Journal of Shahrekord University of Medical Sciences
doi:10.34172/jsums.2022.27 2022;24(4):163-167 http://j.skums.ac.ir

Original Article

Evaluation of Humoral immune responses against coronavirus in healthcare staff in hospitals and medical centers of Shahrekord University of Medical Sciences, Iran

Majid Asadi-Samani[1], Marziyeh Soltani[2], Fereidoun Rahmani[3*], Soleiman Kheiri[4], Mohammad-Taghi Moradi[5]

[1]Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran
[2]Student Research Committee, Shahrekord University of Medical Sciences, Shahrekord, Iran
[3]Department of Infectious Disease, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran
[4]Modeling in Health Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran
[5]Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran

***Corresponding Author:** Fereidoun Rahmani, Department of Infectious Disease, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran, Email: frahmani@skums.ac.ir

Abstract

Background and aims: Due to the different levels of exposure of different people to the coronavirus and different levels of immune response among them, this study was designed to investigate the humoral immune responses against the coronavirus disease 2019 (COVID-19) in healthcare staff in hospitals and medical centers admitting COVID-19 patients.

Methods: In this descriptive-analytical study, which was performed by call-out, the serum levels of IgM and IgG antibodies in 492 staff of hospitals and medical centers were evaluated using ELISA. Then, factors influencing the immune response of participants were determined.

Results: IgG positivity was 11.6% among participants of this study, 19.2% of the staff had a positive polymerase chain reaction (PCR) test, and the IgG positivity rate among them was only 16%. There was no significant relationship between body mass index, underlying diseases, diabetes, immune system-related diseases, herpes simplex virus, workplace, blood type, education level, symptoms, and IgG response ($P>0.05$). Further, the rate of IgG positivity in healthcare staff indicated a significant relationship only with gender ($P=0.005$), history of hospitalization ($P=0.002$) due to COVID-19 and position ($P=0.008$).

Conclusion: This study found that the prevalence of humoral immune response in healthcare staff was lower than the prevalence of the disease based on molecular tests. Based on the results of the present study, it is possible to provide an accurate estimate of the level of involvement and predisposition of healthcare staff in hospital wards and medical centers and to use this information for disease management and control.

Keywords: Coronavirus, Seroprevalence, Epidemiology, Immune responses, Healthcare staff

Received: 16 January 2022, Accepted: 5 Febraury 2022,ePublished: 8 September 2022

Introduction

On December 31, 2019, an unknown epidemic with unexplained infection of the lower respiratory tract began in Wuhan (the capital of Hubei province), China. The total number of cases per day worldwide and outside China was constantly increasing; therefore, the World Health Organization (WHO) considered the disease as a pandemic on March 11, 2020 (1).

The new virus was initially called the coronavirus disease 2019 (COVID-19). Subsequently, the International Committee on Taxonomy of Viruses (ICTV) named the virus the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which belongs to the family of coronaviruses, because of 80% similarity of nucleotides to the causative agent of SARS. This virus, as with the causative agent of SARS, belongs to the Betacoronaviruses and is, therefore, considered as an enveloped positive-strand RNA virus (2). Approximately 30 types of coronaviruses have been identified in humans, mammals, and birds. So far, it has been established that seven types of these viruses have caused infections in humans, which are of two genera: alpha, and beta. The genomes of these viruses encode four structural proteins including nucleocapsid, membrane, envelope, and spike (3,4).

The humoral immune system produces two types of IgM and IgG antibodies against the virus. The presence of IgM antibodies against this virus indicates the acute phase of the disease in the individual, while the presence of IgG antibodies represents a protective immunity against this disease (5-7). Tests based on the presence of antibodies in the serum of patients with COVID-19 can help identify people who have been exposed to the

virus and also develop insights into how the disease has spread, the immune response, and even its lethality. These tests are also useful for tracking people who have tested negative for viral load but are still carriers of the disease (8-11). However, various studies have reported differences in prevalence based on similar molecular and serological examinations (12-14) so that prevalence based on molecular studies is less than that based on urological studies, and serological studies have been considered necessary to more accurately determine the prevalence of the disease (13). In addition, varied rates of immune response have been reported in different communities and occupations (14-17). The present study was designed to investigate the humoral immune responses against COVID-19 among healthcare staff in hospitals and medical centers admitting COVID-19 patients.

