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Monitoring of antibody levels in healthcare workers after inactivated coronavirus disease 19 vaccination

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SUMMARY

OBJECTIVE: Because of the coronavirus disease 19 pandemic, studies on vaccination are being conducted in our country as well as across the world. In this study, the antibody levels in healthcare workers vaccinated with two doses of inactivated vaccine and the factors affecting these levels were investigated.

METHODS: Randomly selected volunteers from healthcare workers, who had been vaccinated with two doses of inactivated vaccine in January to February 2021, were included in the study. Blood samples were drawn twice, 1 month and 6 months after the second dose vaccine (CoronaVac:Sinovac Life Science Co, Ltd, Beijing, China). The antibody levels were determined by the chemiluminescence microparticle immunoassay method using kits for quantitative detection of immunoglobulin class G antibodies to severe acute respiratory syndrome coronavirus 2.

RESULTS: The mean antibody levels of 129 volunteers were 1232.5 (min: 103 to max: 7151) AU/mL in the first month and 403.5 (min: 23 to max: 4963) AU/mL in the sixth month. According to the survey results, 91 (71%) volunteers had not been diagnosed with coronavirus disease 19 before vaccination. The antibody levels 1 month and 6 months after the second dose of vaccination were significantly higher in those who had been diagnosed with coronavirus disease 19 before vaccination than in those who had not. It was found that age, gender, fast food, or healthy nutrition had no effect on antibody levels.

CONCLUSION: Vaccines are very important both to protect against coronavirus disease 19 and to experience only a mild form of the disease. Immunoglobulin class G levels formed after vaccination may be affected by many factors and may decrease over time.

KEYWORDS: Antibody. COVID-19. Healthcare workers. Vaccination.

INTRODUCTION

In late December 2019, a new virus from the coronavirus family was isolated in a group of patients with lower respiratory tract symptoms in Wuhan City, China[1]. This clinical condition was named coronavirus disease 19 (COVID-19) and its causative agent was severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[2]. The disease spread across the world in a short period of time and was declared a pandemic by the World Health Organization on March 12, 2020. The first case in Turkey was detected on March 10, 2020.

In the diagnosis of COVID-19, polymerase chain reaction (PCR) testing is used considering epidemiologic history and symptoms. Especially in asymptomatic cases, additional serological tests are also beneficial to demonstrate the acquiring of immunity in the patient[3]. Antibody tests indirectly support the diagnosis of COVID-19 and determine seroprevalence. At a certain period of time after SARS-CoV-2 infection, antibodies (IgA, IgM, and IgG) are detected in the serum, which have developed against the virus depending on the patient's immune system. SARS-CoV-2 IgG, indicating exposure to the virus, begins to form on the seventh day of the incubation period after contact with the virus, and its level in the serum gradually increases in the second and third weeks. How long the antibodies produced remain at a high level or when they start to decrease is still unclear. It is not yet clear which type of antibodies affect the severity of the disease or to what extent the antibodies are affected.

The study of vaccination, which is an important means of providing immunity, is progressing rapidly worldwide. Many vaccines have been developed since the onset of the pandemic. Sinovac-CoronaVac, developed by Sinovac/China National Pharmaceutical Group, is an inactivated vaccine for COVID-19[4]. The SARS-CoV-2 vaccination program in Turkey was launched on January 11, 2021, with priority given first to healthcare workers and then

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to high-risk groups. In this program, CoronaVac 600 U/0.5 mL (Sinovac Life Science Co., Ltd, Beijing, China) was used, and two doses of the inactivated vaccine were administered intramuscularly 28 days apart[5]. It is known that the inactivated vaccine administration is safe, induces humoral and cellular responses in vaccinated individuals from different age groups, and significantly reduces hospitalization and mortality rates[6].

The aim of this study was to quantify the antibody levels in the first and sixth months after vaccination with an inactivated vaccine, in healthcare workers, and also to investigate various factors that may affect the antibody levels including personal characteristics such as nutritional habits, body mass index (BMI), age, and gender.

