ORIGINAL ARTICLE

CONTENTS

Comprehensive approach to comparative characteristics of clinical and laboratory parameters of the study in children - in 6 months after covid-19 treatment and 6 months after Covid-19 vaccination

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ABSTRACT

Aim: To study and analyze data on clinical and laboratory parameters of investigated children in 6 months after treatment and 6 months after Covid-19 vaccination.
Materials and Methods: A prospective clinical and laboratory examination of a children group (group 1) with identified Covid-19 (n=68) was carried out in comparison with a group (n=31) of Covid-19 vaccinated children (group 2) after 6 months of observation.
Results: The data of Anti-SARS-CoV-2-S1-RBD IgG, non-significant intergroup differences in levels were found (344.71 ± 87.62 versus 315.67 ± 74.91 BAU/ml, p=0.11). Significant differences were found in the children group who was 6 months after Covid-19 treatment and the control group, as well as in the group before and 6 months after Covid-19 vaccination, in the following parameters: IL-2 (p6<0.01; p7<0.01, with a prevalence of 6 and 4 times, respectively); IL-4 (p6<0.01; p7<0.01with a prevalence of 6 and 4.6 times, respectively), IL-10 (p6<0.01; p7<0.01 with a prevalence of 6 and 4.8 times, respectively), Procalcitonin (p6<0.01; p7<0.01, 18 times and 1.4 times respectively).
Conclusions: In our study, according to the data of multivariate linear regression analysis, the level of Anti-SARS-CoV-2-S1-RBD IgG is the dependent variable, and D-dimer, Na level, Total Cholesterol, LF, IL-6, IgG are the influencing factors which determine the COVID-19 anti-infective protection.

KEY WORDS: Covid-19, vaccination, clinical laboratory investigation, children

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INTRODUCTION

COVID-19 vaccination programs have included children much later than adults due to the lack of early data on safety, efficacy, and the relatively mild disease profile in the pediatric population. The children's immune systems are functionally distinct from those of other age groups and the development of vaccines specifically for the pediatric population has largely been limited to titration of primarily vaccines doses developed for adults [1]. However, age-based de-escalation is now being used in the development of new vaccines: once safety and efficacy have been established in the adult population, younger cohorts are enrolled and studied [2].

Several COVID-19 vaccines have been approved for use in children and/or adolescents, including Comirnaty (Pfizer), Spikevax (Moderna), and CoronaVac (Sinovac Biotech). The Pfizer-BioNTech COVID-19 vaccine (BNT162b2) is the only vaccine approved by the Food and Drug Administration (FDA) for children ages 12–17 in the United States. Additionally, in June 2022, the FDA granted emergency use authorization for BNT162b2 and the Moderna vaccine for individuals ages 6 months to 5 years. Although the ingredients in these pediatric vaccines are the same as the adult formulations, the dosage has been adjusted based on age. Although the vaccines work somewhat differently, they all prime a person's immune system to prevent SARS-CoV-2 infection, if infected, to prevent severe curse [3,4].

AIM

To study and analyze data on clinical and laboratory parameters in children over a 6-month period: in those who have recovered from Covid-19 and those vaccinated against Covid-19, with subsequent prediction of anti-infective protection.

MATERIALS AND METHODS

A prospective clinical and laboratory examination of a children group (group 1) with identified Covid-19

Table 1. Anti-SARS-CoV-2-S1-RBD IgG levels in the children studied

Parameters	1 group (n = 68) M ± m	2 group (n = 31) M ± m	p
	6 months after treatment	6 months after vaccination	
Anti-SARS-CoV-2-S1-RBD IgG (BAU/ml)	344,71 ± 87,62	315,67 ± 74,91	0,11

Table 2. Metabolic pool indicators in dynamics

Parameters	Control group(n = 28) M ± m	1 group (n = 68) M ± m	2 group (n = 31) M ± m
		6 months after treatment	6 months after vaccination
Vitamn D3 (30-70, ng/ml)	35,27 ± 4,28	33,63 ± 5,17	34,68 ± 2,91 (p_1=0,54; p_2=0,29)
Zn (12-25, mkmol/l)	16,28 ± 3,05	14,75 ± 4,27	16,47 ± 4,35 (p_1=0,85; p_2=0,07)

Notes: p_1 - significance of differences between the values of indicators 6 months after vaccination and the control group; p_2 - significance of differences between the values of indicators 6 months after vaccination and indicators in patients with COVID 6 months after treatment.

