

**A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF ÇANKIRI KARATEKİN UNIVERSITY**

**INVESTIGATION OF IGM AND IGG ANTIBODY LEVELS IN A
GROUP OF COVID-19 PATIENTS WITH AND WITHOUT
CHRONIC DISEASE IN IRAQ**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
BIOLOGY**

**BY
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ÇANKIRI

2022

INVESTIGATION OF IGM AND IGG ANTIBODY LEVELS IN A GROUP OF
COVID-19 PATIENTS WITH AND WITHOUT CHRONIC DISEASE IN IRAQ

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October 2022

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ABSTRACT

INVESTIGATION OF IGM AND IGG ANTIBODY LEVELS IN A GROUP OF COVID-19 PATIENTS WITH AND WITHOUT CHRONIC DISEASE IN IRAQ

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Master of Science in Biology

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October 2022

A novel Coronavirus known as COVID-19 appeared for the first time in Wuhan city in China in 2019. COVID-19 mainly affects the respiratory system. COVID-19 spread rapidly to over the world causing severe health problems in human. The present study aimed to determine the levels of anti- IgM and anti-IgG antibodies constituted against COVID-19, and to compare the levels of these antibodies in the studied groups involving individuals who do not have any chronic diseases and COVID-19, who do not have any chronic disease but have COVID-19 and COVID-19 patients who have chronic diseases such as heart disease and asthma. A total of 120 serum samples were collected from all these studied groups after the 10th and 20th days. The sociodemographic and clinical information about these 120 individuals were also collected. The blood samples that collected and delivered to the laboratory to get the serum and then stored at -20 °C until starting the work, where all samples were tested for anti-IgM and IgG assessment using the commercial ELISA kit. The results showed that the older age means was in COVID-19 patients with asthma followed by the heart diseases group, and the COVID-19 patients without any chronic diseases group had the smallest age mean. Also, the results showed significantly increased levels of anti-IgM for all COVID-19 patients groups on the tenth day compared to the twentieth day (197.52 ± 24.49 vs. 133.22 ± 71.56 ng/mL for COVID-19 with heart disease group, 175.95 ± 19.62 vs. 97.15 ± 72.40 ng/mL for COVID-19 with asthma group and 157.47 ± 74.0 vs. 110.29 ± 77.93 ng/mL for COVID-19 without any chronic diseases group).

The highest anti-IgM antibody level was in COVID-19 patients with the heart disease group followed by COVID-19 with the asthma group for the tenth and the twentieth days (197.52 ± 24.49 and 175.95 ± 19.62 ng/mL, respectively), while the level of anti-IgM antibody was highest in the COVID-19 patients with heart disease group followed by COVID-19 patients without any chronic diseases group (133.22 ± 71.56 and 110.29 ± 77.93 ng/mL, respectively). In addition, the mean level of anti-IgM antibody was non-significantly increased in males compared to females on the tenth day of collecting, while appeared different significant levels between the males and females on the twentieth day of collecting. Also, the mean level of anti-COVID-19 IgG antibody was significantly increased on the twentieth day compared to the tenth day of collection (399.99 ± 37.82 vs. 176.15 ± 34.03 ng/mL for COVID-19 patients with heart disease group, 367.75 ± 25.96 vs. 141.53 ± 61.76 ng/mL for COVID-19 patients with asthma group and 372.59 ± 55.16 vs. 178.74 ± 50.56 ng/mL for COVID-19 patients without any chronic diseases group). The highest level of anti-IgG antibody was in COVID-19 patients without any chronic diseases group followed by the COVID-19 patients with heart disease group on the tenth day (178.74 ± 50.56 and 176.15 ± 34.03 ng/mL respectively), while on the 20th day the highest anti-IgG antibody level was in COVID-19 patients with heart disease group (399.99 ± 37.82 and 372.59 ± 55.16 ng/mL respectively). In addition, the results showed non-significant decreased levels of anti-IgG antibody in males compared to females on the tenth day of collecting for the COVID-19 patients with heart disease and without any chronic diseases groups (170.26 ± 38.94 vs. 183.21 ± 29.75 ng/mL and 170.74 ± 32.77 vs. 188.34 ± 69.55 ng/mL respectively). While in COVID-19 patients with asthma, the results appeared a significantly increased level of anti-IgG antibody in males compared to females on the tenth day also (208.20 ± 49.0 vs. 128.19 ± 58.60 ng/mL). On the twentieth day, the results were similar to the results of the tenth day (400.63 ± 51.48 vs. 399.23 ± 16.18 ng/mL for COVID-19 patients with the heart disease group, 364.47 ± 73.70 vs. 382.33 ± 24.50 ng/mL for COVID-19 patients without any chronic diseases group and 408.10 ± 35.98 vs. 359.68 ± 18.82 ng/mL for COVID-19 patients with asthma group). In conclusion, the results of a total of 90 individuals who had COVID-19 showed that the older patients with asthma aged had high levels of anti-IgM and anti-IgG antibodies, and the youngest were the COVID-19 patients who also had high levels of anti-IgM and

anti-IgG antibodies. There were various statistically significant differences in anti-IgM antibody levels between the tenth and the twentieth day. For anti-IgM antibody level, it was higher in males' groups compared to females in COVID-19 patients without any chronic diseases and asthma groups, while it appeared different in COVID-19 patients with heart disease. According to gender, the results appeared various significant differences in each group of COVID-19 groups. For anti-IgG antibody, there was a significantly increased level of anti-IgG antibody on the twentieth day compared to the tenth day. The results showed various significant differences in anti-IgG antibody levels in males compared to females on both the tenth and twentieth days.

2022, 70 pages

Keywords: COVID-19, Anti-IgM antibody, Anti-IgG antibody, Chronic heart disease, Chronic asthma

PREFACE AND ACKNOWLEDGEMENTS

I would like to thank my thesis advisor, Asst. Prof. Dr. Müge FIRAT and Co-Advisor, Asst. Prof. Dr. Ali Hafidh ABBAS, for their patience, guidance and understanding.

Ali Abdulrazzaq Tamur ALBU-ATTIYA

Çankırı-2022



ÖZET

IRAK'TA KRONİK HASTALIĞI OLAN VE OLMAYAN BİR GRUP COVID-19 HASTASINDA IGM VE IGG ANTİKOR DÜZEYLERİNİN ARAŞTIRILMASI

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COVID-19 olarak bilinen yeni bir Koronavirüs, ilk kez 2019'da Çin'in Wuhan şehrinde ortaya çıktı. COVID-19, solunum sistemini etkileyen SARS virüslerinden biridir. COVID-19 hızla tüm dünyaya yayılarak insanlarda ciddi sağlık sorunlarına neden olmuştur. Bu çalışmada COVID-19'a karşı oluşan anti-IgM ve anti-IgG antikor düzeylerinin herhangi bir kronik hastalığı olmayan COVID-19 geçirmemiş sağlıklı bireyler ile herhangi bir kronik hastalığı olmayan COVID-19 geçirmiş bireyler, kalp hastalığı ve astım hastaları gibi kronik hastalıkları olan COVID-19 geçirmiş bireylerin yer aldığı çalışma gruplarında karşılaştırılması amaçlanmıştır. 10. ve 20. günlerden sonra çalışılan tüm bu gruplardan toplam 120 serum örneği toplanmıştır. Bu 120 bireyle ilgili sosyodemografik ve klinik bilgiler de toplanmıştır. Serumun ayrılması için toplanan, laboratuvara teslim edilen ve daha sonra çalışmaya başlayana kadar -20 °C'de saklanan kan örnekleri, ticari ELISA kiti kullanılarak anti-IgM ve IgG değerlendirmesi için test edilmiştir. Sonuçlar, ileri yaş ortalamasının astımlı COVID-19 hastalarında ve ardından ısı hastalıkları grubunda olduğunu ve herhangi bir kronik hastalığı olmayan COVID-19 hastalarının en küçük yaş ortalamasına sahip olduğunu göstermiştir. Ayrıca, sonuçlar onuncu günde tüm COVID-19 hasta grupları için yirminci güne kıyasla anlamlı derecede artmış anti-IgM düzeyleri göstermiştir ($197,52 \pm 24,49$ 'a karşı $133,22 \pm$ kalp hastalığı grubuyla COVID-19 için $71,56$ ng/mL, $175,95 \pm 19,62$ 'ye karşı $97,15 \pm$ astım grubuyla COVID-19 için $72,40$ ng/mL ve astım grubu olan COVID-19 için $72,47 \pm 74,0$ 'a karşı $110,29 \pm$ herhangi bir kronik hastalık grubu olmayan COVID-19 için

77,93 ng/mL). En yüksek anti-IgM antikor seviyesi, kalp hastalığı grubundaki COVID-19 hastalarında, ardından onuncu ve yirminci günlerde astım grubundaki COVID-19'dur (sırasıyla $197,52 \pm 24,49$ ve $175,95 \pm 19,62$ ng / mL), anti-IgM antikoru düzeyi kalp hastalığı olan COVID-19 hastalarında en yüksek iken, bunu herhangi bir kronik hastalığı olmayan COVID-19 hastalarında takip ederken (sırasıyla $133,22 \pm 71,56$ ve $110,29 \pm 77,93$ n/ mL). Ek olarak, ortalama anti-IgM antikoru seviyesi, toplanmanın onuncu gününde kadınlara kıyasla erkeklerde anlamlı olmayan bir şekilde artarken, toplamının yirminci gününde erkekler ve kadınlar arasında farklı anlamlı düzeyler ortaya çıkmıştır. Ayrıca, anti-COVID-19 IgG antikorunun ortalama düzeyi, toplanmanın onuncu gününe göre yirminci günde anlamlı olarak artmıştır ($399,99 \pm 37,82$ 'ye karşı $176,15 \pm$ kalp hastalığı olan COVID-19 hastaları için $34,03$ ng/mL, $367,75 \pm 25,96$ 'ya karşı $141,53 \pm$ astım grubu olan COVID-19 hastaları için $61,76$ ng / mL ve astım grubu olan COVID-19 hastaları için $372,59 \pm 55,16$ 'ya karşı $178,74 \pm 50,56$ ng/mL). En yüksek anti-IgG antikoru seviyesi, herhangi bir kronik hastalığı olmayan COVID-19 hastalarında, onu onuncu günde kalp hastalığı olan COVID-19 hastalarında (sırasıyla $178,74 \pm 50,56$ ve $176,15 \pm 34,03$ ng/mL), 20. günde ise en yüksek anti-IgG antikor seviyesi kalp hastalığı olan COVID-19 hastalarında (sırasıyla $399,99 \pm 37,82$ ve $372,59 \pm 55,16$ ng/mL) idi. Ek olarak, sonuçlar, kalp hastalığı olan ve herhangi bir kronik hastalık grubu olmayan COVID-19 hastaları için toplanmanın onuncu gününde kadınlara kıyasla erkeklerde anti-IgG antikor düzeylerinin anlamlı olmayan bir şekilde azaldığını göstermiştir (sırasıyla $170,26 \pm 38,94$ 'e karşı $183,21 \pm 29,75$ ng/mL ve $170,74 \pm 32,77$ 'ye karşı $188,34 \pm 69,55$ ng/mL). Astımlı COVID-19 hastalarında, sonuçlar onuncu günde de kadınlara kıyasla erkeklerde önemli ölçüde artmış bir anti-IgG antikor seviyesi ortaya koymuştur ($208,20 \pm 49,0$ 'a karşı $128,19 \pm 58,60$ ng/mL). Yirminci günde, sonuçlar onuncu günün sonuçlarına benzerdi ($400,63 \pm 51,48$ 'e karşı $399,23 \pm$ kalp hastalığı grubundaki COVID-19 hastaları için $16,18$ ng/mL, astım grubu olan COVID-19 hastaları için $364,47 \pm 73,70$ 'e karşı $382,33 \pm 24,50$ ng/mL ve astım grubu olan COVID-19 hastaları için $408,10 \pm 35,98$ 'e karşı $359,68 \pm 18,82$ ng/mL). Sonuç olarak, COVID-19 hastası olan toplam 90 kişinin sonuçları, astım çağındaki yaşlı hastaların yüksek düzeyde anti-IgM ve anti-IgG antikorlarına sahip olduğunu, en gençlerinin ise yüksek düzeyde anti-IgM ve anti-IgG antikorlarına sahip COVID-19 hastaları olduğunu göstermiştir. Onuncu ve yirminci gün arasında anti-IgM antikor

