparing clinical and laboratory data and cytokine profile parameters in children with influenza, no gender differences were found. There was a statistically significant increase in the level of pro-inflammatory cytokines IL-1β, IL-6, IL-8, TNF-α, INF-α, as well as CRP, anti-inflammatory cytokine IL-4 in influenza in all age categories, in contrast to the control group ($p < 0.05$).*

Conclusion. *Influenza in children of different sexes proceeds classically without a statistical difference in clinical and laboratory parameters and in the level of cytokines.*

Key words: *flu, clinic, cytokines, children*

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ГРИПП У ДЕТЕЙ: КЛИНИЧЕСКИЕ, ЛАБОРАТОРНЫЕ ПОКАЗАТЕЛИ И ПАРАМЕТРЫ ЦИТОКИНОВОГО ПРОФИЛЯ

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РЕЗЮМЕ

Введение. *Болезни органов дыхания занимают ведущее место в структуре патологии детского возраста. Удельный вес гриппа и острой респираторной вирусной инфекции (ОРВИ) среди всех инфекционных болезней составляет 90 %. В литературе описаны гендерные различия в иммунном ответе при респираторных инфекциях у детей, однако существует пробел в описании цитокинового профиля у детей с гриппом.*

Цель исследования. *Провести анализ клинических и лабораторных показателей, а также параметров цитокинового профиля у детей с гриппом.*

Материал и методы. *Проведено одномоментное сравнительное исследование с участием 50 детей от 1 года до 11 лет с диагнозом грипп, которые находились на стационарном лечении в ОГБУЗ «Иркутская областная инфекционная клиническая больница» с декабря 2018 по январь 2019 г. Определялись клинические, лабораторные особенности течения гриппа у детей, продолжительность лечения. Концентрация цитокинов интерлейкина (IL) 1β, IL-4, IL-6, IL-8, фактора некроза опухоли α (TNF-α, tumor necrosis factor α), интерферона (INF) α, INF-γ и высокочувствительного С-реактивного белка (СРБ) в плазме крови определялась методом твердофазного иммуноферментного анализа (ИФА) с использованием диагностических тест-систем производства «Вектор-Бест» (г. Новосибирск) на анализаторе Мультискан EX (Thermo Electron, Германия). Контрольную группу составляли практически здоровые дети без признаков ОРВИ (n = 50; средний возраст 5,3 ± 2,6 года).*

Результаты*. При сравнении клинико-лабораторных данных и параметров цитокинового профиля у детей с гриппом гендерных различий не выявлено. Отмечается статистически значимое повышение уровня провоспалительных цитокинов IL-1β, IL-6, IL-8, TNF-α, INF-α, а также СРБ, противовоспалительного цитокина IL-4 при гриппе во всех возрастных категориях, в отличие от контрольной группы (p < 0,05).*

Заключение. *Грипп у детей разного пола протекает классически без статистической разницы в клинико-лабораторных показателях и в уровне цитокинов.*

Ключевые слова*: грипп, клиническая картина, цитокины, дети*

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INTRODUCTION

Among the aetiologically diverse groups of respiratory viral infections, influenza is a more serious concern and remains an uncontrollable global infection that causes enormous socioeconomic damage. The World Health Organization estimates that influenza and respiratory diseases affect 100 million people (5 to 30 % of the world's population) each year. According to the Ministry of Health of the Russian Federation, economic losses from influenza and acute respiratory viral infection (ARVI) account for 86 % of all damage caused by infectious diseases. The magnitude of the damage caused by influenza and influenza-like infections to public health and the economy of any country can be compared with those for injuries, cardiovascular diseases and malignant neoplasms [1].

Markers of the severe course of pandemic influenza AH1N1pdm09 in adolescents and adults are elevated blood concentrations of cytokines such as tumour necrosis factor alpha (TNF-α) and interleukin (IL) 6 [2]. Similarly, in pandemic influenza complicated by pneumonia, albeit in childhood, there is an increased synthesis of anti-inflammatory mediators and decreased concentration of pro-inflammatory cytokines IL-1β and TNF-α [3]. The same markers are assigned a leading role in the development of cytokine storm in influenza patients [4, 5].

Higher plasma IL-6 and TNF-α concentrations in nasopharyngeal wash samples have been described in overweight children [6, 7]. These children also have higher morbidity and mortality in influenza and a high risk of developing secondary bacterial infections [8].

A similar pattern of pro-inflammatory cytokine secretion of TNF-α and IL-6 has been observed in influenza type A and B in children. In influenza caused by influenza A virus, cytokine output with a predominance of Th2 inflammation is observed; IL-4 output is particularly elevated, which is not observed in influenza B [9].

There is uneven agreement among different authors as to which type of influenza virus variant – seasonal or pandemic – is predominant in childhood. Some authors indicate that seasonal influenza more often causes illness in children under 2 years of age: the infection is particularly severe in children under 6 months of age; pandemic influenza, on the contrary, more often affects children of preschool age, occurs more often in children over 5 years of age [10]. Other authors have reported the opposite: the pandemic variant of influenza is statistically significantly more common in children of the first year of life, while the seasonal variant is more common in children of primary school age [11].

Influenza has moderate form in young children, and only 16 % of cases have a severe course of the disease. Among the symptoms, catarrhal inflammation of the upper respiratory tract and intoxication syndrome predominate [12].