Materials and Methods

This descriptive-analytical cross-sectional study was conducted from March to December 2020. The population of this study consisted of all staff working in hospitals and medical centers of Shahrekord University of Medical Sciences admitting COVID-19 patients in Chaharmahal and Bakhtiari province. A total of 492 individuals who were referred for sampling, and their samples were collected, were included in the study, and their information was collected and registered. Demographic information and possible symptoms of the disease such as fever, fatigue, nausea, headache, dizziness, anorexia, diarrhea, and vomiting were also collected. Moreover, other required information such as the result of the molecular polymerase chain reaction (PCR) test of all staff was collected from relevant authorities and verified and recorded.

Data were collected through a researcher-made sociodemographic checklist. The checklist included items on age, gender, education level, position, workplace, underlying disease, and possible symptoms of the disease such as fever, fatigue, nausea, headache, dizziness, anorexia, diarrhea, and vomiting.

To determine the serum level of antibodies against COVID-19 virus (IgM and IgG), blood samples taken from patients were first centrifuged at 2200 rpm for 10 minutes, and then the serum was isolated from blood cells. To prevent thaw-freeze of the samples, serum samples from one individual were placed in several microtubes at the time of separation. If the tests were performed on the sampling day, the isolated serum samples would enter the work process directly, and if the tests were performed at different times from the sampling day, the isolated sera would store at minus 20°C.

To determine the levels of antibodies in the serum samples, first, the temperature of serum samples, all materials, and reagents were brought to room temperature so that as soon as the test begins, all steps will be performed without stopping. Then, serum samples were diluted with a sample dilution solution based on the type of kit. The first two wells were considered for blank, while the next two wells were considered for negative control, the positive control was then duplicated, and the other wells were used for samples. Afterwards,100 µL of positive control, 100 µL of negative, and 100 µL of diluted specimens were added to the wells of the ELISA kit coated with SARS-CoV virus N (nuclear coating) antigens. The wells were covered with a special plate adhesive and kept at 37°C for 30 minutes. After incubation, the contents of the wells were emptied, and the wells were rinsed 5 times with a ready-to-use washing solution. Then, 100 µL of ready-to-use conjugated enzyme solution was added to the wells, except to the well blank, and the wells were then left at 37°C for 30 minutes. Then, the wells were washed again 5 times with ready-to-use washing solution. After washing, 100 µL of ready-to-use dye solution (containing tetramethylbenzidine and hydrogen peroxide) was poured into all wells, and the wells were placed at room temperature in the dark for 15 minutes. Then, 100 µL of stopper solution (containing normal 1 hydrochloric acid) was added to each well to stop the enzymatic reactions, and the blue color turned yellow. Finally, the optical absorbance of the wells was read by the ELISA reader at 450-nm wavelength up to half an hour after adding the stopper solution, and a 630-nm filter was used as the reference.

To calculate the results, the cutoff value was obtained by the following formula:

For IgM: Cutoff value = Average negative control's optical absorbance + 0.2

For IgG: Cutoff value = Average negative control's optical absorbance + 0.15

Then, to determine the positive and negative results, the index value was calculated by dividing the sample optical absorbance by the cutoff value. According to this formula, values higher than 1.1 were considered positive and lower than 0.9 were considered negative. Samples with an index value of 0.9-1.1 were considered suspicious and retested using fresh serum or plasma after some time.