(n=68) who were in the outpatient department of the city multidisciplinary clinical hospital in Uzhhorod was carried out in comparison with a group(n=31) of Covid-19 vaccinated children (group 2) after 6 months of observation. The control group included healthy children (n=28), identical in age and anthropometric parameters. Clinical and laboratory studies were conducted, which included biochemical, immunological examination, markers of inflammatory and endocrine regulation.

RESULTS

The SARS-CoV-2 mRNA vaccines (BNT162b2) and (mRNA-1273) have been approved by the European Medicines Agency and the US Food and Drug Administration (FDA) for use in children aged 6 months and older. These vaccines offer excellent protection against severe course diseases in children [5].

According to the Ministry of Health of Ukraine, by order No. 1477 dated 17.07.2021, the recommendations of the National Technical Group of Experts on Immunoprophylaxis had approved the possibility of children aged 12 and older vaccination with the Comirnaty, Pfizer-BioNTech vaccines, based on available scientific studies and WHO recommendations with appropriate changes to the instructions for the Comirnaty/Pfizer-BioNTech vaccine. Recommendations for the children from 12 years of age were administered 2 doses of the Comirnaty/Pfizer-BioNTech vaccine, 0.3 ml, with an interval of 21-28 days, with the necessary observance of a 14-day interval between the administration of the COVID-19 vaccine and other diseases, according to the developed [6].

Our study examined the indicators of the child's organism in response to vaccination against SARS-CoV-2 and in comparison with the transferred Covid-19 infection, after 6 months, by determining markers of inflammation, immune response, thyroid status and adipose tissue hormones. Vaccination was carried out as part of the primary vaccination series (i.e., the first two doses administered according to the recommended schedule) and administered before the registered infection. We compared the systemic levels of antibodies (Anti-SARS-CoV-2-S1-RBD IgG) in a subgroup of infected and vaccinated children (Table 1).

When comparing the antibodies data to the spike protein, insignificant differences between the levels were found (344.71 ± 87.62 versus 315.67 ± 74.91 BAU/ml, p=0.11), which indicates the effectiveness of the vaccination in children. Our data are consonant with the many scientists data [7].

Dynamic levels of metabolic pool indicators were investigated (Table 2).

According to Table 2, no significant differences were observed between the levels of vitamin D3 (33.63 ± 5.17 versus 34.68 ± 2.91 and compared with the data of the control group 35.27 ± 4.28 ng/ml, at p1=0.54; p2=0.29) and Zn values (14.75 ± 4.27 versus 16.47 ± 4.35 and compared with the data of the control group 16.28 ± 3.05 μmol/l, at p1=0.85; p2=0.07).

Table 3. Comparative dynamic characteristics of inflammation indicators in the groups of post-COVID and vaccinated children

Parametrs	Control group (n = 28) M ± m	1 group (n = 68) M ± m	2 group (n = 31) M ± m	
		6 months after treatment	Before vaccination	6 months after vaccination
IL-1 (0-11, ng/ml)	0,65 ± 0,06	0,66 ± 0,62	0,68 ± 0,07 (p_5=0,08)	0,67 ± 0,12 (p_6=0,69; p_7=0,43; p_8=0,93)
IL-2 (0-10, pg/ml)	0,34 ± 0,15	1,88 ± 0,89	0,40 ± 0,09 (p_5=0,07)	1,57 ± 0,65 (p_6<0,01; p_7<0,01; p_8=0,09)
IL-4 (up to 0,5, ng/ml)	0,17 ± 0,04	0,99 ± 0,61	0,20 ± 0,08 (p_5=0,08)	0,91 ± 0,42 (p_6<0,01; p_7<0,01; p_8=0,51)
IL-6 (0-10, ng/ml)	0,77 ± 0,04	2,53 ± 1,07	0,69 ± 0,32 (p_5=0,20)	2,18 ± 1,03 (p_6<0,01; p_7<0,01; p_8=0,13)
IL-10 (0-20, pg/ml)	0,48 ± 0,06	2,87 ± 1,92	0,51 ± 0,12 (p_5=0,24)	2,43 ± 1,51 (p_6<0,01; p_7<0,01; p_8=0,26)
γ-IFN (up to 15,0, pg/ml)	8,01 ± 0,32	6,23 ± 4,82	7,48 ± 1,71 (p_5=0,11)	6,73 ± 5,02 (p_6=0,43; p_7=0,18; p_8=0,64)
TNF-α (up to 6, pg/ml)	3,62 ± 0,31	3,17 ± 1,30	3,31 ± 0,86 (p_5=0,08)	3,09 ± 1,66 (p_6=0,52; p_7=0,10; p_8=0,79)
Neopteryn (up to 10, nmol/l)	7,61 ± 1,50	8,68 ± 6,39	6,97 ± 1,41 (p_5=0,10)	8,25 ± 5,74 (p_6=0,23; p_7=0,57; p_8=0,75)
CRP(<3, mg/l)	1,91 ± 0,53	1,64 ± 0,73	2,01 ± 0,39 (p_5=0,41)	1,58 ± 0,82 (p_6=0,01; p_7=0,08; p_8=0,72)
Procalcitonin (0-11, pg/ml)	1,61 ± 0,23	0,09 ± 0,01	1,49 ± 0,35 (p_5=0,13)	1,02 ± 0,02 (p_6<0,01; p_7<0,01; p_8<0,01)
Fibrinigen (2-4, g/l)	2,77 ± 0,33	2,97 ± 0,57	2,84 ± 0,29 (p_5=0,39)	3,02 ± 0,65 (p_6=0,16; p_7=0,07; p_8=0,70)
D-dimer (up to 0,5, mkg/ml)	0,33 ± 0,05	0,28 ± 0,14	0,35 ± 0,03 (p_5=0,07)	0,30 ± 0,09 (p_6=0,01; p_7=0,13; p_8=0,47)