düzeylerinde istatistiksel olarak anlamlı çeşitli farklılıklar vardı. Anti-IgM antikor düzeyi için, herhangi bir kronik hastalığı ve astım grubu olmayan COVID-19 hastalarında erkek gruplarında kadınlara göre daha yüksekken, kalp hastalığı olan COVID-19 hastalarında farklı görünmektedir. Cinsiyete göre, sonuçlar COVID-19 gruplarının her bir grubunda çeşitli önemli farklılıklar ortaya koymuştur. Anti-IgG antikoru için, yirminci günde onuncu güne kıyasla anlamlı derecede artmış bir anti-IgG antikoru seviyesi tespit edilmiştir. Sonuçlar, erkeklerde anti-IgG antikor düzeylerinde, hem onuncu hem de yirminci günlerde kadınlara kıyasla çeşitli önemli farklılıklar göstermiştir.

2022, 70 sayfa

Anahtar Kelimeler: COVID-19, Anti-IgM antikoru, Anti-IgG antikoru, Kronik kalp hastalığı, Kronik astım

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LIST OF SYMBOLS

%	Percent sign
°C	Degree centigrade
SD	Standard deviation
μL	Microliter



LIST OF ABBREVIATIONS

ACE2	Angiotensin-converting enzyme II
APC	Antigen presenting cell
CatB	Cathepsin B
COVID-19	Coronavirus disease 2019
DAMP	Damage associated molecular patterns
DC	Dendritic cells
DsRNA	Double stranded RNA
E	Envelope
FCS	Furin cleavage site
IC	Immune cells
IFN	Interferon
IgG	An immunoglobulin of the class IgG
IgM	An immunoglobulin of the class IgM
IM	Immune system's
M	Membrane
MERS-CoV	Middle east respiratory syndrome
MHC	Major histocompatibility complex
N	Nucleocapsid
NK	Natural Killer cell
NOD	Nucleotide-associated oligomerization domains
PAMP	Pathogen associated molecular patterns
PAMPA	Parallel Artificial Membrane Permeability Assay
PICS	Post-intensive care syndrome
RBD	Receptor binding domain
RIG-I	Retinoic acid inducible gene I
RTC	Replicase transcriptase complex
RT-PCR	Real time polymerase chain reaction
S	Spike
SARS-CoV	Severe acute respiratory syndrome
ssRNA	Single stranded RNA
Tfh	T follicular helper cell
Th1	T helper cell 1
Th17	T helper cell 17
Th2	T helper cell 2
TLR	Toll like receptor
TLRs	Toll-like receptors
TMPRSS2	Transmembrane protease serine 2
TNF	Tumor necrosis factor
WHO	World health organization

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1. INTRODUCTION

Infection with the SARS-CoV-2 coronavirus, which the World Health Organization (WHO) has termed a pandemic, has a substantial social and economic impact globally (Hu *et al.* 2020). By December 31, 2021, the total number of deaths related to COVID-19 had reached five hundred and ninety-four million, while the expected excess deaths were about three hundred and seven times higher, totaling two hundred and eighteen million. The excess mortality rate worldwide from the COVID-19 pandemic has reached three and nineteen deaths for every hundred thousand people (Wang,2022). This outbreak necessitated the quick mobilization of considerable material and human resources in order to contain it and manage sufferers. It is vital to have effective illness diagnostic and monitoring tools with high sensitivity and specificity for the correct management of this pandemic. In this regard, it is critical to emphasize that around 80% of people infected with this virus, but they may not show symptoms, which may be strong and may be mild, therefore establishing a population screening system with cost-effective technologies that are simple to adopt and deploy is critical. The identification of the virus's genetic material using molecular biology methods is the gold standard for diagnosing COVID-19 (Esbin *et al.* 2020). However, this technique has been augmented by useful assessment of antibodies (Abs), particularly IgM and IgG in the serum of COVID-19 suspects or patients (Lisboa *et al.* 2020, Rashid *et al.* 2020, Vashist 2020).

The tests differ in terms of the type of immunoassay used to determine the type of antibodies in the body as it is against this virus. This variation is somewhat influenced by the basis of technology, the specific Ab type, and the mechanism of detection of the antigen and antibody complex structure. These factors, as well as the technology's origin (manufacturing lab), influence its sensitivity and specificity. To detect IgM and IgG Abs against SARS-CoV-2, lateral flow immunoassays have been frequently employed. The preceding is mostly determined by the ease with which the determination is done (no lab is necessary) and the speed with which the result is

obtained. Around 75% of the over 7.5 million samples handled were serological testing (Sofiana *et al.* 2020).

Serological techniques rely on the identification of Abs IgM and IgG, despite the fact that Abs IgA production is critical in the immune system's (IM) response to respiratory infections. Furthermore, these tests do not detect the existence of particular antibodies against the virus. Two In this regard, the use of highly sophisticated diagnostic and surveillance tools to detect elements related to the novel coronavirus is critical, including indirect tests that detect anti-SARS-CoV-2 antibodies, which have been introduced by various platforms but with significant variation in their discovery. New immunological approaches, such as magnetic immunoassay with nanoparticle assistance, are emerging in this setting (Park *et al.* 2013).

In this study, it was aimed to determine the levels of IgG and IgM antibodies against COVID-19 and compare the levels of antibodies in a group of individuals who do not have chronic diseases (healthy) and who have chronic diseases (asthma patients and heart patients) with COVID-19 infection in Iraq.

2. LITERATURE REVIEW

COVID-19, which is caused by SARS-CoV-2, appeared in the year two thousand and nineteen is still ongoing, has caused the deaths of millions of people in the whole world, as a result of this disease has been declared by WHO as a pandemic, as one of its terms is that the body is creating the process of creating so-called antibodies in COVID-19 patients for a period ranging from three to fifteen days. The detection of antibodies, for example IgM and IgG antibodies, in patients with a negative PCR test, or in other words, it is called pseudopodia, helps in the process of diagnosing the COVID-19 virus. It is also known that antigens called IgM are used extensively at the beginning of infection, so that they are called the first line of defense during that before establishing a secondary, high-affinity adaptive IgG response, It is considered one of the types of immunity that is called long, or in other words it is called immune memory in the body (Casadevall and Pirofski 2020).

2.1 History and Epidemiology of Coronaviruses

Coronaviruses (CoVS) were first discovered by scientists in the mid-sixties. Their name is derived from the surface crown-like spikes (S) that they have. CoVs are usually found in animals, so it can be transferred between animals and human (Kahn *et al.* 2005).

Four types caused by the virus called CoVs Which is Alphacoronavirus (alphaCoV), Betacoronavirus (betaCoV), Deltacoronavirus (deltaCoV) and ammacoronavirus (gammaCoV) have caused many recent epidemics, such as severe acute respiratory syndrome (SARS), The detection of SARS-CoV-2 in clinical practice is important to reduce the spread of infection. Reliable diagnosis is provided by nasal swab tests using q-PCR, Serological tests and CT results, on the other hand, before infection, governments of different countries offered many preventions such as quarantine, rules of disguise and closure of public places in order to provide public safety and limit social interactions. Moreover, according to the World Health Organization, precautionary (Artik *et al.* 2021).

COVID-19 is the last CoV infection in human. The virus is specifically called SARS-CoV-2, which was discovered in China and in a city there called Wuhan, where the first cases of infection were discovered there in December in the year two thousand and nineteen. It is a disease with symptoms associated with difficulty breathing and muscle pain, however, in severe patients it generates organ difficulty syndrome, acute respiratory infection, sepsis and poisoning (Huang *et al.* 2020). On March 11, 2020, the World Health Organization (WHO) classified the new coronavirus (COVID-19) outbreak to be a worldwide pandemic. In a press conference, WHO Director-General Dr Tedros Adhanom Ghebreyesus stated that in only two weeks, the number of cases outside of China grew 13-fold, and the number of countries with cases tripled (Cucinotta and Vanelli 2020).

The disease is believed to have been spread in a seafood market in Wuhan, China that sells live animals. Which causes the disease that became called COVID-19, as it spreads rapidly through the air through the respiratory system (McIntosh 2020).

As of March 11, 2020, there were 118,319 confirmed cases and 4,292 deaths (Ghosh *et al.* 2020). According to BBC reporting, the overall number of infected had climbed to 2,063,161 by 16 April, penetrating 185 countries/territories, and had risen to 4,399,070 by May 13th (BBC, 2020e). By May 15, 2020, 453,3255 individuals have been infected with this virus globally, resulting in 303,388 fatalities and infecting more than 188 countries. As of July 16, there were 139,50629 cases worldwide, 597,601 deaths, and 213 countries and territories impacted (Ghosh *et al.* 2020).

