Health Science Monitor
2023; 2(1): 61-72
Published online (http://hsm.umsu.ac.ir)

Original Article

Investigation of specific antibody titer against SARS-CoV-2 virus during hospitalization and one month after discharge in hospitalized children with severe and critical Covid-19 infection

Hamidreza Houshmand [1], Reza Jafary *[2], Javad Rasoli [3], Ahmad Ahmadi [4]

[1] *Assistant Professor of Allergy and Clinical Immunology, Urmia University of Medical Sciences, Urmia, Iran*

[2] *Assistant Professor of Medical Immunology, Cellular and Molecular Research Center, Cellular and Molecular Medicine Research Institute, Urmia University of Medical Sciences, Urmia, Iran*

[3] *Assistant Professor of Epidemiology, Urmia University of Medical Sciences, Urmia, Iran*

[4] *School of Medicine, Urmia University of Medical Sciences, Urmia, Iran*

***Corresponding author:** Reza Jaafary, **Address**: Urmia University of Medical Sciences, Urmia, Iran,
Email: Rezajaafary1983@gmail.com, **Tel**: +9844331937185

Abstract

***Background & Aims*:** The response of the human immune system to the COVID-19 disease is of vital importance in many ways, including determining the place of serological methods in the survival of patients. Therefore, the present study was designed and implemented with the aim of determining the specific antibody titer against SARS CoV-2 during hospitalization and one month after discharge in children hospitalized in Shahid Motahari Hospital in Urmia.

***Materials & Methods*:** In this longitudinal study, hospitalized patients with positive RT-PCR test were included in the study due to COVID-19. Inclusion criteria included age less than 15 years and parental consent, and exclusion criteria included immune system disorder or developmental disorder and negative RT-PCR test. Demographic information and severity of the disease along with specific antibody titer of SARS-CoV-2 were evaluated and extracted for all patients.

***Results*:** The average age of the 40 studied patients was 2.48 years, and 32.5% of them were girls. The average level of IgG during discharge and one month after discharge was equal to 73.66 and 128.36, respectively, and it was significantly higher one month after discharge than during discharge (p<0.001). There was no statistically significant difference between the two sexes in terms of antibody titer during discharge (p=0.77) and one month after discharge (p=0.31). There is no significant relationship between the specific antibody level of SARS-CoV-2 during discharge (r=0.12 and p=0.45) and one month after discharge and length of hospitalization. There was no statistically significant difference between disease severity and antibody titer during discharge (p=0.54) and one month after discharge (p=0.20).

***Conclusion*:** The specific antibody titer of SARS-CoV-2 increases significantly after one month of discharge. There was no significant difference between the level of this antibody during discharge and one month later between girls and boys and people with different severity of the disease. No significant relationship between antibody titer and hospitalization time was observed.

***Keywords*:** Children, COVID-19, Hospitalized patients, Specific antibody

Received 07 October 2022; accepted for publication 21 December 2022

Introduction

The spread of Covid-19 virus in the city of Wuhan (China) during the past two years and its transmission to the whole world including Iran (since February 2018), increased the number of the people infected with the above-mentioned virus in this country since mid-March, 2018(1). Consequently, several specialized centers in different cities were used to take care of this group of patients (2). Coronaviruses are enveloped, non-segmented and single-stranded RNA viruses. They have their origin in animals and belong to the Coronaviridae family and the Nidovirales category. The size of the virus genome ranges from 17 to 91 kilobases. Therefore, they are among the largest RNA viruses. These viruses have two different types of surface proteins and get their name from their appearance. The family of coronaviruses is divided into 4 genera in terms of genotyping and serology including: alpha, beta, gamma, and delta. Human coronaviruses are caused by alpha and beta genera. This large family constitutes the cause of a wide range of viral diseases ranging from colds to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and SARS-CoV (3).

The COVID-19 pandemic has affected the entire world. It is considered to be a serious threat to the public health. According to the first study of the patients who were infected with the new 2CoV-SARS coronavirus, the incubation period of this virus was 5 days on average and ranged from 4 to 7 days. The time interval between a person's exposure to the virus and the appearance of the clinical symptoms is called the incubation period of the virus. Different health organizations in the world have stated different incubation periods for COVID-19 disease. More specifically, the World Health Organization has specified a number in the range of 9 to10 days, and the National Health Commission of China has specified a number in the range of 4 to 10 days. Furthermore, the Center for Disease Control and Prevention of America has specified a number in the range of 4 to 9 days for the above-mentioned period. However, different results have been reported in relation to the length of the Covid-19 epidemic (4-7). It has been confirmed that the coronavirus can be transmitted through the respiratory droplets and the contaminated objects (8, 9). Nonetheless, there is not strong evidence of the intrauterine transmission of this infection (6). According to the results of recent research studies, inhalation of contaminated respiratory droplets, close contact with an infected person or contact with the secretions of a sick person constitute the main routes of the transmission of this virus. That is, first, the infected respiratory droplets are spread in the environment through the infected person's sneeze or cough and land in the mouth or nose of the people who are near the sick person. Next, they are transferred to their lungs. Moreover, a person can contract COVID-19 by touching an infected object or surface. In this way, first, the person touches the contaminated surface. Next, he/she touches his/her mouth, nose and eyes with his/her contaminated hands and the virus enters his/her body. A person’s clinical symptoms show that he/she may cause the highest rate of virus transmission. However, some patients are able to transmit their infection to the others before the appearance of clinical symptoms. Moreover, in some cases, the virus may be present in the patient's feces (10).

