Journal of Wasit for Science and Medicine 2024: 17, (3), 39-50

Study of Human Immunoglobulin G(IgG) and Immunoglobulin M(IgM) in COVID-19 Patients with Chronic Diseases and Non-Chronic Diseases

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Abstract

Coronavirus disease 2019 (COVID-19), an infectious respiratory disease caused by the severe acute respiratory syndrome-coronavirus 2 (SARS-CoV2) in Wuhan, Hubei Province, China in December 2019. The current article study levels of IgG and IgM for the body against COVID-19 in patients with and without chronic diseases. Also, shed light on the influence of chronic diseases in immune response by using ELISA. The results showed that no significant importance for the level of IgG in COVID-19 patients with chronic diseases compared with patients without chronic diseases. While there was a significant increase in IgM levels in COVID-19 patients of chronic diseases compared with control of chronic diseases. In addition, according to age groups, results show that IgM levels can be much higher in patients with chronic diseases compared to patients without chronic diseases. IgM levels may also vary depending on age, it's gave significant importance in age range older than 55 years. However, no significant difference in level IgG in patients of chronic diseases compared with patients without chronic diseases. On the other hand, the level of IgM in COVID-19 patients with chronic diseases significant higher compared with control.

Keywords: IgG, IgM, ELISA, Chronic diseases.

1. Introduction

Coronavirus disease 19 (COVID-19), originated at Wuhan city, Hubei Province of China in early December 2019 has rapidly widespread with confirmed cases in almost every country across the world and has become a new global public health crisis. The etiological agent was designated as

Severe acute respiratory distress syndrome coronavirus 2 (SARS-CoV-2). The virus was originated in bats and transmits through respiratory droplets and surface contact. The World Health Organization coined the term COVID-19 and declared this novel coronavirus disease as a pandemic on 11th of March 2020. The virus is highly contagious, and the incubation period ranges between 2-14 days. The virus infects the human respiratory epithelial cells by binding through Angiotensin-Converting Enzyme 2 (ACE2) receptors [1].

SARS-CoV-2 has been classified as a novel member of the b-coronavirus genus by the whole genome sequencing. It belongs to the subgenus sarbecovirus of the Coronaviridae family. SARSCoV-2 has RNA genome of about 30 kb [2] that encodes 16 non-structural proteins and four structural proteins, including spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins [3]. The S protein of CoVs constitutes the spike on the virion surface, it gives the virion crown-like appearance and plays a vital role in host range determination. Recognition of host receptors, viral binding, fusion, entry, and tissue tropism, as well as the induction of neutralizing antibody and T cell responses. The M protein is responsible for the specific shape of the viral envelope. It forms ribonucleoproteins and mediates inflammatory reactions in host cells. The E protein is a membrane polypeptide that acts as an ion channel (viroporin), it promotes viral pathogenicity while N protein helps viral entry and viral survival in host cells [4].

SARS-CoV-2 infection induces exuberant inflammatory responses by secretion of many of cytokines such as IgG and IgM. Immunoglobulin G (IgG) is one of the most abundant proteins in human serum, accounting for about 10-20 % of plasma protein. It is the major class of the five classes of immunoglobulins in humans [5]. IgG has an approximate molecular weight of 146 Kd and a serum concentration of 9.0 mg/mL. IgG is synthesized mostly in the secondary immune response to pathogens. IgG can activate the classical pathway of the complement system, and it also is highly protective [6]. IgG immunoglobulins are monomeric antibodies in the serum and crucial in maintaining long-term immunity or immunological memory after infection [7] in general, IgG is detectable after 8 days from infection [8].

Immunoglobulin M(IgM), pentamer antibody has a molecular weight of 970 Kd and an average serum concentration of 1.5 mg/ml. It is mainly produced in the primary immune response to infectious agents or antigens [9]. Due to the polyvalent nature of IgMs, they may exhibit higher avidity for

antigen than the bivalent IgG. In addition to neutralizing pathogens, IgM antibodies are highly effective at engaging complement to target lysis of cells and pathogens [10]. IgM is detectable after three to six days from infection [8]. In COVID-19, the first line of immune defence is represented by IgM antibody which can be detected after onset of symptom and increase to its peak level within two to three weeks then begin to decrease while the IgG antibody continues to increment and keep its level beyond seven weeks [11].