The COVID-19 pandemic affected the world for more than 1 year, because at the beginning of 2021, and looking at the last 7 days until January 3, 2021, more than 4 million new cases were reported in the world (4,035,226), which represents A 2% decrease compared to all cases accumulated since the start of the pandemic at the end of 2019. According to a statistical examination of 190 nations and territories, around 3.8 billion COVID-19 infections and illnesses occurred in late 2021, with nearly 44 percent of the world's population infected at least once (Beusekom, 2022).By July 7, 2022, there

were COVID-19 cases in Iraq, with a total of 64,701 confirmed cases, 36,252 recovered, 396 patients in intensive care, and 2,685 recorded fatalities (Abdulkadir 2022)

In both developed and developing countries, viruses in all their forms and types, which may sometimes come from some animals in general, cause some diseases to humans and most of what may affect the respiratory system (Crosta 2021). Scientists consider these viruses to be of a source and origin coming from animals, as they may contain some RNA, and one of their characteristics is that they have a chain called mono, so they can transmit quickly between humans through infection that takes place through some animals, especially those called vertebrates. Therefore, it is spreading rapidly worldwide (Jackson *et al.* 2022). Scientists have proven that this virus when infecting humans, with other viruses that may infect it from Betacoronavirus genera in addition to infecting animals, such as bats, is the same virus, although scientists have not yet discovered the host that may be considered an intermediary that causes its transmission a person in one or more ways (Andersen *et al.* 2020). SARS-CoV-2 exhibits an evolutionary increase of FURIN cleavage site (FCS) on the S protein compared to SARS-CoV-1. FCS is thought to be responsible for the high infectivity and transmissibility as of December 2020. The FCS has played an important role in the development of high pathogenicity in influenza viruses. It is unknown to what degree it has the similar effect on SARS-CoV-2 (Xia *et al.* 2020). As indicated in (Figure 2.1), SARS-CoV-2 comprises four distinct proteins; membrane (M), spike (S), envelope (E) and nucleocapsid (N) and the angiotensin-converting enzyme II (ACE2) on epithelial cells in the lungs interacts to the S protein (Ian *et al.* 2020).

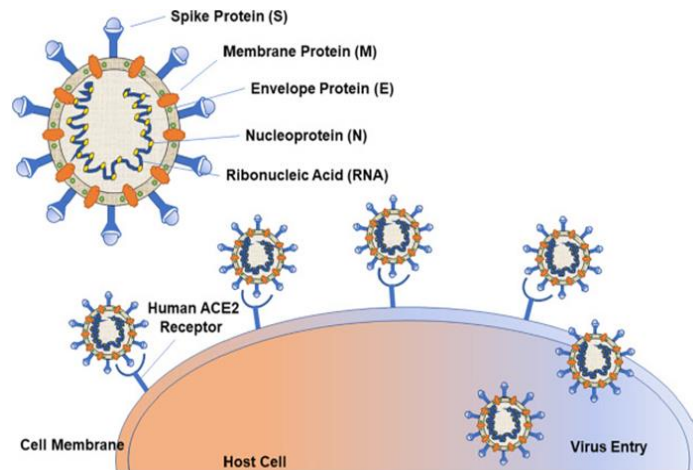


Figure 2.1 SARS-CoV-2 form and structure (Naqvi *et al.* 2020)

2.2 Transmission and Clinical Findings of COVID-19

Contact with an infected person leads to infection through the transmission of droplets from an infected person's sneezing that are loaded with this virus, which leads to its transmission to a healthy person through the air, as well as additional routes of transmission, can all be employed to propagate this virus's infection. Coughs, sneezes, and conversations with sick persons disseminate respiratory droplets that cause contact and droplet transmission. Fomite can be transmitted by touching infected surfaces and then touching your eyes, nose or mouth. This virus can be found on any type of surface and remain that way, perhaps for several hours or days, depending on the environment of that surface in terms of temperature and humidity (Goldman 2020).

Researchers on the one hand, scientists on the one hand, and doctors on the other hand are studying the nature of this virus in terms of other ways of transmission. Airborne transmission is defined as the propagation of an illness by the inhalation of virus-containing droplets that may linger in the air for lengthy periods of time and travel great distances. The potential of a vulnerable person contracting SARS-CoV-2 by breathed aerosols is unknown (Mittal and Seo 2020, Wang *et al.* 2020). This virus has been found in many biological samples such as urine samples, however it has not been determined whether transmission may occur through them (WHO 2020, Wang *et al.* 2020, Cao *et al.* 2020). Also, health variables such as chronic diseases, including

diabetes, chronic liver disorders, and others, may be more susceptible than others to infection with this virus (Folkehelse instituttet 2020).

Comorbidities have an influence on the disease's progression and the extent to which it can be damaging. Fever, a dry cough and exhaustion are symptoms of mild to moderate illnesses. It may cause symptoms that are not common among all patients or are called uncommon, such as discoloration of the fingers or loss of smell and taste, and eventually lead to acute respiratory distress, and then respiratory failure that causes the death of the affected person in severe cases (Renu *et al.* 2020).

According to new research, about one-fifth of those infected with SARS-CoV2 are asymptomatic. This is due to the difficulty of distinguishing between the severity of the symptoms among the infected, and in some cases no symptoms appear in some of the infected, it is difficult to produce reliable numbers (Bianca 2020).

COVID-19 causes long-term affects in one out of every five patients, which are referred to as long COVID-19 effects. COVID is likely to be long lasting in some people due to the severity of their infection, which leads to their stay in the intensive care unit (ICU) department. However, it is still uncertain if COVID-19 or the effects of intensive care are to blame for the symptoms. Infected people who have been in a ICU usually develop a syndrome called post-intensive care (PICS), which is comparable to symptoms of COVID-19. PICS is associated with advanced age, female sex, and the severity of the condition. Many studies have been done on COVID-19 and focus on the duration of the infection, its spread among people, and the symptoms, whether they are obvious or not, but it is not clear how much other symptoms affect the quality of life (Himmels *et al.* 2021).

2.3 Lifecycle of SARS-CoV-2

The mechanism of these viruses prevents the virus from weakening. CoV, exchange pieces of RNA with many other CoV, leading to a large number of new sequences that

the body's immunity does not recognize, in which a protein called S1 interacts which is found on the surface of this virus with an enzyme called angiotensin II (ACE2) on the cells that appear on the lung, where the spike is cleaved through the type of protease with a transmembrane quality serine 2 (TMPRSS2), and then the process of integration inside the cell in the host body. The help/preparation procedure for FURIN proteases, which was recently discovered, ensures that this type of virus performs a penetration process so that it can enter inside the host body within its own cells (Trougakos *et al.* 2021).

To a lesser extent, an enzyme called endogenous cysteine protease aids a process called fusion that it performs within it. The virus releases RNA as soon as it enters the host cell. ORF1a and ORF1b, two huge genomes, are translated, resulting in the synthesis of two big proteins that are rather large like the pp1a protein and pp1ab protein. This type of polyprotein is supplemented with Papain-like proteases (PLpro) and chymotrypsine protease (a serine type Mpro), 3CLpro. (Figure 2.2) shows how the pp1a and pp1ab release non-structural proteins (nsp) with various roles (Folkehelse instituttet 2020, Vellas *et al.* 2020, V'kovski *et al.* 2020).

Non structural proteins (NSPS) are needed by the virus for replication which occurs symmetrically within cells and can form a complex with replication and transcription which is a transcript of the necessary components from a component within the so-called RNA of this virus, which results in the formation of S, E and M in the endoplasmic reticulum. In the Golgi vesicle, viral components develop, culminating in a mature lipid-wrapped virion. The newly emerging virus leaves cells through the process of cellular secretion and binds to additional host cells (Ward 2020).

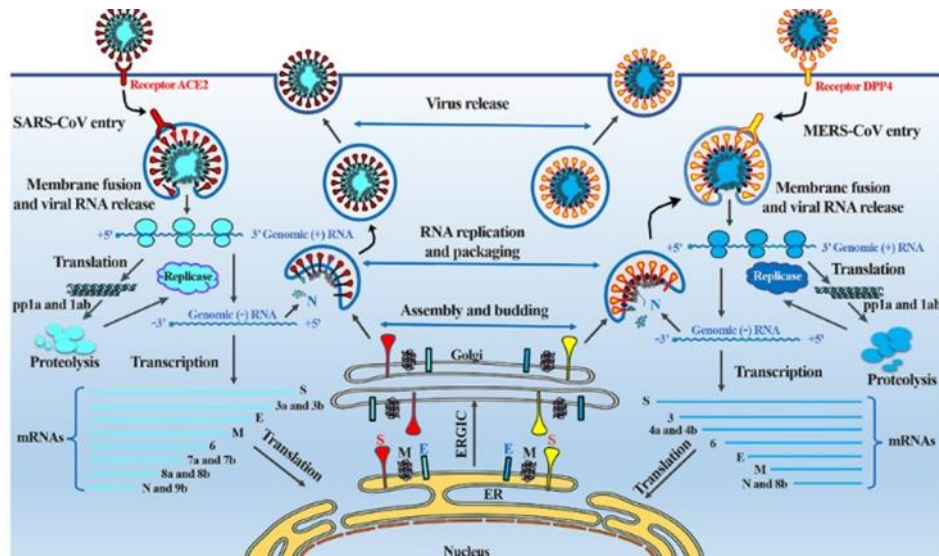


Figure 2.2 SARS-CoV-2 life cycle (Song *et al.* 2018)

2.4 Diagnosis of COVID-19

Swabs from the nasopharyngeal and oropharyngeal cavities are used to diagnose and identify SARS-CoV-2 in patients. The samples are subsequently transported in a transport medium and transmitted to laboratories for viral detection. Depending on the testing capabilities that might be undertaken. However, anyone who has had signs of an acute respiratory infection or other symptoms for more than two days should be checked (Folkehelse instituttet 2020).

There are many ways for the process of doing what are called diagnostic procedures. For the initiation process, the so-called direct detection of the virus used from patient samples through the process of using techniques to amplify DNA, for example Real-time polymerase chain reactions (RT-PCR) (or so-called transcription amplification), or other amplification is called isothermal by Loop (A process that works to discover proteins from inside the virus that is done by culturing it) (LAMP). Second, after a viral infection, immunological diagnostics are employed to identify virus-specific antibodies (Folkehelse instituttet 2020)

RT-PCR tests with excellent specificity and sensitivity are used as global standards for identifying SARS-CoV-2 in samples. It is possible that the specificity is about 95% and the sensitivity is about 80%. Because the specificity depends on the stage of the disease, it is also possible that the sensitivity becomes less in terms of specificity, but if the test is performed shortly after the onset of symptoms, the sensitivity of RT-PCR increases (Folkehelse instituttet 2020)

Antigen rapid-tests, on the other hand, can be used to detect SARS-CoV-2 infections. The majority of quick tests rely on identifying specific viral antigens, which varies depending on the test kit. Antigen testing have a poorer sensitivity, resulting in a low antibody level in the early stages of COVID-19 identification (Folkehelse instituttet 2020).

2.5 Treatment of COVID-19

COVID-19 treatment is not completely specific; nevertheless, recommendations are being created and revised on a regular basis. Vaccines have been created and fast vaccination has begun in the first few months of 2021, however, dosage limitations hamper the acquisition of herd immunity. As it is given to infected people in Norway who need urgent or immediate treatment through so-called immunosuppressants. Treatment with antiviral remdesivir can be administered if the illness is severe (Titlestad *et al.* 2021).

