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Evaluation of humoral immune responses, effective factors on responses and re-infection in recovered COVID-19 patients

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Abstract

Introduction: Despite large studies on the COVID-19 pandemic, little evidence is available on immune response in recovered patients.
Objectives: The aim of this study was to investigate the humoral immune responses (IgM and IgG antibodies) in recovered COVID-19 patients and the role of risk factors and symptoms with respect to the immune responses.
Patients and Methods: In this descriptive-analytical study, which was conducted by call-out method, the serum levels of IgM and IgG antibodies were measured using enzyme-linked immunosorbent assay (ELISA) in 248 recovered patients. Effective factors on immune response were determined. Re-infection was investigated through patient follow-up and using information drawn from the hospital information system. Chi-square, *t* test, ANOVA, and regression analysis in SPSS 15 and Stata 14 were conducted to investigate the relationship between variables.
Results: IgG positivity was 86.3% among our participants. Among those who did not show antibody response to COVID-19 (IgM- and IgG-), the most common symptoms at admission were fever, muscle pain (90.9%), chills and anorexia (81.8%). IgG levels remained positive in recovered patients for over seven months. IgG response showed a significant relationship with body mass index, hospital stay length, smoking, residence place, mortality rate, vomiting, and appetite ($P<0.05$). The re-infection rate after recovery was only 1.6%.
Conclusion: High seroprevalence of IgG antibody against COVID-19 and low re-infection rate in Chaharmahal and Bakhtiari province was observed. In addition, the effects of factors such as fever, muscle pain, chills, vomiting, and anorexia on immune responses were demonstrated. These results can be used to manage disease control efficiently, and follow up the treatment process and re-infection in the recovered patients.

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Introduction

Despite advances in the prevention and treatment of diseases, the emergence of new viral diseases continues to be a serious public health issue. Viral epidemics have occurred in the past 20 years, such as severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002-2003, H1N1 flu in 2009 and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012. In December 2019, an unknown epidemic was reported in Wuhan, China with an unexplained infection of the lower respiratory tract. The experts of International Committee on Taxonomy of Viruses named the virus SARS-CoV-2 because of its similarity to the causative agent of SARS, and the resulting disease was named COVID-19 (1). Coronaviruses are RNA viruses that replicate in the host cytoplasm and evade innate immune sensing in most cell types in birds and mammals (2). Given the presence of living bats and animals in seafood stores in Wuhan, according to genomic research findings, SARS-CoV-2 may be originated

Key point

Evaluation of the immune responses in recovered COVID-19 patients and determining the role of factors influencing immune response and re-infection in these patients can be used to manage disease control efficiently.

from bats or bat droppings that have been exposed to contaminated materials in the store or the surrounding area; subsequent studies, however, increasingly reported the human-to-human transmission of the virus through respiratory droplets and direct contact. Currently, SARS-CoV-2 is being rapidly transmitted among humans and has become an extremely worrying pandemic due to rapid transmission and dramatically increasing incidence as well as potential transmission by asymptomatic carriers (3). Approximately 30 types of coronaviruses have been identified in humans, mammals and birds. It has been established that seven types of the viruses, which are alpha and beta, cause infection in humans. The genomes of these viruses encode four structural proteins, namely, nucleocapsid (N), membrane (M), envelope (E), and spike (S) (4). The S protein exists in the virus envelope, and is an important factor for its binding to the host cell and therefore an important target for antiviral drugs and vaccines. The M and E proteins in the envelope are involved in morphogenesis (formation of new viruses in the host cell). All three types of the proteins are important new coronavirus antigens that produce neutralizing antibodies and develop cellular immunity against the virus. The N protein is located inside the virus and is essential for the synthesis of virus nucleic acid. Research has shown that the N antigen, as the most abundant antigen of this virus, is the best choice to be used in immunological diagnostic techniques (4,5). The presence of IgM antibodies against the virus indicates the acute phase of the disease and the presence of IgG antibody indicates a stable response to it (6,7).