METHODS

After obtaining the approval from the Duzce University Non-Interventional Health Research Ethics Committee dated 01.02.2021 and with the number 2021/16, volunteers who agreed to answer the questionnaire were determined from healthcare workers who had been vaccinated with two doses of the inactivated COVID-19 vaccine (CoronaVac:Sinovac Life Science Co, Ltd, Beijing, China) in January to February 2021. All study participants completed, signed, and returned an informed consent form. Blood samples were drawn from the volunteers twice, in March 2021 and in August 2021, 1 month and 6 months after the second dose of the two vaccine doses that were administered 28 days apart. The blood samples were examined in the Duzce University Faculty of Medicine Medical Microbiology Laboratory. A survey, including their sociodemographic characteristics, whether or not they had been previously diagnosed for COVİD-19, and their nutritional habits, was supplied to the volunteers. Volunteers who did not complete the survey and were vaccinated with the third dose of the COVID-19 vaccine without having a blood sample drawn at 6 months to measure the antibody levels were excluded from the study.

First of all, healthcare workers included in the study were divided into two groups according to whether or not they had been COVID-19 diagnosed, and the difference between antibody levels was investigated.

Determination of the antibody levels

After the collection of blood samples, the serum samples were separated and stored at –20°C until the study was performed. After the serum samples had been placed at room temperature, there was a possibility of binding IgG antibodies, including antibodies against the receptor-binding site of the spike protein S1 subunit of SARS-CoV-2.

The antibody levels were detected (AU/mL) by chemiluminescent microparticle immunoassay (CMIA) (Architect i2000, Abbott, USA) using kits that quantitively determine antibodies.

Statistical analysis

Healthcare workers were selected using a simple random sampling technique. The SPSS 23 program was used for statistical analysis of the data. All data from the study were calculated according to type and using appropriate descriptives (mean, standard deviation, median, width between quarters, and percentage). The Mann-Whitney U test was used to evaluate the factors that might influence the antibody levels in the first and sixth months. The $p<0.05$ was considered significant.

RESULTS

A total of 129 healthcare workers who had been vaccinated with only two doses of inactivated vaccine COVID-19 were included in the study. The flowchart of the cases is shown in Figure 1.

In the study, 76 (59%) healthcare workers were women and 53 (41%) were men, and the mean age was 36.2 (SD 7.6) (min: 20 to max: 60) years. Mean BMI was determined as 25.4 (SD 4.5) (min: 17.6 to max: 40.5). The mean antibody levels of the 129 volunteers included in the study were 1232.5 (min: 103 to max: 7151) AU/mL in the first month and 403.5 (min: 23 to max: 4963) AU/mL in the sixth month. According to the survey results, 91 (71%) volunteers reported that they were not diagnosed with COVID-19 before vaccination. It was found similar ($p=0.439$ and $p=0.299$, respectively) in the mean age and the mean BMI of those diagnosed and not diagnosed with COVID-19, before the first dose of the vaccine. The antibody levels 1 month and 6 months after the second vaccine dose in those diagnosed with COVID-19 before vaccination were significantly higher than those not diagnosed (Table 1).

Some factors that might affect the antibody levels of 91 healthcare workers who were not diagnosed with COVID-19 and had been vaccinated with two doses of the inactivated vaccine were evaluated based on the responses in the survey used (Table 2).

In terms of nutrition, while it was found that the antibody levels were similar in those fed on more fast food and high carbohydrate, the antibody levels were lower in those fed on high probiotic and prebiotic foods than those fed on low probiotic and prebiotic foods.

DISCUSSION

Immunoglobulin G antibodies can be detected in individuals who have been vaccinated against COVID-19 or have been diagnosed with COVID-19. In studies of the antibody levels

formed after both inactivated and mRNA vaccination, IgG antibody levels were reported to be significantly higher in those who have been diagnosed with COVID-19 than in those who have not been diagnosed[7-10]. Yalçın et al., found that IgG antibody levels were higher in people who had COVID-19 and a single dose of vaccine than in people who did not have COVID-19 but were vaccinated with two doses of the vaccine[11]. There are studies reporting that anti-spike IgG antibodies remain stable for 6 months in patients who have had the disease in the past[12]. Similarly, in our study, anti-spike IgG antibody levels were found to be significantly higher in patients who had the disease before vaccination than in patients who had not. This indicates that the antibody response, occurring in those who have undergone the disease, remains positive for some time. In studies on the antibody levels detected approximately 1 month after two doses of vaccine in healthcare workers who were vaccinated with inactivated vaccine without undergoing the disease, Dinç et al., found a mean IgG level of 707.1, and Tekol et al., found this