2.6 A General Overview About Immunity and Immunity Against COVID-19

Viruses, bacteria, fungus, and parasites are all hazardous pathogens the so-called immunity (IM) has many systems that may be considered somewhat complex in addition to many components that scientists consider vital, and some may consider them distinctive as well that carry out the process of defending the body. The innate IM and the adaptive IM are two interconnected branches of the IM. Both systems operate together as a team. Innate immunity is the body's initial line of protection, and it

develops before birth. The IM, which is innate inside the body, is considered the first line in which the body defends itself. It is activated in a short period, sometimes hours and sometimes days, through the process of exposure to what is called antigen. The adaptive system, on the other hand, must be built gradually and is not inherited (Figure 2.3). This system has a remarkable ability to form immunological memories. The innate IM is often generic, but the IM called adaptive is considered the most specialized in addition to its development (Abbas *et al.* 2020).

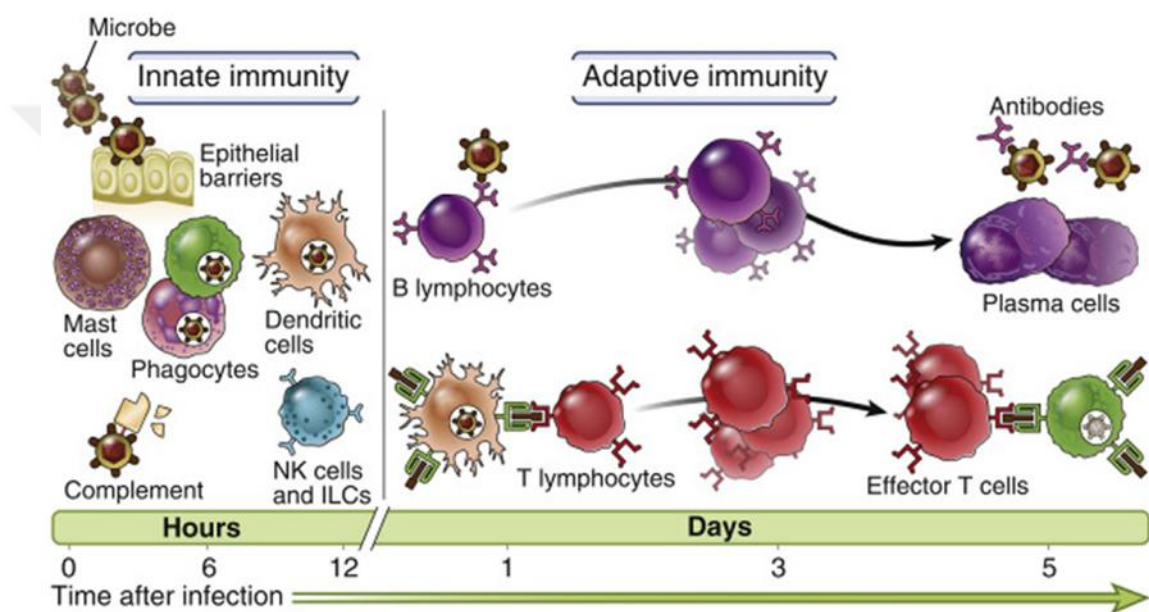


Figure 2.3 Immunity, both innate and adaptive (Sasadeusz *et al.* 2019)

After infection, the innate immune response appears within hours, whereas adaptive immune responses arise later. Different immune cells (IC) are activated by innate and adaptive immunity, which can result in the generation of the so-called cytokines are a type of molecules that can be called messengers, as their components and functions are in a process called intramuscular control by performing a specific process for inhibition. These chemicals attach to cell receptors and set off a complicated chain of events (Abbas *et al.* 2020, Paludan and Mogensen 2022).

Autocrine, which affects the type of cells that are secreted, paracrine, which affects the cells themselves, and the goxtrac affects a type of cell that is adjacent to the affected

cells, but it requires a type of membrane (M) with proteins that are fixed on it, and endocrine glands that influence. On the type of cell being targeted, all of these are possible effects of cytokines (Abbas *et al.* 2020). Inflammatory agents such as interferon / INF-1 type 1, cytokines are separated between these multidirectional proteins. However, depending on the local milieu, a cytokine can have both functions (Su *et al.* 2012, Abbas *et al.* 2020).

2.6.1 Innate immune system

Innate immune system (IIS) is generated by several basic functions, such as inflammation, or as an antiviral response, by the adaptive IIS activation process. Immune memory is not developed in the same way by the IIS as it is by adaptive IIS. Innate IIS responses are very similar in every contact with microorganisms (Abbas *et al.* 2020).

Some types of barriers are epithelial, called dendritic cells (DC) and cells called monocytes, and there are so-called neutrophils, macrophages, and a type of natural killer (NK) cell, in addition to a type of complement system is components that are essential to IIS. The type of molecular patterns that are somehow related to pathogens, PAMPA, Parallel Artificial Membrane Permeability Assay (PAMPSs) that have been evolutionarily conserved in human pathogens are recognized by innate IC. Toll-like receptors (TLRs) and nucleotide-associated oligomerization domains (NOD-) (Abbas *et al.* 2020).

PAMPs are lipopolysaccharides that bind to the TLR on specific antigen-presenting cells (APC) as well as on other cells (Gaudino and Kumar 2019; Abbas *et al.* 2020). Endogenous chemicals termed damage-associated molecular patterns (DAMPs) are released by dying or injured cells and can bind to PRR (Relja and Land. 2020, Abbas *et al.* 2020).

The complement system consists of surface and inner cell M proteins that play a major role in fighting infection and dangerous bacteria. Microbes can be affected by complementary activated proteins by shadowing, cell lysis, or from IC stimulation to generate proinflammatory chemicals (Abbas *et al.* 2020).

2.6.2 Adaptive immune system

The so-called complement system is very important in the process of fighting infection and fighting dangerous virus. The so-called complement system is very important in the process of fighting infection and fighting dangerous bacteria. Antigen-specific receptors are found on B and T lymphocytes. Humoral immunity (mediated by B cells producing antibodies) and cell-mediated immunity (mediated by T lymphocytes) are the two forms of adaptive immune responses.

The so-called naive lymphocytes proliferate, where they develop into so-called "active cells" or sometimes called "memory cells" because they perform the process of bacterial antigen recognition. In contrast to effector cells, they live within memory cells in the absence of antigen, resulting in a greater frequency of memory cells as one ages, as shown in (Figure 2.4) (Abbas *et al.* 2020).

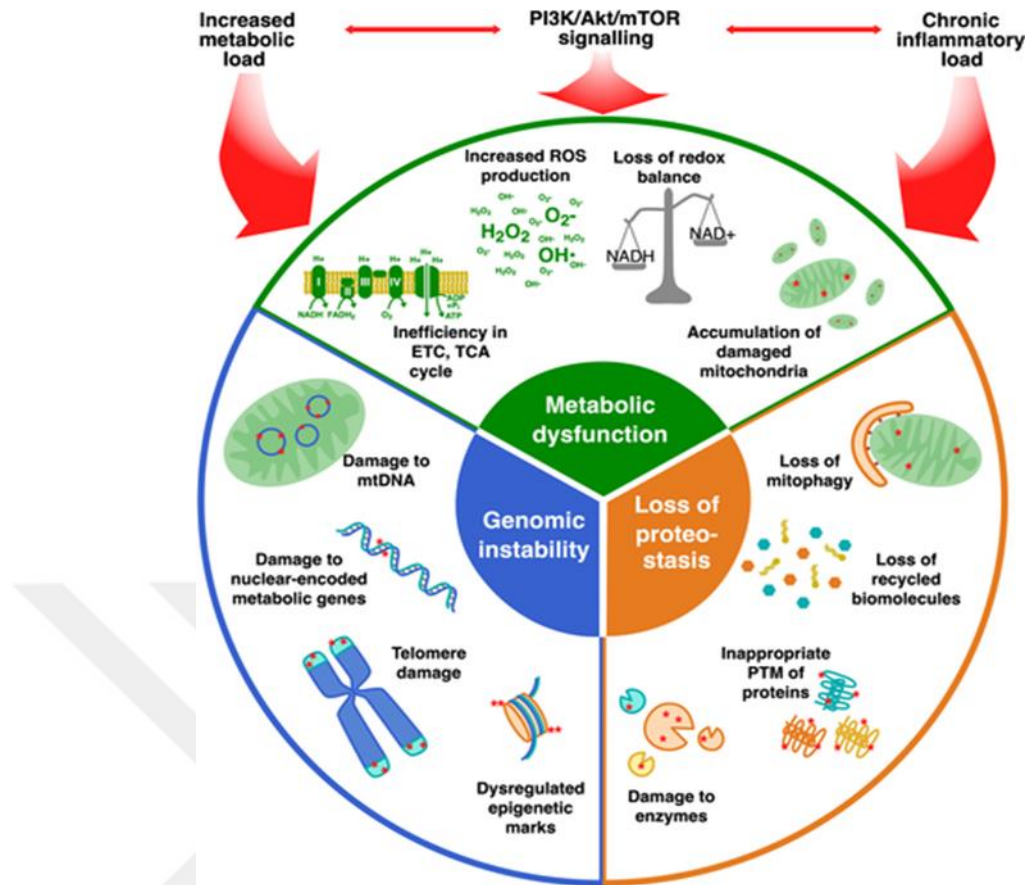


Figure 2.4 The process of changing cells called naive and other cells called memory as a function of age (Quinn *et al.* 2019)

The process that produces the so-called cytokines of a large number of cells, as well as the so-called CD4 helper cells, as well as the so-called T cells, is divided into a large number of subtypes. It also has a function that serves other groups called subgroups for many purposes that protect the body from many types of microbial diseases (Abbas *et al.* 2020).

B cells generate many antibodies and facilitate a type of immunity called humoral. Antibodies, known as immunoglobulins (Ig), are a distinct type of protein that somehow binds to or may be released to certain M cells of the B cells. Similar types of Ig may have various types, including (IgM and IgG), and they also have a number of effects. They have many properties that scientists called physical properties. These cells activate the process of activating B cells and they also turn on the production of so-called antibodies when the process of engaging the antibodies that are bound to M. The

process of stimulating the antibodies takes place, and this in turn leads to a large chain of effector processes that are from Its function is to kill the antigen / microbe (Abbas *et al.* 2020).

Despite the fact that the IM is separated into two parts, both operate together to create an efficient host response. On the surface of APCs is the major histocompatibility complex (MHC), Which in turn presents a specific type and number of antigen-specific fragments that reside intracellularly in the adaptive IM. MHC is also divided into many groups, including: MHC class I, as it is easy to find in all chemicals in the body (Abbas *et al.* 2020).

Despite the fact that lymphocytes are mostly involved in adaptive immunity, they do exhibit certain innate immunity-related characteristics. The morphological and functional characteristics of these cells are comparable to cells called T cells, although there are a variety of somewhat restricted receptors which are T cells in addition to NK-T cells. T cell receptors with limited variety, as well as NK-cell-specific surface components, are expressed by NK-T cells (Abbas *et al.* 2020).