2. Material and Methods

2.1 Study Design

This study included Sixty sample of patients of COVID-19, 30 sample with chronic diseases 16 males and 14 females with age range between 28 to 89 years old. 30 samples without chronic diseases 16 males and 14 females with age range between 17 to 80 years old. All samples of patients were collected from Al-Hayat Center in Al-Zahraa Teaching Hospital, and from external laboratories after confirming their infection using nose swab PCR test, in Wasit Province, Iraq. While, samples of control groups involved 20 samples, 10 control samples with chronic diseases 4 males and 6 females with age range between 30 to 60 years old. Ten control samples without chronic diseases 2 males and 8 females with age range between 16 to 51 years old. Control samples were collected from people that are not infected with covid-19 by used covid-19 rapid test to support the result.

2.2 Samples of collection

Venous blood sample of 5 mL was drawn using a sterile syringe, which can be disposed of after use. Then, 5 mL of blood in a gel tube for the purpose of the immunological study (ELISA) enzyme-linked immune-sorbent assay and left the sample for 15 minutes at room temperature from 20 to 25 °C. Then, samples in a centrifuge approximately 2500-3000 cycles per minute and for 5 minutes to obtain the serum. The serum was distributed into two Eppendorf tubes, after which the tubes were placed in the freezer at a freezing degree of -20 °C.

2.3 Serological Test (ELISA)

This test was used to evaluate the presence of Human Immunoglobulin G(IgG) and Immunoglobulin M(IgM) using enzyme-linked immunosorbent assay technique (ELISA). ELISA kits (Human IgG) for (Monobind Inc.) and (Human IgM) for (ACON Biotech (Hangzhou)) according to the instructions of the manufacturer.

2.4 Statistical Analysis

Data were analysed using the following software, Microsoft excel, IBM SPSSV26. The results reported in this study were expressed as mean ± SD.

3. Results and Discussion

3.1 Comparative Analysis of IgG Levels in patients with and without chronic diseases

IgG levels in covid-19 patients with and without chronic diseases are demonstrates in table 1. The mean of IgG in patients with chronic disease was (52.420) mg/dL, and in patients without chronic disease was (66.571) mg/dL, with non-significant difference (P=0.636).

Table 1: Analysis of IgG Levels measured in mg/dL by ELIZA method in COVID-19 patients with and without chronic diseases.

IgG	Mean	±SD	SE	P-value
Patients with chronic disease	52.420	96.057	17.538	0.636
Patients without chronic disease	66.571	117.753	37.237	

Previous results showed no significant importance for the level of IgG in COVID-19 patients with chronic diseases compared with patients without chronic diseases. IgG (Immunoglobulin G) is a type of antibody that plays a crucial role in the immune system's ability to fight infections, including viral infections like COVID-19. The IgG level in COVID-19 patients, whether they have chronic diseases or not, can be an important factor in understanding the body's immune response to the virus [12]. In patients with chronic diseases, the immune response, including the production of IgG, may be altered compared to those without chronic diseases.

Chronic diseases can affect the immune system in various ways, potentially leading to a weakened or overactive response [13]. IgG plays a more prominent role after the first 2-3 weeks following acute infection, and establishing long-term immune memory, that can persist for several months or years [14]. Importantly, the timing of IgG and IgM antibody occurrence in patients varies greatly, and this variation in timing may be associated with age as well as comorbidity [15]. Hou and co-workers [16] showed that IgG in COVID-19 patients with chronic diseases was generated after one week.