The initial manifestations of COVID-19 are mostly respiratory and cardiovascular. Notwithstanding, neurological complications have been reported in a number of studies. The most common neurological complications, which may arise in the case of these patients, include headache, dizziness, and delirium. These complications are followed by encephalitis which is caused by the disease. In addition, the other complications include Guillain-Barré syndrome, cerebrovascular accidents, and encephalitis. It should be mentioned that sometimes in a number of patients, typical symptoms of the disease such as fever and cough appeared after neurological symptoms, which means that the patient first had symptoms such as headache and dizziness, and then developed common symptoms of COVID-19 such as fever, cough, body pain and other symptoms (11). In most of the viral infections, the amount of antibody response is directly

related to the severity of the infection. In other words, severe infections are more memorable for the immune system of the body. The viral load of corona virus is related to the severity of respiratory disease, systemic inflammatory reaction, and mortality. Moreover, the patients with the severe type of disease produce more antibodies against SARS-COVID (12). The time at which the antibodies are produced in the body differs regarding different viruses or antigens. According to the available information on COVID-19, the onset of antibody response to COVID-19 is slow. There are not enough data on this disease. Nonetheless, it appears that the primary immunoglobulin M antibody response does not peak until day nine after the primary infection, and the immunoglobulin G antibody response does not peak until day eleven (13). Understanding the immune response against the specific neutralizing antibodies of this virus is essential to provide a suitable treatment for it and to develop its vaccine. Studies on SARS virus and MERS virus have shown that different parts of spike protein (S), N terminus (NTD) and receptor binding site (RBD) can be used as the targets for developing the relevant vaccine (14). Long et al. reported that the level of immunoglobulin M and G against nucleoside protein (N) and protein S in the hospitalized patients was positive on the nineteenth day after the onset of symptoms. Moreover, its level decreased within two to three months in asymptomatic patients (15). There is little information on the humoral immune response and virus-neutralizing antibodies and their relationship with the clinical outcomes in COVID-19. Considering the stability of these antibodies in the patients' serum and the lack of internal studies in this field, we decided to investigate the level of spike protein specific antibodies in the children with COVID-19 who were admitted to Motahari Hospital in Urmia (Iran) at the time of their discharge from the hospital and one month after their discharge date.

Materials & Methods

The present study was a longitudinal study. It investigated the serum specific antibody titer in patients with COVID-19. This study involved the Covid-19 patients, whose disease was confirmed by PCR and who were admitted to the hospital.

The inclusion criteria comprised: being a child under 15 years of age with positive PCR, and being admitted to Shahid Motahari Hospital in Urmia, Moreover, the researchers selected the children whose parents had given written consent to their participation in the study.

On the other hand, the exclusion criteria involved being a child whose PCR is negative despite exhibiting the symptoms of COVID-19, having a pulmonary disease other than COVID-19, being a child with immune deficiency, suffering from FTT, having an underlying chronic disease, and being a child whose parents do not give written consent to participation in the study.

After selecting the patients who met the inclusion criteria as the participants of the study, first, 5 cc blood samples were taken from them at the time of their discharge from the hospital and one month after their discharge date (by inviting them to the hospital). Second, the serum level of their SARS-CoV-2 specific antibody was measured twice. Third, one microliter of serum sample was diluted 1:101 using diluting solution. Pishtaz Teb ELISA kits were used to determine the titer of spike protein IgG antibodies. Fourth, the samples were prepared using the protocol and the results were read by an ELISA reader which was capable of measuring light absorption at a wavelength of 450 nm. Finally, a point to point diagram was drawn for the concentration and optical absorption of the samples. The average optical absorption was calculated for the samples.