2.6.3 Immunoglobulin G (IgG)

IgG is a type of antibody. IgG is the form that is considered the most prevalent within the antibodies that can be detected in the circulatory system, as it represents about seventy-five percent of the antibodies in the blood inside the human body of all kinds. Plasma B cells produce and release IgG molecules. There are two paratopes in each IgG antibody (Vidarsson *et al.* 2014). Two heavy chains (VH, CH1 and CH2) and two light chains make up immunoglobulin G (IgG) (VL and CL). Invariant areas (which do not contribute specificity to a given antigen) are C, whereas variable regions (which do) are V. As a result, the antigens bind to the variable regions VL and VH. Only Fab (the antigen-binding component comprised of the VH, VL, CH1 and CL domains) can be utilized to lower the size of IgG as (Figure 2.5) (Piro *et al.* 2016).

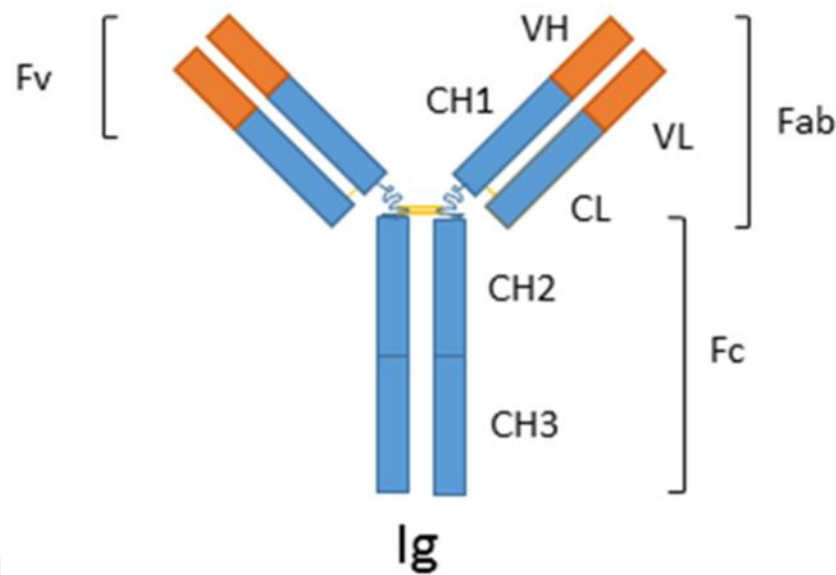


Figure 2.5 Immunoglobulin G (IgG) (Piro *et al.* 2016)

Antibodies make up a large part of humoral immunity. IgG antibodies are the most common form of the type of antibody that is found inside the blood as well as in the fluid outside the cell and they are responsible for controlling infection in bodily tissues. IgG defends the body from infection by binding to many groups of pathogens (Vidarsson *et al.* 2014).

It accomplishes this through a number of mechanisms: IgG-mediated pathogen binding results in their immobility, IgG coating of pathogen surfaces enables for their identification; IgG turns on the classic pathway of the system inside the body called complement, which is a number of chains that lead to the formation of an immune protein and work to eliminate pathogens and toxins are also bound and neutralized by IgG; IgG participates in the so-called cell-dependent cytotoxicity as well as it has the ability to depend on antibodies (ADCC) and it also performs the process of proteolysis within many cells, where it binds to TRIM21 to drive distinctive virions to the juice proteasome cellular; the responses that lead to the type III and type II hypersensitivity reactions that are also associated with IgG (Mallery *et al.* 2010).

IgG antibodies are produced after the antibody response has matured and switched classes, hence they are mostly involved in the secondary immune response (Vidarsson *et al.* 2014). IgG is released as a small monomer, which allows rapid tissue perfusion. It is the unique and only isotope of this antibody that blocks the receptor and allows it to pass through the human placenta, protecting the baby in the womb. Residual IgG absorbed by the placenta, in addition to IgA released in breast milk, offers humoral protection to the infant. Before it happens to IM evolution is specially made for him. Colostrum, particularly bovine colostrum, includes a high amount of IgG. IgG occurs From twenty-four hours to forty-eight hours after the antigen stimulation process is completed in those who have had preexisting immunity to a virus (Finkelman 2007).

As a result, the infant has the same antibodies as the mother during the first six months of life, and the kid can protect itself against any infection his mother had previously in her life until these antibodies are degraded. Immunoglobulins and their groups are also very necessary for all children who are highly susceptible to infections, as is the case with respiratory diseases. IgG can play a very important role in the control process that takes place inside the body to improve its responses and sensitivity as well. Systemic anaphylaxis can occur in two ways, according to Finkelman (Finkelman 2007).

Antigens that are normally crossed with IgE antigens bind in one way or another to the mast cell receptor called FcRI, and this in turn leads to the release of a factor that leads to activation of platelets. It also leads to the release of a so-called histamine, which can lead in some way to systemic anaphylaxis in mice via the traditional route (PAF). Antigens generate complexes with IgG in the alternative route, which a receptor called FcRIII junctional macrophage that stimulates the release of so-called PAF is only (Finkelman 2007).

An antigen of any type may act before it can bind to IgE, which in turn is bound to mast cells. IgG antibodies can prevent IgE anaphylaxis. As a result, IgG antibodies can mediate systemic anaphylaxis generated by high amounts of antigen while blocking systemic anaphylaxis induced by little amounts (Finkelman 2007).

IgG is a specific group of proteins with a spherical shape with a large size and it consists of four peptide chains that have a specific molecular weight that may reach approximately one hundred and fifty kilodaltons (Janeway *et al.* 2001). It has a quadrilateral structure with two chains that have the character of congruence (gamma) and it is heavy so that it reaches approximately fifty kilodaltons (Antibody Basics 2014).

The disulfide bonds (which are characterized by heavy chains) are connected to each other as well as to other light chains. The resultant tetramer contains two identical halves that create a Y-like structure when combined. Each fork end has an antigen binding site that is identical. The picture to the left depicts many things, whether regions or areas, but they are specific to the so-called typical IgG. Within the constant region of heavy chain, asparagine 297 is one of the sites formed within the binding of nitrogen glycosylation and is also conserved in the Fc sections of IgGs (Cobb 2019). Four polypeptide chains (two heavy chains and two light chains) are joined together by disulfide bridges in a typical IgG molecule. It also has a variable constant light region (VL), and the heavy chain is made up of a single VH chain connected to three fixed regions (CH1, CH2, and CH3). The Fc region is crucial for IM function because it binds A and G proteins as in (Figure 2.6) (Murphy *et al.* 2016).

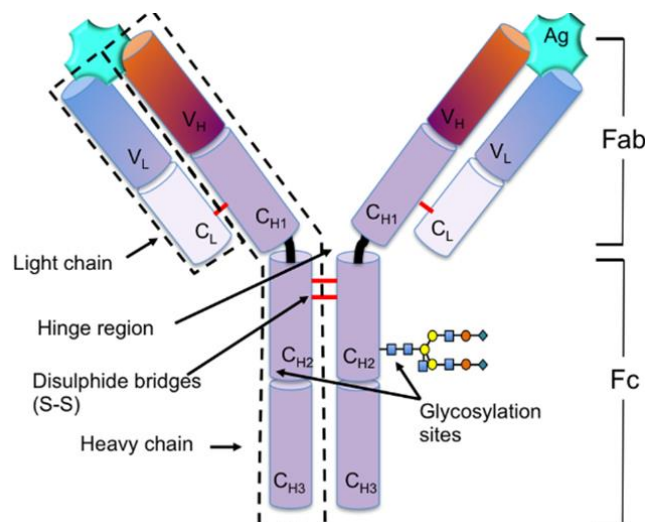


Figure 2.6 IgG Structure (Murphy *et al.* 2016)

The so-called N-glycans that are linked in one way or another within the site may be in most cases of a complex type of so-called pantene structures that are characterized by having a fucosylated nucleus (Parekh *et al.* 1985). In addition, the N-glycans include a section of GlcNAc and a section of sialic acid residues that are bound in small numbers (Stadlmann *et al.* 2008). Several autoimmune, viral, and metabolic illnesses have been connected to the N-glycan composition of IgG (De Haan *et al.* 2019).

Within the human body, there are four classes of IgG (one, two, three and four) whose names derive from the amount of available serum and its arrangement, with IgG1 being the most abundant) (Table 2.1) (Vidarsson *et al.* 2014).

Table 2.1 Categories of IgG antigens inside the body (Vidarsson *et al.* 2014)

Name	%	Crosses the placenta with ease	Activator of complements	On phagocytic cells, it binds to the Fc receptor	The half-life / days
IgG 1	66	Yes (1.47)*	Second-highest	A strong bond	21
IgG 2	23	(0.8)*	It is considered to be the third and it is considered the highest	Affinity but very low	21
IgG 3	7	Yes (1.17)*	Highest	A strong bond	7
IgG 4	4	Yes (1.15)*	No	Affinities in the middle	21

The affinity of IgG for Fc receptors on phagocytic cells varies depending on the species and class of the antibody. The distinct biological features of each of the four IgG classes are influenced by hinge region structure. Despite the fact that their Fc regions are almost identical, sometimes they differ in their structure in regions called hinges in addition to their regions slightly different because they have distinct but conflicting features in some of the subsections found in IgG (Collins *et al.* 2013).

According to this concept, IgG3 originates in the early stages within the reaction. Despite its low affinity, IgG3 permits IgG-mediated defenses to collaborate with IgM-mediated defenses in removing foreign antigens. IgG1 and IgG2 with a greater affinity are created as a result. The intensity from the following so-called inflammatory processes is determined through the so-called relative equilibrium associated with these

subclasses, or in other words, immune complexes can develop. Eventually if the antigen persists, a process is triggered by which the so-called high-affinity IgG4 is synthesized, to do helps control FcR-mediated activities and thus reduce inflammation. It's possible that the differential capacity of various IgG subclasses explain their work as they carry out a repair process that is and explain why the vaccine given by donors after leaving the body is weak, as it performs a number of reactions within the antibodies (Gao *et al.* 2004).

- **Immunoglobulin M (IgM)**

IgM is one of a large number of species that are similar to so-called antibodies because they are generally produced by cells in vertebrates. IgM is one of the largest types of antibodies and is one of the body's first line of defense in case of exposure to the virus (Alberts *et al.* 2002). The spleen, which houses the plasma responsible for its synthesis (Williams and O'Connell 2008, Capolunghi *et al.* 2013).

IgM was discovered in the year one thousand nine hundred and thirty-seven, when scientists vaccinated strong animals in the first place, the horses were hyper-immune with pneumococci of polysaccharide character, which made them generate antibodies with a molecular weight estimated at nine hundred and ninety thousand daltons, substantially greater than the ordinary rabbit –globulin (Kabat 1939).

Due to its large size, the new antibody was initially named -macroglobulin, and later IgM-M in the following designation. The domains of normal immunoglobulin V vary greatly, reflecting their protective role against a wide range of microorganisms considered infectious, and this variability has hampered extensive structural research on IgM (Waldenstrom 1943).

