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The course of COVID-19 in school-aged children depends on the state of humoral immunity against diphtheria

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Introduction: There is evidence that high titers of diphtheria antibodies are more often associated with an asymptomatic or less severe course of COVID-19. The aim of the study was to determine the features of the COVID-19 course in school-aged children depending on the state of postvaccination immunity against diphtheria toxin.

Methods. In total, 90 children aged 6 to 18 years were examined: 60 patients with confirmed SARS-CoV-2 infection and 30 children without signs of the disease (control group). There were 20 patients with a mild COVID-19 course, 31 patients with moderate disease severity and 9 patients with severe disease severity. All children were tested for the level of immunoglobulin G (IgG) against diphtheria toxin by immune-enzymatic analysis.

Results. Total serum immunoglobulin G against diphtheria toxin was 0.431 (0.113; 0.828) IU/mL. Antitoxic immunoglobulin G was 2.5 times higher in children in the control group than in patients with SARS-CoV-2 infection. A decreased level of immunoglobulin G was associated with increased disease severity. In children with specific IgG 0.01-0.2 IU/mL revaccination is recommended; therefore, 4.2% of the children in the control group, 6.8% with a mild COVID-19 course, 50% with moderate disease severity and 100% of children with severe COVID-19 needed a booster dose of diphtheria toxoid. A moderate negative correlation was observed between the level of immunoglobulin G against diphtheria toxin and ESR (r=-0.38, p=0.004), CRP (r=-0.32, p=0.021), D-dimer (r =-0.35, p=0.009), duration of hyperthermia (r=-0.49, p=0.003), and duration of treatment (r=-0.43, p=0.012).

Conclusions. Children with manifested SARS-CoV-2 infection had significantly lower levels of immunoglobulin G against diphtheria toxin compared to the control group, indicating a lower level of humoral immunity. Children with a mild course of COVID-19 had a significantly higher level of specific IgG against diphtheria toxin than those with a moderate and severe disease course.

Keywords: CHILD; COVID-19; DIPHTHERIA; IMMUNITY, HUMORAL

INTRODUCTION

Among all patients with COVID-19 in Ukraine, children account for 5%. Since the beginning of the pandemic, more than 153,000 children have been infected in Ukraine, and more than 50 of them have died. A significant proportion of people infected with SARS-CoV-2 are asymptomatic, while others develop severe respiratory distress syndrome (1). Therefore, it is important to establish factors that influence the course of coronavirus disease, morbidity and mortality. There is evidence that high titers of diphtheria antibodies are more often associated with an asymptomatic or less severe course of COVID-19 (2, 3). These statements are based on the study of the relationship between coronavirus proteins and some pathogenic microorganisms. In particular,

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the structure of the diphtheria toxin is similar to that of the SARS-CoV-2 protein, so the administration of diphtheria-tetanus toxoid can have a protective effect against SARS-CoV-2 (4). Worldwide, children receive several vaccinations against diphtheria and tetanus, including three to four doses during the first year of life (depending on the country of residence) and one dose between the ages of 4 and 6 years. In addition, a low antigenic dose in the form of ADT-m-toxoid is also administered at the age of 9 to 16 years. There is evidence of a high correlation between the response of memory T cells to spike-S1 and the nuclecapsid of the SARS-CoV-2 virus and proteins of pathogens of other infections, including Corynebacterium diphtheria. Numerous cross-reactive T and B-cell epitopes between antigens have also been detected in vaccines against tetanus, diphtheria, pertussis and SARS-CoV-2 (2).

In this way, it is likely that children can be protected from SARS-CoV-2 through the cross-immunity caused by vaccination against diphtheria (5, 6). The aim of the study was to determine the features of the course of COVID-19 in school-aged children depending on the state of postvaccination immunity against diphtheria toxin by evaluating the level of specific immunoglobulins G against diphtheria toxin in their blood serum.

