Archives of Razi Institute, Vol. 77, No. 3 (2022) 1191-1197

DOI: 10.22092/ARI.2022.357515.2054

Original Article

Evaluation of IgM and IgG in COVID-19 Recovered Patients in Iraq

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Received 17 January 2022; Accepted 1 April 2022
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Abstract

Severe acute respiratory syndrome coronavirus-2 is a major threat to health care worldwide with high morbidity and mortality. Therefore, understanding the role of immune mechanisms and humoral response is vital in this disease. The present study aimed to investigate the relationship between Immunoglobulins (IgM, IgG) in COVID-19 recovered patients with age, gender, and severity of the disease. The duration of effect of antibody levels and protection against re-infection has also been evaluated in the patients. Three groups participated in this study; group 1: 0-14 days after recovery, group 2: 2 months after recovery, group 3: 3 months after recovery, group 4: 4-6 months after recovery, group 5: more than 6 months. The nasopharyngeal swab was used to confirm recovery by Real-Time Polymerase Chain Reaction (RT-PCR) technique. IgM and IgG antibody levels were evaluated using Enzyme-Linked Immuno Fluorescent Assay (ELIFA) technique. The results indicated that the IgM levels increased for one month during the seven days after infection and then decreased in most patients ($P \leq 0.05$). The mean of IgG in group 1 increased compared to those of other studied groups. A significant decrease was observed in group 2 compared to group 1, as well as in group 3 compared to groups 1, and 2. Also, a significant difference existed between group 4 compared to groups 1, 2, and 3. Finally, significant differences were noticed between group 5 compared to groups 1, 2, 3, and 4 ($P \leq 0.05$). No significant differences were observed in antibodies level between male, and female COVID-19 recovered patients in groups 1, 2, 3, 4, and 5 ($P \leq 0.05$). Finally, highly significant differences in IgG levels between mild, moderate, and severe subgroups in groups 1 and 2. The present study demonstrated that IgM and IgG against SARS-CoV-2 appeared in the early stages of the disease and decreased after 1 month and failed to maintain high levels during the 6-month observation.

Keywords: COVID-19, IgM, IgG, Recovered Patients, Severity

1. Introduction

In recent years, a new pandemic of Severe Acute Respiratory Syndrome coronavirus-2 (SARS-CoV-2) has spread around the world caused by a new β-coronavirus under the family Coronaviridae which is an enveloped, non-segmented, positive-sense RNA virus. SARS-CoV1 and MERS-CoV have been identified in the past. The diameter of the virus is about (65–125 nm), with crown-like spikes on the outer surface (1). The spikes are divided into four main proteins including spike (S) glycoprotein, envelope (E) glycoprotein, membrane (M) glycoprotein, and nucleocapsid (N) protein, and also several auxiliary proteins. The S glycoprotein facilitates the binding of enveloped viruses to host cells by specific receptor angiotensin-converting enzyme 2 (ACE2) expressed in lower respiratory tract cells (2). SARS-CoV-2 is mainly transmitted through respiratory droplets of infected people (2).

The symptoms of COVID -19 infection include mild to severe pneumonia, fever, dry cough, fatigue,

difficulty breathing, chest pain, kidney failure, or death (3). The disease progresses to severe pneumonia with focal and systemic hyper-inflammation caused by a cytokine storm in some cases (4, 5).

Some environmental factors such as climate, culture, pollution, social and health care organizations as well as comorbidities cardiovascular diseases such as hypertension, cancer, diabetes, immunodeficiency, and genetic differences affect SARS-CoV-2 infection (6-10).

The SARS-CoV-2 Spike Antigen (S. Ag) stimulates the immune system to produce specific IgM and IgG antibodies in the bloodstream of patients (11). In general, IgM develops in the early and acute stages of the disease and then decreases; however, the IgG titer increases in the lateral phase and remains in the serum for months (12). The IgG and IgM levels are associated with the antibody kinetics (decreased IgM and appearance of IgG) that develop within a few days after infection (13, 14). Additionally, the positivity of the serological test might not be noticeable in all patients, and the response of antibodies to other human coronaviruses decreases over time (15). The WHO divided patients with COVID-19 into different groups based on the severity of the disease; mild case: individuals who have one or more symptoms (fever, cough, sore throat, nausea, headache, muscle pain, vomiting, diarrhea, and loss of taste and smell) without dyspnea, moderate case: patients with low oxygen saturation (SpO_2)≥94% on room air at sea level, and severe case: patients with SpO_2 <94%, pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) <300 mm Hg, a respiratory rate >30 breaths/min and/or lung infiltration >50%. In the present study, the level of IgM and IgG and the stability of antibodies in COVID-19 recovered patients were evaluated at different time intervals.