In this study, the demographic characteristics included age and sex. The children under 15 years of age were examined separately and in relation to each other in terms of their age and gender. This examination aimed to differentiate the course of the COVID-19 disease based on the provided protocol. The criteria for entering the severe phase of the disease involved: rapid progression of respiratory symptoms, Tachypnea (RR>50) and shortness of breath,

PaO2/FiO2 ≤300 mmHg, SpO2<90%, increased involvement of more than 50% of the lung in CT scan. At this stage, patients may exhibit a number of laboratory symptoms such as lymphopenia exacerbation, progressive increase in D-dimer or ferritin (more than 500 ng/dl) or LDH>245 U/l or increase in the liver enzymes or triglycerides, and signs of occurrence or exacerbation of organ failure. Moreover, there is the possibility of an increase in CRP, a decrease in the number of platelets, and a sharp decrease in the number of eosinophils. Furthermore, it is possible to observe the involvement of 5 lung lobes with less than one third of the volume of each lobe or the involvement of three lobes to a greater extent. Usually, the pulmonary involvement is bilateral. The extent of lung involvement in CT scan in severe cases of the disease is often greater than its involvement in moderate cases. Furthermore, several of the studies, which have focused on this disease, have reported that more than 50% of the entire lung is involved. Eventually, the bilateral diffuse infiltration may be observed as white lung. The criteria for entering the critical phase of the disease involve: being a patient with symptoms of respiratory failure: SpO2 ≤88% despite non-invasive oxygen therapy treatments, being a patient with shock symptoms, being a patient in need or under mechanical ventilation, being a patient with multiple organ failure. At this stage, patients may exhibit a number of laboratory symptoms including the worsening of lymphopenia, severe increase in inflammation markers (D-dimer>1000, IL6), Ferritin>1000ngdl, troponin, NT-proBNP, advanced cytopenia, severe increase in markers of multi-organ failure/damage which can be related to renal enzymes, thrombocytopenia, increased BUN/Cr, and coagulation disorders. Moreover, it is possible to observe the diffuse bilateral lung involvement which is consistent with ARDS. Finally, at this stage, patients may exhibit certain symptoms including pleural effusion, lymphadenopathy, increased bronchial wall thickness, and structural changes in the lungs.

In this study, centrality and dispersion indices (mean and standard deviation), were used to perform the quantitative data analysis. Moreover, the qualitative variables frequency and frequency percentage were calculated in the present study. Furthermore, appropriate statistical tables and graphs were used to provide the results of the data analysis. Finally, a number of parametric statistical tests such as t-test, ANOVA, and chi-square or their non-parametric equivalents were used to make comparisons according to the data distribution. The significance level for all of the judgments was less than 0.05. All of the analyses were performed using SPSS 21 software.

Results

This study involved a total number of 40 children with severe and critical COVID-19 who were hospitalized in Shahid Motahari Hospital. The average IgG levels during their discharge from the hospital and one month after their discharge date were 73.66 ± 55.03 and 128.36 ± 69.96, respectively (Table 1).

1-4 Comparison of specific antibody titer against SARS-CoV-2 one month after discharge date of the children who were hospitalized in Shahid Motahari hospital in Urmia according to demographic characteristics in the present study, 13 (32.5%) of the 40 children (i.e. participants) were girls and 27 (67.5%) of them were boys (Chart 1).

Table 1. Average IgG level at the time of the patients' discharge from the hospital and one month after their discharge date

		Mean ± standard deviation
IgG level	At the time of discharge from hospital	73.66 ± 55.03
	One month after discharge date	128.36 ± 69.96

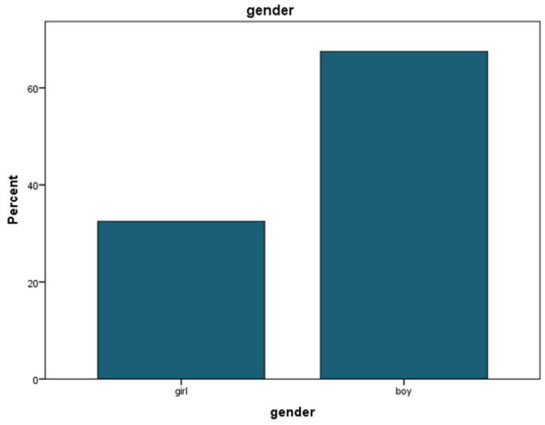

Chart 1. Frequency of gender among the patients.

The independent-samples t-test was used to compare the specific antibody titer during the participants' discharge from the hospital and one month after their discharge date between the two sexes. Based on the results of this test, there were not any significant differences between the two sexes in terms of antibody titer during their discharge from the hospital (p=0.77) and one month after their discharge date (p=0.31) (Table 2).

Table 2. Comparison between two sexes in terms of specific antibody titer during the patients' discharge from the hospital and one month after their discharge date

		Girl	Boy	*p* value
		Mean ± standard deviation		
Antibody titer	At the time of discharge from the hospital	77.34±57.33	71.89±54.92	0.77
	One month after the discharge date	88.64±31.33	62.41±13.30	0.31

The patients' average age was 2.48 ± 2.18 years. Moreover, their minimum age and maximum age were one year and 10 years respectively (Chart 2).

