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Antibody formation as an immune response in patients infected with SARS Covid-19

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ABSTRACT

This study recorded a total of 90 samples divided into three groups, 30 confirmed patients with Coronavirus (COVID-19) for the first group. They were admitted to Al-Hussein Teaching Hospital in Dhi Qar Governorate, affiliated with the Iraqi Ministry of Health, for the period between October 27 and November 26, 2021. 30 patients had passed the acute infection period and their blood samples were collected during routine clinical trials. Tests. All recorded cases of SARS-CoV-2 infection were confirmed using standard RT-qPCR testing on throat swab samples from the respiratory tract. For all enrolled patients, date of onset of disease, clinical classification of severity, RNA test results during the hospitalization period, and Personal demographic information was obtained from clinical records The highly purified receptor binding domain (RBD) of SARS CoV-2 protein was expressed to detect the presence of IgM, IgG, and C-reactive protein, respectively Using a fluorescence-based POCT immunoassay The current study showed that there was a .analyzer (Ichroma™ II) significant increase in the level of IgG antibodies in the group of patients who had passed the acute stage of the Coronavirus (COVID-19), which recorded a significant increase in the levels of IgM antibodies in the serum of patients who were still suffering from severe symptoms of Covid-19 infection. 19 The current study also showed a significant increase in the level of C-reactive protein in the group of patients who were still in the acute stage of infection compared to patients who had passed the stage of acute symptoms of infection. Laboratory tests were conducted to find out the effect of infection with the Coronavirus on some criteria and to know the correlation between some criteria in patients, by evaluating the diagnostic ability and adopting immunological tests as a biological marker for COVID-19 patients

Introduction

Devastating illnesses produced by the human coronaviruses MERS-CoV, SARS-CoV, and SARS-CoV-2 include Middle East Respiratory Syndrome (MERS), Severe Acute Respiratory Syndrome (SARS), and Coronavirus Infectious Disease 2019 (COVID-19) (1).The latter is in charge of an outbreak that began in Wuhan City, China, in December 2019. The World Health Organization (WHO) first classified it as a Public Health Emergency of International Concern on January 30, 2020 (2), then on March 11, 2020, it was classified as a pandemic (3). As of March 26, 2020, the WHO had received more than 2 million confirmed cases of COVID-19(4). The prefix "nido-" means "nest" and coronaviruses are grouped as a family under the Nidovirales order because they replicate using a nested set of mRNAs. The coronavirus subfamily also includes the genera of alpha, beta, gamma, and delta coronaviruses. Beta coronaviruses (HCoV-HKU1, HCoV-OC43, Middle East respiratory syndrome coronavirus [MERS-CoV], severe acute respiratory syndrome coronavirus [SARS-CoV]), SARS-CoV-2, and alpha coronaviruses (HCoV-229E and HCoV-NL63) are the two genera into which the human coronaviruses (HCoVs) fall (5,6). The name "coronavirus" comes from the medium-sized, enclosed, positive-stranded RNA viruses' distinctive crown-like appearance in electron micrographs (7, 8). The "common cold" is a mild upper respiratory infection brought on by an endemic HCoV with an incubation period of 2–5 days. Upper respiratory tract infections are frequently brought on by endemic HCoVs. Bronchitis and pneumonia are uncommon illnesses of the lower respiratory tract. Patients infected with SARS-CoV frequently exhibit symptoms of fever, headache, and myalgias after an incubation period of typically four to five days.Cough and dyspnea are two respiratory symptoms that typically appear a few days to a week following the start of an infection. In 20–30% of instances, atypical pneumonia and respiratory impairment take place. With the exception of a higher percentage of patients progressing to respiratory deterioration and distress, the incubation time and clinical course of MERS are comparable to those of SARS The SARS-CoV-2 infection's incubation period and clinical trajectory are presumably comparable to those of SARS. A mean incubation duration of 5.2 days was first reported by Li et al. [9]. Early on in the course of an illness, fever and cough are usually recorded [10,11]. Dyspnea, respiratory distress, and a positive chest X-ray are other signs of infections [12]. About a week after the onset of the initial symptoms, lower respiratory problems frequently appear [10]. The transmission of infection is mainly person to person through respiratory droplets. Faecal–oral route is possible. The presence of the virus has been confirmed in sputum, pharyngeal swabs and faeces [13]. The median incubation period of COVID-19 is 5.2 days; most patients will develop symptoms in 11.5 to 15.5 days. Therefore, it has been recommended to quarantine those exposed to infection for 14 days[14].The SARS-CoV-2 infection enters the host cells through the S spike protein by binding to ACE2. The high infectivity of the virus is related to mutations in the receptor binding domain and acquisition of a furan cleavage site in the S spike protein. The virus interaction with ACE2 may down regulate the anti-inflammatory function and heighten angiotensin II effects in predisposed patients [15] Age and sex have been shown to affect the severity of complications of COVID-19. The rates of hospitalization and death are less than 0.1% in children but increase to 10% or more in older patients. Men are more likely to develop severe complications compared to women as a consequence of SARS-CoV-2infection [16]. Diagnostic tests of Individuals, who are suspected of SARS-CoV-2 infection, mainly rely on real-time reverse transcription polymerase chain reaction (rRT-PCR) of viral genetic material collected in nasopharyngeal. In contrast to the PCR test, serologic assays demonstrate the presence of an immune reaction against the virus through the detection of immunoglobulins directed against SARS-CoV-2 structural proteins. The immune system of a CoViD-19 patient produces antibodies to SARS-CoV-2 within days to a few weeks following viral infection(17) detection of SARS-CoV-2 RNAs is a standard approach COVID-19 diagnosis. However, there is an urgent need for reliable and rapid serodiagnostic methods to screen SARS-CoV-2-infected people including those without obvious symptoms. Most emerging studies have described serological tests based on detection of SARS-CoV-2-specific IgM and IgG. Although detection of SARS-CoV-2-specific IgA in serum has been reported in a few papers, analyzes of IgA levels in a larger number of COVID-19 patients are still lacking19(Ma *et al.*, 2020) .This study examined the immune tests IgG, IgM, C-reactive protein, and myoglobin for people during the infection period and after the infection period, and compared them to healthy people.

