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Persistence assessment of SARS-CoV-2-specific IgG antibody in recovered COVID-19 individuals and its association with clinical symptoms and disease severity: A prospective longitudinal cohort study

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ABSTRACT

Background: Antibodies play an important role in neutralizing invading pathogens and protecting the host against re-infection. Thus, the accurate assessment of antibodies during a pandemic can provide important evidence for monitoring pathogen exposure, understanding the role of antibodies in protective immunity, and helping vaccine development.

Methods: In this study, 96 west Iranian recovered COVID-19 subjects were recruited and, based on clinical symptoms and disease severity, categorized into three different groups: mild, moderate, and severe. In addition, the presence and dynamic change of SARS-CoV-2-specific IgG antibody three, four-, and six months post symptom onset (PSO) were measured. Also, the association between IgG antibody titer with clinical symptoms and disease severity was examined.

Results: Although in real-time RT-PCR-positive samples negative IgG antibody results were found, most subjects mount humoral immune responses that could raise a robust SARS-CoV-2-specific IgG antibody. Furthermore, this antibody persisted in the serum of most recovered COVID-19 subjects at least six months PSO and demonstrated little to no decrease. Also, specific IgG antibody titer was strongly correlated with clinical symptoms and disease severity.

Conclusions: These results provide an insight into the presence and persistence of the SARS-CoV-2-specific IgG antibody. Although serological tests could not be used as the primary diagnostic test, they may support real-time RT-PCR results. Also, they could be used for diagnosing COVID-19 subjects tested later outside of the optimal period. Thus, the SARS-CoV-2-specific IgG antibody is an excellent marker of COVID-19 infection or vaccination and provides an additional diagnostic tool for verifying results and helps monitor and control COVID-19 spread.

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1. Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) that emerged in late 2019 called coronavirus disease 2019 (COVID-19) [1]. This virus caused an acute respiratory disease that threatens human health and public safety [1]. The worldwide outbreak of COVID-19 prompted the World Health Organization to declare a pandemic on 11 March 2020 [2]. According to the Johns Hopkins Coronavirus Resource Center, SARS-CoV-2 during the COVID-19 pandemic infected>169 million people worldwide and caused>3.5 million deaths so far. It has shown that all ages of the population are susceptible to SARS-CoV-2 infection [3]. COVID-19 infected patients show a wide range of clinical symptoms varying from asymptomatic infection (no or mild symptoms like influenza clinical presentations) to more severe forms of the disease. Common early symptoms of COVID-19 disease are fever, chills, coughing, malaise, myalgia, and headache. Severe forms of the disease consist of pyrexia, cough, dyspnea, serious pneumonia, sometimes followed by respiratory and multiple organ failure, which could be associated with death [4–6]. During COVID-19 infection, the SARS-CoV-2 receptor-binding domain (RBD) binds to its receptor angiotensin-converting enzyme-2 (ACE-2) and facilitates human cells entry [7,8]. Although the nucleic acid detection of COVID-19 in infected patients is rapid and specific to define infection, it might underestimate the proportion of infected patients due to the virus shedding window and testing sensitivity [9,10].

Antibodies play an important role in neutralizing invading pathogens like viruses and bacteria and protecting the host against re-infection. Thus, the accurate assessment of antibodies during a pandemic can provide important evidence for monitoring pathogen exposure, understanding the role of antibodies in protective immunity, and helping vaccine development [11,12]. Therefore, we aimed to assess the presence and persistence of IgG against SARS-COV-2, the levels of IgG among recovered COVID-19 individuals three, four, and six months post-symptom onset (PSO), and its association with sex, age, clinical symptoms, and disease severity.

2. Materials and methods

2.1. Ethical considerations

Before the study and sample collection, the Ethics Committee of Kurdistan University of Medical Sciences (MUK), Sanandaj, Iran, approved this study protocol (IR.MUK.REC.1399.136), informed written consent was obtained from all participants, and a questionnaire was completed.