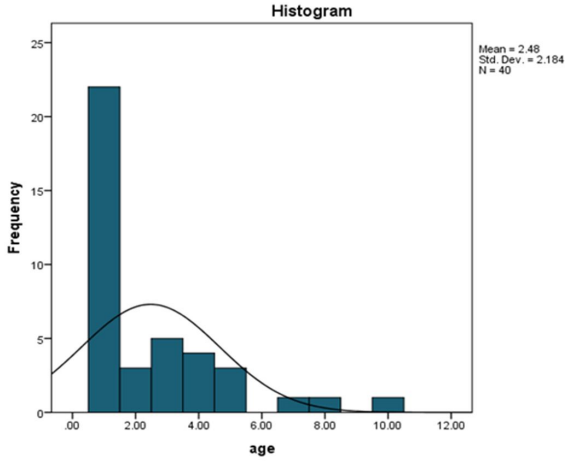

Chart 2. Patients' age distribution

Pearson's correlation coefficient was used to investigate the relationship between the patients' age and their level of SARS-CoV-2-specific antibody during their discharge from the hospital and one month after their discharge date. The results of the study showed that there was a strong direct correlation between the level of SARS-CoV-2-specific antibody during the participants' discharge from the hospital and their age. That is, an increase in their age was accompanied by an increase in their antibody level (r=0.34 and 02 (p=0.00) (Chart 3). Nonetheless, there was not a significant correlation between the patients' specific antibody level of SARS-CoV-2 and their age one month after their discharge date (r=0.06 and p=0.73) (Chart 4).

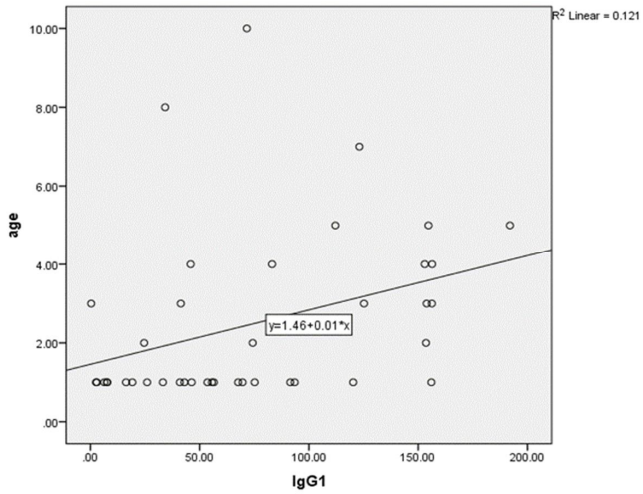

Chart 3. Examining the relationship between the patients' age and the level of their SARS-CoV-2-specific antibody during their discharge from the hospital

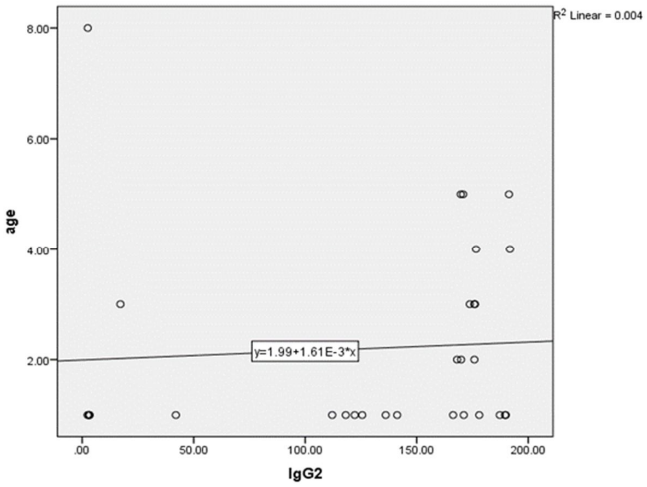

Chart 4. Examining the relationship between the patients' age and the level of their SARS-CoV-2specific antibody one month after their discharge date

Comparison of specific antibody titer against SARS-CoV-2 at the time of the children's discharge from the hospital and one month after their discharge date (i.e. the children who were admitted to Shahid Motahari Hospital in Urmia):

A paired-samples t-test was used in order to compare the specific antibody titer during the patients' discharge from the hospital and one month after their discharge date. The results of this test showed that there was a significant increase in these patients' specific antibody titer one month after their discharge date compared to the time of their discharge from the hospital (P>0.001) (Table 3).

