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Detection of IgM and IgG antibodies in patients with COVID-19 disease

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Abstract---The present study was planned to estimate some parameters linked covid 19 in Iraqi patients and these parameters includes serum levels of immunoglobulins (IgM and IgG) in the serum of the patients and comparing it with the healthy control group. This study aimed to determine the IgM and IgG responses against severe acute respiratory syndrome coronavirus (SARS-CoV)-2 in coronavirus disease 2019 (COVID-19) patients with varying illness severities. The study was conducted between 66 adult patients with COVID-19 and compared with 22 age and gender-matched healthy subjects as controls. All patients were diagnosed and confirmed to be positive for COVID-19 by Real Time-Polymerase Chain Reaction (RT-PCR). , the blood samples have been collected from patients in Al-Zahraa teaching hospital and martyr fairus hospital and some private laboratories, with diagnosis of COVID-19 , from October 2021 - January 2022. The age range in both groups was 14–72 years. The patients were classified into mild group (22 patients),moderates group (22 patient), and severe group (22 patients). Serum samples were collected from all participants and tested for the immunoglobulines levels by ELISA (enzyme-linked immunosorbent assay) method. Further data include age, sex, occupation, residency, number of COVID19 infection, smoking history and any clinical illness obtained by a study form plate along with patient concepts and ethical approval •The IgM levels were higher in patients in the severe group as compare with other group and the IgG were no significant difference among the mild,moderate,and severe group. we observed that the IgM antibody response to SARS-CoV-2 occurred earlier and peaked earlier than the IgG antibody response; the IgM antibody response began to decline at week 3 of the illness, while the IgG antibody response persisted and was maintained in patients with COVID-19.

Keywords---COVID-19, immunoglobulin G, immunoglobulin M, SARS-CoV-2.

Introduction

The novel coronavirus, severe acute respiratory syndrome coronavirus (SARS-CoV)-2, has been identified as the causative pathogen of coronavirus disease 2019 (COVID-19).(1,2,3,4) This disease has been called a public health emergency of international concern by the World Health Organization (WHO). Since December 2019, a serious outbreak of the disease has spread via human-to-human transmission from China to more than 200 countries and territories worldwide.(5,6) The numbers of infected cases and deaths associated with COVID-19 are still increasing daily. As of 27 August 2022, SARS-CoV-2 has caused 605 098 736 confirmed cases and 6 485 974 deaths worldwide according to the WHO.(6) The diagnosis of COVID-19 is dependent mainly on clinical characteristics, CT imaging and a few laboratory tests. Although some symptoms and laboratory parameters have indicative values in confirmed patients, they are not unique to SARSCoV-2 infection. Before the publication of the seventh edition of the 'Guideline of diagnosis and treatment for COVID-19' by the Chinese National Health Commission, laboratory diagnosis of confirmed patients was carried out by detecting viral RNA in throat swab or nasal swab specimens using real-time reverse transcription polymerase chain reaction (RT-PCR) assays.(7)

This method does not require live virus to be present in the specimens, but the turnaround times of the current real-time RT-PCR assays are long, and these assays need to be performed in certified laboratories. A high percentage of false-negative results were reported because of the quality of sample collection and multiple preparation steps, limiting the role of this assay for outbreak containment.(8,9,10,11) Therefore, accurate, convenient and rapid methods are acutely needed for the diagnosis of COVID-19. SARS-CoV-2 shares similar clinical genetic and epidemiological features with SARS and Middle East respiratory syndrome (MERS).(12,13) Thus, the process of generating antibodies against SARSCoV-2 might be similar, and the detection of both IgM and IgG antibodies could provide information on the time course of virus infection.(10,14) Following a SARS infection, IgM is detectable after 3–6 days, and IgG is detectable after 8 days.(15) Most recently, serological tests for virusspecific IgM and IgG antibodies against SARS-CoV2 have been developed, and similar serological responses were observed in one COVID-19 patient.(11,16) Rapid and specific antibody detection could offer information for confirmation or exclusion of SARS-CoV-2 infection in suspected patients and has been recommended by the newest 'Guideline of diagnosis and treatment for COVID-19' issued by the Chinese National Health Commission.(17) Most COVID-19 patients have a mild illness and recover quickly after appropriate clinical intervention. Some COVID-19 patients develop severe SARS, multiple organ failure and even death over a short period of time.(5,18,19,20) Previous studies have reported that massive inflammatory responses induce the over activity of T cells, and leads to severe immune injury during SARS-CoV-2 infection.(5,18,21) However, the humoural immune response to COVID-19 is still largely unknown.