Patients with this disease refer to medical centers with non-specific symptoms such as fever, headache, muscle pain, dry cough and shortness of breath; however, a substantial proportion of the patients report infrequent symptoms such as diarrhea (gastrointestinal disorders), acute kidney injury, liver failure, and heart failure that are difficult to treat and recover from (8). The high potential of this virus for human-to-human transmission on the one hand and international travel on the other hand have increased the incidence of the disease in different countries. Besides that, many carriers are asymptomatic, which necessitates prevention and screening to control the devastating effects of the disease.

Tests based on the presence of antibodies in serum can help identify individuals who have been exposed to the virus, gain insight into how the disease has spread and whether it is life-threatening or not, and appropriately understand the immune system's response rate to the disease and determine the probability of its recurrence. In addition, these tests are useful to identify people who have tested negative for viral load but are still carriers of the disease. Furthermore, it may be useful to identify people who have exhibited a complete immune response to the disease after recovery for plasma therapy of patients under treatment. Serological assays have certain advantages (9,10).

Few studies have so far investigated the level of immune response in recovered patients, and most studies have addressed the community exposed to the disease, immune response across the community, and the epidemic, and comparatively examined the incidence rate of the disease using various screening methods.

Objectives

The aim of this study was to investigate the serum levels of antibodies against coronavirus in recovered COVID-19 patients to understand the patients' immune response more accurately.

Patients and Methods

Study design

This cross-sectional, descriptive-analytical study was conducted from September 2020 to November 2020 on recovered COVID-19 inpatients in Chaharmahal and Bakhtiari province. Data on all recovered inpatients were drawn from the hospital information system (HIS) and all the patients were called out. Finally, a total of 248 individuals who referred for sampling and their samples were collected were enrolled in the study and their data were collected.

Data were collected using a researcher-made sociodemographic checklist and by referring to the patients' medical files during the course of treatment. The data included age, gender, education level, occupation, marital status, residence place, city, main street, medical and psychiatric comorbidities, history of contact with the infected individual, weight, height, and symptoms of the disease experienced after recovery.

To determine the serum level of antibodies against coronavirus (IgM and IgG), blood samples of the 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 would be poured into several micro-tubes as soon as they were collected. If the tests were performed on the sampling day, the isolated serum samples would be processed immediately, and if the tests were performed on a day other than the sampling day, the isolated serum samples were stored at -20°C.

To determine the levels of antibodies in the serum samples, first the temperature of serum samples, all materials and reagents was brought to room temperature thereby all steps were carried out non-stop as soon as the test started. Then, serum samples were diluted using a dilution solution based on the type of kit. The first two wells were considered as blank, the next two wells as negative control, and then the positive control was introduced in duplicate and the other wells were specified for samples. One hundred microliters of positive control,

100 µL of negative control, and 100 µL of diluted samples were added to the wells of enzyme-linked immunosorbent assay (ELISA) kit coated with SARS-CoV-2 (N-coating) antigens. The wells were covered with plate adhesive and left at 37°C for 30 minutes. After incubation, the contents of the wells were evacuated and the wells were washed five times with a ready-to-use washing solution. Then, 100 µL of ready-to-use conjugated enzyme solution was added to the wells, except for the blank well, and left at 37°C for 30 minutes. Afterwards, the wells were washed five times further 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 left in the dark at room temperature for 15 minutes. Then, 100 µL of stopper solution (containing 1N hydrochloric acid) was added to each well to stop the enzymatic reactions and turn the blue color into yellow color. Finally, the optical absorbance of the wells was read by the ELISA reader with a 450-nm filter up to half an hour after adding the stopper solution and a 630-nm filter was used as the reference filter.

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

For IgM:

Cutoff value = average optical densities of negative control + 0.2

For IgG:

Cutoff value = average optical densities of negative control + 0.15

Then, to determine the positive and negative results, the index was calculated by dividing the sample's optical absorbance by the cut-off value. Based on this formula, values higher than 1.1 were considered positive and those 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, its sensitivity for measuring IgM and IgG antibodies against SARS-CoV-2 is 85.4 (7-14 days after the onset of symptoms) and 94.1% and its specificity 99.4% and 98.3%, respectively.