Table 3. Comparison between the patients' specific antibody titer during their discharge from the hospital and one month after their discharge date

		Mean ± standard deviation	*p* value
Specific antibody titer	At the time of discharge from hospital	78.29±56.22	<0.001
	One month after discharge date	128.36±96.69	

Comparison of specific antibody titer against SARS-CoV-2 one month after the discharge date of children who were hospitalized in Shahid Motahari Hospital in Urmia according to the length of their hospitalization:

Pearson's correlation coefficient was used to investigate the relationship between the length of the patients' hospitalization and the level of their SARS-CoV-2-specific antibody during their discharge from the hospital and one month after their discharge date. The results of the investigation showed that there was not a significant correlation between the patients' level of SARS-CoV-2-specific antibody during their discharge from the hospital (r = -0.12 and p=0.45) (Chart 5) and one month after their discharge date and the length of their hospitalization (r = -0.18 and p=0.31) (Chart 6).

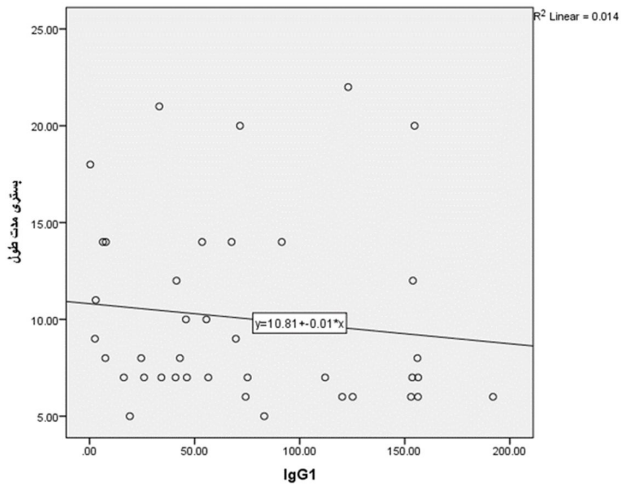

Chart 5. Examination of the relationship between the length of the patients' hospitalization and the level of their specific antibodies during their discharge from the hospital

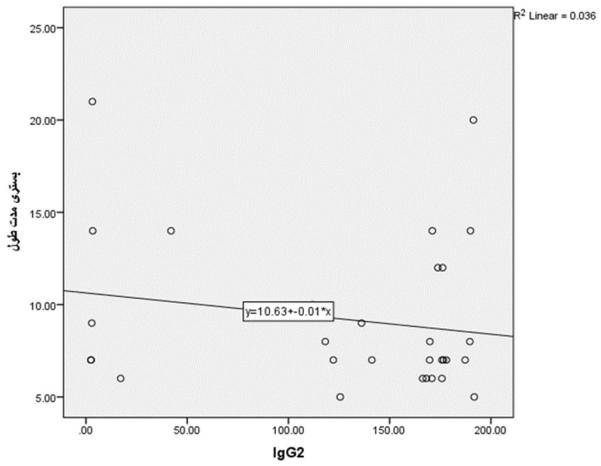

Chart 6. Examining the relationship between the length of the patients' hospitalization and their specific antibody level one month after their discharge date

Comparison of specific antibody titer against SARS-CoV-2 one month after the discharge date of the children who were hospitalized in Shahid Motahari Hospital in Urmia according to the severity of the disease (critical, severe):

In this study, 13 children (32.5%) were in critical conditions and 27 children (67.5%) were in severe disease conditions (Chart 7).

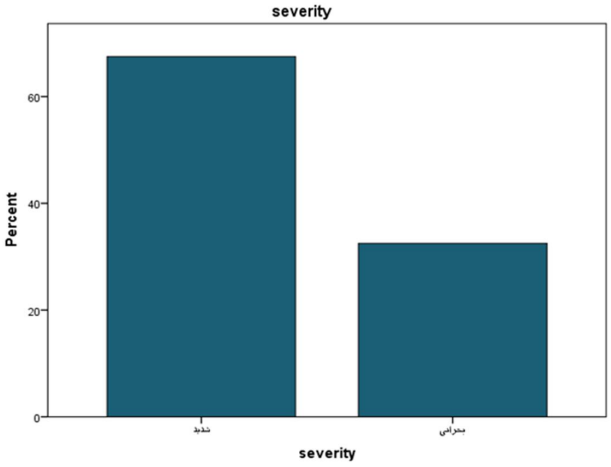

Chart 7. Patients' disease severity

In order to compare the specific antibody titer during the patients' discharge from the hospital and one month after their discharge date according to the severity of the disease (critical, severe), an independent-samples t-test was used. The results of this test showed that there was not a significant difference between the severity of the patients' disease and their antibody titer during their discharge from the hospital (p=0.54) and one month after their discharge date (p=0.20) (Table 4).