Materials and Methods

Samples collection

This study was carried out in alzahraa hospital and Fairouz Hospital in the city of Wasit province. This study included the collection of 88 blood samples, which were obtained from various areas in Wasit province during the period from the October 2021 - January 2022, The blood samples were collected from (covid-19) patients who attended alzahraa and Fairouz Hospital and some private laboratories. The present work has been accepted via the local ethics committee at scientific research by ethical approval of health, higher education and scientific research ministry in Iraq, all patients' take part in the study were already informed about the aim of the study, agreed, and signed consent. Before blood sampling, personal information for each patient was obtained, including name, age, gender, smoking, family history, disease duration and the type of therapy regime. all blood samples were placed in a cool-Box under aseptic condition, Serum was isolated by centrifugation at 3000 rpm for 10 min and the serum was divided into aliquots in eppendorf tubes and this tube was stored in the freezer at (-80°C), Serum levels,(IgM) and(IgG) in the samples were measured by Enzyme Linked Immunosorbent Assay (ELISA) using The Kits From(CUSABIO, CHINA) at wavelength of 450 nm .

Study Groups

The total number of participants in the study was eighty eight people between men and women, study groups included the following:

- **Group 1:** sixty six people with covid-19 They were diagnosed by (R T-PCR), their samples were collected from Fairouz Hospital / and al Zahraa Teaching Hospital /wasit and some private laboratories . Their clinical information were obtained from their files.
- **Group 2:** twenty two people apparently healthy people were classified as control group.

Statistical analysis

Data were entered, coded, and analyzed in SPSS (statistical package for social sciences) software program version 26. Data analysis were done using different tests. Frequency and percentages were used for the description of categorical variables. The mean and standard deviation were used to describe the continuous variables. Both Chi-square and Fisher's exact test were used for the assessment of the association between categorical variables. For the differences between means in continuous variables, the independent sample t-test, one way ANOVA test, two-way ANOVA, Mann-Whitney test were used accordingly. Spearman correlation coefficient was used to assess the presence of correlation in non-normally distributed variables. A P-value equal to or less than 0.05 was considered significant. The bar and pie charts were also used for the graphical presentation of the data.

Results and Discussion

A total of 88 people were recruited for this study to determine the IgM and IgG responses against severe acute respiratory syndrome coronavirus. Of those participants, there were 22 persons with negative (RT-PCR) test (mean no COVID-19). Other 66 patients with positive PCR were divided into 3 equal groups (22 patients) who were classified as severe, moderate, and mild cases. The mean and standard deviation (SD) of the 88 persons who participated in the study were 49.55±17.11 years old (minimum age =14, and the maximum =72 years old).Other sociodemographic features presented by the participants were presented in (table 1). More than half of them (53.4%) were females and the majority (83%) were living in urban places. The prominent blood group among the sample was A+ (33%) followed by O+ (29.5%). Among the selected 66 (COVID-19) patients, about 42 (63.6%) were on steroid treatment while only 18 (27.3%) were on antiviral treatment (Remdesivir). There are 5 (7.6%) of patients who suffered from secondary bacterial infection. Only 4 (6.1%) of those COVID-19 patients ended with death (table 2). There was no significant difference between the patients and control groups concerning age and sex distribution. socio-demographic features are presented in (Table 1) and Frequency distribution of (COVID-19) disease history of the patients are presented in (Table 2).

Table 1
Frequency distribution of the socio-demographic features of 88 participants

Variables	Categories	Frequency	Percent
Gender	Female	47	53.4%
	Male	41	46.6%
Place of living	Rural	15	17.0%
	Urban	73	83.0%
Smoking status	Non-smoker	70	79.5%
	Smoker	18	20.5%
Job-status	Governmental	23	26.1%
	Self-employer	23	26.1%
	Housewife	37	42.0%
	Retired	4	4.5%
	Student	1	1.1%
COVID-19 vaccination	Non-vaccinated	76	86.4%
	Vaccinated	12	13.6%
Blood group	AB+	10	11.4%
	AB-	2	2.3%
	A+	29	33.0%
	A-	1	1.1%
	B+	18	20.5%
	B-	2	2.3%
	O+	26	29.5%

Table 2
Frequency distribution of COVID-19 disease history among 66 infected patients

Variables	Categories	Frequency	Percent
COVID-19 disease severity	Severe Moderate Mild	22 22 22	33.3% 33.3% 33.3%
Steroid drugs	No steroid Steroid	24 42	36.4% 63.6%
Antiviral drugs	No-remdesivir Remdesivir	48 18	72.7% 27.3%
Secondary bacterial growth	No bacterial growth Bacterial growth	61 5	92.4% 7.6%
Disease outcome	Cure Death	62 4	93.9% 6.1%

The results of the laboratory investigation made for the whole sample including IgM antibody, and IgG antibody values were described in (table 3) according to their health status. The mean IgG titer was found to be 0.724 U/ml for the (non-COVID-19) sample but it reaches 3.646 U/ml in those with severe (COVID-19). Regarding the mean IgM titer, it appears to be 0.825 U/ml and 5.123 U/ml in the same previous order.