The peak level was reached in three weeks and was maintained at a high level for an extended period even over 48 days. Meanwhile, IgM was generated in COVID-19 patients in one week after symptom onset. Then peak level was reached in two to three weeks after that the level was decreased. The detectable levels of IgG and IgM antibodies could provide information regarding serological convention over the

disease course. As the detection of IgM antibody indicates recent exposure to SARS-CoV-2 and the detection of IgG antibody in the absence of detectable IgM antibody indicates prior virus exposure. Also, the current study revealed that the IgG level in critical cases was lower than those in both mild and severe cases (critical vs. mild, P = 0.0397; critical vs. severe, P = 0.026). This might be because of the high disease activity and/or a compromised immune response in critical cases. In contrast, in the mild group patients, IgG was maintained at a high level. Besides, male aged over 55 years old multiple pre-existing comorbidities and obesity appear commonly associated with increased disease severity and/or mortality [17].

On the other hand, in study by Rizqoh and co-workers [18], indicated that the comorbid factors of type 2 diabetes mellitus in COVID-19 patients do not affect the level of IgM and IgG antibodies. Furthermore, the difference between IgM and IgG antibody levels in COVID-19 patients with comorbid type 2 DM and patients without comorbid type 2 DM is insignificant. However, this result is different from previous studies, it was found that serum levels of IgG and IgM in patients with diabetes mellitus were lower than in those without diabetes mellitus. Due to the condition of hyperglycemia which is characteristic of diabetic patients. Type 2 diabetes mellitus occurs due to insulin resistance in human cells. Consequently, the mechanism of type 2 DM impact in decreasing IgM and IgG levels is due to hyperglycemia inhibiting the production of IgM and IgG in splenocytes [19].

Ma et al., [20] found that serum IgG levels in moderate and severe COVID-19 patients without chronic diseases was significantly higher than in mild cases, while no significant difference was observed between severe and moderate patients. Liu et al., [21] showed that severe cases of COVID-19 tended to have a more vigorous IgG response against SARS-CoV-2 compared with mild cases. Notably, some patients with mild disease had a robust IgG antibody response from nine days after symptom onset, while a few mild cases did not generate adequate IgG antibodies.

3.2 Comparative Analysis of IgM Levels in patients with chronic diseases and in control group

The current research aim is to measure the serum level of Immunoglobulin M (IgM) in COVID-19 patients, and how it may differ between those with and without chronic diseases. IgM is one of the five classes of immunoglobulins found in humans. It is typically the first antibody produced in response to an antigen and can be detected

relatively early in the course of an infection. Table (2) shows that the IgM level in COVID-19 patients with chronic diseases was 51.244 mg/dL. While IgM level in control of chronic diseases was (3.297) mg/dL.

Table 2: Analysis of IgM Levels measured in mg/dL by ELIZA method in COVID-19 patients with chronic diseases and in control group.

IgM	Mean	±SD	SE	P-value
Patients of chronic disease	51.244	18.361	15.402	0.004
Control of chronic disease	3.297	1.670	0.528	

Table two shows a significant increase in IgM levels in COVID-19 patients of chronic diseases compared with control of chronic diseases. IgM antibody is the first line of immune defence which can be detectable within three days after viral infection. The IgM antibody can be detected after onset of symptom and increase to its peak level within two to three weeks then begin to decrease [22]. The exact IgM levels in COVID-19 patients with chronic diseases can vary widely depending on the specific disease, its severity, and other individual factors. There is ongoing research to determine if there's a significant difference in IgM levels between COVID-19 patients with and without chronic diseases [23]. Elawamy et al., [24] found that there is a significant rise of IgM concentration in asymptomatic cases of COVID-19 when compared to controls ($p = 0.0001$). Also, IgM concentration is rise in symptomatic cases of COVID-19 when compared to controls but not significant ($p = 0.1079$).

In addition, Assaid et al.,[25] indicated that patients with comorbidities had lower median IgM levels than those without comorbidities at two weeks after symptom onset ($P = 0.384$). Whereas the median antibody level was nearly similar in both patient groups at the other follow-up time points ($P > 0.05$). It should be noted that factors such as gender, age, and comorbidities may have an impact on the antiviral humoral response and its persistence over time.