Based on the kit's instructions, the sensitivity of the kits for IgM and IgG antibodies against SARS-CoV-2 was 85.4% (between 7-14 days after the onset of symptoms) and 94.1%, respectively, while their specificity was 99.4% and 98.3%, respectively.

The data in the present study were analyzed using SPSS 15 software. Qualitative data were analyzed in terms of frequency and percentage. The chi-square, *t* test, and analysis of variance (ANOVA) were used to investigate the relationship between variables.

Results

Out of 492 staff working in hospitals and medical centers affiliated to Shahrekord University of Medical Sciences admitting COVID-19 patients, 314 (64.1%) were women. The mean age of staff was 39.02 ± 8.70 years, and most (46.6%) of the participants were nurses. Only 2.7% of staff reported having an underlying disease, and only 0.8% reported diabetes. Most (79.1%) of the participants had

academic education. Among 260 staff for whom a PCR test was done using nasal swabs, 19.2% had a positive PCR test. The rate of IgG positivity among staff was 11.6%, and 4.9% of the staff had a positive IgM response. Furthermore, among the participants, only 1.6% reported a history of hospitalization due to COVID-19.

Table 1 indicates no significant relationship between education level, body mass index, blood type, underlying diseases, diabetes, herpes history, workplace, and job position in staff with IgG response ($P > 0.05$). Further, IgG positivity had a significant relationship only with gender, history of hospitalization due to the disease ($P < 0.05$) , and position (P = 0.008), so that the rate of positive serological response in male staff was higher than that in female staff ($P = 0.005$). In addition, the positive serological response in staff who were severely ill and were hospitalized was higher than the staff who were mildly ill and treated as outpatient ($P = 0.002$). Furthermore, the rate of positive serological response in administrative staff was higher than other staff groups ($P = 0.008$).

In the present study, the most common symptoms reported in serological tests were fatigue (44.2%), headache (38.5%), muscle pain (30.8%), sore throat (25%), and cough (21.2%). Among the studied symptoms, none of them showed a significant relationship with IgG response ($P > 0.05$).

Discussion

The aim of this study was to investigate the serum level of antibodies against COVID-19 virus in staff working in hospitals and medical centers of Shahrekord University of Medical Sciences admitting COVID-19 patients in Chaharmahal and Bakhtiari province to provide the healthcare system and policymakers with a better understanding of the humoral immune response in the staff. The IgG-positivity rate (serum prevalence of IgG antibody against COVID-19) in medical staff was 11.6%, which is lower than the prevalence of the disease based on molecular tests.

According to the results of the present study, 19.2% of the staff working in hospitals and medical centers of Shahrekord University of Medical Sciences admitting COVID-19 patients had a positive PCR test. However, among staff who tested positive for PCR, the IgG-positivity rate (seroprevalence of IgG antibody against COVID-19) was only 16%. Moreover, the rate of IgG positivity (serum prevalence of IgG antibody against COVID 19) in the whole population under study was 11.6%. A study in Pakistan examined the prevalence of serum-specific anti-SARS-CoV-2 antibodies in three groups: healthcare staff (working in various hospitals), industrial workers (consisting of the pharmaceutical industry and hardware companies), and the general population. The antibody positivity for the whole study population was 36%, which was partly unexpected for creating herd immunity. Moreover, the prevalence and immune response in industrial workers was 50.3%, in healthcare staff 13.2%, and in the general population 34% (17). In a study in India, the seroprevalence of anti-SARS-CoV-2 IgG antibody was reported to be 1.6% among healthcare staff, and the IgG antibody positivity was not significantly different among employees who were more frequently exposed to the disease compared to those who were less frequently exposed or not exposed (15).