Methods for the development of IgM-producing tumors in animals were discovered in the 1960s, which in turn led to a source of homologous IgM including IgM. It has recently been used in tissue culture production of immunoglobulin genes modified to

create IgM with micro-mutations, allowing researchers to reveal the molecular requirements for desired properties (Potter 2007).

Light chains and heavy chains are two types of immunoglobulins. Chain of the lights (λ or κ). It consists of two hundred and twenty proteins of the so-called amino acids, in a variable range, VL (A piece of about one hundred and ten of these amino acids), and a constant domain, CL (A piece of about one hundred and ten of these amino acids). The heavy chain of IgM is a type of protein of 576 amino acids with within a variable domain, there are four multiple but separate domains that are stable C μ (1, 2, 3, 4, Each $\sim$ one hundred and ten within the number of amino acids. Five asparagine residues in the heavy chain contain oligosaccharides (Figure 2.7) and (Figure 2.8) (Monica *et al.* 1995).

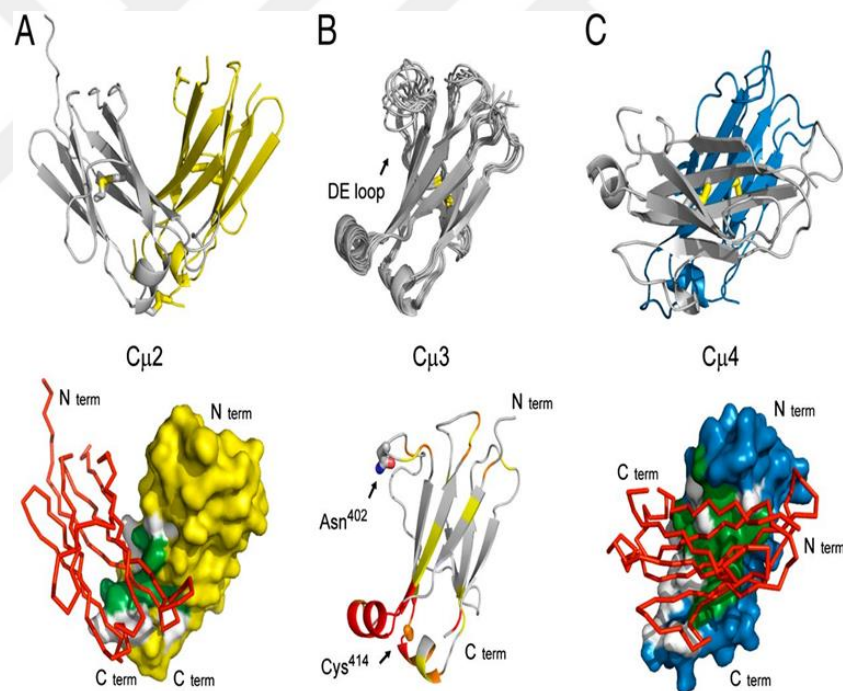


Figure 2.7 Individual Fc domain structures in IgM (Müller *et al.* 2013)

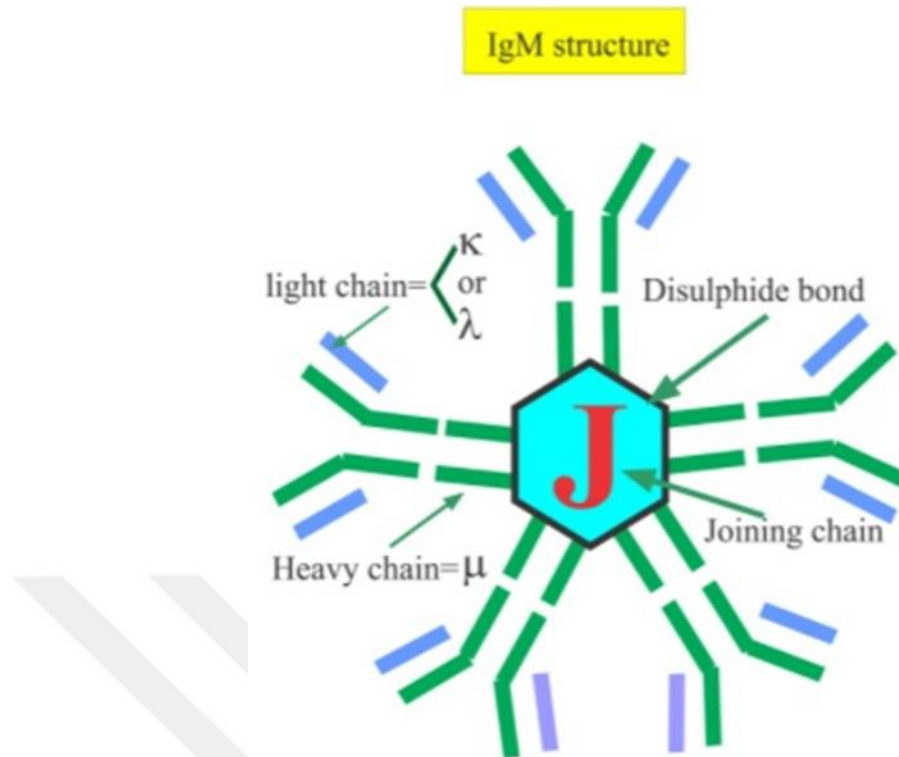


Figure 2.8 IgM structure (Sowers *et al.* 2022)

As indicated in figure 2.5, the variable modulator, L, has many variable domains (VH or VL). In another region, it is considered constant C μ (1, 2, 3,4) (Heyman and Shulman 2016). A called cysteine, which in turn mediates a process that makes connections called disulfide, is displayed between a number of chains that are used as red arrowheads, so it produces through it an arrow head that has a double character of red color in order to take place in it the bonded to cysteine disulfide (Heyman and Shulman 2016).

Based on sedimentation velocity and appearance in electron micrographs, IgM, which is a polymer with five monomers (μ L) 2, has other disulfide bonds located between other domains called C 3 and between sections called tail (Milstein *et al.* 1975). The existence of a third protein, the J chain, is also established in pentameric IgM. The J-string was discovered because it is an ingredient which may be bonded in several ways, but are covalently covalent within the so-called IgA polymers and within the IgM polymers as well (Halpern and Koshland 1970, Mestecky *et al.* 1971). The J-chain is an acid-shaped protein as it is small in size, consisting of one hundred and thirty-seven types of amino

acids. Also, this chain is in the process of forming a number of disulfide bonds with the so-called cysteine when the tail is cut to join two chains (Frutiger *et al.* 1992). J chain was thought to be vital in the formation of polymeric immunoglobulins, and it is true that polymerization of IgA is substantially (but not completely) dependent on J chain (Johansen *et al.* 2000, Sorensen *et al.* 2000). In the absence of the J chain, polymeric IgM develops effectively (Fazel 1997). Pentamer is the most common type of human and mouse IgM (Schwager and Hadji-Azmi 1984).

Through the use of several in the expression of the so-called DNA, which consists of a hexagonal type, which is an important type of IgM types found in animals that are generated through many conditions in order to synthesize it in cells whose characteristics are that it lacks the preparation of the J chain, either Through the process of manufacturing so-called intracellular IgM of the J series (Cataneo and Neuberger 1987). Or it may be done by a process called IgM generation without the so-called cysteine in the heavy chain tail (Collins *et al.* 2002).

Sometimes the availability of this cysteine may differ in order to form a number of bonds called disulfide among a number of chains that are considered heavy as heavy chains in a certain way, but it is large within the heavy chains and light. In another case, which may be called a heavy chain, the bonds of the only quality between the chains are cysteine as it is in the hinge, which may be connected to a number of chains, each chain may be through only one other chain (Davis *et al.* 1989).

The role of cysteine in the C μ 3 domain is more complicated. Five-shaped IgM motifs. Within these models, each chain is expected to be connected to two additional chains. However, none of the motifs is sufficiently well explained for the polymeric IgM structure. Disulfide link between the C μ 2 domains, for example, is predicted to be needed for the creation of disulfide-bonded polymeric IgM. It is possible to work on the formation of the so-called polymeric IgM that binds to the sulfide even if one of the cysteine number three is not present. These studies reveal that in models where each chain interacts with just two other chains, some chemicals interact with only two other molecules. On the other hand, it is indicated that there are three types of cysteine for the

linking coin between these chains that the chains can link three other chains. In a similar vein (Wiersma and Shulman 1995).

- **COVID-19 antibodies**

Infection with the SARS-CoV-2 virus causes a type of immune response, whether humoral or otherwise, which leads to a process through which work is done to develop a type of antibody whose action is against antigens specific to a type of virus, such as a type called (N) and another type called spike (S). Among these antibodies are the so-called S1 subunit and spike receptor binding region (RBD). The so-called antibody test can detect its presence in the serum present in the blood within days after its entry and may continue for weeks after the infection is completed and may be severe at this time. However, it may not be appropriate to use this test to detect severe SARS-CoV-2 infection. But this test can help public health scientists understand the epidemiology, more specifically SARS-CoV-2, because it identifies the person infected in advance. The immune correlates used for protection are sometimes not fully understood, but research suggests that antibody production after infection may lead to immunity up to six months into the future (LumLey *et al.* 2020, Hall *et al.* 2021). However, it's unclear how SARS-CoV-2 variations could affect protection against infection in the future (Abdool *et al.* 2021). Antibodies to SARS-CoV-2 in infected patients' serum might be a valid target for COVID-19 diagnosis. During viral infection, the body creates particular antibodies (IgM and IgG) (Racine *et al.* 2007). After that, IgM antibody was found in the serum of SARS-CoV patients. IgG antibodies were discovered after infection for 8 days (Wan *et al.* 2003, Lee *et al.* 2010) and the infection was observed for 3-6 days. Anti-SARS-CoV-2 Because they belong to the same viral family and have a similar surface shape, the body's manufacturing process may be comparable to that of SARS-CoV-2 as (Figure 2.9) (Wrapp *et al.* 2020).