Table 4. Comparison of specific antibody titer during the patients' discharge from the hospital and one month after their discharge date according to disease severity

		Critical	Sever	*p* value
		Mean ± standard deviation		
Specific antibody titer	At the time of discharge from the hospital	65.88±61.30	77.41±52.57	0.54
	One month after the discharge date	103.52±88.53	139.00±59.71	0.20

Discussion

The corona virus resulted in a pandemic in 2019 and infected a large number of people. The infected people exhibited a number of symptoms such as fever, dry cough, shortness of breath, and acute respiratory distress syndrome. This disease is caused by SARS-CoV-2, which belongs to the class of beta coronaviruses (23, 24). The characteristics of the antibody response of the human immune system to this disease are of vital importance due to various reasons including the awareness about the progress of vaccines, the determination of strategies which are related to them, and the provision of a suitable guide for determining the role of serological methods in the survival of the affected patients (25, 26).

Therefore, the present study was conducted to determine the specific antibody titer against Sars coronavirus one month after the discharge date of the children who were admitted to Shahid Motahari Hospital in Urmia. In the present study, 32.5% of patients were female and 67.5% of them were male. The patients' average age was 2.48 ± 2.18 years.

Tom Woudenberg et al. (27) conducted a study to determine the COVID-19 patients' humoral immunity. Similar to the results of our study, the majority of the patients in their study were male. Moreover, the results of the study which was carried out by Emelie Marklund (16) are in line with the results of our study. Based on the results of the pertinent study, the majority of the patients were male. The results of the present study showed that the increase in the patients' age was accompanied by an increase in their antibody level. Likewise, the results of the study which was conducted by Wang et al. (28) in 2021 in China, showed that the antibody titer in patients increased with age. Moreover, Wu et al. (29) found that middle-aged and elderly patients had higher antibody levels in comparison with the young patients. These results support the results of the present study. These results may stem from the further progress and development of the immune system with age. That is age provokes a stronger response against the disease. Based on the results of our study, the specific antibody titer was significantly increased one month after the patients' discharge date compared to the time of their discharge from the hospital ($p<0.001$). Moreover, there was not a statistically significant difference between the severity of the disease (critical and severe) and antibody titer during the patients' discharge from the hospital ($p=0.54$) and one month after their discharge date ($p=0.20$). Ekasit Kowitdamrong (17), conducted a study to investigate the antibody response in patients with COVID-19 with different disease severity levels. The results of the study indicated that the patients who were in the severe condition of the disease had higher S1-specific antibody titer in comparison with the patients who were in a less severe condition. In our study, there was not a significant difference between severe and critical patients. Moreover, we did not compare the specific antibody levels of patients who were in the severe and critical conditions with the specific antibody levels of the patients who were in the mild condition of the disease. Therefore, our results are not comparable with the results of the above-mentioned study. Moreover, in the aforementioned study, the level of specific IgG increased during a one-month period of time. This result supports our results. On the other hand, based on the results of the study by Ekasit Kowitdamrong (17), the patients with moderate and severe disease had higher levels of IgA and IgG antibodies compared to the patients with mild disease in the 15-day follow-up. One of the reasons behind the

difference between the present study and the above-mentioned studies is the low sample size of the present study. Moreover, most of the patients in our study were people who suffered from the mild severity of the disease. A number of the previous studies have shown that the production of antibodies against this virus is low in the mild form of the disease (28, 30). Therefore, this difference can produce the results which are different from the results of the other studies. In our study, there was not a significant relationship between the patients' age and gender and their specific antibody level of coronavirus 2 one month after their discharge date. Our results are in line with the results of the study by Ekasit Kowitdamrong (17) which indicated that there was not a significant relationship between the age and gender and the antibody level of the patients who were in the severe condition of the disease. Likewise, our results support the results of the study by Ashraf Hassan Alzaab et al (20) which showed that there was an increase in IgG levels a few days after the onset of the disease to a few months after the disease. The results of this study showed that the increase in specific antibody level was significant in a few weeks after the disease compared to the first few days of the disease. Moreover, the increase in the level of antibody in a few months after the disease was significant compared to the onset of the disease. Nonetheless, the increase in the level of antibody a few months after the disease was not significant compared to a few weeks after the disease. Antibodies are an important and vital part of the immune system response and disease control in viral infections. However, their ability to respond to new pathogens can be widely underestimated. The results of studies of influenza have shown that elderly people have higher levels of IgG and IgA compared to young people. This result stems from the elderly people's contact with different types of influenza over time. Nonetheless, their ability to produce antibodies against the new viruses decreases. Contrary to these results, children have a greater ability to produce antibodies against new viruses and to fight against them (31, 32). In addition to their neutralizing properties, antibodies play a role in some other functions of the human immune system including antibody-dependent cellular cytotoxicity and antibody-dependent cellular phagocytosis (33). Several studies, which have been conducted to examine the long-term response of the immune system to the infection of COVID-19, are of particular importance since they determine the causes of the viruses which escape from the immune system and result in the re-infection of people. A study which was conducted in Iceland indicated that the immune response to this virus produced by the immune system through IgG can exist in a long-term period of time which is at least four months (34). Another study, which was conducted by Wu et al. in Wuhan (China) during a 36-week period of time, examined the patients' IgG and IgM levels. The results of this study showed that 70% of patients had a positive IgG response to the COVID virus after a six-month period of time (19) Moreover, based on the results, the IgG level reached its maximum in weeks four to five and remained constant after that time (35). The present study had a number of limitations. The most important limitation of the study was the lack of the patients' long-term follow-up. Moreover, the examination of patients who are in the less severe condition of the disease can provide a better understanding of the ways of responding to this disease and generating immunity against it. Nonetheless, this study was not able to focus on this group of patients due to its focus on the hospitalized patients.