In the disease pattern of influenza in children, acetonemic syndrome is observed in 26.3 % of cases, as well as isolated cases of neurotoxicosis and haemorrhagic syndrome [13, 14].

Nowadays, it has been proved that influenza type A, especially the virus subtype containing neuraminidase N2, is associated with a more severe course of infection, more often complicated by secondary bacterial infection [15, 16].

In respiratory viral infections, oxidative stress is actively involved in the mechanisms of sustaining homeostatic disturbances; antioxidant drugs such as vitamin C, N-acetylcysteine, quercetin, glutathione, fat-soluble vitamins and polyunsaturated fatty acids have proven themselves in clinical trials in influenza, pneumonia and other respiratory diseases [17, 18].

Male subjects have higher synthesis of pro-inflammatory cytokines such as IL-6 and TNF-α; female subjects have high synthesis of the anti-inflammatory cytokine IL-10. Gender differences in immune response have been attributed to hormonal factors. There is speculation that women's defense against infections is associated with the pro-inflammatory effects of estradiol, while men's susceptibility to infections is associated with immunosuppression as a consequence of testosterone effects, possibly involving specific receptors [19].

There is a significant difference between girls and boys in the concentration of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), neutrophils in inflammatory processes in children with pneumonia, pyelonephritis, bronchiolitis [20].

Recently, there is a limited number of studies devoted to the analysis of cytokine profile parameters in children with influenza; there is also insufficient information on gender differences in acute respiratory viral infections in children.

THE AIM OF THE STUDY

To analyse clinical and laboratory parameters and to assess cytokine profile parameters in children with influenza.

MATERIALS AND METHODS

A one-stage comparative study was conducted with the participation of 50 children aged 1 to 11 years old, who from December 2018 to January 2019 were admitted for inpatient treatment in the Irkutsk Regional Infectious Diseases Clinical Hospital with a diagnosis of influenza.

Distribution of children by age groups was performed according to the periodisation of childhood using the scheme of N.P. Gundobin: early childhood – from 1 to 3 years; preschool age, middle childhood or the 1st period of childhood – from 3 to 7 years; younger school age, older childhood or the 2nd period of childhood – girls from 7 to 11 years, boys from 7 to 12 years.

The clinical features of the influenza course in children were analysed with consideration of the disease duration, number of days spent in hospital, main and concomitant diagnoses, presence of complications, disease outcome,

and history of influenza vaccine prophylaxis. Disease pattern was assessed by complaints, occurrence, nature and duration of symptoms of rhinitis, cough, intoxication (lethargy, refusal to eat, nausea or vomiting at the peak of fever, general weakness, sleep disturbance) and fever (body temperature increase from 37.2 to 38.0 °C – subfebrile fever; from 38.1 to 39.0 °C – febrile fever; from 38.1 to 39.0 °C – febrile fever; 39.1 to 40.0 °C – pyretic fever), other clinical manifestations (rash, cramps, sore throat, headache, muscle pain), deviations of laboratory indicators of general and biochemical blood tests and general urinalysis on admission, volume and duration of infusion, antiviral and antibacterial drug therapy throughout the disease.

The concentration of highly sensitive CRP and cytokines IL-1β, IL-4, IL-6, IL-8, TNF-α, INF-α, INF-γ in blood serum was determined by solid-phase enzyme immunoassay (EIA) using diagnostic test systems produced by Vector-Best (Novosibirsk) on a MultiscanEX microplate photometer (Thermo Electron, Germany). Blood sampling to determine the level of CRP and cytokines was performed in the first 3 days of the disease at admission to the hospital. The main group consisted of children diagnosed with influenza (n = 50), control group – of 50 practically healthy children without signs of ARVI or 14 days after acute viral infection (mean age of children – 5.3 ± 2.6 years). The study protocol was approved by the Biomedical Ethics Committee of Scientific Centre for Family Health and Human Reproduction Problems (excerpt from the minutes of the meeting No. 8.4 dated November 02, 2018).

Analysis and statistical processing of information were performed using MS Excel (Microsoft Corp., USA) and Statistica 6.0 application software package (StatSoft Inc., USA). The confidence interval with significance level of 95 % (95 % CI) for frequencies and fractions was calculated using an online calculator at Vassar Stats: Website for Statistical Computation[1]. Qualitative characters are presented as absolute (n) and relative values (P), quantitative characters are presented with median (Me) plus 25th and 75th quartiles (Q25; Q75). In the analysis of qualitative characters, the relative frequency of the characteristic (prevalence) P and 95 % CI were assessed. Statistical significance of intergroup differences in qualitative characters was assessed using the χ^2 criterion: at $P_{abs.} < 10$ – with Yates correction, at $P_{abs.} < 5$ – using Fisher's two-sided exact test. Statistical significance of two unrelated groups was assessed by Mann – Whitney test (U-test). The critical level of statistical significance in testing statistical hypotheses about the existence of differences between groups (p) was considered to be 0.05.

RESULTS AND DISCUSSION

In the study group, the sex distribution was almost equal: the proportion of boys was 52 % (n = 26), girls – 48 % (n = 24). The mean age of the children was 3 ± 2.6 years.