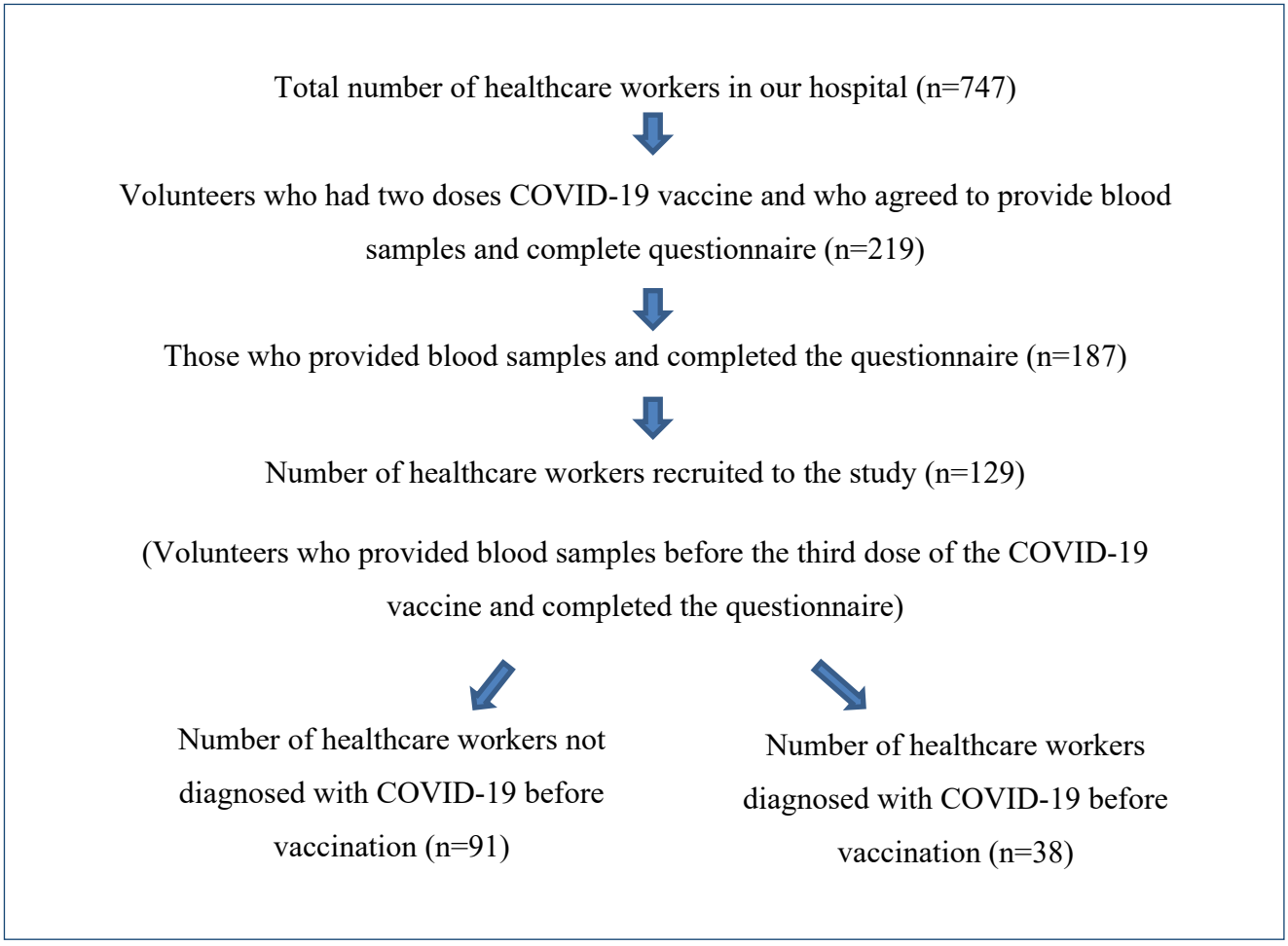

Figure 1. The flowchart of cases.

Table 1. Antibody levels (AU/mL) in those with and without diagnoses with coronavirus disease 19 before vaccination.