In this study, on August 15, 2021 (late peak of the coronavirus outbreak in Iran where the highest increase in the prevalence of delta strain was observed), re-infection of patients under study was investigated through follow-up and information drawn from the health information system.

Statistical analysis

Data analysis was conducted using SPSS version 15. Qualitative data were expressed as frequency and percentage. Chi-square, t test, ANOVA and regression analysis in SPSS 15 and STATA 14 were conducted to investigate the relationship between variables. $P<0.05$ was considered as statistically significant.

Results

Patients' demographic characteristics

Among 248 admitted patients, 134 (54%) were women. The mean age of patients was 54.47±17.08 (range; 4-94) years, with no significant difference in age between men and women ($P=0.114$). The mean body mass index (BMI) of patients was 27.16±4.97 (kg/m^2), with no significant difference between men and women ($P=0.058$). Around 11.89% of the patients were married, most (58.04%) of them had under high school diploma education and were housewives. Most (27.4%) of patients were self-employed, followed by students and civil servants. About 57.3% of the participants reported having underlying disease, so that 16.5% of them had diabetes, 13.7% had autoimmune diseases, and 7.7% had neurological diseases. The prevalence of underlying diseases was 67.2% and 45.6% in men and women, respectively, with diabetes being the most common underlying disease in both genders. Only 4% of patients reported having herpes simplex virus (HSV) during the last six months, during the disease and before testing. The rates of diabetes, neurological disease and HSV infection was significantly higher in women than in men ($P<0.05$). The rate of contact with first-degree relatives with COVID-19 after recovery and prior to testing was 7.3% in all participants. However, men had contact with infected people than women more frequently (15% versus 2.4%, $P<0.001$). Only 4.46% of patients reported to be smoker. Mean hospital stay length in our patients was 7.03±4.34 days. Mean hospital stay length was longer in women than in men; however, no statistically significant difference in hospital stay length was observed between the genders ($P=0.468$, Table 1). Only 2.4% of the patients were reported to die after follow-up. However, the cause of death in patients after recovery was old age and heart and neurological diseases. Mortality rate was higher in men than in women (3.51% versus 1.49%, $P=0.04$).

Disease severity at admission based on CT scan result and oxygen saturation

Among 169 patients with known CT scan result and oxygen saturation percentage, 82.84% had less than 93% oxygen saturation and only 26.63% had positive CT scan result. There was a significant difference in oxygen saturation between men and women ($P=0.001$). Reduction of oxygen saturation to less than 93% was more frequent in women than in men (87% versus 76.8%).

The prevalence of immune response in recovered patients

A total of 214 individuals out of the 248 patients were positive for COVID-19-specific IgG. The frequency of positive antibodies in our patients was 86.3% (87.3% in men and 85.1% in women). There was no significant difference in IgM and IgG positivity between men and women ($P<0.05$). During the study, 21.77% of patients were in the recovery phase (IgM+ and IgG+); however,

Table 1. Demographic characteristics of participants

Variables	No. (%) or Mean ± SD (range)
Age (y)	54.47 ± 17.08 (4-94)
Gender	
Female	134 (54.3)
Male	114 (45.7)
Marital status	
Single	27 (10.9)
Marriage	221 (89.1)
Height (cm)	166.71± 12.33 (110-198)
Weight (kg)	76.01± 16.52 (15-130)
BMI (kg/m^2)	27.16± 4.97 (12.3-44)
Hospital stay length (day)	7.03± 4.34 (1-31)
Hospitalization duration until testing (day)	59.83± 56.19 (1-221)
Smoking	
Yes	10 (4.5)
Residence place	
Shahrekord	179 (72.2)
Saman	7 (2.8)
Farsan	30 (12.1)
Ben	17 (4.9)
Shalamzar	9 (3.6)
Kohrang	6 (2.4)
Mortality rate after recovery and discharge	6 (2.4)
Education level	
Illiterate	58 (25.9)
Under academic education	130 (58)
Academic	36 (16.1)
Occupation	
Housewife	134 (54)
Student	22 (8.9)
Civil servant	17 (6.9)
Self-employed	68 (27.4)
Related diseases	142 (63.4)
Autoimmune diseases	34 (13.7)
Diabetes	41 (16.5)
Neuropsychological diseases	19 (7.7)
Contact with infected first-degree relatives	18 (7.3)
HSV infection*	10 (4)

*History of herpes simplex virus infection acquisition in the last six months before coronavirus infection

no IgM positivity alone was observed in cases of IgG negativity.