2.2. Study population

This prospective longitudinal cohort study was performed on 96 recovered COVID-19 subjects in three different groups with mild (n = 31), moderate (n = 33), and severe (n = 32) clinical symptoms. The COVID-19 case selection and classification based on clinical symptoms and disease severity were defined according to the Clinical management of COVID-19 guidance [4]. The inclusion criteria in all three groups are given in Table 1, and the exclusion criteria were non-participation in each stage and having contraindications to venous blood sampling. Exposure was COVID-19 disease measured by positive nasopharyngeal real-time reverse transcription PCR (real-time RT-PCR) test and other criteria listed in Table 1. The outcome in this study was the level of SARS-CoV-2-specific IgG antibody in three, four, and six months PSO. The questionnaire consisted of three parts: the first part: demographic information of people including age, sex, underlying disease; Part 2: Clinical symptoms at the time of illness, including clinical symptoms, real-time RT-PCR test results, a computerized tomography (CT) scan results, length of hospital stay; Part 3: Serological test results. The first two sections' information was completed at the time of referral and before sampling based on the hospital file and self-reported individuals. The information in the third section was also completed during IgG antibody testing.

Table 1
Inclusion and exclusion criteria of patients in three different groups.

Inclusion criteria	Groups		
	Mild (n = 30)	**Moderate (n = 31)**	**Severe (n = 30)**
	N (%)	N (%)	N (%)
	-Positive PCR test	- Positive PCR test	- Positive PCR test
	-Outpatient treatment at home	- The duration of hospitalization is less than one week	- The duration of hospitalization is more than one week
	-Having symptoms in favor of COVID-19 disease	- Positive CT-scan	- Positive CT-scan
	- Oxygen saturation percentage higher than 93%	- Oxygen saturation percentage between 90 and 93%	- Oxygen saturation percentage less than 90%
	- Being over 18 years old	-Shortness of breath or feeling of pain and pressure in the chest	-Severe shortness of breath (RR > 30)
	- Resident of the city where the study was conducted (Sanandaj, western Iran)	- Being over 18 years old	- Being over 18 years old
	-Positive IgG test at the beginning of the study	- Resident of the city where the study was conducted (Sanandaj, western Iran)	- Resident of the city where the study was conducted (Sanandaj, western Iran)
	- Willingness to participate in the study	-Positive IgG test at the beginning of the study	-Positive IgG test at the beginning of the study
		- Willingness to participate in the study	- Willingness to participate in the study

2.3. Sample collection, serum separation, and Enzyme-linked immunosorbent assays

From each participant, 3 mL of peripheral blood samples were drawn in a serum separator tube (SST). Serum samples were collected and stored at −20 °C until analysis. The serum level of IgG against the S1 domain of SARS-CoV-2 spike protein was assayed by the commercially available ELISA kit (EUROIMMUN Medizinische Labordiagnostika AG). Optical densities were gained using an automated ELISA reader processing system (Synergy HTX Plate Reader-BioTek Instruments, USA). All calibrator and positive and negative controls were assayed in triplicate, and values were calculated and measured according to the manufacturer's instructions.

2.4. Data analysis method

All analyses were performed with SPSS software v20.0 (IBM Corp., Armonk, NY, USA). Due to the non-parametric distribution of the data, the Kruskal-Wallis test was used to compare IgG levels in three groups. First, Mann-Whitney and Kruskal-Wallis tests were used to compare IgG levels by demographic variables and underlying disease records. Second, a Chi-square test was used to compare the qualitative results of the tests. Finally, mean ± standard deviation (Mean ± SD) was used to report IgG level by time and participating groups.

3. Results

3.1. Characteristics of the recovered COVID-19 subjects

Due to the negative IgG antibody titer in the first measurement or three months PSO, five subjects consist of two subjects in the severe group, two subjects in the moderate group, and one in the mild group were excluded from the study. Finally, 91 subjects participated in all three periods of IgG antibody measurement in three different groups: severe, moderate, and mild. In the third stage measurement, a 74-year-

Table 2
Characteristics of individuals with COVID-19 participants in the study.