The diagnosis of influenza was verified by polymerase chain reaction comprehensive diagnosis with typing of influenza A/B strains in 96 % of children (n = 48) in the study group. Influenza A serotype H1N1sw2009 predominated in the etiologic structure: it was observed in 76 % of cases (n = 38). Influenza AH3N2 was the second most frequently observed – 16 % (n = 8). Clinically, influenza A was diagnosed in 4 % of patients (n = 2), mixed viral infection influenza AH1N1 + respiratory syncytial mixtovirus infection in 2 % (n = 1), and influenza A H1N1 + bocavirus mixtovirus infection in 2 % (n = 1).

Along with the main diagnosis, concomitant pathology was also considered: acute intestinal infection (rota- and norovirus etiology) in 16 % of cases (n = 8), atopic dermatitis in 2 % (n = 1), hypochromic anaemia in 6 % (n = 3). Complications of the underlying disease were observed in 30 % of patients (n = 15): acetone vomiting syndrome or ketoacidosis syndrome in 12 % (n = 6), pneumonia in 16 % (n = 8), acute obstructive bronchitis in 2 % (n = 1).

The mean duration of children's admission to hospital was 5 ± 1.6 days. In analysing the vaccination history, it was found that influenza vaccination was performed in 16 % of cases (n = 8), 72 % of patients (n = 36) were not vaccinated against influenza, and 12 % of patients (n = 6) had no information about vaccination.

Clinical features of the influenza course in children were assessed by the presence of complaints and objective examination data. The prevalence of complaints and clinical manifestations in the study group were as follows: pharyngeal hyperemia – 96 % (n = 48), rhinorrhea – 66 % (n = 33), cough – 84 % (n = 42), sore throat and pain when swallowing – 6 % (n = 3), back pain abdominal and loose stools – 8 % (n = 4), intoxication syndrome – 38 % (n = 19), including fever – 92 % (n = 46), lethargy, drowsiness – 44 % (n = 22), weakness – 34 % (n = 17), vomiting – 28 % (n = 14), decreased appetite – 26 % (n = 13), headache – 8 % (n = 4), convulsions – 4 % (n = 2), ear pain and dizziness – in single cases, muscle and joint pain was not observed in anyone. In describing the nature and duration of respiratory symptoms, children diagnosed with influenza were observed to have the following predominant symptoms: mucous runny nose – 72 % (n = 36) with a duration of 5 ± 2.48 days; dry cough – 84 % (n = 42) and wet cough – 6 % (n = 3). The median fever was 38.5 ± 0.68° and the duration of fever was 2 ± 1.45 days.

The clinic of influenza in children corresponds to the forme pleine: mucous runny nose, dry cough, fever, and hyperemia of the pharynx predominate in 96 % of cases. No statistical differences were revealed cause-specific to the gender of the child in terms of both the prevalence of clinical manifestations (Table 1) and the duration of the main symptoms (Table 2).

No differences between the gender groups were also revealed while comparing the main haematological and biochemical parameters of blood and urine in children with influenza (Table 3).

In the epidemic season of 2009, according to the findings of S.A. Chavanina et al. (2011), among the peculiarities of seasonal influenza it was observed that in 25 % of cases

[1] http://vassarstats.net/

patients were being admitted to hospital on the 6–9th day from the onset of the disease, often against the background of already developed complications. The height of fever was higher in children with seasonal influenza AH1N1 (mean – 39.2 °C vs. 37.9 °C in pandemic influenza A H1N1sw2009); marked intoxication and haemorrhagic syndrome were statistically significantly more prevalent in seasonal influenza AH1N1 [21]. The analysis of haematological changes revealed a high incidence of leukocytosis in patients with influenza AH1N1 (33.3 %) [22], while in our study we obtained data revealing that leukocytosis in children with influenza was observed in 6 % of cases and accelerated ESR syndrome in 42 % of cases.

The duration of infusion, antiviral and antibiotic therapy for influenza in children of different sexes did not differ. All hospitalized children were discharged with recovery.

Determination of cytokine and high-sensitivity CRP concentrations in children with influenza during the first days of hospitalisation was compared with a group of healthy children standardised by the copy-pair method. The method is based on the selection for each observation unit of the group under study by one or more characteristics, in this case – by gender, age; this method of selection is expedient for studying rare phenomena. The levels of CRP and cytokine profile parameters studied in healthy children were within the reference range according to the test system manufacturer's instructions: CRP < 5 pg/ml; IL-1β < 11 pg/mL; IL-4 < 4 pg/mL; IL-6 < 10 pg/mL; IL-8 < 12 pg/mL; TNF-α < 6 pg/mL; INF-α < 11 pg/mL; INF-γ < 20 pg/mL. In the serum of children with influenza, the concentrations of IL-1β, IL-4, IL-6, IL-8, TNF-α, INF-γ and CRP were significantly higher than the upper limit of the range.

A number of literature sources provide age and gender differences in the levels of pro- and anti-inflammatory cytokines in children, which is important to consider when interpreting cytokine status parameters [2, 21, 23].

No statistically significant differences were revealed by analysing cytokine levels associated with influenza in children of different genders (Table 4).