2- Materials and methods

2-1.CoViD-19 patient samples

Serum samples (n = 90) whose ages ranged between 18 and 80 years. were obtained from patients at Al-Hussein Teaching Hospital in Nasiriyah City. CoViD-19 patients were confirmed by RT-qPCR and CT imaging at the Molecular Biology Unit at Al-Hussein Teaching Hospital in Nasiriyah City according to the approval of Thi-Qar Health Department/Training Center. and Human Development/Research Committee in their letter No. 66/2021 dated 10/25/2021 collected from the end of October 2021 to November 2021. In total, 30 out of 90 serum samples from cases collected within 10 days after the first symptoms appeared (range 4–9 days), and there were 30 of these collected 10 or more days after the onset of first symptoms (range 10–28 days). Control samples not associated with SARS-CoV-2 (n = 30) obtained from the same hospital were included**.**

2-2-Serological tests for IgG, IgM, C-reactive protein by Ichroma II TEST

Serum tests for IgG, IgM, and C-reactive protein were performed according to the protocol for the IChroma™ II test

2-2-1Detection of IgG,IgM & C-reactive protein against SARS-CoV-2

Serum tests for IgG,IgM & CRP were performed according to the protocol for the IChroma™ II test by making a hole in the upper part of the buffer, for detection by inserting the empty sample collection tool. Adding 10 microliters of Covid-19 patient serum from the sample using the sample collection tool. Assembling the sample collector and detection buffer into one device. The vial was shaken more than 10 times until the sample was removed from the sample collector by inversion. The buffer and sample mixture was used within 30 seconds. The cap was removed from the top of the collecting tube. Two drops of reagent were blotted onto a paper towel before being applied to the cartridge. Only two drops of the mixture were then loaded onto the sample cartridge. The cartridge was left at room temperature for 3 Minutes before inserting the device into the holder. Immediately scan the cartridge loaded with the sample at the end of the incubation period. For the purpose of testing accuracy, by inserting the cartridge loaded with the sample into the cartridge holder of the device to perform ichroma™ tests. To ensure the correct orientation of the cartridge before pushing it fully into the cartridge holder. A special cartridge is marked with an arrow for this purpose. Press the “Select” button or press the “Start” button on the ichroma™ tests device to start the scanning process. Starts the ichroma™ tester to scan the sample-loaded cartridge. The test result is read on the ichroma™ tester display.