2. Materials and Methods

2.1. Study Design and Participants

A total of 150 COVID-19 recovered patients, in addition to 70 healthy controls between 15-81 years were selected from both genders. Swap and blood samples were collected from patients in the Department of Educational Laboratories, Clinical Immunology Section in Medical City, AL-Ataa hospital, Al-Resafa, Baghdad, Iraq from January to September 2021. The recovery of patients was confirmed by examining an internist and using EURORealTime SARS-CoV-2 (EUROIMMUN, Germany). Recovered patients were evaluated based on recovery time in different groups including; group 1: 0-14 days after recovery, group 2: 2 months after recovery, group 3: 3 months after recovery, group 4: 4-6 months after recovery, and group 5: more than 6 months.

2.2. Detection of COVID-19 Specific IgG and IgM

A total of five blood samples were taken from each patient according to the time after recovery and serum levels of IgM, IgG, and COVID-19 specific antibodies were determined using the ELIFA technique by MiniVidaas device from Biomerieux Company. Also, the IgM and IgG kits were manufactured by Euroimmune Company, Germany. The cutoff value for IgM and IgG positivity was ≥ 1.0 AU/mL, while negative < 1.0 AU/mL.

2.3. Statistical Analysis

The results were analyzed using SPSS software (version 20). The significant differences in mean ± SD were assessed by the Independent Samples t-Test. Also, the correlation between different parameters such as the concentration of IgM and IgG between gender, age, and severity of diseases were estimated. A P-value ≤ 0.05 is considered statistically significant.

3. Results

Demographic characteristics of 150 COVID-19 recovered patients including 86 men (57.3 %) and 64 women (42.7%) were examined. The age range of the participants was between 15 to 81 years. They were divided into three groups according to the severity of the disease. They were also divided into five groups according to the time after recovery.

The present study investigated the association between COVID-19 and age in recovered patients. These results clarified statistically significant differences P=0.001 (P> 0.05) between studied groups according to age as presented in table 1.

Also, table 1 presents the distribution of COVID-19 according to insignificant differences *P*=0.07 (*P*>0.05) between COVID-19 recovered patients with gender.

A significantly increased *P*=0.007 (*P*≤ 0.05) in IgM level was observed between COVID-19 recovered patients and healthy controls. Also, a significant increase *P*=0.0001 (*P*≤0.05) was observed in IgG between COVID-19 recovered patients and the control group (Table 2).

Table 3 indicates the periodic changes in IgM levels. The mean of IgM increased in group 1 (2.008±0.246), in contrast, IgM in other studied groups were 0.696±0.07, 0.319±0.025, 0.242±0.034, 0.140±0.009, respectively. IgM significantly decreased *P*=0.002 in group 2 (0.696±0.075) compared to group 1 (2.008±0.246). Also, a significant decrease *P*=0.001, 0.001 was noticed between group 3 (0.319±0.025) compared to group 2 (0.696±0.075), and group 1 (2.008±0.246). A significant difference *P*=0.002, *P*=0.002 existed between group 4 compared to groups 2 and 3. Finally, significant differences were observed between group 4 compared to groups 1, 2, 3, and 4 (*P*=0.001,0.0001, 0.0001, 0.002).

The mean of IgG decreased in group 1 (51.07±2.1), while, IgG in other studied groups was 27.08±1.334, 14.47±0.769, 8.05±0.486, and 3.17±0.314, respectively. A significant decrease *P*=0.0001 was also observed in group 2 compared to group 1, as well as *P*=0.0001, *P*=0.0001 in group 3 compared to groups 1, and 2. Also, a significant difference *P*=0.0001 was noticed in group 4 compared to groups 1, 2, and 3. Finally, significant differences *P*=0.0001 were reported between group 5 compared to groups 1, 2, 3, and 4 as represented in table 3.

No significant differences *P*=0.091, 0.953, 0.471, 0.149, 0.519 and 0.0.347 were observed in IgM level between male and female COVID-19 recovered patients in group 1, 2, 3, 4, 5 and control group (Table 4)

Table 1. Characteristics of COVID-19 recovered patients and controls

Participants Characteristics		COVID-19	Control	*P*-value
		No.	%	
Age (year)	<20 years	9	6.0	0.001**
	20---29	32	21.3	
	30---39	48	32.0	
	40---49	25	16.7	
	50---59	12	8.0	
	60---69	17	11.3	
	=>70 years	7	4.7	
	Mean±SE (Range)	39.3±1.314 (7-81)	37.6±1.553 (16-72)	0.455
Gender	Male	86	57.3	0.07
	Female	64	42.7	
Severity	Mild	45	30.0 - -	0.37
	Moderate	58	38.7 - -	
	Severe	47	31.3 - -	