Table 3
Descriptive statistics of IgG, and IgM antibodies among all health status categories of the study sample (88)

Disease status	IgG (U/ml)		IgM (U/ml)	
	Mean (Standard deviation)	(Min.-Max.)	Mean (Standard deviation)	(Min.-Max.)
Severe COVID-19	3.646(1.068)	1.588-5.501	5.123(1.194)	3.261-6.640
Moderate COVID-19	3.244(1.147)	0.680-4.607	3.921(1.589)	1.457-6.406
Mild COVID-19	2.985(1.190)	1.325-4.990	3.079(0.972)	1.021-4.457
No COVID-19	0.724(0.191)	0.413-1.109	0.825(0.085)	0.686-0.972
Total sample	2.650(1.500)	0.413-5.501	3.237(1.918)	0.686-6.640

There were significant differences in the means of the two selected variables between samples with (COVID-19), and those without the infection. The calculated *P*-value was less than 0.001 for the three investigated variables as shown in (table 4).

Table 4
Mean differences of IgG, and IgM between COVID-19 control(non-COVID-19) participants

Variables	COVID-19	Not COVID-19	*P*-value (Mann-Whitney Test)
IgG	3.292 (1.1520)	0.724 (0.191)	<0.001
IgM	4.041 (1.515)	0.825 (0.085)	<0.001

(Table 5) shows that there is no significant difference (*P*-value=0.159) between the COVID-19 classes regarding the IgG value. The means were 3.646, 3.244, and 2.985 for patients with severe, moderate, and mild (COVID-19), respectively this results agreed with(22).who observed The positive rates of IgG detection were not significantly different among the mild, severe and critical groups. Also, study agreed with study by(23).

Table 5
Mean differences and descriptive statistics of IgG among (66) COVID-19 patients

COVID-19	Mean	Standard Deviation	95% Confidence Interval for Mean		*P*-value (One way ANOVA test)
			Lower Bound	Upper Bound	
Severe	3.646	1.068	3.172	4.120	0.159
Moderate	3.244	1.147	2.736	3.753	
Mild	2.985	1.190	2.457	3.513	
Total	3.29	1.152	3.008	3.575	

There was a significant difference between (COVID-19), classes concerning IgM value (<0.001) as shown in (table 6). , quantitative analyses of antibody levels over the disease course revealed that SARS-CoV-2-specific IgM levels were higher in patients in the severe group, as compared with the other groups, which might be because of high disease activity and/or a compromised immune response in these patients.The present study agrees with a study done by(24).who show the severe cases of (COVID-19) tended to have a more vigorous response in IgM antibodies to (COVID-19) illness. also The increased IgM level in the deceased case group might be related to the higher disease severity in these patients and indicate a poor prognosis. In summary, we observed that the IgM antibody response to (SARS-CoV-2), occurred earlier and peaked earlier than the IgG antibody response; the IgM antibody response began to decline at week 3 of the illness, while the IgG antibody response persisted and was maintained in patients with (COVID-19).

Table 6
Mean differences and descriptive statistics of IgM among (66) COVID-19 patients

COVID-19	Mean	Standard Deviation	95% Confidence Interval for Mean		*P*-value (One way ANOVA test)
			Lower Bound	Upper Bound	
Severe	5.123	1.194	4.594	5.652	<0.001
Moderate	3.921	1.589	3.216	4.626	

Mild	3.079	0.972	2.648	3.510	
Total	4.041	1.515	3.669	4.414	

Table 7 There are no significant differences between patients who died and those who recovered from (COVID-19), in IgG levels p-value 0.110 also in IgM levels p-value 0.471

Table 7
Differences in mean IgG, and IgM according to disease outcome

Variables	Disease outcome	N	Mean	Std. Deviation	P- value (independent sample t-test)
IgG	Recovery	18	3.818	1.069	0.110
	Death	4	2.870	0.725	
IgM	Recovery	18	5.034	1.166	0.471
	Death	4	5.525	1.416	

Conclusions

Patients in the severe group had greater IgM levels than those in other groups, whereas there was no discernible difference in IgG levels across the mild, moderate, and severe groups. we observed that the IgM antibody response to SARS-CoV-2 occurred earlier and peaked earlier than the IgG antibody response; the IgM antibody response began to decline at week 3 of the illness, while the IgG antibody response persisted and was maintained in patients with COVID-19.

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