Variables	N	Groups			P-value
		Severe (n = 30) N (%)	**Moderate (n = 31)** N (%)	**Mild (n = 30)** N (%)	
Sex					
Male	44 (48.4)	17 (56.7)	11 (35.5)	16 (53.3)	0.204*
Female	47 (51.6)	13 (43.3)	20 (64.5)	14 (46.7)	
Underlying diseases					
Yes	29 (31.9)	12 (40.0)	13 (41.9)	4 (13.3)	0.029*
No	62 (68.1)	18 (60.0)	18 (58.1)	26 (86.7)	
CT-scan result					
Symptomatic lung	62	30 (1 0 0)	26 (83.9)	6 (20.0)	
Asymptomatic lung	9	0	1 (3.2)	8 (26.8)	-**
Not done	20	0	4 (12.9)	16 (53.3)	
Contact History					
Yes	35 (38.5)	7 (23.3)	17 (54.8)	11 (36.6)	0.040*
NO/not know	56 (61.5)	23 (76.7)	14 (45.2)	19 (63.4)	
		Mean (SD)	**Mean (SD)**	**Mean (SD)**	
Age (year)		51.7 (10.7)	40.5 (10.9)	38.5 (12.3)	0.001#
IgG antibody level after 3 months		5.8 (1.5)	4.0 (1.8)	3.5 (2.1)	0.001#
IgG antibody level after 4 months		5.6 (1.6)	4.6 (2.6)	3.7 (2.1)	0.001#
IgG antibody level after 6 months✓		5.7 (1.4)	4.2 (2.2)	3.3 (2.0)	<0.001##
Length of hospitalization (day)		6.6 (2.2)	2.5 (1.3)	0	0.001##

*Based on the Chi-square test.
** The test assumption for Chi-square is not set.
† If the changes in the second IgG were<5% of the initial measurement of the IgG, it was considered unchanged.
#Based on the Kruskal-Wallis test performed. Then ran the Mann–Whitney *U* test as a post-hoc based on Bonferroni adjustment. Except for the comparison between mild and moderate, other comparisons were significant in the post hoc test ($P < 0.0166$)
##Based on Mann-Whitney *U* test comprise performed between the severe and mild group.
✓ The measurement of the third stage of IgG was not performed in one of the severe cases and one of the moderate cases due to death.

old female from the severe group and a 42-year-old male from the moderate group was absent due to death unrelated to COVID-19. Of all participants in three groups, 51.6% (n = 44) were female, and 31.9% (n = 29) had a history of underlying diseases. Mean ± SD age in the three severe, moderate, and mild groups were 51.7 ± 10.7, 40.5 ± 10.9, and 38.5 ± 12.3, respectively, which was statistically significant. The age of the severe group was higher than the other two groups ($p = 0.001$).

3.2. Association of antibody titer with clinical symptoms and disease severity

According to Table 2, the mean ± SD titer of IgG antibody in the first measurement (three months PSO) in the three severe, moderate, and mild groups was 5.8 ± 1.5 4.0 ± 1.8, and 3.5 ± 2.1, respectively. Thus, the level of IgG antibody in the severe group was higher than the other two groups ($p = 0.001$). Also, the mean ± SD titer of IgG antibody four months and six months after the infection was higher in the severe group than the other groups ($p < 0.05$).

According to Table 3, the mean ± SD changes of IgG antibody in the severe group three, four, and six months PSO was not statistically significant ($p = 0.279$). Also, mean ± SD changes of IgG antibody in the moderate group during three, four, and six months PSO increased but not statistically significant ($p = 0.854$). However, the mean ± SD changes of IgG antibody in the mild group during three, four, and six months PSO were reduced significantly ($p = 0.048$). Figs. 1 and 2 show the changes in IgG antibody levels of participants of the study. IgG antibody levels of 4 (4.4%) participants reached below the positive range of six months PSO. In each severe and mild group, only one patient recovered from COVID-19 had a history of COVID-19 symptoms again after six months. In moderate cases, none of the 31 improvements showed any suspicious symptoms.

3.3. Association of antibody titer with sex and age

In this study, the relationship between antibody titer and sex of the subjects was investigated as a whole. The results were also evaluated in three different groups. The results showed no significant relationship between COVID-19 specific antibody titer and the sex of the studied subjects (Table 4). Also, the relationship between the ages of the subjects with antibody titers was compared. The IgG antibody titer showed no relationship with the ages of the studied subjects (Table 4).

4. Discussion:

Several studies showed the rapid waning of antibody titer in recovered COVID-19 individuals [13–18]. They were even suggesting that COVID-19 infection could occur without seroconversion. Consistently, antibody titers were noted to wane both in patients with mild and severe infection [13–18]. This evidence raised the possibility that humoral immunity to this new coronavirus may be very short-lived.