*Significant difference between proportions using Pearson's chi-squared test at the level of 0.05
#Significant difference between two independent means using Student's t-test at the level of 0.05
NO. Number; S.E.: Standard Error; *P*-value: Probability value

Table 2. Immunoglobulin levels in COVID-19 recovered patients and controls

Igs level	COVID-19 (n=150)	Control (n=70)	*P*-value
	Mean±SE (Range)	Mean±SE (Range)	
IgM (AU/mL)	2.008±0.604 (0.01-13.0)	0.10±0.021 (0.01-0.90)	0.007#
IgG (AU/mL)	51.07±2.10 (10.72-179.40)	0.16±0.028 (0.01-0.90)	0.0001#

#Significant difference between two independent means using Students t-test at the level of 0.05
NO. Number; S.E.: Standard Error; *P*-value: Probability value

Table 3. The concentration of IgM and IgG (Au/mL) in COVID-19 recovered patients

Studied Groups	COVID-19	*P*-value compared with			
	Mean±SE (Range)	0-14d	2m	3m	4-6m
After 0-14 Days (n=150)					
IgM (AU/mL)	2.008±0.246 (0.01-13.0)				
IgG (AU/mL)	51.07±2.10(10.72-179.40)				
After 2 months (n=146)					
IgM (AU/mL)	0.696±0.075 (0.01-5.17)	0.002#			
IgG (AU/mL)	27.08±1.334 (2.23-79.0)	0.0001#			
After 3 months (n=142)					
IgM (AU/mL)	0.319±0.025 (0.02-0.90)	0.001#	0.001#		
IgG (AU/mL)	14.47±0.769 (0.22-72.80)	0.0001#	0.0001#		
After 4-6 months (n=135)					
IgM (AU/mL)	0.242±0.034 (0.02-2.94)	0.001#	0.002#	0.857	
IgG (AU/mL)	8.05±0.486 (0.01-72.80)	0.0001#	0.0001#	0.0001#	
Over 6 months (n=129)					
IgM (AU/mL)	0.140±0.009 (0.01-0.82)	0.001#	0.0001#	0.0001#	0.002#
IgG (AU/mL)	3.17±0.314 (0.09-17.53)	0.0001#	0.0001#	0.0001#	0.0001#

#Significant difference between two dependent means using Paired t-test at the level of 0.05
N. Number; S.E.: Standard Error; *P*-value: Probability value; Au/mL: Arbitrary unit per milliliter

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Table 4. Serum immunoglobulin levels and gender in COVID-19 recovered patients

Studied Groups	COVID-19				*P*-value
	Male		Female		
	No	Mean±SD	No	Mean±SD	
After 0-14 Days (n=150)					
IgM (AU/mL)	86	3.40±9.41	64	1.33±2.75	*P*=0.091
IgG (AU/mL)	86	54.67±26.74	64	46.24±23.65	*P*=0.047#
After 1 month (n=146)					
IgM (AU/mL)	85	0.58±0.94	61	0.57±0.91	*P*=0.953
IgG (AU/mL)	85	28.37±17.24	61	25.28±14.38	*P*=0.255
After 2 months (n=142)					
IgM (AU/mL)	82	0.32±0.35	60	0.28±0.33	*P*=0.471
IgG (AU/mL)	82	13.63±10.57	60	11.76±7.24	*P*=0.241
After 4-6 months (n=135)					
IgM (AU/mL)	77	0.24±0.29	58	0.37±0.73	*P*=0.149
IgG (AU/mL)	77	6.88±6.06	58	9.60±13.42	*P*=116
After 6 months (n=129)					
IgM (AU/mL)	75	0.14±0.12	54	0.16±0.10	*P*=0.519
IgG (AU/mL)	75	2.63±3.47	54	2.48±2.85	*P*=0.799
Control (n=70)					
IgM (AU/mL)	34	0.12±0.23	36	0.08±0.12	*P*=0.347
IgG (AU/mL)	34	0.24±0.28	36	0.08±0.17	*P*=0.004#

N. Number; S.E.: Standard Error; *P*-value: Probability value; Au/mL: Arbitrary unit per milliliter.

A significant increase of *P*=0.047, and *P*=0.004 (*P*≤ 0.05) was observed in IgG levels between men and women in group 1 and the control group. Also, no significant differences existed between men and women in groups 2, 3, 4, and 5 (*P*=0.255, 0.241, 0.116, and 0.799).