Immune response in patients based on the duration from onset of symptoms

At testing, over three months had elapsed since the admission of 23.4% of the patients and the data showed that 81% of them had positive IgG levels. Moreover, all five patients who had recovered for over seven months had positive IgG levels.

Our results revealed that IgG positivity in patients who had recovered for one week to two months until testing showed an increasing trend and in patients who had recovered for over two months until testing showed a decreasing trend. The highest positive IgG level was observed in patients who had recovered for four weeks to two months until testing (Table 2, Figure 1).

Patients' clinical symptoms based on the type of serological response

Based on the results of serological tests, patients were classified into three groups (IgM+ and IgG+), (IgM- and IgG+) and (IgM- and IgG-). Patients' signs and symptoms at the onset of the disease and serological tests were investigated in different serological groups. Among patients who did not show antibody response to COVID-19 (IgM- and IgG-), the most common symptoms at admission were fever, muscle pain (90.9%), chills, anorexia (81.8%) and vomiting (72.7%), and the most common symptoms at testing were anorexia (34.5%), fatigue, and muscle pain (31%). Among those who showed a positive antibody response (IgM+ and IgG+; IgM- and IgG+), anorexia was the most common symptom at admission and fatigue was the most common symptom at testing. Long-term symptoms in patients at complete recovery phase included fatigue, cough, and muscle pain. In addition, none of the patients whose antibody response was negative for COVID-19 had HSV infection in the last six months before testing; however, HSV infection was reported in 13% of patients with positive antibody response.

Relationship between patients' underlying disease and the prevalence of immune response

The prevalence of immune response was higher in individuals with underlying disease, immune system disorders, diabetes, and neurological disorders than in those with negative immune response (Table 3; $P<0.05$). Relationship between other variables and the prevalence of immune response

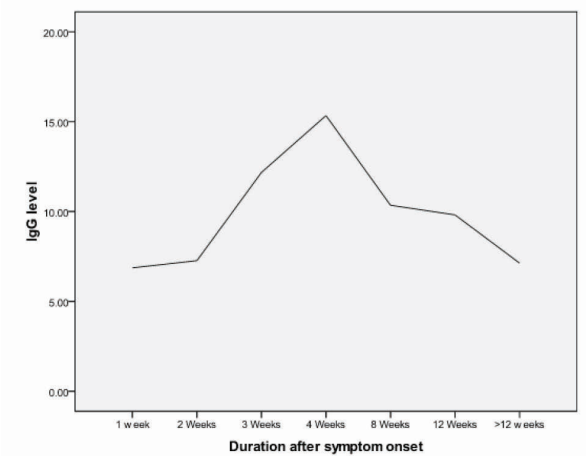

Figure 1. Immune system response to COVID-19 by duration after onset of symptoms.

Table 2. Frequency of immune response to COVID-19 by hospitalization duration until testing

Hospitalization duration until testing	Immune response result	Antibodies	
		IgM, No. (%)	IgG, No. (%)
1 Week	Positive	7 (36.8)	14 (73.7)
	Negative	12 (63.2)	5 (26.3)
2 Weeks	Positive	10 (28.6)	26 (74.3)
	Negative	25 (71.4)	9 (25.7)
3 Weeks	Positive	16 (40)	37 (92.5)
	Negative	24 (60)	3 (7.5)
4 Weeks	Positive	3 (60)	5 (100)
	Negative	2 (40)	-
5-8 Weeks	Positive	9 (20.9)	41 (95.3)
	Negative	34 (79.1)	2 (4.7)
9-12 Weeks	Positive	8 (16.7)	43 (89.6)
	Negative	40 (83.3)	5 (10.4)
≥13 Weeks	Positive	2 (4.3)	47 (81)
	Negative	56 (96.6)	11 (19)