Since this study was conducted from March to December 2020, when the prevalence of the disease in studies conducted among a high-risk population was reported to be about 20%, the prevalence was expectable, and compared to the national rate, no significant difference was observed. This suggests balanced COVID-19 management in the province and its healthcare staff in fighting the disease. Differences in prevalence based on molecular and serological studies have been reported in different studies (12-14) as in the present study. Some studies have underreported the prevalence based on molecular studies due to lower false negatives and have recommended that it is necessary to use serological studies to more accurately determine the prevalence of the disease (13). However, in studies where the seroprevalence was higher in the non-medical population, the prevalence based on serological tests was more dependable, but when medical staff were evaluated, the results of serological tests were different from those of other populations. The difference in prevalence based on molecular and serological tests can be due to differences in the serological response of different individuals exposed to the virus. In addition to this, some studies and hypotheses are based on the fact that the immune systems of people who have been exposed to the virus for a long time but adhere to health protocols do not respond strongly to the virus. As a result, their seroprevalence will be lower than molecular tests' results because the supply of the virus to the immune system occurs in a longer time but with a lower rate of the virus.

In the present study, at the time of serological testing, the most common reported symptoms were fatigue, headache, and muscle aches. The symptoms reported in the present study are consistent with the results obtained in other studies (18-20). It is, therefore, necessary for hospital management officials and policymakers to give more attention to the symptoms and to take measures to eliminate them to increase the efficiency of the staff.

Despite serological assessments of the disease, the need for vaccination is still felt in the community. In this regard, a study in the United Kingdom highlighted the immunity of people with previous infection and the importance of seroepidemiological studies to guide herd immunity and vaccination planning (16). A study in Pakistan also found that the minimum antibody positive for protective immunity was 60%-70%. That study considered the high seroprevalence of specific antibodies against SARS-CoV-2 to be effective in promoting protective immunity; however, it emphasized taking preventive measures and efforts to manufacture vaccines (17).

Table 1. Relationship between the studied variables and the immunity response in staff

Variables	Groups and subgroups	Serum IgG Positivity No. (%)	Serum IgG Negativity No. (%)	*P* value
Gender	Female	27 (8.6)	286 (91.4)	0.005
	Male	30 (17)	146 (83.0)	
Education level	Illiterate	1 (100)	-	0.405*
	Under high school diploma	4 (30.8)	9 (69.2)	
	Academic	19 (36.5)	33 (63.5)	
BMI	Underweight	0 (0)	0 (0)	0.885
	Normal	7 (36.8)	12 (63.2)	
	Overweight	12 (32.4)	25 (67.6)	
	Obese	4 (40)	6 (60)	
Blood type	O	11 (31.4)	24 (68.6)	0.762*
	A	4 (30.8)	9 (69.2)	
	B	6 (42.9)	8 (57.1)	
	AB	2 (50)	2 (50)	
Underlying disease	Yes	1 (9.1)	10 (90.9)	1*
Diabetes	Yes	0 (0)	3 (100)	1*
History of herpes infection	Yes	1 (33.3)	2 (66.7)	1*
Position	Physician	2 (6.3)	30 (93.8)	0.008*
	Nurse and assistant nurse	10 (4.8)	198 (95.2)	
	Laboratory staff	2 (10)	18 (90)	
	Midwife	0 (0)	11 (100)	
	Radiology technologist	1 (6.7)	14 (93.3)	
	Health worker and healthcare support worker	0 (0)	15 (100)	
	Secretary	1 (6.2)	15 (93.8)	
	Attendant	4 (7.5)	49 (92.5)	
	Administrative staff	6 (28.6)	15 (71.4)	
	Environmental health staff	3 (20)	12 (80)	
	Other	7 (17.9)	32 (82.1)	
Workplace	Emergency	2 (8.7)	21 (91.3)	0.178*
	CCU	0	18 (100)	
	Administrative	4 (28.6)	10 (71.4)	
	NICU	1 (7.7)	12 (92.3)	
	Dialysis	0	9 (100)	
	Internal medicine	1 (9.1)	10 (90.9)	
	Labor	0	7 (100)	
	Laboratory	2 (25)	6 (75)	
	Endoscopy	0	6 (100)	
	Infectious disease	3 (17.6)	14 (82.4)	
	Pediatrics	0	8 (100)	
	ICU	0	30 (100)	
	Surgery	1 (12.5)	7 (87.5)	
	Radiology and CT scan	1 (16.7)	5 (83.3)	
	High risk	0	5 (100)	
	Others	6 (20.7)	23 (79.3)	