Therefore, in this prospective longitudinal cohort study, we analyzed the level and dynamic changes of SARS-CoV-2-specific IgG antibody among 96 recovered COVID-19 subjects categorized into three different groups based on clinical symptoms and disease severity. In this study, we observed that most recovered COVID-19 subjects could raise SARS-CoV-2-specific IgG antibody PSO. Also, IgG levels against SARS-CoV-2 did not decrease four months after the infection, and it is persisting at least six months PSO in the serum. Besides, patients with severe COVID-19 disease are more likely to mount robust IgG antibody responses than those with mild and moderate cases. The IgG level and duration of antibody persistence were strongly correlated with the clinical symptoms and disease severity. Consistent with our results, other studies have shown that most recovered COVID-19 individuals could raise SARS-CoV-2-specific antibodies [19–22]. Approximately 90% of COVID-19 infected individuals elicit neutralizing antibodies to SARS-CoV-2 spike and RBD antigens PSO. These antibodies are detected in the blood of recovered COVID-19 individuals by 10–15 days PSO. Neutralizing antibodies have been shown to target the RBD that binds to ACE2 and access human cells [23–25]. Several other studies showed that IgG levels against SARS-CoV-2 did not decrease four months after the infection, and it is persisting after six months PSO [19–32]. People with mild to moderate infection mount a strong IgG response to COVID-19 antigens at the beginning of the infection, and this IgG titer is relatively stable over five months and then rises down. Also, they showed a positive relationship between antibody titer with the clinical course of the disease [32–34].

This prospective longitudinal cohort study showed that most recovered COVID-19 individuals mount humoral immune responses and could raise robust SARS-CoV-2-specific IgG antibody PSO. Also, IgG levels against SARS-CoV-2 did not decrease four months PSO and persisted at least six months. However, the IgG titer has begun to decline from the fourth to sixth months in some cases, especially in mild symptoms, and false-negative cases will be observed from six months PSO. Besides, our results indicate that severe cases are more likely to mount robust IgG

Table 3
Comparison of serological changes of COVID-19 infected individuals.

		IgG antibody level			P-value
		After 3 months Mean (SD)	**After 4 months** Mean (SD)	**After 6 months** Mean (SD)	
Group	Severe	5.7 (1.5)	5.7 (1.6)	5.9 (1.4)	0.279*
	Moderate	3.9 (1.8)	4.5 (2.6)	4.2 (2.2)	0.852*
	Mild	3.5 (2.1)	3.7 (2.1)	3.3 (2.0)	0.048*
Time between diagnosis and first serology test††	4 months	4.3 (2.1)	4.6 (2.3)	4.5 (2.2)	0.338*
	3 months	4.5 (2.1)	4.6 (2.3)	4.3 (2.2)	0.347*
Group	SevereVsModerate	5.7 (1.5)	5.7 (1.6)	5.9 (1.4)	0.020**
		3.9 (1.8)	4.5 (2.6)	4.2 (2.2)	
	P-value✓	<0.001	0.071	0.001	
	SevereVsMild	5.7 (1.5)	5.7 (1.6)	5.9 (1.4)	0.001**
		3.5 (2.1)	3.7 (2.1)	3.3 (2.0)	
	P-value✓	<0.001	<0.001	<0.001	
	ModerateVsMild	3.9 (1.8)	4.5 (2.6)	4.2 (2.2)	0.090**
		3.5 (2.1)	3.7 (2.1)	3.3 (2.0)	
	P-value✓	0.319	0.140	0.091	

*Based on the Friedman test performed, the Wilcoxon test was then run as a post-hoc based on Bonferroni adjustment. Except for the comparison between IgG level 2 and 3 in the Mild group ($p = 0.004$), other comparisons were not significant in the post hoc test ($P > 0.0166$).
**Based on Repeated measure ANOVA test contrast, with control of Age and Underlying disease variables.
†The measurement of the third stage of IgG was not performed in one of the severe cases and one of the moderate cases due to death.
††The measurement of the third stage of IgG was not performed in two cases of that's the time between diagnosis and first serology test was 4 months.
✓ unpaired T-test was performed and based on Bonferroni adjustment decided to significance at $P < 0.0166$.