Notes: p5 - significance of differences between the values of indicators before vaccination and the control group; p6 - significance of differences between the values before vaccination and after vaccination; p7 - significance of differences between the values of indicators 6 months after vaccination and the control group; p8 - significance of differences between the values of indicators 6 months after vaccination and the indicators in patients who had COVID, 6 months after treatment.

We will also consider a number of cytokines and other markers of the inflammatory reaction of the child'sorganism and their comparative dynamic characteristics in the studied groups (Table 3).

According to table 3, significant differences were found in the control and 6 months after treatment groups and in the group before vaccination and 6 months after in the following parameters: IL-2 ((p6<0.01; p7<0.01, with a prevalence of 6 and 4 times, respectively); IL-4 (p6<0.01; p7<0.01 with a prevalence of 6 and 4.6 times, respectively), IL-6 (p6<0.01; p7<0.01 with a prevalence of 3.3 and 3.2 times, respectively), IL-10 (p6<0.01; p7<0.01 with a prevalence of 6 and 4.8 times, respectively). The level of Procalcitonin presented significant differences in both groups (p6<0.01; p7<0.01;), in post-COVID children the level decreased in 18 times, of the vaccinated group in 1.4 times, which was a significant difference between the parameters, p8<0.01, but all values were corresponded to the reference values. A significant decrease in the C-reactive protein indicators (p6=0.01) and D-dimer (p6=0.01) were observed in 6 months after vaccination children, but, again, within the reference values. Let's consider the immunogram in children of the studied groups (Table 4)

According to Table 4, no significant differences between the immunogram indicators were found, which indicates intergroup identity or closeness of the immunogram values in children both after Covid-19 treatment and 6 months after Covid-19 vaccination. Many articles have been published on the topic of metabolic adaptation during COVID-19, so it is justified to study the level of adipose tissue hormones in the studied contingent (Table 5).

According to table 5, there are significant increases in Adiponectin levels (p6<0.01;p7<0.01;p8<0.01) in both groups and between them, with variation within the reference range. A significant increase in Ferritin levels (p7=0.002;p8=0.05) was noted in both groups and a significant decrease in Leptin values (p7=0.01; p8<0.01) For optimal interpretation and understanding of the obtained results of the study of children data in Covid-19 diagnosing multivariate linear regression analysis was performed. Regression analysis is a section of mathematical statistics devoted to methods of analyzing the dependence of one value to another. Unlike correlation analysis, regression analysis not only indicates the existence of a relationship between an independent variable and one or more dependent variables, but also allows us to determine this relationship quantitatively. Independent variables are called regressors or predictors, and dependent variables are called criteria. The terminology of dependent and independent variables reflects only the mathematical relationship between variables, and not the cause-and-effect relationships. Classical linear regression analysis is based on a system of provisions on the properties of the regression model, the implementation of which guarantees obtaining optimal estimates of the parameters and the regression function. Using the binary logistic regression method, it is possible to study the dependence of dichotomous (binary, i.e. those that have only 2 categorical values) variables on independent variables, the data can have any type of scale. Therefore, the relationships established during regression analysis can sometimes be mistakenly interpreted as cause-and-effect [8]. We present a developed mathematical model based on the statistically significant data we obtained and the ability to predict the dynamics of the dependent variable, in particular, the levels of ΔAnti-SARS-CoV-2-S1-RBD IgG, in accordance with the change in the studied influencing factors.

