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Evaluation of Immune Response in Asymptomatic Children with Parents withCOVID-19

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Abstract

Objective: From a public health perspective, understanding the pathogenesis and transmission of SARS-CoV-2 in children is important both in understanding the role of the pediatric population in the transmission and spread dynamics of the epidemic, and in controlling the severity of the pandemic. The present study investigated the role of children in the spread of COVID-19 infection.

Methods: Children, who applied to the Pediatrics Outpatient Clinic for routine check-ups between May and June 2021, with no symptoms and were not tested with RT-PCR for COVID-19 although their parents and close relatives were diagnosed with COVID-19 in the last 6 months, were evaluated prospectively.

Results: Thirty-five cases, 20 of whom were male, were included in the study. While 17 of the children had contact with only 1 case, 18 of them had contact with 2 or more cases. While the mean antibody values for IgG of the subjects with one contact were 1.55±1.93 S/C and IgG-spike was 910.6±1512.02 AU/mL, the mean antibody values for IgG of the subjects with 2 or more contacts were 2.21±2.07 S/C and 1289.15±1750.49 AU/mL for IgG-spike. Twelve of the cases came into contact with patients in autumn, 21 in winter, and 2 in spring.

Conclusion: As the virus spreads in the community, the dynamics and clinical features of the disease will change. There should be data with more cases in a wider geographical distribution. However, studies show that children are not the primary source of households to date. It was observed that the severity of the disease and more than one contact did not affect the severity of the disease in terms of antibody level.

Keywords: covid-19, child, spike IgG, antibody, immune response

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Ebeveynleri COVID-19 Geçiren Asemptomatik Çocuklarda İmmün Yanıtın Değerlendirilmesi

Öz

Amaç: Halk sağlığı açısından çocuklarda SARS-CoV-2'nin patogenezini ve bulaşmasını anlamak, hem salgının bulaşma ve yayılma dinamiklerinde pediatrik popülasyonun rolünü anlamada hem de pandeminin şiddetini kontrol etmekte önemlidir. Bu çalışmada çocukların COVID-19 enfeksiyonunun yayılımındaki rolü araştırıldı.

Yöntemler: Mayıs- Haziran 2021 arasında Pediatri Polikliniğine rutin kontrolleri için başvuran ve öyküsünde son 6 ay içinde ebeveynleri ve yakın çevresinde COVİD-19 tanısı alıp kendisinde herhangi bir bulgu olmayan RT-PCR yapılmayan çocuklar prospektif olarak değerlendirildi.

Bulgular: Çalışmaya 20'si erkek 35 olgu alındı. Çocukların 17'si sadece 1 olgu ile temaslı iken, 18'i iki ve üzerinde olgu ile temaslı idi. Bir temaslı olguların ortalama antikor değerleri IgG için 1,55±1,93 S/C, IgG-spike 910,6± 1512,02 AU/mL, iken 2 ve üzeri temaslı olguların ortalama antikor değerleri ise IgG için 2,21±2,07 S/C, IgG-spike için 1289,15±1750,49 AU/mL idi. Olguların 12'si sonbahar, 21'i kış, 2'si ise ilkbahar mevsiminde temaslı idi.

Sonuç: Virüsün toplum içinde yayılımı arttıkça, bulaşın, hastalığın dinamikleri ve klinik özellikleri değişecektir. Daha geniş bir coğrafi dağılımda daha fazla vaka içeren verilerin olması gerekmektedir. Ancak, yapılan çalışmalar, bugüne kadar çocukların hane halkının birincil kaynağı olmadığını göstermektedir. Hastalık şiddetinin ve birden fazla temasın antikor düzeyi açısından hastalığın şiddetini etkilemediği görülmüştür.

Anahtar kelimeler: COVİD-19, çocuk, spike IgG, antikor, immün yanıt.

INTRODUCTION

COVID-19, caused by the new type of coronavirus (SARS-CoV2), which was first detected in China's Wuhan province in December 2019, spread in a short time and caused a pandemic. Severe acute respiratory syndrome secondary to the disease is still a major problem. According to the May 2021 data of the World Health Organization (WHO), more than 165 million people were infected in 206 countries, over 3.4 million people were lost and over 1.4 billion people were vaccinated with the start of vaccination studies[1]. A significant number of patients and deaths have been reached worldwide, and unfortunately this increase still continues[2,3].

According to recent studies, children make up a small proportion of COVID-19 cases[2]. While some studies indicate that children do not play an important role in domestic transmission of SARS-CoV-2, some studies have concluded that children are not contagious with Covid-19. However, in some studies, it has been reported that children are mostly asymptomatic and are the driving force behind the spread of the disease[4]. Children are mostly infected from symptomatic adult households and the disease is relatively mild. Studies show that one third of children have the disease without any symptoms[5,6]. The immunological basis of the mild course of the disease is still unclear, but decreased ACE2 expression in the pediatric inspiratory epithelium and acquired innate immunity are suggested to be among the causes of this course[7].