TABLE 1

MAIN CLINICAL SYMPTOMS IN CHILDREN WITH INFLUENZA ACCORDING TO GENDER (*n* = 50)

Indices	Influenza A, boys (*n* = 26)	Influenza A, girls (*n* = 24)	*P* (Fisher's exact criterion)
Mucous rhinitis	19 (73 %)	17 (70.8 %)	0.554
Dry cough	17 (65.4 %)	19 (79.2 %)	0.222
Fever	15 (57.7 %)	22 (91.6 %)	0.539
Intoxication	10 (38.5 %)	9 (37.5 %)	0.588
Pharynx hyperemic	25 (96.2 %)	23 (95.8 %)	0.734
Drowsiness, lethargy	11 (42.3 %)	11 (45.8 %)	0.513
Weakness	9 (34.6 %)	8 (33.3 %)	0.581
Vomiting	6 (23.1 %)	7 (29.2 %)	0.433

Note. * – differences are statistically significant at $p < 0.05$.

TABLE 2

DURATION OF MAJOR CLINICAL PARAMETERS IN CHILDREN WITH INFLUENZA ACCORDING TO GENDER

Duration of symptoms (days)	Influenza A, boys (*n* = 26) Me (Q25; Q75)	Influenza A, girls (*n* = 24) Me (Q25; Q75)	*p* (U-test)
Rhinitis	5 (3; 6)	5 (1.5; 5)	0.302
Cough	5 (4; 6)	5 (4; 7)	0.380
Fever	2 (2; 4.25)	2 (1; 3)	0.482

Note. * – differences are statistically significant at $p < 0.05$.

TABLE 3

LABORATORY PARAMETERS IN CHILDREN WITH INFLUENZA ACCORDING TO GENDER

Indices	Influenza A, boys (n = 26) Me (Q25; Q75)	Influenza A, girls (n = 24) Me (Q25; Q75)	p (U-test)
Erythrocytes, $\times 10^{12}$	4.35 (4.06; 4.70)	4.36 (4.07; 4.79)	0.917
Hemoglobin, g/L	120 (113.00; 127.00)	120 (113.25; 127.00)	0.796
Platelets, $\times 10^9$	226 (191.75; 250.75)	226 (187.25; 252.25)	0.358
Leukocytes, $\times 10^9$	3.57 (2.73; 5.00)	3.59 (2.74; 4.93)	0.796
Stab neutrophils, $\times 10^9$	1.84 (0.98; 3.34)	1.84 (0.98; 3.16)	0.876
Segmented neutrophils, $\times 10^9$	0.84 (0; 2.27)	0.00 (0.00; 1.37)	0.515
Lymphocytes, $\times 10^9$	1.32 (1.08; 1.89)	1.32 (1.09; 1.88)	0.764
Monocytes, $\times 10^9$	0.43 (0.34; 3.00)	0.48 (0.34; 3.00)	0.287
Eosinophils, $\times 10^9$	0.03 (0.01; 0.06)	0.03 (0.01; 0.07)	0.392
ESR, mm/h	12 (5.00; 19.00)	12 (5.00; 19.00)	0.179
C-reactive protein, mg/L	17.10 (3.87; 18.30)	15.10 (5.57; 18.10)	0.926
Total protein, g/L	67.10 (60.25; 70.93)	67.60 (60.63; 70.98)	0.983
Glucose, mmol/L	4.02 (3.52; 4.58)	4.02 (3.54; 4.49)	0.664
AST, U/L	51.25 (38.15; 68.38)	51.25 (38.36; 67.53)	0.529
ALT, U/L	15.25 (10.95; 21.25)	15.25 (10.85; 20.95)	0.860
Creatinine, μmol/L	44.9 (41.12; 53.15)	45.35 (41.33; 53.25)	0.278
Urea, mmol/L	1.53 (0.00; 3.86)	1,53 (0.00; 3.79)	0.967
Amylase, U/L	0 (0.00; 34.30)	0 (0.00; 35.10)	0.332
Urine specific gravity, g/L	1020 (1013.75; 1020.00)	1020 (1015; 1020)	0.470
Ketones in urine, mg/dL	0 (0.00; 0.50)	0 (0.00; 0.50)	0.489

Note. ESR – erythrocyte sedimentation rate; AST – aspartate aminotransferase; ALT – alanine aminotransferase; * – differences are statistically significant at $p < 0.05$.

TABLE 4

CYTOKINE LEVELS IN CHILDREN WITH INFLUENZA CAUSE-SPECIFIC TO GENDER

Cytokine concentration (pg/mL)	Influenza A, boys (n = 26) Me (Q25; Q75)	Influenza A, girls (n = 24) Me (Q25; Q75)	p (U-test)
IL-1β	8.10 (3.25; 12.45)	8.30 (2.75; 21.80)	0.853
IL-4	3.80 (1.95; 6.60)	3.2 (1.92; 4.45)	0.593
IL-6	18.30 (13.42; 32.77)	22.50 (14.20; 41.82)	0.509
IL-8	157.55 (72.02; 233.17)	183.70 (102.85; 287.30)	0.361
TNF-α	2.25 (1.70; 4.03)	2.55 (1.30; 4.52)	0.961
IFN-α	27.35 (4.90; 54.80)	21.4 (13.75; 59.03)	0.838
IFN-γ	1.60 (0.85; 5.50)	1.2 (0.35; 3.17)	0.289

Note. * – differences are statistically significant at $p < 0.05$.

Analysis of cytokine and CRP levels in influenza among children of different ages revealed that children of primary school age have increased synthesis of IL-1β, IL-6, IL-8, IFN-α, in contrast to young children, but no statistically significant differences were revealed ($p > 0.05$) (Table 5).

Increased synthesis of IL-4, TNF-α, IFN-γ was observed in young children, in contrast to preschool and primary school children; no statistically significant differences were revealed ($p > 0.05$) (Table 5).