Note. BMI: Body mass index; CCU: *Critical care* unit; NICU: *Neonatal intensive care unit*; ICU: *Intensive care unit*; CT: Computed tomography.
*It has been analyzed by Fisher's Exact Test.

Conclusion

The results of the present study demonstrated that the prevalence of humoral immune response in the staff was lower than the prevalence of the disease based on molecular tests. The results obtained in this study can be used to estimate the serum level of COVID-19 virus-neutralizing antibodies among staff working in hospitals and medical centers affiliated with Shahrekord University

of Medical Sciences admitting COVID-19 patients to more accurately predict their predisposition, gain information on disease progression, and manage human resources in hospitals and medical centers. Moreover, since fatigue, headache, and muscle pain were the most important symptoms in the medical staff for a long time after the disease, it is necessary for the healthcare system to take certain measures to overcome the symptoms.

Acknowledgments

We would like to thank the Vice Chancellor for Research and Technology of the University, all staff in different departments involved in conducting the study, and our participants.

Authors' Contribution

FR and MAS designed the study. MAS, MS, and MTM carried out the experiments. SK analyzed the data. All authors contributed to writing the draft. All authors read and approved the final proof.

Conflict of Interests

The authors declare that they have no conflict of interest regarding the authorship or publication of this article.

Ethical Approval

The protocol of the study was approved by the Ethics Committee of Shahrekord University of Medical Sciences (IR.SKUMS.REC.1399.047) and informed consent was obtained from all participants and the principles of data confidentiality were meticulously observed by the authors.

Funding/Support

This article was derived from an approved research project no. 5427, which was carried out with the support of the Vice Chancellor for Research and Technology of Shahrekord University of Medical Sciences.