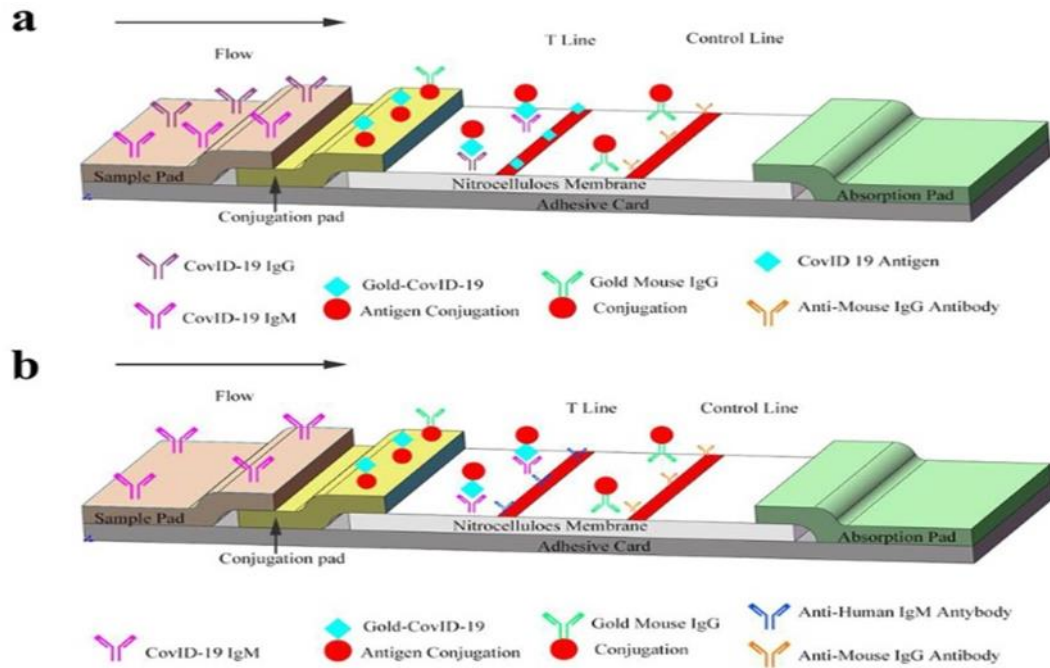


Figure 2.9 COVID-19 antibodies (Wrapp *et al.* 2020)

Following SARS-CoV-2 infection, virtually all immunocompetent people have an adaptive immune response, the process of eliciting the type of immune responses, the humoral type, in addition to the cellular ones, which are considered antiviral, is carried out through the mediation of humoral and T-immunity (Grifoni *et al.* 2020, Robbiani *et al.* 2020, Suthar *et al.* 2020). The immunological response to SARS-CoV-2 is fast becoming more understood. Antibodies against type S and type N proteins are part of the humoral response in humans. S1 and S2 are two subunits of the S protein. The RBD, which facilitates viral binding to susceptible cells, is found in the S1 subunit. Neutralizing antibodies mostly target RBD. Antibodies against S and its components, including IgM, IgG, and IgA, It will be found inside the serum within one to three weeks after the injury occurred (Wolfel *et al.* 2020, Qu *et al.* 2020). IgM and IgG antibodies can form almost simultaneously (Qu *et al.* 2020) but, IgM (and IgA) antibodies degrade faster than IgG antibodies (Qu *et al.* 2020, Iyer *et al.* 2020). Although the clinically significant importance of detecting serum IgA in SARS-CoV-2 infection is uncertain, the secretion of IgA type performs a process by which surfaces called mucous are protected from infection by neutralizing the viruses within respiratory system such as SARS-CoV-2 (Matuchansky *et al.* 2021).

IgG antibodies, and more specifically the type S and type N of the so-called proteins, generally persist for a period of up to months after an infection has occurred in the body, but the exact age may be uncertain (Dan *et al.* 2020). Seroreversion has been recorded in people with moderate illness (Milani *et al.* 2020). People infected with either one or more diseases have a stronger antibody response, with higher titer, longer persistence of IgM, IgG, and IgA antibodies (Milani *et al.* 2020, Rijkers *et al.* 2020).

Antibody persistence varies depending on the test (Choe *et al.* 2020), while other research has found that about five percent to ten percent of people do not develop antibodies to the so-called IgG, which are detectable by scientists after the body has been infected with it (Petersen *et al.* 2020, Kaufman *et al.* 2020). Although neutralizing antibodies may not be found in individuals with moderate or asymptomatic illness (Payne *et al.* 2020), Although certain antibodies are lost after a certain period of time due to persistence of a type of memory cell called B, the body appears to mount the humoral immune response and remain preserved within it (Ogega *et al.* 2020). RBD is the main target of SARS-CoV-2 antibody, which limits SARS-CoV-2 virus replication in vitro (Suthar *et al.* 2020, Robbiani *et al.* 2020).

In order to better understand the kinetic process in the antibody, as well as the longevity of responses to so-called humoral immunity, and in order to correlate a number of levels of antibodies that may be bound in addition to neutralizing antibodies, as well as serological alternatives for immune protection. Tests are done for antibodies, whose conditions are that they are quantitatively binding, and a process can be carried out in order to track them and to calibrate them, but this is according to the so-called international standard (Gundlapalli *et al.* 2020).

In a research conducted by scientists collectively in Britain, it was done for the recipients, and the follow-up was done for people who had a type of antibody to SARS-CoV-2, as well as without it, for an average of two hundred days, after they were tested for the detection of antibodies positive, and the rate that was detected for the rate of modified and recurrent infection was eleven percent in the event that it scientists have

carried out an operation to compare it with people who had previously been confirmed to have been infected with the SARS-type virus (LumLey *et al.* 2020).

Discover a major analysis of commercial lab results associated with medical claims and electronic medical records data conducted in the USA that people with antibodies had a 90 percent lower risk of infection than people without antibodies (Harvey *et al.* 2021), Over a period of roughly six weeks, a large number of participants who were infected with the vaccine had an eighty-two percent lower chance of contracting SARS-CoV-2, according to another study of US military recruits (Letizia *et al.* 2020).

Experiments on nonhuman primates back up the findings in humans. An experimentally infected rhesus macaque, which had developed an immune response as well as a linear and cellular type, was protected from infection if it reoccurred after 35 day (Chandrashekar *et al.* 2020).

A number of other studies revealed that pure IgG in animals infected with SARS-CoV-2 macaques successfully protected naive macaques if infected, and protective titers may be determined using a number of potentially binding and neutralizing antibodies. It was also associated with high neutralising and anti-S-binding antibody titers utilised for more efficient infection prevention according to results from two vaccination studies (Feng *et al.* 2021, Gilbert *et al.* 2021).

A number of these findings, in humans and in primates considered non-human, indicate that an infection with SARS-CoV-2 generates antibodies that may provide adequate protection but against infection otherwise transmitted by SARS-CoV-2. It is also still in protection and its extent and duration is unknown. With endemic seasonal coronaviruses, life-long immunity has not been observed (Edridge *et al.* 2020).

A number of researchers have discovered a type of antibody that can be detected in people who have been infected with the SARS-CoV-1 and MERS-CoV virus, eighteen to twenty-four months after infection (Wu *et al.* 2007, Alshukairi *et al.* 2016), while a

small investigation of MERS-CoV-infected individuals found neutralizing antibody for 34 months (Payne *et al.* 2016). It is also not known if these patients have another infection with the SARS-CoV-2 virus because they may spread the virus to other people or if there is a change in the clinical spectrum from what the original disease before that (Payne *et al.* 2016).

2.7 Vaccination Against COVID-19

The RBD type of SARS-S CoV-2 protein may interact with a site of a number of ACE-2 receptors in constituent cells within the human body, and this is the first step that allows the process to lead to virus entry. But it is possible to avoid infection and disease that are caused by SARS-CoV-2 via preventing SARS-CoV-2 from attaching to ACE-2 receptors in the respiratory system in the human body (Cromer *et al.* 2021).

The interaction of SARS-S CoV-2's protein with vaccine research has focused on a number of your ACE-2 receptor sites. However, vaccine candidates who have achieved EUA or FDA type approval, or who are in the late phases of research, aim to neutralize antibodies against type S protein or type RBD (Poland *et al.* 2020). Some data that were taken from two phases from a number of experiments that are considered to be effective in mRNA vaccine and in group studies indicated that it has an efficacy of up to ninety-five percent after a series of two doses of vaccination (Baden *et al.* 2020, Polack *et al.* 2020, Thompson *et al.* 2021).

It is not known whether infection provides the same level of protection as immunization. The process of causing SARS-CoV-2 infection in order to develop antibodies against viral proteins like type N and type S proteins. Antibody testing is affected by vaccine-induced antibody formation. Prior to the release of the vaccine, to determine prior SARS-CoV-2 exposure, a SARS-CoV-2 antibody test that detects any of the N, S, or RBD antibodies might be employed. Serological screening for a specific target antigen vaccination (type S and S subunits, including RBD) might result in a positive result, but not for other non-target proteins (Krammer *et al.* 2021, Fotis *et al.* 2021).

When performing the process of interpreting the results of antibody testing, the process of taking into account first the history of vaccination and then the presence of previous infection with SARS-CoV-2 should be taken into account. These tests must have anti-SARS-CoV-2 antibodies and then evaluate for IgM and evaluate for IgG in one of the two viral proteins, type S or type N. A positive type test for S IgM and/or IgG may imply past infection and then COVID-19 vaccination is a vaccine that encodes a spike protein or part of it. In the case of a person who has a history of this disease by doing the vaccination against COVID-19, this external test code must be used to specifically assess the type of anti-N IgM / IgG in order to check the symptoms of the previous infection. If antibody tests for specific proteins are sufficiently established, when adopting immunisation, checking for antibodies suggesting past illness might be a beneficial public health technique (or antigenic) programmes (Krammer *et al.* 2021, Fotis *et al.* 2021).

Although antigen-specific antibodies can be used in antibody tests, the antibodies generated may react in response to mutually distinct proteins and thus may not provide sufficient information on the presence of antigen-specific antibodies. It is not always clear whether antigens frequently used in the FDA EUA antibody test detect only antibodies to those antigens or to other antigens. Although current EUA indications do not rule out these tests in vaccinated patients, none of the tests that are now permitted have been approved to perform a process to determine immunity and determine the level of protection in recipients of the vaccine used for COVID-19 (Krammer *et al.* 2021, Fotis *et al.* 2021). SARS-CoV-2-specific CD4⁺ and CD8⁺ T-cell responses, as well as neutralizing antibodies, are elicited by COVID-19 vaccinations. T-cell responses may have a role in host protection against SARS-CoV-2 variations, despite the fact that antibodies generated by existing COVID-19 mRNA vaccines have exhibited decreased neutralizing activity against SARS-CoV-2 variants as (Figure 2.10) (Noh *et al.* 2021).

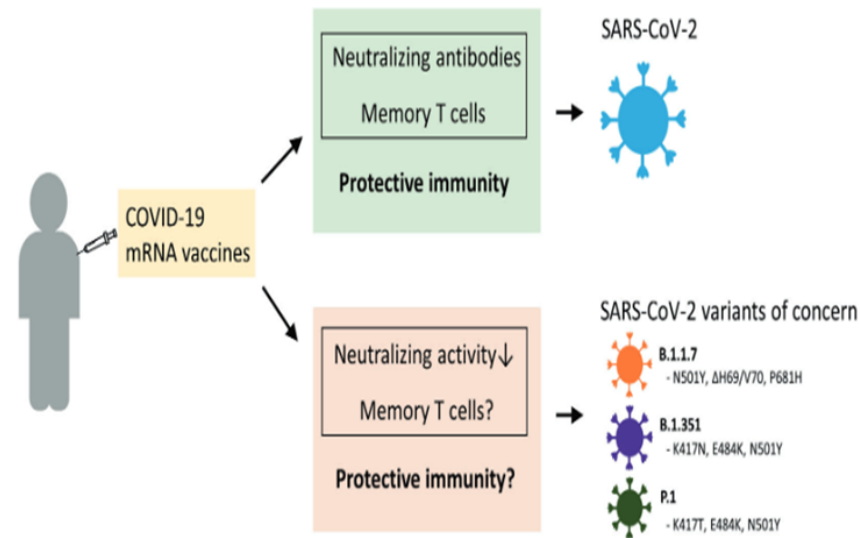


Figure 2.10 SARS-CoV-2-specific T-cell responses (Noh *et al.* 2021)

Comirnaty vaccine from Pfizer-BioNtech - the first vaccine to be licensed in the European Union: on December 21, 2020 by the European Medicines Agency (EMA).