In our study, the Anti-SARS-CoV-2-S1-RBD test is the dependent variable, and D-Dimer, Na level, Total Cholesterol, APh, IL-6, IgG are the influencing factors. Formule:

ΔAnti-SARS-CoV-2-S1-RBD IgG = 28.08 + 6.93*D-Dimer – 0.17*Na + 2.09*Total Cholesterol – 0.05*APh + 0.76*IL-6 – 0.42*IgG.

An increase in the D-Dimer level by 1 μg/ml initiates an increase in the anti-SARS-CoV-2-S1-RBD IgG level by 6.93 BAU/ml; with an increase in the Na concentration by 1 mmol/l, the anti-SARS-CoV-2-S1-RBD IgG level will decrease by 0.17 BAU/ml; with an increase in the concentration of total cholesterol by 1 mmol/l, the level of anti-SARS-CoV-2-S1-RBD will decrease by 2.09 BAU/ml; an increase in the concentration of alkaline phosphatase by 1 U/l will be accompanied by a decrease in the level of anti-SARS-CoV-2-S1-RBD by 0.05 BAU/ml; with an increase in the concentration of IL-6 by 1 pg/ml, the level of anti-SARS-CoV-2-S1-RBD IgG will increase by 0.76 BAU/ml; an increase in the level of IgG by 1 g/l will contribute to a decrease in the level of anti-SARS-CoV-2-S1-RBD IgG by 0.42 BAU/ml.

DISCUSSION

Several previous studies of vaccine efficacy against long-term outcomes of COVID-19 have mostly demonstrated protective effects with a wide range of effect estimates, but some have not demonstrated an overall protective effect. [9,10] The methodology and data included in the previous studies were heterogeneous and had limitations. The study populations were rarely based on well-defined populations and often included small numbers of participants.[11,12] Analysis of the differential effects for different numbers of doses of the

Table 4. Dynamic indicators of the immunogram in the studied children

Parametrs	Control group (n = 28) M ± m	1 group (n = 68) M ± m	2 group (n = 31) M ± m	
		6 months after treatment	Before vaccination	6 months after vaccination
Ig M (0,31-1,79, g/l)	1,37 ± 0,06	1,53 ± 0,44	1,39 ± 0,24 (p_5=0,67)	1,41 ± 0,34 (p_6=0,79; p_7=0,54; p_8=0,18)
Ig G (6,98-15,49, g/l)	11,02 ± 0,07	10,21 ± 2,29	10,95 ± 0,46 (p_5=0,43)	10,08 ± 2,63 (p_6=0,08; p_7=0,06; p_8=0,80)
Ig E (Up to 120 IU/ml)	15,38 ± 5,07	13,61 ± 4,24	14,42 ± 6,01 (p_5=0,51)	14,63 ± 5,31 (p_6=0,89; p_7=0,58; p_8=0,31)
Ig A (0,61-3,48, g/l)	1,69 ± 0,43	1,75 ± 0,44	1,73 ± 0,52 (p_5=0,75)	1,77 ± 0,63 (p_6=0,79; p_7=0,58; p_8=0,86)

Notes: p5 - significance of differences between the values of indicators before vaccination and the control group; p6 - significance of differences between the values before vaccination and after vaccination; p7 - significance of differences between the values of indicators 6 months after vaccination and the control group; p8 - significance of differences between the values of indicators 6 months after vaccination and the indicators in patients who had COVID, 6 months after treatment.

Table 5. Analysis of metabolic homeostasis indicators in the studied groups of children

Parametrs	Control group (n = 28) M ± m	1 group (n = 68) M ± m	2 group (n = 31) M ± m	
		6 months after treatment	Before vaccination	6 months after vaccination
Feritin (7-140, ng/ml)	77,07 ± 10,40	96,81 ± 20,67	82,37 ± 12,11 (p_5=0,08)	88,41 ± 15,35 (p_6=0,09; p_7=0,002; p_8=0,05)
Adiponektin (5-18,6 mkg/ml)	7,73 ± 0,86	8,35 ± 8,62	8,95 ± 3,24 (p_5=0,06)	12,14 ± 2,61 (p_6<0,01; p_7<0,01; p_8<0,01)
Leptin (2,05-11,09, ng/ml)	6,97 ± 0,32	4,08 ± 0,61	6,53 ± 1,71 (p_5=0,19)	6,19 ± 1,17 (p_6=0,37; p_7=0,01; p_8<0,01)
C-peptide (0,81-3,85, ng/ml)	1,43 ± 0,08	1,56 ± 0,43	1,61 ± 0,62 (p_5=0,13)	1,58 ± 0,71 (p_6=0,86; p_7=0,27; p_8=0,86)