References

1. World Health Organization (WHO). WHO Director-General's Opening Remarks at the Media Briefing on COVID-19 - 11 March 2020. Geneva: WHO; 2020. Available from: https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-COVID-19---11-march-2020.
2. Wang H, Li X, Li T, Zhang S, Wang L, Wu X, et al. The genetic sequence, origin, and diagnosis of SARS-CoV-2. Eur J Clin Microbiol Infect Dis. 2020;39(9):1629-35. doi: 10.1007/s10096-020-03899-4.
3. Ahmed SF, Quadeer AA, McKay MR. Preliminary identification of potential vaccine targets for the COVID-19 coronavirus (SARS-CoV-2) based on SARS-CoV immunological studies. Viruses. 2020;12(3):254. doi: 10.3390/v12030254.
4. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell. 2020;181(2):281-92.e6. doi: 10.1016/j.cell.2020.02.058.
5. Infantino M, Grossi V, Lari B, Bambi R, Perri A, Manneschi M, et al. Diagnostic accuracy of an automated chemiluminescent immunoassay for anti-SARS-CoV-2 IgM and IgG antibodies: an Italian experience. J Med Virol. 2020;92(9):1671-5. doi: 10.1002/jmv.25932.
6. To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis. 2020;20(5):565-74. doi: 10.1016/s1473-3099(20)30196-1.
7. Zhang L, Pang R, Xue X, Bao J, Ye S, Dai Y, et al. Anti-SARS-CoV-2 virus antibody levels in convalescent plasma of six donors who have recovered from COVID-19. Aging (Albany NY). 2020;12(8):6536-42. doi: 10.18632/aging.103102.
8. Haveri A, Smura T, Kuivanen S, Österlund P, Hepojoki J, Ikonen N, et al. Serological and molecular findings during SARS-CoV-2 infection: the first case study in Finland, January to February 2020. Euro Surveill. 2020;25(11). doi: 10.2807/1560-7917.es.2020.25.11.2000266.
9. Infantino M, Damiani A, Gobbi FL, Grossi V, Lari B, Macchia D, et al. Serological assays for SARS-CoV-2 infectious disease: benefits, limitations and perspectives. Isr Med Assoc J. 2020;22(4):203-10.
10. Stadlbauer D, Amanat F, Chromikova V, Jiang K, Strohmeier S, Arunkumar GA, et al. SARS-CoV-2 seroconversion in humans: a detailed protocol for a serological assay, antigen production, and test setup. Curr Protoc Microbiol. 2020;57(1):e100. doi: 10.1002/cpmc.100.
11. Xu Y, Xiao M, Liu X, Xu S, Du T, Xu J, et al. Significance of serology testing to assist timely diagnosis of SARS-CoV-2 infections: implication from a family cluster. Emerg Microbes Infect. 2020;9(1):924-7. doi: 10.1080/22221751.2020.1752610.
12. Bendavid E, Mulaney B, Sood N, Shah S, Bromley-Dulfano R, Lai C, et al. COVID-19 antibody seroprevalence in Santa Clara County, California. Int J Epidemiol. 2021;50(2):410-9. doi: 10.1093/ije/dyab010.
13. Lou B, Li TD, Zheng SF, Su YY, Li ZY, Liu W, et al. Serology characteristics of SARS-CoV-2 infection after exposure and post-symptom onset. Eur Respir J. 2020;56(2):2000763. doi: 10.1183/13993003.00763-2020.
14. Poustchi H, Darvishian M, Mohammadi Z, Shayanrad A, Delavari A, Bahadorimonfared A, et al. SARS-CoV-2 antibody seroprevalence in the general population and high-risk occupational groups across 18 cities in Iran: a population-based cross-sectional study. Lancet Infect Dis. 2021;21(4):473-81. doi: 10.1016/s1473-3099(20)30858-6.
15. Hunter BR, Dbeibo L, Weaver CS, Beeler C, Saysana M, Zimmerman MK, et al. Seroprevalence of severe acute respiratory coronavirus virus 2 (SARS-CoV-2) antibodies among healthcare workers with differing levels of coronavirus disease 2019 (COVID-19) patient exposure. Infect Control Hosp Epidemiol. 2020;41(12):1441-2. doi: 10.1017/ice.2020.390.
16. Lumley SF, O'Donnell D, Stoesser NE, Matthews PC, Howarth A, Hatch SB, et al. Antibody status and incidence of SARS-CoV-2 infection in health care workers. N Engl J Med. 2021;384(6):533-40. doi: 10.1056/NEJMoa2034545.
17. Zaidi S, Rizwan F, Riaz Q, Siddiqui A, Khawaja S, Imam M, et al. Seroprevalence of anti-SARS-CoV-2 antibodies in residents of Karachi-challenges in acquiring herd immunity for COVID 19. J Public Health (Oxf). 2021;43(1):3-8. doi: 10.1093/pubmed/fdaa170.
18. Kamal M, Abo Omirah M, Hussein A, Saeed H. Assessment and characterisation of post-COVID-19 manifestations. Int J Clin Pract. 2021;75(3):e13746. doi: 10.1111/ijcp.13746.
19. Raveendran AV, Jayadevan R, Sashidharan S. Long COVID: an overview. Diabetes Metab Syndr. 2021;15(3):869-75. doi: 10.1016/j.dsx.2021.04.007.
20. Taquet M, Geddes JR, Husain M, Luciano S, Harrison PJ. 6-month neurological and psychiatric outcomes in 236 379 survivors of COVID-19: a retrospective cohort study using electronic health records. Lancet Psychiatry. 2021;8(5):416-27. doi: 10.1016/s2215-0366(21)00084-5.