Groups	One month after the second vaccine dose			Six months after the second vaccine dose		
	Median	IQR	p-value	Median	IQR	p-value
Diagnosed with coronavirus disease 19 before vaccination (n=38)	1076.5	1383.2	**0.004**	446.0	519.4	**<0.001**
Not diagnosed with coronavirus disease 19 before vaccination (n=91)	805.0	768.7		140.8	155.7	

IQR: interquartile range. Significant p-value are indicated in bold.

level to be 171.3 and 89.85 4 months after the second dose[10-13]. In our study, the mean IgG level after 1 month was 805 AU/mL, and after 6 months, it was 140.8 AU/mL. The detected antibody levels showed a decrease over time.

It is known that there are many risk factors that determine being infected or the severity of the disease. For example, male gender, age over 50 years, chronic diseases such as hypertension, heart disease, diabetes, malignancy, chronic lung disease, kidney disease, living in nursing and rehabilitation centers, and staying in crowded environments such as schools, prisons, and immigrant camps are risk factors for COVID-19[14]. Vural et al., reported a decrease in the antibody levels with age, compared with vaccinated individuals under 40 and over 40 years of age[7]. Yiğit et al., also found that the younger the age of vaccinated healthcare workers, the higher the anti-SARS-CoV-2 immunoglobulin G level[15]. Bayram et al., studied the

Table 2. In those with no diagnosis of coronavirus disease 19 before vaccination, antibody levels 1 month and 6 months after vaccination, and the relationships with certain factors.

Groups	One month after the second vaccine dose			Six months after the second vaccine dose		
	Median	IQR	p-value	Median	IQR	p-value
Profession						
Doctor (n=48)	642.7	761.9	0.469	153.7	149.4	0.994
Others (n=43)	864.1	806.7		138.4	168.0	
Age group						
Below 35 years (n=39)	801.6	745.3	0.873	136.7	155.7	0.854
35 years and above (n=52)	818.9	831.4		143.4	160.4	
Gender						
Female (n=55)	891.8	860.9	0.403	135.1	179.3	0.792
Male (n=36)	632.8	664.2		153.7	113.8	
Body mass index						
Thin-normal (n=50)	860.3	788.2	0.661	133.0	158.1	0.873
Overweight-obese (n=41)	653.2	771.0		161.5	167.0	
Use of antibiotics in the past 6 months						
Yes (n=72)	844.7	697.9	0.257	155.8	151.9	0.232
No (n=19)	632.2	833.4		122.7	185.5	
Using vitamins (one or more of vitamin C, vitamin D, and fish oil)						
Yes (n=45)	805.0	799.0	0.353	123.9	122.4	0.079
No (n=46)	829.1	859.5		170.9	188.8	
Presence of chronic disease						
Yes (n=25)	958.0	653.5	0.722	182.6	208.6	0.513
No (n=66)	651.5	760.6		268.8	117.3	
Fed on carbohydrate						
Below average (n=61)	939.8	723.9	0.098	140.8	177.9	0.960
Above average (n=30)	598.6	631.3		149.9	298.5	
Fed on fast food						
Below average (n=53)	811.0	692.9	0.803	140.8	127.9	0.907
Above average (n=38)	803.3	846.9		141.0	193.3	
Fed on probiotic and prebiotic food						
Below average (n=58)	**905.4**	658.0	**0.019**	171.4	168.0	0.073
Above average (n=33)	**513.3**	817.6		111.7	114.0	

IQR: interquartile range. Values with statistically significant differences were bolded.

post-vaccination antibody levels in healthcare workers aged 18–34 years and found that it was higher in the older age groups[9]. Dinç et al., in their study examining post-vaccination antibody levels in healthcare workers who had not yet been diagnosed with COVID-19, found that the antibody levels were slightly lower in those over 40 years of age than in those under 40 years of age[10]. In our study, healthcare workers in the over and under 35 age groups were found to have similar antibody levels (Table 2). It is suggested that these levels were similar because all healthcare workers included in our study were under 60 years of age.

Although there are studies in which the antibody levels were higher in women than in men[11,15,16], studies with similar rates have also been reported as in our study[10]. This suggests that gender alone may not be an indicator.