TABLE 5

CYTOKINE AND C-REACTIVE PROTEIN LEVELS IN CHILDREN WITH INFLUENZA CAUSE-SPECIFIC TO AGE

Indices	Influenza A, Me (Q25; Q75)	*p* (U-test)
IL-1β[1]	5.50 (12.89; 28.64)	0.614
IL-1β[2]	6.60 (9.29; 17.26)	0.159
IL-1β[3]	14.50 (23.85; 63.29)	0.299
IL-4[1]	3.60 (1.95; 4.33)	0.470
IL-4[2]	3.35 (2.70; 5.02)	0.792
IL-4[3]	3.45 (1.86; 4.94)	0.760
IL-6[1]	14.80 (28.59; 63.55)	0.626
IL-6[2]	18.90 (11.00; 20.43)	0.278
IL-6[3]	31.60 (44.40; 117.85)	0.215
IL-8[1]	119.65 (88.71; 197.13)	0.581
IL-8[2]	199.24 (70.69; 131.32)	0.121
IL-8[3]	247.80 (76.87; 204.04)	0.161
TNF-α[1]	3.15 (3.15; 7.00)	0.277
TNF-α[2]	1.75 (1.18; 2.20)	0.769
TNF-α[3]	2.70 (15.69; 41.66)	0.117
IFN-γ[1]	2.55 (2.93; 6.51)	0.284
IFN-γ[2]	1.20 (2.19; 4.07)	0.953
IFN-γ[3]	1.40 (2.75; 7.31)	0.451
IFN-α[1]	31.85 (17.04; 37.86)	0.338
IFN-α[2]	17.00 (43.39; 80.61)	0.364
IFN-α[3]	45.75 (45.96; 121.99)	0.230
CRP [1]	14.55 (5.615; 12.477)	0.322
CRP [2]	17.20 (5.84; 10.85)	0.953
CRP [3]	15.25 (5.95; 15.80)	0.439

Note. [1] – age category of children from 1 year to 2 years 11 months 29 days ($n = 14$); [2] – age category of children from 3 years to 6 years 11 months 29 days ($n = 22$); [3] – age category of children from 7 to 11 years inclusive ($n = 14$); * – differences are statistically significant at $p < 0.05$.

Preschool children have increased synthesis of CRP, as opposed to children of other age categories; no statistically significant differences were revealed ($p > 0.05$) (Table 5). The pro-inflammatory cytokines IL-1β, IL-6, IL-8 and anti-inflammatory IFN-α are elevated in serum among children with seasonal influenza compared to healthy controls, with IL-6 concentration statistically significantly increased in all age groups ($p < 0.05$). In the age group of 7–11 years, a more significant increase was revealed in serum IL-1β – 14.50 (23.85; 63.29) pg/ml, IL-8 – 247 (76.87; 204.04) pg/ml, IFN-α – 45.75 (45.96; 121.99) pg/ml, compared to children of early and preschool age. Such results in school-age children can probably be explained both by a more efficient realisation of phagocytic neutrophil functions and by a more pronounced activity of inflammation. An increase in serum interleukins 1-β, IL-8 and INF-α is considered by some authors as unfavourable prognostic signs against the development of pneumonia and its prolonged and complicated course [21, 24, 25]. The interpretation of the increase in pro-inflammatory cytokine levels, according to the literature data, has a contradictory character and further requires the analysis of the relationship of systemic concentrations of IL-1β, IL-6, IL-8 and TNF-α with the severity and duration of intoxication syndrome, the peculiarities of the influenza course.

One of the key cytokines in the implementation of antiviral and anti-infectious immunity is IL-4, which provides induction of immune response through the humoral pathway. The cell-mediated Th1-type response is most effective in viral infections; therefore, in a number of diseases, IL-4 hyperproduction against the background of IFN-γ decrease is considered as an unfavourable prognostic sign [26]. However, there is a study that revealed increased levels of the anti-inflammatory cytokine IL-4 to be prognostically favourable in the course of pneumonia in preterm neonates, in contrast, an increase in TNF-α was associated with an unfavourable outcome of the disease [27]. Among children with influenza, a statistically significant increase in IL-4 was revealed in all age groups, in contrast to the control group ($p < 0.05$). The IFN-γ level during influenza in children of the 2nd and 3rd age groups was higher than in healthy children, and at early age had no statistically significant differences in the influenza acute period, which can be considered a failure of the interferon system in children under 3 years of age and can be regarded as a decrease in antiviral defense and as a predictor of a complicated, prolonged course of viral infection in this age group.

The analysis of CRP level as a marker of the acute phase of inflammation is of great diagnostic significance in bacterial infections, while in viral diseases the increase of CRP is determined less frequently, and a significant increase in serum CRP in influenza may be a prognostically unfavourable sign against the development of bacterial complications.

We have studied the relationship between the parameters of cytokine status and CRP in children with influenza as a cause-specific complication of pneumonia

(Table 6). Consequently, in children with uncomplicated influenza pneumonia a positive correlation between CRP and IL-1β levels was revealed, which is evidence of inflammation activity and timely activation of innate immunity factors. The negative correlation between CRP and IFN-γ levels in children with influenza without pneumonia is evidence of insufficiency of the interferon system in childhood during the acute phase of viral infection.