Table 3. Frequency of underlying variables in serological groups

Underlying variables		Serological groups				*P* value
		IgM- IgG-	IgM+ IgG-	IgM+ IgG+	IgM- IgG+	
Related diseases	Yes	18 (62.1)	0	27 (57.4)	97 (65.5)	>0.05
	No	11 (37.9)	0	20 (42.6)	51 (34.5)	
Autoimmune diseases	Yes	7 (24.1)	0	3 (6.4)	24 (16.2)	>0.05
	No	22 (75.9)	0	44 (93.6)	124 (83.8)	
Diabetes	Yes	4 (13.8)	0	12 (25.5)	25 (16.9)	>0.05
	No	25 (86.2)	0	35 (74.5)	123 (83.1)	
Neuropsychological diseases	Yes	3 (10.3)	0	2 (4.3)	14 (9.5)	>0.05
	No	26 (89.7)	0	45 (95.7)	134 (90.5)	

Note: Comparisons between serological groups were performed using Chi-square test in Stata 14.

Patients with an oxygen saturation below 93% at admission were more likely to show immune response at testing, therefore 84.1% of patients with IgG+ had less than 93% oxygen saturation at admission ($P>0.05$). However, based on the CT scan results, most (72%) of the patients with reportedly negative pulmonary involvement had positive IgG after recovery ($P>0.05$).

The results of the present study showed that IgG positivity was significantly associated with BMI, hospital stay length, smoking, residence place, mortality rate, and vomiting and appetite ($P<0.05$; Table 4).

There was a significant relationship between BMI and IgG response so that positive IgG response was higher in overweight patients than in other (lean, normal weight and obese) ones ($P<0.05$). The prevalence of IgG response was higher in people hospitalized for 3-6 days than in those hospitalized for a shorter or longer period ($P<0.05$; Figure 2).

Statistical analysis using Mann-Whitney U test

The rate of positive IgG response was higher in Shahrekord than in other counties of the province and the lowest rate of positive IgG response was reported from Farsan and Koohrang counties.

Among the symptoms, only vomiting and appetite were significantly associated with IgG response, thereby patients who reported vomiting at admission and testing had a lower IgG positivity ($P<0.05$). Moreover, patients who reported normal appetite at testing had a higher IgG positivity ($P<0.05$).

Re-infection

Among our patients, only four cases of re-infection were confirmed, out of whom three had positive serology test and one had negative serology test (Table 5).

Discussion

The outbreak of coronavirus has become a major concern across the world. To deal with the global health issue, several teams around the world have begun testing of population samples to assess the prevalence of the disease in terms of the prevalence of SARS-CoV-2 antibodies, and preliminary findings have led to inconsistent evidence on the prevalence of the disease based on molecular tests (7,10-12). Besides that, the exact level of immune response has not yet been definitely determined in recovered

Table 4. Relationship between seroprevalence of COVID-19 antibodies and studied variables