PATIENTS AND METHODS

The study was conducted in the Ternopil Municipal Children's Communal Hospital. Patient safety rules and ethical principles of conducting scientific medical research with human participation (2000) were applied. Written informed consent was obtained from the parents of the examined patients. The Bioethics Commission of I. Horbachevsky Ternopil National Medical University granted permission to conduct this study (protocol No. 61 dated November 13, 2020). An examination of 90 children aged 6 to 18 years was conducted, among which 60 had clinical manifestations of laboratory-confirmed SARS-CoV-2 infection and 30 had no signs of COVID-19 (with a negative antigen rapid test).The etiology of the disease was confirmed in 63.33% by PCR, in 21.67% by rapid antigen test (in the presence of a positive epidemiological anamnesis), and in 15.00% by the presence of positive immunoglobulins M (7).

The clinical course of the disease and laboratory indicators (C-reactive protein, ESR, D-dimer, leukocytes) were evaluated. The criteria of the mild COVID-19 course were subfebrile fever, diarrheal syndrome without signs of dehydration, catarrhal syndrome without lower respiratory tract involvement, and changes in taste or smell (8, 9). Moderate course criteria were hyperthermia above subfebrile, manifestations of dehydration, and signs of pneumonia without disturbing saturation. Patients with severe disease were treated in the intensive care unit, had signs of severe respiratory failure, and needed additional oxygen supply; no patients needed mechanical ventilation, and nobody died. The children in the control group were not hospitalized, and their general condition was satisfactory. According to the severity of the course of the disease, 3 groups were formed: 20 children with a mild course of COVID-19 made up the first group, 31 patients with a moderate course made up the second group, and 9 patients with a severe course of the disease formed the third group. Children without any manifestations of infectious diseases (30 people) made up the control group. A total of 33,3% (n=20) of children with signs of COVID-19 had comorbidities. There was no significant difference in the frequency of comorbidities in the groups of children with different courses of COVID-19 ($p = 0.066$, $x2 = 5.43$). Among comorbidities were allergic diseases (30%), followed by chronic adenoiditis and tonsillitis (20%), obstructive bronchitis, helminth infections and being overweight (10% each), and finally (5% each) urinary tract infections without renal failure, chronic gastritis, congenital heart disease without heart failure, and chronic ear disease. No children had received immunosuppressive therapy in the past.

Blood samples for laboratory tests were taken on the day of seeking medical care, before corticosteroid and antimicrobial therapy was prescribed. According to the manufacturer's instructions, after sampling, venous blood was centrifuged, and serum samples were stored at 2-8°C for up to 7 days, with longer storage at -20°C. Lipemic, hemolyzed, and bacterially contaminated specimens were not used. Quantitative determination of the level of immunoglobulin G (Ig G) against diphtheria toxin was carried out in all children by immunoenzymatic analysis (Diphtheria Elisa Ig G, IBL, Germany). The results were evaluated as follows: less than 0.01 IU/ml - recommended basic immunization, 0.01-0.1 IU/ml - recommended booster vaccination, and more than 0.1 IU/ml - good immunity.

Statistical analysis was performed with the help of the "Stat Plus" program, and the assessment of the normality of the distribution of features in the variation series was performed according to the Shapiro-Wilk test. Under the condition of correct distribution of values ($p>0.05$), quantitative data were presented in the form of mean ± SD (standard deviation); in the case of incorrect distribution ($p < 0.05$ for the Shapiro-Wilk test), quantitative data were presented in the form of median and ICR (lower and upper quartile). A comparison of frequency indicators in observation groups was performed using the $\chi 2$ criterion. Quantitative indicators were assessed by the Kruskal–Wallis and Mann–Whitney tests. The Spearman correlation coefficient was used.

RESULTS

The average age of the observed children was (11,47±3.80) years, the control group – (11,57±3,27) years, the children of the first group (with a mild course of COVID-19) – (12,00±3,92)) years, patients of the second group (with a moderate course of COVID-19) – (11,54±4,05) years, of the third group (severe course of COVID-19) – (9,67±4,39 years) (p=0,490). There was no significant difference between the gender composition in the groups of this study (x2=4,97; p=0,174). The total serum immunoglobulin G against diphtheria toxin was 0.431 (0.113; 0.828) IU/ml. The mean level of immunoglobulin G against diphtheria toxin in children in the control group was 0.51 (0.38; 0.87) IU/ml, and in children with manifestations of SARS-CoV-2 infection, it was 0.21 (0.09; 0.46) IU/ml (p=0.007).