Conclusion

The specific antibody titer of SARS-CoV-2 increased significantly after one month of the patients' discharge from the hospital. There was not a significant difference between the level of this antibody of the girls, boys, and the people with different severity of the disease during their discharge from the hospital and one month after their discharge date. Moreover, there was not a significant relationship between the patients' antibody titer and their hospitalization time.

Offers

The future studies should involve larger samples and need to focus on the adult and child patients in

order to evaluate the humoral response of the immune system in a satisfactory way. Moreover, there is a need for the studies that include longer follow-up periods in order to expedite the treatment process of patients with this disease.

Acknowledgments

None declared.

Conflict of interest

The authors have no conflict of interest in this study.

Funding/support

The Urmia University of Medical Sciences financially supported this study.

Data availability

The raw data supporting the conclusions of this article are available from the authors upon reasonable request.

References

1. Chen T, Wu D, Chen H, Yan W, Yang D, Chen G, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. Br Med J 2020;368(1123):249-73.
2. Ye G, Pan Z, Pan Y, Deng Q, Chen L, Li J, et al. Clinical characteristics of severe acute respiratory syndrome coronavirus 2 reactivation. Journal of Infection. 2020;80(5):e14-e7.
3. Farnoosh G, Alishiri G, Zijoud SH, Dorostkar R, Farahani AJ. Understanding the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease (COVID-19) based on available evidence-a narrative review. Journal of Military Medicine 2020;22(1):1-11.
4. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus–infected pneumonia. The New England Journal of Medicine. 2020;72(11):1211-25.
5. Wang Y, Guo H, Lu Z, et al. Characteristics of patients with coronavirus disease (COVID-19) confirmed using an IgM-IgG antibody test. Journal of Medical Virology. 2020;92(10):204-10.
6. Tavakoli A, Vahdat K, Keshavarz M. Novel coronavirus disease 2019 (COVID-19): an emerging infectious disease in the 21st century. Iranian Sout Medical Journal. 2020;22(6):432-50.
7. Chavez S, Long B, Koyfman A, Liang SY. Coronavirus Disease (COVID-19): A primer for emergency physicians. The American Journal of Emergency Medicine. 2020;11(3):371-89.
8. Lam TT-Y, Shum MH-H, Zhu H-C, Tong Y-G, Ni X-B, Liao Y-S, et al. Identification of 2019-nCoV related coronaviruses in Malayan pangolins in southern China. BioRxiv. 2020;5(23):148-62.
9. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The lancet. 2020;395(10224):565-74.
10. Control CfD, Prevention. Coronavirus Disease 2019: COVID-19. 2020;13(2):28-48.
11. Ahmad I, Rathore FA. Neurological manifestations and complications of COVID-19: A literature review. Journal of Clinical Neuroscience. 2020;341(38):189-201.
12. Combes AJ, Courau T, Kuhn NF, Hu KH, Ray A, Chen WS, et al. Global absence and targeting of protective immune states in severe COVID-19. Nature. 2021;591(7848):124-30.
13. Xie J, Ding C, Li J, Wang Y, Guo H, Lu Z, et al. Characteristics of patients with coronavirus disease (COVID-19) confirmed using an IgM-IgG antibody test. Journal of Medical Virology. 2020;92(10):2004-10.
14. Jiang S, Hillyer C, Du L. Neutralizing antibodies against SARS-CoV-2 and other human coronaviruses. Trends Immunol 2020;41(5):355-9.
15. Long Q-X, Liu B-Z, Deng H-J, Wu G-C, Deng K, Chen Y-K, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med 2020;26(6):845-8.
16. Marklund E, Leach S, Axelsson H, Nyström K, Norder H, Bemark M, et al. Serum-IgG responses to SARS-CoV-2 after mild and severe COVID-19 infection and analysis of IgG non-responders. PLoS One. 2020;15(10):e0241104.