2-2-2 Measurement the level of anti-SARS-CoV-2 IgM, IgG antibodies

The current study showed that there is a significant increase in the level of IgG antibodies in the group of patients who have passed the acute stage of COVID-19 in comparison with the group of patients who are still in the acute stage of infection with figuer (2-1). While this study also showed a COVID-19 and the control group significant increase in the levels of IgM antibodies in the serum of patients who still suffer from acute symptoms of infection with Covid 19 compared to the control group On the other hand, its and the group of people who crossed the acute infection stage level decreased in the group of those in the post-acute phase of infection when compared with the previous two groups figure (2-2).

2-2-3Measurement the level of C-reactive protein

The current study showed that there was a significant increase in the level of C-reactive protein in the group of patients who are still in the acute stage of infection compared with patients who exceeded the stage of acute symptoms of infection, while the lowest level was observed in the control group Figure (2-3).

2-2-4Measurement the level of Myoglobin

It was found through the results of this study that there were no significant differences in the level of myoglobin in both groups of patients and control, as it was the highest level in the group of acute infection patients and less than in the control group, then the group after acute symptoms. High levels of myoglobin in the blood in our study could be a significant and effective indicator of poor outcomes in Covid-19 patients. For the purpose of validating the clinical value of myoglobin, we recommend strengthening these results in other groups of Covid-19 patients for myoglobin as a biomarker for a worse prognosis in Covid-19.

Correlation between myoglobin and other parameter

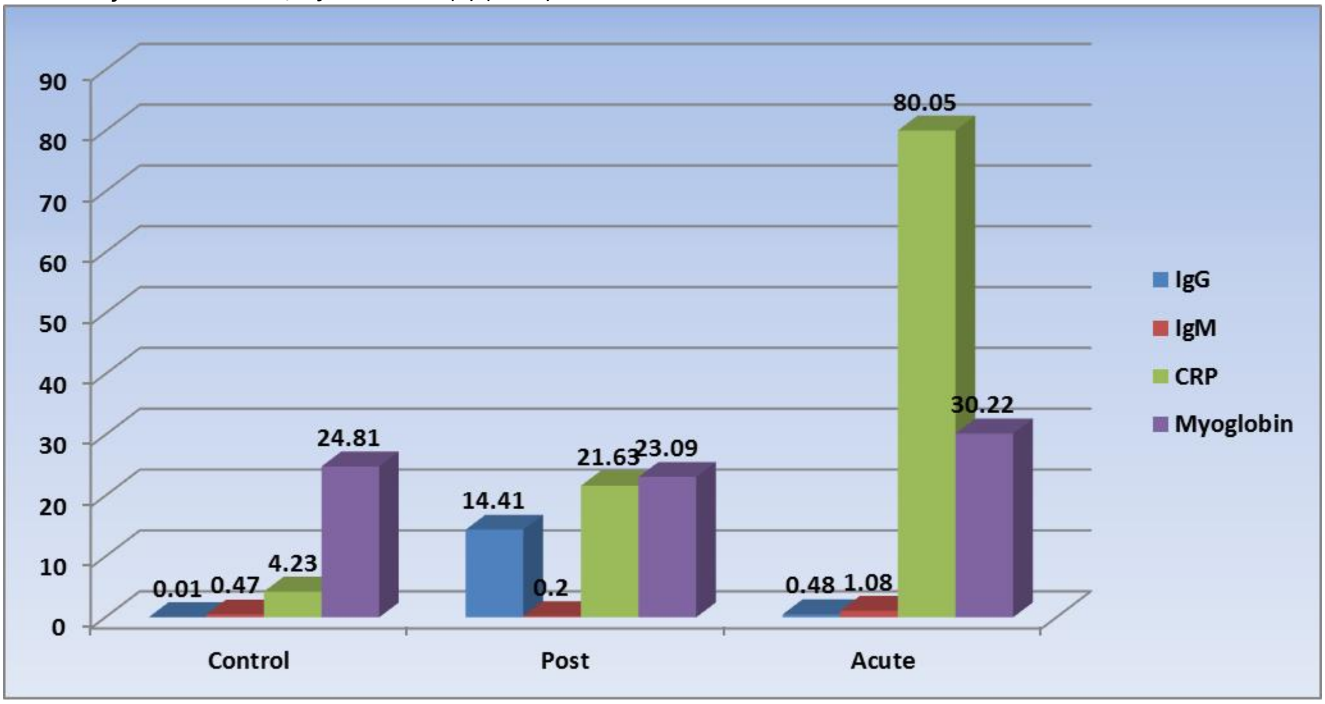
Through studying the correlation coefficient between myoglobin and the rest of the parameters in this study, it was found that there is a weak direct relationship between myoglobin and C-reactive protein. It was also shown that myoglobin is weakly inversely related to IgG. It is weakly proportional to IgM, as it was (R<0.25, 0.25<R>0.75, 0.75<R>1), respectively, as in Table (2-2)