Immunoglobulins G against diphtheria toxin levels and their distribution in the observation groups are presented in Table 1. The level of antitoxic immunoglobulin G was 2.5 times higher in children in the control group than in patients with manifestations of SARS-CoV-2 infection. A de-

TABLE 1. **Indicators of class G immunoglobulins against diphtheria toxin in observation groups**

Indicator	Control group, n=30	Children with the signs of SARS-CoV-2-infection, n=60			H, p
		I group	II group	III group	
Immunoglobulins G against diphtheria toxin, IU/ml	0,52 [0,38; 0,87]	0,44 [0,21; 0,83]	0,11 [0,07; 0,31]	0,08 [0,06; 0,09]	H=13,07; p=0,005*

Note 1. * – a statistically significant difference.
Note 2. Validity of differences between groups: p_{k-II}=0,013, p_{k-III}=0,011, p_{I-II}=0,022, p_{I-III}=0,008

TABLE 2. **The level of immunoglobulin G against diphtheria toxin in blood serum in the observation groups**

The level of Ig G against diphtheria toxin, IU/ml	Control group, n=30	I group, n =20	II group, n=31	III group, n=9	χ^2; p
<0,01 IU/ml Basic vaccination recommended	0%	0%	0%	0%	χ^2=24,12 p<0,001*
0,01–0,1 IU/ml Booster vaccination recommended	4,2%	6,8%	50,0%	100,0%	
>0,1 IU/ml Good immunity	95,8%	93,2%	50,0%	0%	

Note * – a statistically significant difference.

TABLE 3. **Basic clinical and laboratory characteristics of children with symptoms of COVID-19**

Patients' groups	The first group, n = 20	The second group, n=31	The third group, n=9	χ^2, p/ H, p	p<0,05*
Presence of hyperthermic syndrome	18 (90,00%)	28 (90,32%)	9 (100,00%)	χ^2=0,96 P=0,617	–
Duration of hyperthermic syndrome, days	2,0 [1,0; 4,0]	6,0 [3,3; 8,0]	9,5 [7,0; 10,0]	H=14,17 P <0,001*	p1-2=0,008* p1-3=0,001* p2-3=0,031*
Duration of treatment, days	5,0 [3,0; 7,3]	9,0 [5,5; 10,0]	14,0 [10,0; 16,0]	H=15,03 P <0,001*	p1-2<0,014* p1-3<0,001* p2-3<0,008*
C-reactive protein, mg/l	4,25 [2,95; 5,30]	5,40 [3,52; 11,3]	33,5 [21,9; 51,3]	H=38,69 P <0,001*	p1-3<0,001* p2-3=0,002* p1-2=0,067
ESR, mm/h	6,5 [3,0; 10,0]	12 [5,0; 17,7]	21,5 [15,6; 27,0]	H=34,60 P <0,001*	p1-2=0,028* p1-3<0,001* p2-3=0,005*
D-dimer, mg/l	94,1 [47,3; 255,3]	269,7 [142,0; 535,8]	2000,0 [1881,5; 3112,9]	H=34,47 P <0,001*	p1-2=0,005* p1-3<0,001* p2-3=0,002*
The number of WBC, 10^9/l	4,7 [4,2; 7,5]	8,5 [4,7; 12,0]	15,1 [9,3; 26,7]	H=12,47 P=0,006*	p1-2=0,031* p1-3=0,011* p2-3=0,058

Note * - a statistically significant difference

TABLE 4. **Correlation of the WBC count, D-dimer level, ESR, CRP level, duration of hyperthermia and duration of treatment depending on the level of specific immunoglobulin G against diphtheria toxin**