17. Kowitdamrong E, Puthanakit T, Jantarabenjakul W, Prompetchara E, Suchartlikitwong P, Putcharoen O, et al. Antibody responses to SARS-CoV-2 in patients with differing severities of coronavirus disease 2019. PLoS One. 2020;15(10):e0240502.
18. Sun B, Feng Y, Mo X, Zheng P, Wang Q, Li P, et al. Kinetics of SARS-CoV-2 specific IgM and IgG responses in COVID-19 patients. Emerg Microbes & Infect 2020;9(1):940-8.
19. Chen Y, Tong X, Li Y, Gu B, Yan J, Liu Y, et al. A comprehensive, longitudinal analysis of humoral responses specific to four recombinant antigens of SARS-CoV-2 in severe and non-severe COVID-19 patients. PLoS Pathog 2020;16(9):e1008796.
20. Alzaabi AH, Ahmed LA, Rabooy AE, Zaabi AA, Alkaabi M, AlMahmoud F, et al. Longitudinal changes in IgG levels among COVID-19 recovered patients: A prospective cohort study. PLoS One. 2021;16(6):e0251159.
21. Muecksch F, Wise H, Batchelor B, Squires M, Semple E, Richardson C, et al. Longitudinal serological analysis and neutralizing antibody levels in coronavirus disease 2019 convalescent patients. The Journal of Infectious Diseases. 2021;223(3):389-98.
22. Wang K, Long Q-X, Deng H-J, Hu J, Gao Q-Z, Zhang G-J, et al. Longitudinal dynamics of the neutralizing antibody response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Clinical Infectious Diseases. 2021;73(3):e531-e9.
23. Skoog H, Withrow K, Jeyarajan H, Greene B, Batra H, Cox D, et al. Tracheotomy in the SARS-CoV-2 pandemic. Head & Neck. 2020;42(7):1392-6.
24. Wax RS, Christian MD. Practical recommendations for critical care and anesthesiology teams caring for novel coronavirus (2019-nCoV) patients. Canadian Journal of Anesthesia. 2020;67(5):568-76.
25. Davies NG, Kucharski AJ, Eggo RM, Gimma A, Edmunds WJ, Jombart T, et al. Effects of non-pharmaceutical interventions on COVID-19 cases, deaths, and demand for hospital services in the UK: a modelling study. Lancet. 2020;5(7):e375-e85.
26. Ferguson NM, Laydon D, Nedjati-Gilani G, Imai N, Ainslie K, Baguelin M, et al. Impact of non-pharmaceutical interventions (NPIs) to reduce COVID-19 mortality and healthcare demand. Latin American Journal of Pharmacy. 2020;11(4):132-7.
27. Woudenberg T, Pelleau S, Anna F, Attia M, Donnadieu F, Gravet A, et al. Humoral immunity to SARS-CoV-2 and seasonal coronaviruses in children and adults in north-eastern France. EBioMed. 2021;70:103495.
28. Wang X, Guo X, Xin Q, Pan Y, Hu Y, Li J, et al. Neutralizing antibodies responses to SARS-CoV-2 in COVID-19 inpatients and convalescent patients. Clinical Infectious Diseases. 2020;3(62):37-57.
29. Wu F, Wang A, Liu M, Wang Q, Chen J, Xia S, et al. Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. medRxiv. 2020;32(8): 20047365.
30. Klein SL, Pekosz A, Park H-S, Ursin RL, Shapiro JR, Benner SE, et al. Sex, age, and hospitalization drive antibody responses in a COVID-19 convalescent plasma donor population. The Journal of Clinical Investigation. 2020;130(11):6141-50.
31. Carsetti R, Quintarelli C, Quinti I, Mortari EP, Zumla A, Ippolito G, et al. The immune system of children: the key to understanding SARS-CoV-2 susceptibility. Lancet. 2020;4(6):414-6.
32. Henry C, Zheng N-Y, Huang M, Cabanov A, Rojas KT, Kaur K, et al. Influenza virus vaccination elicits poorly adapted B cell responses in elderly individuals. Cell Host & Microbe. 2019;25(3):357-66. e6.
33. Arnold KB, Chung AW. Prospects from systems serology research. Immun. 2018;153(3):279-89.
34. Gudbjartsson DF, Norddahl GL, Melsted P, Gunnarsdottir K, Holm H, Eythorsson E, et al. Humoral immune response to SARS-CoV-2 in Iceland. The New England Journal of Medicine. 2020;383(18):1724-34.
35. Wu J, Liang B, Chen C, Wang H, Fang Y, Shen S, et al. SARS-CoV-2 infection induces sustained humoral immune responses in convalescent patients following symptomatic COVID-19. Nat Commun. 2021;12(1):1-9.