Notes: p5 - significance of differences between the values of indicators before vaccination and the control group; p6 - significance of differences between the values before vaccination and after vaccination; p7 - significance of differences between the values of indicators 6 months after vaccination and the control group; p8 - significance of differences between the values of indicators 6 months after vaccination and the indicators in patients who had COVID, 6 months after treatment.

COVID-19 vaccine has not always been performed.[13] A sound and informed understanding of the immune response nuances of to both, infection and vaccination, is essential for scientific research, which can be used to improve or develop new vaccines that are better able to control and prevent the spread of COVID-19 infection.[14].

The pediatric trial of the AstraZeneca COVID-19 vaccine was stopped after the detection of thrombosis with thrombocytopenia syndrome, a rare but serious

adverse event that affects mainly young people (3.4 per 100,000 people) [15]. Along with the deployment of vaccination programs for adolescents and children, vaccination of adults working in kindergartens, schools and health care facilities should be encouraged to provide indirect protection to children, minimizing transmission of SARS-CoV-2 [15].

It was noted that routine vaccination of healthy and non-contact children should not be stopped. On the contrary, it is necessary to continue primary vaccination of infants and young children according to routine programs to prevent the threat of outbreaks and epidemics, such as measles, polio, tetanus, diphtheria, etc. [16].

As described above, age-dependent molecular differences in the immune response to COVID-19 likely contribute to a milder course of infection in children. It is clear that adaptive immune responses change significantly with age. Studies should determine whether COVID-19 vaccination in children of different ages elicits different immune responses compared to adult patients to further optimize the efficacy of vaccination [16].

CONCLUSIONS

1. When comparing the data of antibodies to the Spike Protein, non-significant intergroup differences in levels were found (344.71 ± 87.62 versus 315.67 ± 74.91 BAU/ml, p=0.11), which indicates the effectiveness of the vaccination in children.
2. No significant differences were observed between the levels of Vitamin D3 (33.63 ± 5.17 versus 34.68 ± 2.91 and compared with the data of the control group 35.27 ± 4.28 ng/ml, at p1=0.54; p2=0.29) and Zn values (14.75 ± 4.27 versus 16.47 ± 4.35 and compared with the data of the control group 16.28 ± 3.05 µmol/l, at p1=0.85; p2=0.07).
3. Significant differences were found in the children group who was 6 months after Covid-19 treatment and the control group, as well as in the group before and 6 months after Covid-19 vaccination, in the following parameters: IL-2 (p6<0.01; p7<0.01, with a prevalence of 6 and 4 times, respectively); IL-4 (p6<0.01; p7<0.01with a prevalence of 6 and 4.6 times, respectively), IL-6 (p6<0.01; p7<0.01 with a prevalence of 3.3 and 3.2 times, respectively), IL-10 (p6<0.01; p7<0.01 with a prevalence of 6 and 4.8 times, respectively). The level of Procalcitonin presented significant differences in both groups (p6<0.01; p7<0.01;), in post-covid children the level decreased by 18 times, in the vaccinated group by 1.4 times, which was a significant difference between the indicators (p8<0.01), but all values corresponded to the reference values. A significant decrease in the indicators of CRP(p6=0.01) and D-Dimer (p6=0.01) was observed in post-vaccinated children after 6 months, but, again, within the reference values.
4. Significant increases in Adiponectin levels were found (p6<0.01; p7<0.01; p8<0.01) in both groups and between them, with variation within the reference range. A significant increase in Ferritin levels was noted (p7=0.002; p8=0.05) in both groups and a significant decrease in Leptin values (p7=0.01; p8<0.01) .
5. In our study, according to the data of multivariate linear regression analysis, the level of Anti-SARS-CoV-2-S1-RBD IgG is the dependent variable, and D-dimer, Na level, Total Cholesterol, LF, IL-6, IgG are the influencing factors that determine the value of the increase or decrease in the test of COVID-19 anti-infective protection

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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