3.Results

The current study showed that there is a significant increase in the level of IgG antibodies in the group of patients who have passed the acute stage of Coronavirus (COVID-19) compared to the group of patients who are still in the acute stage of Coronavirus infection. Coronavirus (COVID-19). 19 and the control group Figure (3-1). While this study also showed a significant increase in the levels of IgM antibodies in the serum of patients who were still suffering from severe symptoms of Covid-19 infection compared to the control group and the group of people who had crossed the acute stage of infection Figure (3-1). The current study also showed a significant increase in the level of C-reactive protein in the group of patients who were still in the acute stage of infection compared to patients who had passed the stage of acute symptoms of infection, while its lowest level was recorded in the control group Figure (3-1). It was also noted in the results of this study that there were no statistically significant differences in the level of myoglobin in both groups of patients and control, as the level was highest in the group of patients with acute infection and lower than in the group of patients with acute infection. The control group, then the group after acute symptoms

TUBEL(2-1)The correlation between Myoglobin and other parameter

		N	Mean	Std. Error	L.S.D
IgG	**Control**	**30**	**0.01^{b}**	**0.001**	**3.31**
	post	**30**	**14.41^{a}**	**1.75**	
	patient	**30**	**0.48^{b}**	**0.12**	
IgM	**Control**	**30**	**0.47^{b}**	**0.01**	**0.07**
	post	**30**	**0.20^{c}**	**0.03**	
	patient	**30**	**1.08^{a}**	**0.06**	
CRP	**Control**	**30**	**4.23^{c}**	**0.24**	**7.57**
	post	**30**	**21.63^{b}**	**3.11**	
	patient	**30**	**80.05^{a}**	**17.39**	
Myoglobin	**Control**	**30**	**24.81^{a}**	**1.41**	**10.29**
	post	**30**	**23.09^{a}**	**5.04**	
	patient	**30**	**30.22^{a}**	**10.62**	


90
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Control
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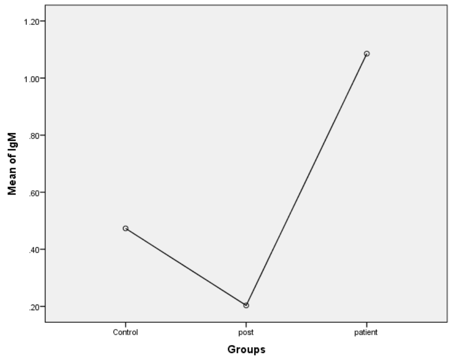
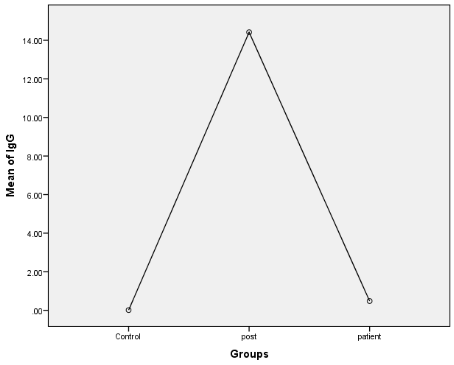
Acute
IgG
IgM
CRP
Myoglobin