Obesity has been reported to cause lower levels of antibodies to COVID-19 compared with healthy-weight individuals[17]. Pellini et al., found that the antibody levels were significantly higher in thin and normal-weight individuals than in overweight and obese individuals[16]. Franca et al., also reported a negative correlation between BMI and antibody levels[18]. Similar to our study, Dinç et al., found that the antibody levels did not differ in normal weight and obese healthcare workers[10]. These results indicate that more comprehensive studies are needed to determine the effects of obesity on antibody levels. It is known that several chronic diseases such as obesity also affect antibody levels. Bayram et al., and Dinç et al., found that patients with chronic diseases and hypertension had lower IgG levels against COVID-19 compared with healthy individuals[9,10]. Although no statistically significant difference was found in the patients with chronic diseases in our study, it was observed that the antibody levels were lower, especially in the sixth month. These results indicate that chronic diseases may have an inhibitory effect on the immune system of individuals.

It is reported that the type of nutrition and use of vitamin supplements have no effect on COVID-19 infection, but consumption of water and adequate and balanced nutrition are important for the treatment of disease[19]. It is known that the use of vitamin D and vitamin C may also be beneficial in prophylaxis and treatment[20,21]. Nutrition is shown to be important in reducing mortality from COVID-19 because high-carbohydrate nutrition leads to obesity, which negatively affects the prognosis of the disease. In our literature search, we could not find any study on how the antibody levels formed after vaccination are affected by nutrition. In our study, no statistically significant difference was found between the antibody levels of those who were fed high carbohydrate and more fast food compared with those who were fed less of these foods (Table 2). Those who consumed more foods containing probiotics (e.g., yogurt, cabbage, and kefir) and prebiotics (e.g., garlic, onion, and fruit) were found to have lower antibody levels. As IgG levels alone cannot be an indicator of the overall immune system, it was considered that more comprehensive studies are also needed in which cellular immunity parameters can be determined by assessing nutrition habits.

Therefore, vaccines serve as the most important shield in protecting people against COVID-19 and in alleviating the disease. Post-vaccination IgG levels can be affected by many factors, especially the presence of chronic disease, and they decrease over time. As there is no definitive value for the level of protective antibodies, it is important to continue vaccinations with differently produced technologies and not to forget reminder doses in order to maintain protection. More comprehensive studies on the effects of nutrition on antibodies are needed.

Limitations of the study

As third-dose vaccinations were not being considered at the time the ethics committee approval of the study was obtained, the study was initiated to monitor the antibody levels of two doses of vaccine for 1 year. However, due to the introduction of the third dose of the vaccine in August 2021, the antibody levels generated by two doses of the inactivated COVID-19 vaccine could only be monitored for 6 months rather than the planned 12 months.

ETHICS

Approval of the Duzce University Non-Interventional Health Research Ethics Committee dated 01.02.2021 and with the number 2021/16.

AUTHORS' CONTRIBUTIONS

EÇ: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **CEÖ**: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – review & editing. **ŞÖ**: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – review & editing. **NI**: Data curation, Formal Analysis, Investigation, Writing – original draft, Writing – review & editing. **DY**: Data curation, Formal Analysis, Investigation, Writing – original draft, Writing – review & editing. **GK**: Data curation, Formal Analysis, Supervision, Writing – review & editing. **PD**: Data curation, Formal Analysis, Supervision, Writing – review & editing. **İŞ**: Writing – review & editing.