Variables		IgM response		IgG response	
		Positive cases No. (%)	OR (95% CI)	Positive cases No. (%)	OR (95% CI)
Gender	Female	26 (19.4)	1	117 (87.3)	1
	Male	29 (25.4)	1.417 (0.78-2.58)	97 (85.3)	0.829 (0.40-1.71)
Marital status	Single	3 (11.1)	1	21 (77.8)	1
	Married	52 (23.5)	2.462 (0.71-8.51)	193 (87.3)	1.969 (0.73-5.30)
Age groups (y)	<30	4 (18.2)	0.704 (0.22-2.28)	16 (72.7)	0.471 (0.159-1.40)
	30-39	7 (25.9)	1.108 (0.42-2.94)	21 (77.8)	0.618 (0.21-1.78)
	40-49	11 (24.4)	1.025 (0.45-2.33)	41 (91.1)	1.809 (0.57-5.79)
	50-59	9 (16.7)	0.633 (0.27-1.48)	51 (94.4)	3 (0.828-10.87)
	>60	24 (24)	1	85 (85)	1
Body mass index (kg/m^2)	Underweight	1 (12.5)	0.532 (0.06-4.80)	5 (62.5)	0.177 (0.03-0.97)*
	Normal weight	14 (20.9)	0.985 (0.41-2.39)	54 (80.6)	0.442 (0.15-1.33)
	Overweight	21 (21.6)	1.030 (0.45-2.34)	89 (91.8)	1.184 (0.37-3.82)
	Obese	11 (21.2)	1	47 (90.4)	1
Smoking	No	47 (22)	1	190 (88.8)	1
	Yes	0	-	5 (50)	0.126 (0.03-0.47)*
Mortality	Health	55 (22.7)	1	211 (87.2)	1
	Died	0	-	3 (50)	0.147 (0.03-0.76)*
Related diseases	No	20 (24.4)	1	71 (86.6)	1
	Yes	27 (19)	0.728 (0.38-1.40)	124 (87.3)	1.067 (0.48-2.39)
Autoimmune diseases	No	44 (23.2)	1	168 (88.4)	1
	Yes	3 (8.8)	0.321 (0.09-1.10)	27 (79.4)	0.505 (0.2-1.3)
Diabetes	No	35 (19.1)	1	158 (86.3)	1
	Yes	12 (29.3)	1.75 (0.81-3.77)	37 (90.2)	1.464 (0.48-4.46)
History of contact with COVID patients	No	40 (19.4)	1	179 (86.9)	1
	Yes	7 (38.9)	2.64 (0.96-7.24)	16 (88.9)	1.207 (0.26-5.54)
Herpes simplex virus infection	No	25 (37.3)	1	56 (83.6)	1
	Yes	3 (30)	0.72 (0.17-3.04)	10 (100)	-

Only the association of cases with asterisk with the seroprevalence of antibody response was statistically significant (P<0.05).

COVID-19 patients. A study in the United Kingdom highlighted the immunity of individuals with previous infection and the importance of seroepidemiological studies to guide herd immunity and vaccination planning

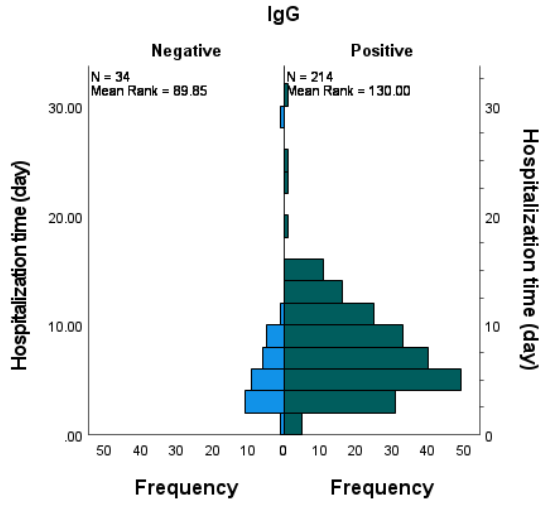

Figure 2. Frequency of IgG response by hospital stay length

(13). In 2020, a research team in Wuhan, China examined the serum levels of IgG and IgM antibodies against SARS-CoV-2 in 338 COVID-19 patients by quantitative luminescence (chemiluminescent immunoassay). That study highlighted the importance of assessing the level of specific antibodies against SARS-CoV-2 to determine the severity and prognosis of the disease (14). In a study on humoral immune response, duration of immune response and development of protective immunity in inpatients with COVID-19, serum levels of IgG, IgA and IgM antibodies against SARS-CoV-2 were assessed using ELISA. That study showed that the response of the antibodies increased and decreased three weeks after infection and the virus neutralization activity correlated with the IgG?titer. Moreover, the level of antibodies in the bloodstream can be traced up to six months after symptoms onset. Most infected people have protective immunity until a few months that reduces the severity of the disease (15).