Hou and partners [16], reported that IgM levels in COVID-19 patients with chronic diseases in the severe and critical groups were higher than those in the mild group (severe vs. mild, $P = 0.0084$; critical vs. mild, $P = 0.031$). Because of high disease activity and/or a compromised immune response in these patients. This is related to the higher disease severity in these patients and indicates a poor prognosis. Alternately, cytokine storm, severe immune dysfunction and other comorbidities might be the important risk factors in these cases [26].

3.3 Analysis of IgM Levels in COVID-19 patients with and without chronic diseases according to age group

The current study demonstrated IgM level in COVID-19 patients with and without chronic diseases. The means of IgM level in patients with chronic disease were (19.222) mg/dL, (12.792) mg/dL and (78.828) mg/dL with significant difference (P=0.0239). In age ranges (15-34), (35-54) and older than 55 years respectively. While, in patients without chronic disease, the means of IgM level were (5.239) mg/dL, (3.658) mg/dL and (5.601) mg/dL with non-significant difference (P=0.812) as shown in table 3.

Table 3: Analysis of IgM Levels in COVID-19 patients with and without chronic diseases according to age group.

Para meter	Patients of Chronic disease			Patients of Non-Chronic disease		
IgM	Mean	±SD	P-value	Mean	±SD	P-value
15-34 Years	19.222	12.571	0.0239	5.239	8.044	0.812
35-54 Years	12.792	8.660		3.658	1.352	
more 55 Years	78.828	8.654		5.601	6.509	

According to age groups, results show that IgM levels can be much higher (78.828) mg/dL in patients with chronic diseases compared to patients without chronic diseases, and that IgM levels may also vary depending on age. it gave significant importance (p= 0.0239) in age range older than 55 years old. Bai et al., [27] showed that severe/critical cases of COVID-19 patients with chronic diseases and lymphocytopenia who older than 65 years had a higher SARS-CoV-2-specific IgM level. The patients with lymphocytopenia had a higher level of SARS-CoV-2-specific IgM.

Lymphocytopenia is common in COVID-19 patients and might be a critical factor associated with lung injury, disease severity, and mortality [28]. In COVID-19 patients, the counts of peripheral CD4 and CD8 T lymphocytes are substantially reduced, and their immuno-status is hyperactivated, indicating severe immune injury in these patients [29].

On the other hand, Ghasemi [30] found the effect of age on the mean IgM levels at different time intervals. The patients below 60 years old group had a much lower IgM than over 60 groups at T0, which was statistically significant. However, after the third and six months the younger than 60 years old groups showed slightly higher IgM than those with age older 60. older individuals over 60 years old had higher levels of IgM levels at the beginning of infection and lower levels six months after infection. One of the elements influencing the humoral immune response

is antigen persistence in the patients' bodies [31]. The median length of viral persistence is generally greater in older individuals and patients with severe symptoms. Because of this phenomenon, people exposed to SARS-CoV-2 antigens for prolonged periods, maintain higher antibody levels. As previously shown, the initial IgM in over 65 years old participants is higher than others, but the decay rate is quicker [32]. Moreover, Mitani et al., [33] revealed a significant decrease of the IgM with age ($P<0.0001$), this decreases due to several factors such as gender, age, smoking, and comorbidities.

In addition, Parker et al.,[34] indicated that IgM responses to the S1, Spike, and NP proteins all demonstrated higher levels in patients aged between 41 and 60 in comparison to the below 40 or over 70 age groupings. Nevertheless, these finding that older males are more prone to severe disease and death, suggests that delayed antibody production is associated with severe disease and death in older patients over 60 but not in younger individuals below 40 years old. A potential explanation for this disparity is that in younger individuals, more robust innate immune responses help to limit virus replication during early infection, reducing the overall viral burden and subsequently delaying the production of Antibody responses. Conversely, advanced age is associated with blunted innate immune responses, which in combination with delayed antibody production likely accounts for the higher risk of severe disease [35].

4. Conclusion

Level of IgG in COVID-19 patients has a significant importance for patients with chronic diseases compared with patients without chronic diseases. IgM levels were significant higher in COVID-19 patients of chronic diseases compared with control of chronic diseases. According to age groups, IgM levels is perhaps much higher in patients with chronic diseases in age range more than 55 years old compared to patients without chronic diseases.

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