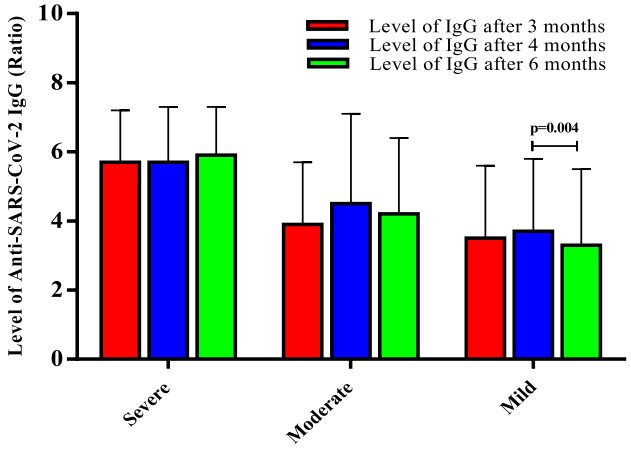

Fig. 1. Mean (standard deviation) changes of IgG antibody titer during six months in severe, moderate, and mild groups of recovered COVID-19 individuals. Most recovered COVID-19 subjects could raise specific IgG antibody levels against SARS-CoV-2 post symptom onset (PSO). Also, serum levels of IgG against SARS-CoV-2 persisting at least six months PSO. Besides, patients with severe COVID-19 disease are mount robust IgG antibody responses than those with mild and moderate cases. The IgG level and duration of antibody persistence were strongly correlated with the clinical symptoms and disease severity. Based on the Friedman test performed, the Wilcoxon test was then run as a post-hoc based on Bonferroni adjustment. Except for the comparison between IgG level four and six months PSO in the mild group ($p = 0.004$), other comparisons were not significant in the post hoc test ($P > 0.0166$).

antibody responses than those with mild and moderate cases. These results provide insight into the interaction between the virus and host immune systems, the presence and duration of SARS-CoV-2 antibodies, and its association with clinical symptoms. Thus, SARS-CoV-2-specific IgG antibody measurement was more suitable for epidemiologic studies, although false-negatives cases will be observed since the fourth month. Finally, it is important to note that the commercially available ELISA kit used in this study relies on detecting IgG antibodies against SARS-CoV-2 Spike protein. The Spike protein is present in all forms of COVID-19 available vaccines, such as mRNA, adenoviral, inactivated, and others [35,36]. Therefore, all vaccinated individuals without a previous or recent history of COVID-19 infection will be seropositive for anti-Spike IgG.

5. Limitations of our study

Our study has some limitations—first, lack of measurement of the initial IgM, IgA, and IgG titer in the first month PSO. Second, we did not detect antibodies by virus-neutralization tests; therefore, the neutralizing activities of these antibodies are unknown. Third, quantitative viral load monitoring was not available.

6. Conclusions and future perspective

These results provide insight into the presence and persistence of the SARS-CoV-2-specific IgG antibody. In addition, a positive correlation between IgG antibody titer and its duration with the clinical symptoms of disease indicates that cases with mild or moderate symptoms require