In the present study, which was conducted on recovered COVID-19 inpatients, the mean age of the patients was 54.47 ± 17.08 years, indicating that most patients admitted due to COVID-19 were middle-aged. The mean BMI

Table 5. Coronavirus re-infection after recovery

Demographic characteristics	Recurrence based on serology results			
	Negative result	Positive result		
	Patient 1	Patient 2	Patient 3	Patient 4
Age (y)	37	50	56	23
Gender	Female	Female	Female	Male
Body mass index (kg/m^2)	31.1	29.3	24.2	31.7
History of smoking	No	No	No	No
History of underlying disease	No	No	No	No
Related diseases	Pulmonary diseases	Pulmonary diseases	Diabetes	No
Date at initial infection	20 May 2020	19 June 2020	24 July 2020	4 May 2020
Relapse date	26 June 2021	8 November 2020	4 June 2021	24 August 2020
Time elapsed from initial infection to re-infection (month)	12	5	11	4

of the patients was 27.16 ± 4.97 kg/m^2, which indicates overweight of the patients. Approximately 57.3% of our patients reported having underlying disease and diabetes was the most common underlying disease in both men and women, indicating that diabetics have a higher susceptibility to hospitalization after coronavirus infection. The results of risk factor assessment showed that men were more frequently in contact with infected people than women, which indicates the need for more training of men to observe social distance. In the present study, the mean hospital stay length was 7.03 ± 4.34 days, which can be taken into account in managing hospitalized patients. The oxygen saturation and CT scans of patients showed that 82.84% of them had less than 93% oxygen saturation and only 26.63% had positive CT scan result. More clearly, the oxygen saturation below 93% can be a more reliable indicator in hospitalized patients. The most common symptoms at admission were anorexia (83.12%), fever (76.62%), muscle pain (75.32%), cough, chills (72.73%) and fatigue (70.13%).

In this study, the rate of IgG positivity (seroprevalence of IgG antibody against COVID-19) was 86.3%. At testing, over three months had elapsed since the admission of most (23.4%) patients and the data showed that 81% of them had positive IgG levels. All five patients who had recovered for over seven months had positive IgG levels. Based on our results, IgG level was positive in patients who had recovered for one week to two months from admission until testing, and decreased in patients who had recovered for more than 2 months. The highest positive IgG level was observed in patients who had recovered for four weeks to two months from admission until testing. These results indicate that the immune response develops in most COVID-19 patients and can last up to seven months, and it is therefore necessary to take certain measures to prevent the disease. A study in China showed that IgM levels began to rise during the first week after infection, peaked within the second week and then decreased. IgG levels began to rise after the first week and then remained constant for a long time (14). In 2020, Australian researchers collected blood samples from 25 COVID-19 patients since the onset of infection until 242 days after diagnosis. They observed antibody decline about two weeks after infection; however, they observed memory B cells, which produces antibodies, in their bloodstream. These cells specifically target the coronavirus S protein. In addition, they reported that COVID-19 survivors could rely on their immune system to prevent re-infection for at least eight months (16).

In the present study, the most common symptoms in individuals who did not show antibody response to COVID-19 (IgM- and IgG-) were fever, muscle pain, chills, anorexia, and vomiting at admission and anorexia at testing; it is therefore necessary to take certain measures for patients who refer to hospital and experience muscle pain, chills, vomiting and anorexia to prevent re-infection.

In the present study, the majority of patients who had less than 93% oxygen saturation at admission had a significantly higher prevalence of immune response at testing. However, according to the CT scan results, the majority (72%) of them with negative pulmonary involvement were IgG positive after recovery, but the difference was not statistically significant. In this regard, a study on the prevalence of immune responses showed that the level of antibody positivity in groups of patients with different severity of the disease (moderate, severe, critical) did not differ significantly; however, the results showed that IgM levels were higher than moderate in severe and critical cases of the disease, while IgG levels were lower in critical cases than in severe and moderate cases (14).

In the present study, IgG response showed a significant relationship with BMI, hospital stay length, smoking, residence place, mortality rate and some of the studied symptoms. There was a significant relationship between BMI and IgG response so that positive IgG response was higher in overweight people than in other individuals. People with high BMI and advanced age have a more inflamed immune system than others. Inflammation and the production of inflammatory cytokines are effective in switching the humoral immune response to the production of IgG antibodies. It seems that aging and weight gain

play an effective role in inducing the IgG immune response. However, IgG positivity may not constantly lead to improved or efficient immune response, because the levels of B cells decrease with aging but the levels of immunoglobulins with low affinity in the bloodstream increase. The production of immunoglobulins with low affinity can be derived from switching the production of IgD and IgM to IgG by naive B cells and to IgA by memory B cells (17,18).