No significant relationship *P*=0.423 at *P*>0.05 was reported in IgM levels with severity in all studied groups. Whereas, significant differences *P*=0.0001, and *P*=0.0001 (*P*≤ 0.05) were observed in IgG levels between mild, moderate, and severe subgroups in groups 1 and 2, respectively. While no significant differences (*P*>0.05) were noted in IgG levels of other studied groups as shown in table 5.

Table 5. Serum immunoglobulin levels and severity of disease in COVID-19 recovered patients

Studied group	Mild		Moderate		Severe		*P*-value
	No	Mean±SE	No	Mean±SE	No	Mean±SE	
After 0-14 Days (n=150)							
IgM (AU/mL)	45	1.57±0.469	58	2.44±0.439	47	3.60±1.873	0.423
IgG (AU/mL)		3 30.63±1.55		42.39±1.704		81.36±2.88	0.0001^
After 1 Month (n=146)							
IgM (AU/mL)	43	0.60±1.16	58	0.74±0.145	45	0.743±0.137	0.123
IgG (AU/mL)		16.98±1.258		22.55±1.218		42.55±2.619	0.0001^
After 2 Months (n=142)							
IgM (AU/mL)	45	0.26±0.049	57	0.35±0.049	44	0.336 ±0.045	0.441
IgG (AU/mL)		1 12.37±1.71		12.69±1.059		18.805±1.396	0.854
After 4-6 Months (n=135)							
IgM (AU/mL)	38	0.198±0.064	54	0.23±0.058	43	0.30±0.076	0.364
IgG (AU/mL)		1 6.033±0.920		7.44±0.718		11.70±1.545	0.200
Over 6 months (n=129)							
IgM (AU/mL)	35	0.128±0.018	51	0.13±0.011	43	0.16±0.021	0.207
IgG (AU/mL)		2.23±0.488		2.54±0.448		6.323±0.529	0.679

^Significant difference among three independent means using ANOVA-test at the level of 0.05
N. Number; S.E.: Standard Error; *P*-value: Probability value; Au/mL: Arbitrary unit per milliliter

4. Discussion

Due to the emerging diseases caused by SARS-CoV-2, studies on the immunogenicity of this disease and the persistence of antibodies produced against it seem necessary.

In the present cohort study, the recovery of 150 patients with COVID-19 was confirmed by negative PCR. The results of this study were consistent with those of a previous study which reported COVID-19 affects all ages (16). Furthermore, the present study confirmed that no significant difference was observed between men and women which was similar to different studies that recorded approximately the same proportion (17, 18).

The effect of antibodies against SARS-CoV-2 has been studied. IgM was detected in blood within 14 days after recovery which was consistent with other studies that reported from 5 days to 1 month (19, 20). IgG was detected within 14 days and increased to maximum level during one month of the onset of symptoms then gradually decreased to the lowest level after 6 months of recovery which was supported by other studies (19, 21-26). Therefore, IgG protects against COVID-19 disease for a period (25). Furthermore, the level of IgG correlated with severity meaning that in severe cases it was relatively high compared to mild ones (19, 27, 28). The IgG provides good protection due to forming a memory B and T cells; however, it is temporary

protection as it fails to provide good protection against the new strain of the virus.

Our results revealed that the persistence of COVID-19 specific IgG and titers depended on the severity of COVID-19. Severe COVID-19 is caused by uncontrolled virus replication which leads to excessive inflammation and overproduction of antibodies (29). COVID-19 viral load also played a key role in the severity of COVID-19 and patients with severe symptoms had higher viral loads than mild or moderate cases (29). Also, humoral immune response to COVID-19 may be associated with cytokine storms including interleukin-1 (IL-1), IL-6, and interferon-γ (30, 31). Time-dependent changes in IgG levels should be focused on to further investigate the immunity created against this disease.

Authors' Contribution

Study concept and design: S. M. D.
Acquisition of data: I. K. A. J.
Analysis and interpretation of data: S. M. D.
Drafting of the manuscript: I. K. A. J.
Critical revision of the manuscript for important intellectual content: S. M. D.
Statistical analysis: I. K. A. J.
Administrative, technical, and material support: S. M. D.

Ethics

This study was approved by the Ethics Committee of the Department of Biology, College of Science, Mustansiriyah University, and Unit of Clinical Immunology, Baghdad Medical City, Iraq. Also, all participants provided informed consent.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgment

The authors would like to express their sincere gratitude to the Ministry of Higher Education and Scientific Research, Mustansiriyah University, and the Head of Educational Laboratories, Unit of Clinical Immunology, Baghdad Medical City, Iraq as well as all participants for their contribution to conducting the study.

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