Influenza with pneumonia in children was characterised by a statistically significant direct correlation between CRP and IL-4 levels, which indicates deviation of Th0-lymphocyte differentiation by $CD4^+$/Th2-type and is a determining factor affecting the severity of clinical manifestations and outcome of influenza infection [28, 29].

CONCLUSION

Clinical manifestations of influenza in children aged 1 to 11 years, admitted to an infectious diseases hospital, correspond to the classic pattern/forme pleine: mucous runny nose, dry cough, fever, and pharyngeal hyperaemia predominate in 96 % of cases. Complications of the underlying disease were observed in 30 % of patients ($n = 15$) in the form of acetonemic vomiting syndrome or ketoacidosis syndrome, pneumonia, obstructive syndrome. No statistically significant differences were revealed in children with influenza cause-specific to gender, prevalence of clinical manifestations, duration of main symptoms, duration and intensity of infusion, antiviral and antibacterial therapy. The results of the analysis of islet-inflammatory, general clinical, biochemical indices of blood, urine, parameters of cytokine profile in children with influenza also revealed no statistically significant differences.

The study established the peculiarities of cytokine status in children during the acute phase of influenza. The increased concentration of CRP, systemic levels of pro-inflammatory cytokines IL-1β, IL-6, IL-8 and anti-inflammatory IFN-α in influenza in children in all age groups, especially at the age of 7–11 years, requires further additional analysis and identification of the relationship of IL-1β, IL-6, IL-8 and TNF-α levels with the features of the disease pattern, in particular, with the severity of intoxication syndrome, in order to determine the degree of influence of each cytokine on the course and outcome of influenza in childhood.

The absence of statistically significant differences in IFN-γ levels at an early age during the acute period of viral infection compared with healthy children is alarming considering the risk of prolonged and complicated course of influenza, especially if the decrease in IFN-γ is accompanied by increased IL-4 levels. Increased systemic IL-4 level along with high concentration of CRP can be considered statistically significant signs of influenza complicated by pneumonia in childhood.

The revealed peculiarities of cytokine status in influenza among children allow to make some approximation to the understanding of the nature of the infectious process. Studies in this area are worth continuing, as they will have practical significance: identification of markers of adverse influenza course, development of a personalized approach to the treatment of acute respiratory infections in children under 3 years of age and timely prescription of medicinal products based on interferon and its inducers for prophylactic and therapeutic purposes, as well as the development of immunorehabilitation programmes.

Conflict of interest
The authors of this article declare no conflicts of interest.

TABLE 6

CORRELATION BETWEEN C-REACTIVE PROTEIN AND CYTOKINE LEVELS IN CHILDREN WITH INFLUENZA CAUSED-SPECIFIC TO THE PRESENCE OF PNEUMONIA COMPLICATION

Cytokines	Children with influenza without complications ($n = 42$)		Children with influenza complicated by pneumonia ($n = 8$)	
	r	*p*	*r*	*p*
IL-1β	0.309	0.008*	–0.142	0.735
IL-4	0.094	0.257	0.754	0.031*
IL-6	0.206	0.155	0.261	0.530
IL-8	0.217	0.123	0.428	0.289
TNF-α	0.265	0.088	0.144	0.732
IFN-α	–0.066	0.284	–0.595	0.119
IFN-γ	–0.477	0.001*	–0.239	0.567

Note. *r* – Spearman correlation coefficient; *p* – statistical significance of differences; * – differences are statistically significant at $p < 0.05$.

REFERENCES

1. *Influenza and other acute respiratory viral infections during the ongoing COVID-19 pandemic: Prevention and treatment. Guidelines*. Moscow, 2022. (In Russ.). [*Грипп и другие ОРВИ в период продолжающейся пандемии COVID 19: профилактика и лечение.* Методические рекомендации. М., 2022].

2. Ivanov VV, Shipilov MV. Inflammatory cytokines and their importance in influenza pH1N1. *Medical News of North Caucasus*. 2012; 4: 70-72. (In Russ.). [Иванов В.В., Шипилов М.В. Провоспатительные цитокины и их значение при гриппе pH1N1. *Медицинский вестник Северного Кавказа*. 2012; 4: 70-72].

3. Miromanova NA, Baranchugova TS. Clinical and epidemiological analysis of the course of highly pathogenic influenza A H1N1 in children of the Transbaikal region. *Aktual'nye voprosy klinicheskoy I eksperimental'noy meditsiny: Materialy X yubileynoy nauchno-prakticheskoy konferentsii molodykh uchenykh*. Saint Petersburg; 2010: 187-188. (In Russ.). [Мироманова Н.А., Баранчугова Т.С. Клинико-эпидемиологический анализ течения высокопатогенного гриппа А H1N1 у детей Забайкальского края. *Актуальные вопросы клинической и экспериментальной медицины: Материалы X юбилейной научно-практической конференции молодых учёных*. СПб.; 2010: 187-188].