Despite all these uncertainties, investigation of immunity against SARS-CoV-2 in children and adult groups seems to be a key element in identifying vaccine candidates and understanding the severity and susceptibility of disease in sick individuals. In this study, we aimed to evaluate immunoglobulin G (IgG) type antibody levels against COVID-19 and COVID-19 spike protein in asymptomatic children who applied to the Pediatrics Outpatient Clinic of our hospital and whose parents were diagnosed

with COVID-19 within 6 months, according to variables such as age, gender, season, and the number of contacts.

METHODS

For the study, after receiving the approval of the Local Ethics Committee, dated 07.05.2021 and numbered 754, the children who applied to the Pediatrics Outpatient Clinic for routine controls between May and June 2021, who had no symptoms and were not tested by RT-PCR although their parents and close relatives were positive for COVID-19 by RT-PCR (reverse transcription polymerase chain reaction) in the last 6 months, were evaluated prospectively. A "Parent Informed Voluntary Con-sent" form for "non-interventional clinical trials" was obtained from parents of all children.

To obtain serum, blood samples taken from the back of the hand or from the antecubital region were centrifuged at 1500g for 10 minutes after coagulation at room temperature, and the serum samples were separated and stored at -70^{0}C until tested for antibody levels. The antibody levels were measured with the Abbott Architect i2000SR (Abbott Diagnostics, USA) using the original kits (Abbott SARS-CoV-2 IgG, which is designed to detect IgG antibodies against the nucleocapsid protein of SARS CoV-2 and SARS- CoV-2 IgG II, which is designed to detect IgG antibodies against the receptor binding region [RBD] of the spike protein S1 subunit of SARS CoV-2) were tested after running two-level quality control. Both antibody tests are immunoassays based on the chemiluminescent microparticle method. The results were determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product with the signal of the threshold value previously obtained by calibration.

The results of the samples obtained with the Abbott Architect i2000SR were evaluated as the cut-off index "S/C" for anti-SARS CoV-2 IgG antibodies and as the cut-off "AU/mL" for anti-SARS CoV-2 IgG II antibodies. S/C <1.4 value in the samples were interpreted as non-reactive, that is, "Negative for Anti-SARS CoV-2 IgG antibodies", S/C ≥ 1.4 is interpreted as reactive; that is "positive for anti-SARS CoV-2 IgG antibodies". AU/mL < 50.0 was "negative for anti SARS-CoV-2 IgG II (spike anti-body) antibodies", and AU/mL ≥ 50.0 was interpreted as "positive for anti SARS-CoV-2 IgG II antibodies".

In our study, the severity of the disease was classified as mild for those who spent at home without going to the hospital, as moderate for those who went to the hospital and received medical help and were not hospitalized, and as severe for those who were hospitalized, according to their parent's statements.

Statistical Analysis

SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) was used for data analysis. The suitability of numerical variables to the normal distribution was examined using visual (histogram and probability graphs) and analytical methods (Shapiro-Wilk tests). Mann Whitney U Test was used when the normal distribution condition was not met in pairwise group comparisons for numerical variables. The Kruskal-Wallis Test was used to analyze differences in continuous variables between groups. Statistical significance level was accepted as p value less than 0.05.

RESULTS

The study included 35 cases. Twenty of the cases were male (57%). The mean age of the cases was 75.7±57.7 months (7-201 months), and IgG values were 1.89±2.01 S/C (0.03-8.67) and IgG-spike values were 1105±1626 AU/ mL (0.6-7658). While 17 (49%) of the children were in contact with only 1 case, 18 (51%) were in contact with 2 or more cases. Antibody values of cases in contact with 1 person were

1.55±1.93 S/C (0.03-6.46) for IgG and 910.6±1512.02 AU/mL (0.6-4649) for IgG-spike. Antibody values of cases in contact with 2 or more people were 2.21±2.07 S/C (0.04-8.67) for IgG and 1289.15±1750.49 AU/mL (2.3-7658) for IgG-spike. When the groups were compared in terms of the number of contacts, no significant difference was found in terms of antibody levels in both IgG (p=0.16) and IgG-spike group (p=0.19) (Table I).

Table I: Antibody levels in groups formed according to the number of contacts.

	Group 1 (1contact) *N*=17			Group 2 (2 or more contacts) *N*=18			p value*
	Median	Min-Max	IQR	Median	Min-Max	IQR	
Age (Mont)	63	7-179	88	71.5	8-201	104	0.90
IgG	0.98	0.03-6.46	2.82	2.18	0.04-8.67	1.81	0.16
IgG-spike	164.6	0.6-4648.2	1460.2	1183.2	2.3-7658.2	1680.9	0.19

**p<0.05 significiant, Mann-Whitney U Test.*

Twelve (34%) of the cases came into contact with the patients in autumn, 21 (60%) in winter, and 2 (6%) in spring months. The antibody values of the subjects who came into contact in autumn were 1.42±1.29 S/C (0.04-3.58) for IgG and 805.04±770.88 AU/mL (2.3-2133) for IgG-spike, and antibody values of the subjects who came into contact during the winter months were 2.23 ± 2.34 S/C (0.03-8.67) for IgG, 1374.90±1983.04 AU/mL (0.6-7658) for IgG-spike. The antibody values of the cases that came into contact in the spring were 1.14±1.58 S/C (0.03-2.26) for IgG and 75.95±105.85 AU/mL (1.1-150. 8) for IgG-spike. When the groups were compared in terms of the season of contact, no significant difference was found in terms of antibody levels in both IgG group (p=0.68) and IgG-spike group (p=0.33) (Table II).