REFERENCES

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382(8):727-33. https://doi.org/10.1056/NEJMoa2001017
2. Ebinger JE, Fert-Bober J, Printsev I, Wu M, Sun N, Prostko JC, et al. Antibody responses to the BNT162b2 mRNA vaccine in individuals previously infected with SARS-CoV-2. Nat Med. 2021;27(6):981-4. https://doi.org/10.1038/s41591-021-01325-6
3. Miller TE, Garcia Beltran WF, Bard AZ, Gogakos T, Anahtar MN, Astudillo MG, et al. Clinical sensitivity and interpretation of PCR and serological COVID-19 diagnostics for patients presenting to the hospital. FASEB J. 2020;34(10):13877-84. https://doi.org/10.1096/fj.202001700RR
4. Karamese M, Tutuncu EE. The effectiveness of inactivated SARS-CoV-2 vaccine (CoronaVac) on antibody response in participants aged 65 years and older. J Med Virol. 2022;94(1):173-7. https://doi.org/10.1002/jmv.27289
5. Çolak A, Baysoy A, Fidan M, İşbilen Başok B. Body mass index, age, and gender affect CoronaVac vaccine antibody response. Forbes J Med. 2022;3(2):215-7. https://doi.org/10.4274/forbes.galenos.2022.10337
6. Wu Z, Hu Y, Xu M, Chen Z, Yang W, Jiang Z, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. Lancet Infect Dis. 2021;21(6):803-12. https://doi.org/10.1016/S1473-3099(20)30987-7
7. Vural S, Hacıbekiroğlu M, Yıldır FR, Vural P. Immunological response after vaccine in a group of health-care workers with and without COVID-19 during pandemia. ANKEM J. 2021;35(2):45-52.
8. Salvaggio M, Fusina F, Albani F, Salvaggio M, Beschi R, Ferrari E, et al. Antibody response after BNT162b2 vaccination in healthcare workers previously exposed and not exposed to SARS-CoV-2. J Clin Med. 2021;10(18):4204. https://doi.org/10.3390/jcm10184204
9. Bayram A, Demirbakan H, Günel Karadeniz P, Erdoğan M, Koçer I. Quantitation of antibodies against SARS-CoV-2 spike protein after two doses of CoronaVac in healthcare workers. J Med Virol. 2021;93(9):5560-7. https://doi.org/10.1002/jmv.27098
10. Dinc HO, Saltoglu N, Can G, Balkan II, Budak B, Ozbey D, et al. Inactive SARS-CoV-2 vaccine generates high antibody responses in healthcare workers with and without prior infection. Vaccine. 2022;40(1):52-8. https://doi.org/10.1016/j.vaccine.2021.11.051
11. Yalçın TY, Topçu DI, Doğan Ö, Aydın S, Sarı N, Erol Ç, et al. Immunogenicity after two doses of inactivated virus vaccine in healthcare workers with and without previous COVID-19 infection: prospective observational study. J Med Virol. 2022;94(1):279-86. https://doi.org/10.1002/jmv.27316
12. Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, Faliti CE, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science. 2021;371(6529):eabf4063. https://doi.org/10.1126/science.abf4063
13. Demir Tekol S, Altıntaş MM, Yılmaz E, Saracoğlu K, Demirhan R. Detection and evaluation of antibodies to SARS CoV-2 spike protein in healthcare workers after ınactivated COVID-19 (CoronaVac) vaccination. South Clin Ist Euras. 2021;32(3):217-22. https://doi.org/10.14744/scie.2021.94899
14. Lauer SA, Grantz KH, Bi Q, Jones FK, Zheng Q, Meredith HR, et al. The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. Ann Intern Med. 2020;172(9):577-82. https://doi.org/10.7326/M20-0504
15. Yigit M, Ozkaya-Parlakay A, Cosgun Y, Ince YE, Bulut YE, Senel E. Should a third booster dose be scheduled after two doses of CoronaVac? A single-center experience. J Med Virol. 2022;94(1):287-90. https://doi.org/10.1002/jmv.27318
16. Pellini R, Venuti A, Pimpinelli F, Abril E, Blandino G, Campo F, et al. Obesity may hamper SARS-CoV-2 vaccine immunogenicity. medRxiv [Preprint]. 2021. https://doi.org/10.1101/2021.02.24.21251664.
17. Watanabe M, Balena A, Tuccinardi D, Tozzi R, Risi R, Masi D, et al. Central obesity, smoking habit, and hypertension are associated with lower antibody titres in response to COVID-19 mRNA vaccine. Diabetes Metab Res Rev. 2022;38(1):e3465. https://doi.org/10.1002/dmrr.3465
18. Frasca D, Reidy L, Cray C, Diaz A, Romero M, Kahl K, et al. Influence of obesity on serum levels of SARS-CoV-2-specific antibodies in COVID-19 patients. PLoS One. 2021;16(3):e0245424. https://doi.org/10.1371/journal.pone.0245424
19. Muslu M, Özçelik Ersü D. Nutritional treatment and its importance during new coronavirus (SARS-CoV-2/COVID-19) pandemia. Bes Diy Derg. 2020;48(1):73-82. https://doi.org/10.33076/2020.BDD.1341.
20. Feyaerts AF, Luyten W. Vitamin C as prophylaxis and adjunctive medical treatment for COVID-19? Nutrition. 2020;79-80:110948. https://doi.org/10.1016/j.nut.2020.110948
21. Mercola J. Grant WB, Wagner CL. Evidence regarding vitamin D and risk of COVID-19 and its severity. Nutrients. 2020;12:3361. https://doi.org/10.3390/nu12113361