Indicator	0,01 – 0,1 IU/ml	>0,1 IU/ml	p
The number of WBC, 10^9/l	12,6 [5,2; 18,4]	5,7 [4,7; 7,4]	P=0,020*
ESR, mm/h	13,5 [10,0; 19,0]	5,0 [3,0; 7,0]	P<0,001*
D-dimer, mg/l	191,3 [155,0; 277,2]	76,0 [52,0; 119,5]	P=0,004*
CRP, mg/l	14,1 [5,1; 23,3]	3,4 [2,1; 4,1]	P=0,002*
Duration of hyperthermia, days	7,0 [6,0; 9,5]	3,0 [1,0; 7,5]	P=0,012*
Duration of treatment, days	10,0 [8,5; 14,0]	7,0 [3,5; 9,5]	P=0,008*

Note. * - statistically significant difference

gree of decrease in immunoglobulin G level follows an increase in the severity of the disease.

In our study, 3.33% of children in the control group and 28.85% of children with manifestations of SARS-CoV-2 infection (p=0.004) had an incomplete course of vaccination (according to age). Among patients with COVID-19, 10% of children with a mild course, 29.63% with a moderate course, and 100% of children with a severe course of the disease had incomplete vaccination against diphtheria (p<0.001, x2 = 28.65). Children with the level of specific immunoglobulins G, such as 0,01–0,1 IU/ml, needed booster vaccination (revaccination). In total, 4.17% of children in the control group needed booster vaccination, 6.8% in the first group, and 50% and 100% of children in the second and third groups (patients with moderate and severe course of the disease) needed a booster dose of diphtheria toxoid, respectively.

There was no significant difference in the presence of hyperthermic syndrome in all groups of patients with COVID-19 (p=0.617, x2=0.96). The duration of hyperthermic syndrome increased with the severity of the disease. In children with a more severe course of the disease, the duration of treatment increased, and there were higher indicators of ESR, CRP, D-dimer, and the number of leukocytes, which indirectly indicate the severity of the course of COVID-19 (10,11). The ratio of the number of WBCs, ESR, D-dimer, CRP, the duration of hyperthermia, and the duration of treatment depending on the level of specific immunoglobulins G against diphtheria toxin is shown in Table 4. In children with manifestations of coronavirus infection, a reduced number of antibodies to diphtheria toxin, an increase in the number of leukocytes, an increase in ESR, D-dimer, CRP, duration of hyperthermia and duration of treatment are previously reported as indicators of a more severe course of this disease (10). In our study, a medium-strength negative correlation was observed between the level of immunoglobulin G against diphtheria toxin and ESR (r=-0.38, p=0.004), CRP (r=-0.32, p=0.021), D-dimer (r =-0.35, p=0.009), duration of hyperthermia (r=-0.49, p=0.003), and duration of treatment (r=-0.43, p=0.012).

DISCUSSION

According to the national vaccination calendar approved by the Ministry of Health of Ukraine, children under the age of 18 receive 6 vaccinations against diphtheria: at the age of 2, 4, and 6 months, at 18 months, and at 6 and 16 years. Therefore, school-age children from 6 to 17 years old should have 5 or more vaccinations against this infection according to the vaccination calendar. The main reason for incomplete immunization was the refusal of parents to carry it out on time and their underestimation of the risks of violating the vaccination schedule.

According to the instructions for the assay, the result of each sample was evaluated in three ways: <0.01 IU/ml-basic immunization recommended, 0.01-0.1 IU/ml-booster immunization recommended, and >0.1 IU/ml-good immunity. In our opinion, booster vaccination in this case means an additional dose of vaccine to form sufficient immunity after laboratory confirmation of a low concentration of specific antibodies (or revaccination), indicating an insufficient titer of protective antibodies against this disease. Similar terminology is used in Europe and in the USA (4, 12, 13). According to the national vaccination calendar approved by the Ministry of Health of Ukraine, vaccinations against diphtheria and tetanus are also carried out for adults every 10 years. In the current literature, there is some information that the frequency of severe forms of COVID-19 was less than 54% in people who received a diphtheria booster (12, 13, 14, 15). Other findings indicate that there is a high correlation between T-cell responses to SARS-CoV-2-spike-S1 and nucleocapsid and Tdap proteins. Further study of the immune response against diphtheria and other vaccine-controlled infections as prognostic biomarkers for the occurrence of similar lesions in this infectious process and the possibility of using nonspecific vaccination as one of the methods of immune protection in this disease is needed.