Mean of IgM
Groups
Control
post
patient


Mean of IgG
Groups
Control
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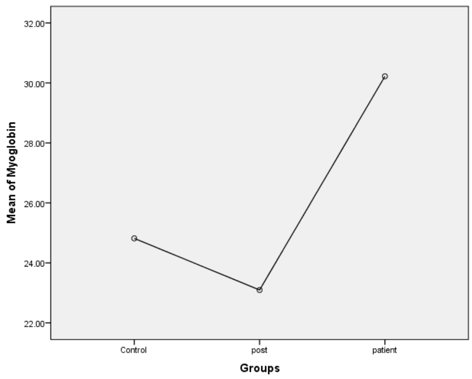
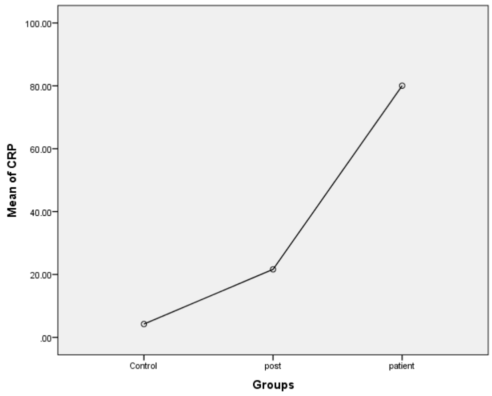

Mean of Myoglobin
Groups
Control
post
patient


Mean of CRP
Groups
Control
post
patient

Correlation value	correlation type
R=0	No correlation
0.00< R <0.25	weak extreme
0.25 < R < 0.75	moderate extreme
0.75 < R < 1	strong extreme
R=1	perfect
Negative value	inversely

Level of relationship between myoglobin and other parameters

		CRP	IgG	IgM
Myoglobin	**Pearson Correlation**	**0.01**	**- 0.05**	**0.13**
	Sig. (2-tailed)	**0.89**	**0.67**	**0.27**

TUBEL (2-2) Level of relationship between myoglobin and other parameters:-

Discussion

Human serum mainly consists of proteins (albumin: 70% by weight; IgG: 14%, IgA: 3%, IgM: 2%; carrier: 6%; fibrinogen: 2%), lipids (phospholipids). ; glycerol), and cholesterol of the(Goulart *et al.*, 2021). Acommon observation in COVID-19 is that levels of IgM and IgG antibodies vary over time after infection. This is to be expected because antibodies are produced late after infection, and early IgM is later replaced by IgG antibodies and levels of antibodies in the blood generally decline. It decreases over time after the infection resolves and readily and antibody(Shah *et al.*, 2021). A positive serological result and negative PCR may represent previous infection with SARS-CoV-2 after clearance of the virus The.(Beavis *et al.*, 2020) Mean IgG antibody levels are approximately similar between females and males at different follow-up times. Patients over 50 years of age had a significantly higher level of IgG antibodies compared to those under 50 years of age at all follow-up time points. Participants with comorbidities had higher mean IgG antibodies higher.(Assaid *et al.*, 2023). The average IgG level is higher in patients with pulmonary embolism compared to patients without it Patients produce a better quality repertoire of polyclonal antibodies to RBD over time. During the progression of the disease, a smaller decrease in the rate of antibody formation is observed, which indicates an improvement in the avidity or quality of the antibodies. Therefore, the rate of deviation can be calculated and plotted as a function of the days of symptom onset the(Schasfoort *et al.*, 2021) . The liver produces the inflammatory protein CPR in acute cases and may be elevated in many conditions, such as inflammation, cardiovascular disease, and infection. In their meta-analysis of 13 studies, elevated CRP was associated with severe COVID-19 (Soiza, Donaldson and Myint, 2018). Sadeghi et al conducted a study to evaluate the relationship between CRP and COVID-19 infection, and the results indicated that a patient with a CRP level > 64.75 mg/L was more likely to develop the severe form of the disease. In other words, ROC analysis confirmed that CRP is a valuable indicator of coronavirus progression and severity of(Sadeghi-Haddad-Zavareh *et al.*, 2021). Also, the study conducted by Chen et al, which suggests that CRP can be used to predict the severity of pneumonia caused by the Coronavirus (COVID-19). To our knowledge, this is the first study to evaluate the prognostic ability of CRP in estimating the severity of pneumonia caused by COVID-19 of SARS-CoV-2(Chen *et al.*, 2020). and based on the above and the results of our current study, which aims to evaluate the immunoglobulin level. . IgG, IgM, C-reactive protein, and myoglobin in the serum of patients infected with the Coronavirus in the acute infection stage and the symptom relief stage, and comparing their rates with those who recovered and those who were not infected, it was shown, through the results of the current study, that they match previous studies and research conducted on COVID-19 patients, which are related to immune tests. For serum and measuring the level of C-reactive protein, it is therefore possible to adopt serological tests as a diagnostic marker for those infected with Covid-19.

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