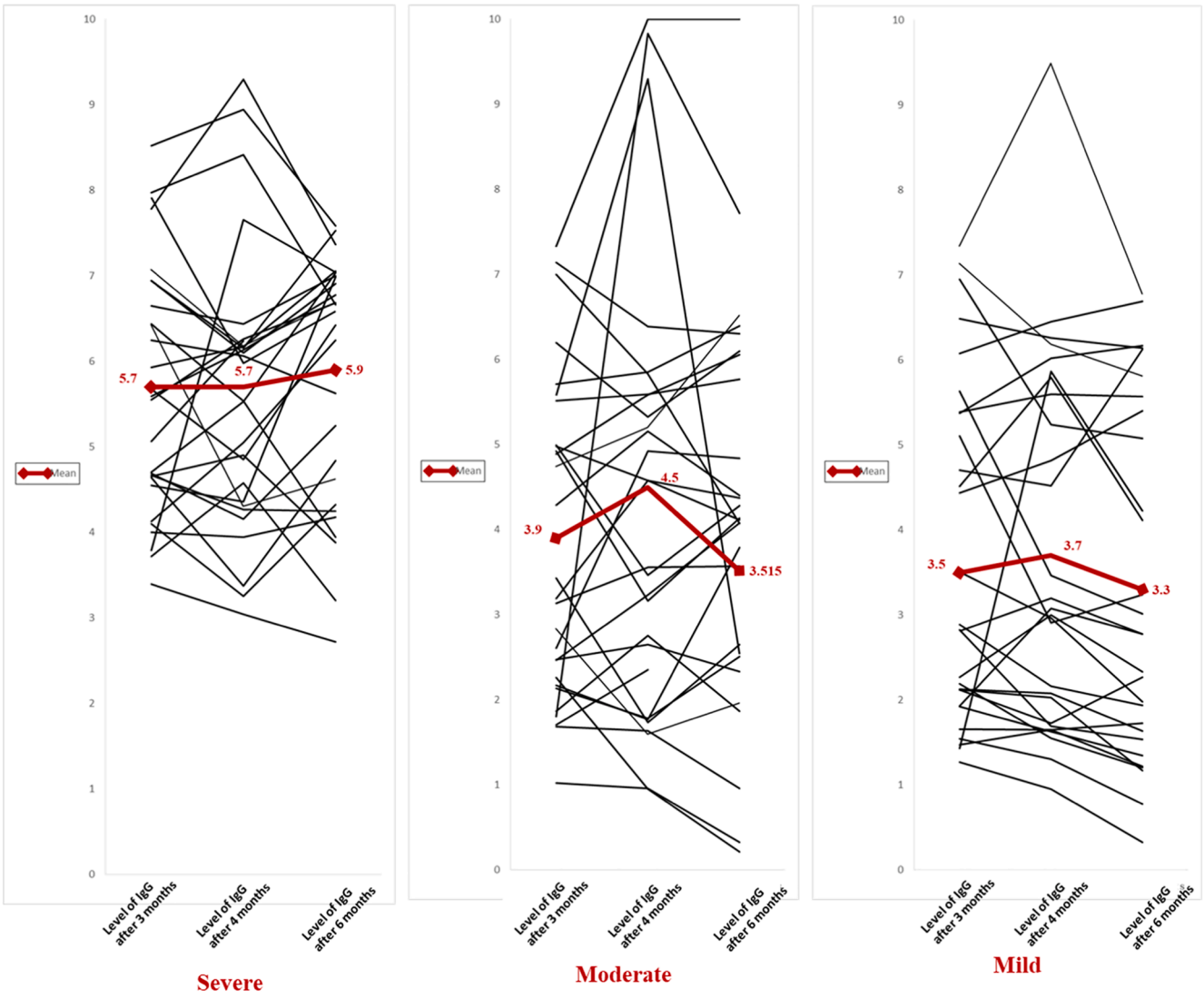

Fig. 2. Changes in IgG antibody levels of severe, moderate, and mild participants three, four, and six months post-symptom onset. The figure shows the trend of changes in IgG levels of each recovered COVID-19 individuals. It aims to show changes in each individual in the three groups of mild, moderate, and severe during three, four, and six months PSO, which changes in time.

Table 4
Comparison of serological changes and its association with sex and age in COVID-19 infected individuals.

P-value*	**IgG antibody level** **After 6 months**Mean (SD)	**After 4 months**Mean (SD)	**After 3 months**Mean (SD)		
0.115*	4.2 (2.4)	4.7 (2.6)	4.3 (2.3)	Male (43)	Sex (N)
0.901*	4.7 (2.0)	4.6 (2.1)	4.5 (1.8)	Female (46)	
	0.453	0.984	0.574	P-value#	
0.121	4.0 (2.5)	4.2 (3.0)	4.7 (2.4)	<=29 (9)	Age (N)
0.945	4.1 (2.4)	4.0 (2.1)	3.7 (2.0)	30–39 (31)	
0.191	3.9 (1.8)	4.4 (2.0)	4.0 (1.9)	40–49 (26)	
0.670	5.3 (1.9)	5.6 (2.6)	5.1 (2.0)	50–59 (10)	
0.584	5.8 (1.6)	6.1 (1.8)	5.9 (1.6)	>=60 (13)	

* Based on Friedman test performed.
#Based on U Mann-Whitney test performed.

more urgent vaccination. Although serological tests could not be used as the primary diagnostic test, they may support real-time RT-PCR results. Also, they could be used for diagnosing COVID-19 subjects tested later outside of the optimal period. Thus, the SARS-CoV-2-specific IgG antibody is an excellent marker of previous and recent infection, COVID-19 vaccination, and provides an additional diagnostic tool for verifying results and helps monitor and control COVID-19 spread. These results highlight the importance of serological testing to achieve more accurate estimates of the extent of the COVID-19 spread for future studies.

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