CONCLUSIONS

The indicator of antitoxic immunoglobulin G was 2.5 times higher in children in the control group than in patients with manifestations of SARS-CoV-2 infection, which indicates a lower level of humoral immunity against diphtheria in this group of patients. Children with a mild course of the disease had a significantly higher level of specific immunoglobulins against diphtheria toxin (which indicates a higher level of humoral immunity against this pathogen) compared to groups of children with a moderate and severe course of

the disease. Against the background of low levels of immunoglobulins G to diphtheria toxin, there is an increase in the level of pro-inflammatory markers (number of leukocytes, ESR, CRP) and D-dimer, duration of the hyperthermic syndrome and duration of treatment, which also indicates a more severe course of the disease caused by COVID-19 (i.e., A lower level of humoral immunity against diphtheria is associated with an increase in the severity of the disease caused by COVID-19.

Vaccination against diphtheria, as a rule, is carried out together with vaccination against tetanus with a combined vaccine. Therefore, it is advisable to study humoral immunity against tetanus and its impact on the course of coronavirus infection.

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SAŽETAK

Klinički tijek i težina COVID-19 bolesti u djece školske dobi ovisi o stanju humoralne imunosti protiv difterije

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Uvod: Postoje dokazi da su visoki titri antitijela na difteriju češće povezani s asimptomatskim ili lakšim tijekom bolesti COVID-19. Cilj istraživanja bio je utvrditi značajke tijeka COVID-19 kod djece školske dobi ovisno o stanju imuniteta nakon cijepljenja protiv toksina difterije.

Metode: Ukupno je pregledano 90 djece u dobi od 6 do 18 godina: 60 pacijenata s potvrđenom infekcijom SARS-CoV-2 i 30 djece bez znakova bolesti (kontrolna skupina). COVID-19 blažeg kliničkog tijeka je bio u 20 pacijenata, srednje teškog u 31 bolesnika i težeg u 9 bolesnika. Svoj djeci je imunoenzimskom analizom određena razina imunoglobulina G (IgG) protiv toksina difterije.

Rezultati: Ukupni serumski IgG protiv toksina difterije bio je 0,431 (0,113; 0,828) IU/mL. Antitoksični IgG bio je 2,5 puta veći u djece u kontrolnoj skupini nego u bolesnika s infekcijom SARS-CoV-2. Smanjena razina antitoksičnog IgG bila je povezana s povećanom težinom bolesti. U djece sa specifičnim IgG 0,01-0,2 IU/mL preporučuje se ponovno cijepljenje; stoga je 4,2% djece u kontrolnoj skupini, 6,8% s blagim tijekom bolesti COVID-19, 50% s umjerenom težinom bolesti i 100% djece s teškim oblikom bolesti COVID-19 trebalo docjepljivanje toksoida difterije. Umjerena negativna korelacija zabilježena je između razine IgG protiv toksina difterije i ESR (r=-0,38, p=0,004), CRP (r=-0,32, p=0,021), D-dimera (r=-0,35, p =0,009), trajanje hipertermije (r=-0,49, p=0,003) i trajanje liječenja (r=-0,43, p=0,012).

Zaključak: Djeca s manifestiranom infekcijom SARS-CoV-2 imala su značajno niže razine IgG protiv toksina difterije u usporedbi s kontrolnom skupinom, što ukazuje na nižu razinu humoralne imunosti. Djeca s blagim tijekom bolesti COVID-19 imala su značajno višu razinu specifičnih IgG protiv toksina difterije od djece s umjerenim i teškim tijekom bolesti.

Ključne riječi: DIJETE; DIFTERIJA; IMUNOST, HUMORALNA