4. Hagau N, Slavcovici A, Gonganau DN, Oltean S, Dirzu DS, Brezoszki ES. Clinical aspects and cytokine response in severe H1N1 influenza A virus infection. *Crit Care Med*. 2010; 14(6): 203. doi: 10.1186/cc9324

5. Lai C, Wang X, Yang P. Cytokines network and influenza virus infection. *Clin Microbial*. 2014; 3(147): 3. doi: 10.4172/2327-5073.1000147

6. Arias-Bravo G, Valderrama G, Inostroza J, Tapia C, Toro-Ascuy D, Ramilo O, et al. Overnutrition, nasopharyngeal pathogenic bacteria and proinflammatory cytokines in infants with viral lower respiratory tract infections. *Int J Environ Res Public Health*. 2022; 19: 8781. doi: 10.3390/ijerph19148781

7. Luzina EV, Lareva NV. Severe respiratory complications as a cause of poor outcome of influenza A (H1N1sw2009) in obese patients. *Pulmonologiya*. 2011; (3): 96-100. (In Russ.). [Лузина Е.В., Ларева Н.В. Тяжелые респираторные осложнения как причина неблагоприятного исхода при гриппе А (H1N1sw2009) у больных с ожирением. *Пульмонология*. 2011; 3: 96-100]. doi: 10.18093/0869-0189-2011-0-3-96-100

8. Garcia-Sastre A. Induction and evasion of type I interferon responses by influenza viruses. *Virus Res*. 2011; 162: 12-18. doi: 10.1016/j.virusres.2011.10.017

9. Masatoki S, Mitsuaki H, Peter FW. Differences in serum cytokine levels between influenza virus A and B infections in children. *Cytokine*. 2009; 47(1): 65-68. doi: 10.1016/j.cyto.2009.05.003

10. Principi N, Esposito S. Severe influenza in children: incidence and risk factors. *Expert Revi Anti Infect Ther*. 2016; 14(10): 961-968. doi: 10.1080/14787210.2016.1227701

11. Lobzin YuV, Babachenko IV, Vasiliev VV, Uskov AN. Features of influenza in children possibility of modern management and prevention. *Consilium Medicum*. 2016; 18(3): 12-17. (In Russ.). [Лобзин Ю.В., Бабаченко И.В., Васильев В.В., Усков А.Н. Особенности гриппа у детей, современные возможности лечения и профилактики. *Consilium Medicum*. 2016; 18(3): 12-17]. doi: 10.26442/2075-1753_2016.3.12-17

12. Kochkina SS, Lerner EV, Bakhareva TB, Kremneva NYu, Ryabikova ES. Features of influenza in 2019 in young children in Yaroslavl. *Children Infections*. 2019; 18 (Special Issue): 47. (In Russ.). [Кочкина С.С., Лернер Е.В., Бахарева Т.Б., Кремнева Н.Ю., Рябикова Е.С. Особенности гриппа в 2019 году у детей раннего возраста в г. Ярославле. *Детские инфекции*. 2019; 18 (Спецвыпуск): 47].

13. Kelesheva IYu, Petrova AG, Rychkova LV, Moskaleva EV. Retrospective analysis of the clinical course of influenza in obese children. *Children Infections*. 2019; 18(S): 70-71. (In Russ.). [Келешева И.Ю., Петрова А.Г., Рычкова Л.В., Москалева Е.В. Ретроспективный анализ клинического течения гриппа у детей с ожирением. *Детские инфекции*. 2019; 18(S): 70-71]. doi: 10.22627/2072-8107-2019-18-1S

14. Petrova AG, Rychkova LV, Vanyarkina AS, Kelesheva IYu, Moskaleva EV, Novikova EA. Clinical laboratory features of influenza in children with obesity. *Clinical Practice in Pediatrics*. 2020; 15(4): 8-14. (In Russ.). [Петрова А.Г., Рычкова Л.В., Ваняркина А.С., Келешева И.Ю., Москалёва Е.В., Новикова Е.А. Клинико-лабораторные характеристики гриппа у детей с ожирением. *Вопросы практической педиатрии*. 2020; 15(4): 8-14]. doi: 10.20953/1817-7646-2020-4-8-14

15. Skryabina AA, Nikiforov VV, Shakhmardanov MZ, Zastrozhin MS. Bacterial complications of influenza (literature review). *Lechaschi vrach*. 2022; 1(11): 48-54. (In Russ.). [Скрябина А.А., Никифоров В.В., Шахмарданов М.З., Застрожин М.С. Бактериальные осложнения гриппа (обзор литературы). *Лечащий врач*. 2022; 11(25): 48-54]. doi: 10.51793/OS.2022.25.11.008

16. Abramovich ML, Ploskireva AA. Features of hematological parameters in acute respiratory infections in children of different ages. *Lechaschi vrach*. 2015. [Абрамович М.Л., Плоскирева А.А. Особенности гематологических показателей при острых респираторных инфекциях у детей разного возраста. *Лечащий врач*. 2015]. URL: https://www.lvrach.ru/2015/11/15436342 [дата доступа: 20.07.2023].