Table II: Antibody levels in contact groups according to seasons.

	Autumn *N*=12			Winter *N*=21			Spring *N*=2			p value*
	Median	Min-Max	IQR	Median	Min-Max	IQR	Median	Min-Max	IQR	
IgG	1.22	0.04-3.58	2.43	1.21	0.03-8.67	2.8	1.14	0.03-2.26	-	0.68
IgG-spike	646.7	2.3-2133.3	1281.9	377.3	0.6-7658.2	1830.8	75.9	1.1-150.8	-	0.33

**p<0.05 significiant, Kruskal Wallis Test.*

While the IgG levels of the subjects who came into contact with the group with mild disease were 1.93±2.19 S/C (0.03-6.46), IgG-spike values were 1452.16±1689.06 AU/mL (2.6-4649). The IgG levels of the subjects who came into contact with the group with moderate disease were 1.54±1.38 S/C (0.03-4.93), and IgG-spike values were 624.76±758.23 AU/mL (0.6 -2133). While the IgG levels of the subjects who came into contact with the severely diseased group were 5.59±4.34 S/C (2.52-8.67); IgG-spike values were 4483.56±4489.63 AU/mL (1309-7658). When the disease severity was compared between the groups, no significant difference was found in terms of antibody levels in both IgG antibody group (p=0.11) and IgG-spike antibody group (p=0.23) (Table III).

Table III: Antibody levels of the cases according to the severity of the disease contacted.

	Mild *N=11*			Moderate *N*=22			Severe *N*=2			p value*
	Median	Min-Max	IQR	Median	Min-Max	IQR	Median	Min-Max	IQR	
IgG	1.09	0.03-6.46	3.44	1.22	0.03-4.93	2.17	5,59	2.52-8.67	-	0.23
IgG-spike	1057.5	2.6-4648.8	2232.3	301.45	0.6-2133.3	1366.1	4483.6	1308.9-7658.2	-	0.11

**p<0.05 significiant, Kruskal Wallis Test.*

DISCUSSION

Understanding the role of the pediatric population in the transmission dynamics of the pandemic is necessary because children can be an important carrier in the spread of the epidemic. Although some studies suggest that children, who are often asymptomatic, are the driving force behind the spread of COVID-19, there is no clear data on this subject[4,8].

Although it has been stated in recent studies that transmission of COVID-19 among children is one of the various factors driving the pandemic[7], it is actually parents who transmit the disease to children[9-11]. Generally, young children participate in fewer daily social activities than adolescents; therefore they have less contact with carriers or patients. This may contribute to a lower risk of catching COVID-1912.

Although no statistically significant relationship was found in our study, the antibody values of the cases that came into contact with 2 or more people were slightly higher. Similarly, although it was not significant, the antibody values of the subjects who came into contact during the winter months were higher. In addition, the antibody values in the group that came into contact with severe patients were higher, but not significant.

Considering that both young children and adults do not have adaptive specific immunity to this novel virus, the mild clinical course in young children can be explained by the more dominant innate immunity compared to adults. Additionally, poor ability to trigger an acute inflammatory response to SARS-CoV-2 may contribute to better outcomes in children. However, this does not completely exclude the possibility of serious consequences or even death, especially in children with underlying diseases[13].

Comprehensive studies on why children have a milder disease course will shed light on immunotherapy in the treatment and prevention of the disease[11]. However, it is stated in some publications that antibody response is not suitable in the diagnosis and management of cases. There is limited data on the occurrence of secondary antibody response to SARS-CoV-23. Since antibodies in immunoassay systems have just appeared on the market, studies are scarcely any. In addition, SARS-CoV-2 antibody tests determine total antibody levels and cannot distinguish between viral specific IgM and IgG. Therefore, our study is valuable in terms of demonstrating the antibody response after infection in children, even though the sample is small. Our study showed that the detected antibody levels were not significant in any group. Therefore, our results are in line with publications showing that children are not the primary source of infection in the household[3,13].

CONCLUSION

The pathogenesis of SARS-COV-2 remains an important priority for future research. Understanding the immune response to COVID 19 through comprehensive studies is the cornerstone of disease control. The small sample size is a limitation of our study. Studies

with larger case numbers in a wider geographical distribution are needed. It was concluded that the determination of seroprevalence, the presence of pre-vaccine antibody levels, and the use of antibody tests in symptomatic patients with negative RT-PCR may be useful in detecting patients.

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