The study of effects of obesity on serum levels of coronavirus antibodies in Crohn's disease patients showed that leptin, which is associated with body fat and BMI, increased in overweight patients. Leptin induces the secretion of pro-inflammatory cytokines (IL-6 and TNF) in B cells by activating the JAK2/STAT3 and P38/MAPK/ERK1/2 signaling pathways (19,20).

In this study, the rate of positive IgG response was higher in Shahrekord than in other studied counties and the lowest rate of positive IgG response was observed in Farsan and Koohrang counties, which could be due to the fact that the likelihood of contact with infected people and system involvement is higher in more populated areas. In areas with a lower immune response, prevention and vaccination measures should be given priority. Among the symptoms, only vomiting and appetite were significantly associated with IgG response, i.e., patients who reported vomiting at admission and testing had significantly lower IgG-positive levels. In patients who report vomiting among other symptoms, it is necessary to give further attention to following up the treatment process and re-infection. Besides that, patients who reported normal appetite at testing had a significantly higher rate of IgG positivity; therefore, anorexia after recovery can be considered a risk factor for lower IgG response and consequently recurrence of the disease. Patients who reported normal appetite at testing had a significantly higher rate of IgG positivity; therefore, anorexia after recovery can be considered a risk factor for lower IgG response and consequently the possibility of recurrence of the disease.

Conclusion

Serological assays can be used to detect suspected or asymptomatic patients, to determine the extent of the immune response in patients, indicators affecting the immune response, and the likelihood of re-infection, to identify those who have acquired immunity to the disease, and to manage disease prevention and treatment. In the present study, the seroprevalence of IgG antibody against COVID-19 in Chaharmahal and Bakhtiari province was obtained 86.3%. Based on our results, it is possible to obtain an accurate estimate of the rate of immune response of patients and to determine more effective risk factors for the development of immune response in patients. In addition, measures have been taken to manage risk factors and symptoms of the disease that could lead to enhanced immune response. Given the results of the present study, the role of factors such as fever, muscle pain, chills, vomiting and anorexia in the immune system response can be taken into consideration. Patients who have fever, muscle pain, and chills at admission may show a weaker immune response after recovery. Anorexia after recovery can be considered a risk factor for lower IgG response and subsequent recurrence. Obviously, people with higher appetites receive more nutrients that strengthen their immune systems compared to people with anorexia and lean individuals. Moreover, patients who report vomiting as an initial symptom at admission are less likely to be IgG positive and need more attention in terms of follow-up and re-infection. Smoking is another factor for weakening of the immune response in COVID-19 patients, which needs to be taken into account in these patients. Finally, in people whose immune system response is negative after recovery, it is necessary for them to adhere to health protocols more seriously, to receive vaccines, and to make necessary efforts to increase the immune response. Furthermore, as emphasized in other studies, fatigue, cough and muscle pain are the most important post-COVID-19 symptoms, for which certain measures should be taken in the community.

Limitations of the study

We invited the recovered patients to participate in this study through callout. One of the limitations of this study was failure to invite the patients to participate in the study in person. However, we did our best to enroll a sufficient number of patients.

Authors' contribution

Conceptualization: MAS and MTM. Methodology: MAS, MTM, and SK. Validation: MAS, and SK. Formal Analysis: SK. Investigation: MAS, MS, DA, FI, and FAS. Resources: MAS, DA, ans MTM. Data Curation: MS, SK, and FAS. Writing—Original Draft Preparation: MAS, MS, FI, SK, and FAS. Writing—Review and Editing: All authors. Visualization: MAS and MTM. Supervision: MAS, MTM, and SK. Project Administration: MAS and MTM. Funding Acquisition: MAS.

Data availability statement

The authors confirm that all relevant data are included in the manuscript.

Ethical issues

The study protocol followed the principles of the Declaration of Helsinki. The institutional ethics committee at Shahrekord University of Medical Sciences approved the entire study protocol (Ethical code#IR.SKUMS.REC.1399.073), and written informed consent to participate in the study was obtained from all participants.

Conflicts of interest

The authors declare that they have no competing interests.

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