17. Darenskaya MA, Kolesnikova LI, Kolesnikov SI. The association of respiratory viruses with oxidative stress and antioxidants. implications for the COVID-19 pandemic. *Current Pharmaceutical Design*. 2021; 27(13): 1618-1627. (In Russ.). [Даренская М.А., Колесникова Л.И., Колесников С.И. Ассоциация респираторных вирусов с окислительным стрессом и антиоксидантами. Последствия для пандемии COVID-19. *Current Pharmaceutical Design*. 2021; 27(13): 1618-1627]. doi: 10.2174/1381612827666210222113351

18. Darenskaya MA, Kolesnikova LI, Kolesnikov SI. COVID-19: oxidative stress and the relevance of antioxidant therapy. *Annals of the Russian Academy of Medical sciences*. 2020; 75(4): 318-325. (In Russ.). [Даренская М.А., Колесникова Л.И., Колесников С.И. COVID-19: окислительный стресс и актуальность антиоксидантной терапии. *Вестник РАМН*. 2020; 75(4): 318-325]. doi: 10.15690/vramn1360

19. Casimir GJ, Mulier S, Hanssens L, Zylberberg K, Duchateau J. Gender differences in inflammatory markers in children. *Shock*. 2010; 33(3): 258-262. doi: 10.1097/SHK.0b013e3181b2b36b

20. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol*. 2016; 16: 626-638. doi: 10.1038/nri.2016.90

21. Chavanina SA, Bogomolova IK, Levchenko NV. Clinical course and immunological indicators in children with pneumonia in the period of grippe A/H1N1/09 pandemic. *Siberian Medical Review*. 2011; 6: 21-24. (In Russ.). [Чаванина С.А., Богомолова И.К., Левченко Н.В. Клиническое течение и иммунологические показатели при пневмониях у детей в период эпидемии гриппа А/H1H1/09. *Сибирское медицинское обозрение*. 2011; 6: 21-24].

22. Golovacheva EG, Afanasyeva VS, Osidak LV, Afanasieva OI, Obraztsova EV, Koroleva EG, et al. The dynamics of the immune response to influenza in children treated with interferon. *Children Infections*. 2017; 16(1): 7-12. (In Russ.). [Головачева Е.Г., Афанасьева В.С., Осидак Л.В., Афанасьева О.И., Образцова Е.В., Королева Е.Г., и др. Особенности динамики иммунного ответа при гриппе у детей на фоне интерферонотерапии. *Детские инфекции*. 2017; 1: 7-12]. doi: 10.22627/2072-8107-2017-16-1-7-12

23. Kolesnikova NV, Kondratieva EI, Nesterova IV, Gaprindaschvili EG, Ponomarenko YuB, Asecretova TV, et al. Age and sexual features of some cytokines healthy children. *Kuban Scientific Medical Bulletin*. 2011; 6(129): 68-72. (In Russ.). [Колесникова Н.В., Кондратьева Е.И., Нестерова И.В., Гаприндашвили Е.Г., Пономаренко Ю.Б., Асекретова Т.В., и др. Возрастные и половые особенности некоторых цитокинов крови здоровых детей. *Кубанский научный медицинский вестник*. 2011; 6(129): 68-72].

24. Chavanina SA, Bogomolova IK, Levchenko NV. Changes in cytokine levels during the complicated course of influenza A/H1N1/09 in children. *Children Infections*. 2012; 11: 90-91. (In Russ.). [Чаванина С.А., Богомолова И.К., Левченко Н.В. Изменение уровней цитокинов при осложненном течении гриппа A/H1N1/09 у детей. *Детские инфекции*. 2012; 11: 90-91].

25. Sovalkin VI, Sokolova TF, Sabitova ON. The content of TNF-α, IL-1β, IL-8 in patients with different clinical risk factors for prolonged community-acquired pneumonia and their prognostic significance. *Siberian Medical Journal*. 2013; 122(7): 56-60. (In Russ.). [Совалкин В.И., Соколова Т.Ф., Сабитова О.Н. Содержание ФНО-α, ИЛ-1β, ИЛ-8 у больных с различными клиническими факторами риска затяжной внебольничной пневмонии и их прогностическая значимость. *Сибирский медицинский журнал*. 2013; 122(7): 56-60].

26. Kasokhov TB, Sokhiyeva FA, Tsorayeva ZA, Mazur AI, Tsareva AA. Indicators of the cytokine profile in children with respiratory allergies. *Effective Pharmacotherapy*. 2019; 15(37): 14-16. (In Russ.). [Касохов Т.Б., Сохиева Ф.А., Цораева З.А., Мазур А.И., Царева А.А. Показатели цитокинового профиля у детей с респираторными аллергозами. *Эффективная фармакотерапия*. 2019; 15(37): 14-16]. doi: 10.33978/2307-3586-2019-15-37-14-16

27. Zhuravleva LN, Novikova VI. The role of cytokines in the pathogenesis of pneumonia in preterm newborns. *International Journal of Immonopathology, Allergology, Infectology*. 2018; 3: 33-38. (In Russ.). [Журавлева Л.Н., Новикова В.И. Роль цитокинов в патогенезе пневмоний у недоношенных новорожденных. *Иммунопатология, аллергология, инфектология*. 2018; 3: 33-38]. doi: 10.14427/jipai.2018.3.33

28. Zheleznikova GF. Cytokines as predictors of infection course and outcome. *Cytokines & Inflammation*. 2009; 8(1): 10-17. (In Russ.). [Железникова Г.Ф. Цитокины как предикторы течения и исхода инфекций. *Цитокины и воспаление*. 2009; 8(1): 10-17].

29. Golovacheva EG, Afanasyeva OI, Osidak LV, Obraztsova EV, Voloshchuk LV. The influence of the interferon system on the direction of polarization of the immune response during influenza in children. *Pediatrician*. 2014; 3: 51-57. (In Russ.). [Головачева Е.Г., Афанасьева О.И., Осидак Л.В., Образцова Е.В., Волощук Л.В. Влияние системы интерферона на направленность поляризации иммунного реагирования при гриппе у детей. *Педиатр*. 2014; 3: 51-57].

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