- Spikevax Vaccine (Moderna): On January 6, 2021, licensed by EMA.
- Vaxzevria Vaccine (AstraZeneca): On January 29, 2021, licensed by EMA.
- Janssen Vaccine (Johnson and Johnson): On March 11, 2021, licensed by EMA.
- Novavax Vaccine (Novavax): On December 20, 2021, licensed by EMA.

2.8 Public Health and Clinical Practice Considerations

According to growing data, the existence after infection, the production of antibodies provides some protection against reinfection. Here are several examples of evidence: (Hall *et al.* 2021). A number of studies have found that an outbreak of antibodies detectable before to infection is related with a reduced likelihood of infection (Letizia *et al.* 2020, Addetia *et al.* 2020, Pray *et al.* 2020, Letizia *et al.* 2021, To *et al.* 2020). The

pre-existing detectable antibody corresponds to a lower frequency of infection, according to data from epidemiological studies (Abdool *et al.* 2021, Letizia *et al.* 2020, Addetia *et al.* 2020, Pray *et al.* 2020, Letizia *et al.* 2021, Grifoni *et al.* 2020) viral neutralization demonstrated with serum from people who have been infected (Robbiani *et al.* 2020, Suthar *et al.* 2020). Many researches indicate that vaccination, which is produced by the body through the formation of antibodies, can reduce the incidence of disease (Suthar *et al.* 2020, Baden *et al.* 2020, Polack *et al.* 2020). The administration of monoclonal antibodies has been linked to a reduction in disease severity and even to the prevention of exogenous infection (Cohen *et al.* 2021). While it is not known to what extent and for how long to protect people who have antibodies that may be detected for reinfection with SARS-CoV-2, or what concentrations of antibodies may be required to provide this protection, cohort studies show a reduction of between eighty percent to ninety percent in the event of infection. In antibody-positive individuals, for at least six months following infection (Hall *et al.* 2021, Abdool Karim *et al.* 2021, LumLey *et al.* 2020).

A number of long-term studies carried out in order to follow-up through the patient currently being conducted to analyse antibody levels before and after vaccination or infection in order to determine the association between a variety of reactions that may fall below a particular threshold and vaccine failure or reinfection. The link between antibodies and protection against reinfection is expected to be elucidated in these long-term patient follow-up investigations. The type of adaptive immunity mediated by T cells after infection, although not fully understood, is considered to have a major role in the future protection against infection by the virus, especially the emerging SARS-CoV-2 (Stephens 2020).

It is currently unknown whether and how the process of viral evolution and the formation of a number of novel SARS-CoV-2 variants may impact so-called infection immunity. According to a study done in the United Kingdom, the risk of reinfection with the Delta variant was greater than the risk of reinfection with the Alpha variant with the Alpha variant among patients who had original infections >180 days previous to reinfection (Public Health England 2021).

2.9 The Current State of Global Antibody Testing

Although N-type protein is the type of immune protein most observed, S-type protein may be necessary for the virus to enter the body and into cells and may also be present in one way or another on the surface of the virus. These antigens use the full-length S1 and S2 proteins, in part in the S1 domain or in the RBD type, in an antibody assay. Since the N-type is more conserved than the S-type across coronaviruses, and the RBD is then more conserved in the S-type than in the S1-type or S-type at full length, the target of this protein may dictate cross-reactivity and specificity. Antigen target selection may aid in addressing several components of the immune response. Antibodies against RBD are closely associated with functional features such as the virus's capacity to neutralize it (Suthar *et al.* 2020). The differential reaction of S- and N-specific antibodies can be used in sero-research to identify prior infection from vaccination, especially in vaccines that produce antibodies against the S protein (Cohen *et al.* 2021, Harvey *et al.* 2021, Hall *et al.* 2021).

Various assays may be used to evaluate different aspects of the adaptive immune response and antibody activity. The tests are classified into two types: binding antibodies and neutralising antibodies. Detection of antibody binding: These experiments use pure SARS-CoV-2 proteins rather than live virus and may be performed in facilities with lower biosafety requirements (e.g., BSL-2). Specific reagents can be used to discriminate between different antibody types, such as IgG, IgM, and IgA. Following infection, IgM and IgG antibodies to SARS-CoV-2 may be detected. While IgM is most effective in identifying recent infections, it generally becomes undetectable within a few weeks to months; on the other hand, IgG can be found for extended periods of time. Immunoglobulin type A is required for mucosal immunity and may be found in a variety of mucosal secretions, including saliva, albeit its presence in this ambiguous situation is critical. Many antibody-binding assays, depending on their complexity, can be completed in less than thirty minutes in the field yet require several hours in the laboratory (Galipeau *et al.* 2020).

Individuals who are affected receive timely and efficient treatment. Because COVID-19 and SARS-CoV are related, their aim and symptoms of infection are quite similar. As a result, M globulin (IgM) will be the first line of protection against COVID-19 vaccination in 1-6 days and fade in 7-14 days (Poland 2020). After 8 days, high-affinity IgG, which is required for long-term protection and immunological memory, develops (Lee *et al.* 2020). Furthermore, early IgM can defend different organs in the human body against COVID-19 invasion, and the presence of IgM indicates recent COVID-19 exposure. The presence of IgG in the blood indicates that the patient was exposed to COVID-19 and that the body has developed strong immunity to it over time. As a result, rapid IgM and IgG detection can provide information on the date and stage of infection, as well as references for COVID-19 broad screening, diagnosis, and treatment, as well as appropriate protective references for health-care workers. As a result, every COVID-19 patient who has positive IgM and IgM or IgG antibodies may be categorised as confirmed. IgM/IgG detection, on the other hand, is as diversified as IgM/IgG combination detection (Jacofsky *et al.* 2020). Because the diffusion rate of AuNPs is size dependent, the smallest particle size and narrowest size distribution were preferred for practically all applications. Furthermore, smaller particles have a larger specific surface area, allowing for greater protein coupling, as (Figure 2.11) (Liu *et al.* 2020).

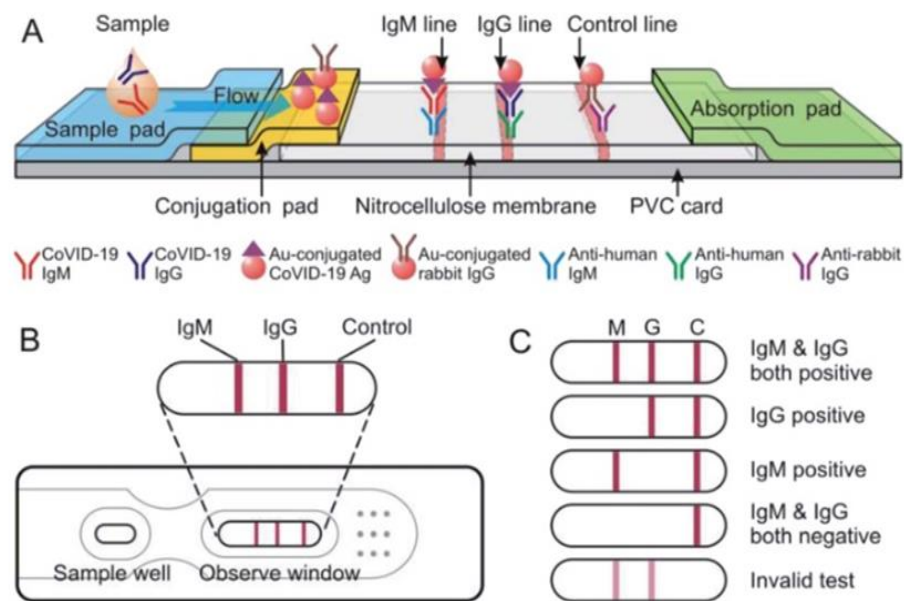


Figure 2.11 The COVID-19 IgM/IgG fast test strip is shown schematically: (A) COVID-19 IgM/IgG fast test strip preparation and premise, (B) Products

that have been assembled, (C) A diagram illustrating the test findings (Liu *et al.* 2020)

Point of care (POC) tests are diagnostic tests that can offer results in minutes rather than hours and are done at or near a sample collecting location. POC antibody tests are typically lateral flow devices that detect IgG, IgM, or total antibody in whole blood utilising the body's fingertips. The process of laboratory-type tests uses lateral flow methods, ELISA, or chemiluminescence tests in order to detect antibodies in serum as well as in plasma, whole blood and dried blood spots, which may need qualified laboratory scientists and specific apparatus in some cases. Some tests detect total Ig antibodies, while others detect IgG type and IgM type completely and separately. While most tests detect antibodies to a type of protein called S or another type called N, other so-called tests detect antibodies to each type of immunoglobulin. Which evaluates the process of tests for neutralizing antibodies as it has a functional ability of these antibodies and works to prevent viral infection of the type called SARS-CoV-2 in the laboratory. When incubated with serum or plasma, these assays identify viral growth suppression in cell culture (McCance *et al.* 2022).

COVID-19 IgG/IgM tests detect antibodies in response to COVID-19 vaccines, according to ongoing research. The performance of the CTK Rapid COVID-19 IgG/IgM Test in detecting antibody responses after COVID-19 vaccination was evaluated in a group of 20 people who had no detectable levels of SARS-CoV-2 antibody before vaccination. The COVID-19 IgG/IgM fast test yielded a positive result in all 20 individuals (100%) 9 to 16 days after immunization, indicating the test's great sensitivity (Lewis *et al.* 2020) as (Table 2.2).

Table 2.2 Preliminary data for post-vaccine COVID-19 IgG/IgM test (Lewis *et al.* 2020)

Days after vaccination	Positive for IgG	Positive for IgM	Positive for IgG or IgM
7	0% (0/20)	0% (0/20)	0% (0/20)
14	85% (17/20)	70% (14/20)	95% (19/ 20)
17	100% (20/20)	85% (17/20)	100% (20/20)

3. MATERIALS AND METHODS

3.1 Material

3.1.1 Sample collection

As a result of the examination, 120 blood samples (serum), including 30 COVID-19 patients having asthma and 30 COVID-19 patients having heart diseases, and 30 patients diagnosed with COVID-19 only, were taken from cases diagnosed with the COVID-19 from Baghdad Teaching hospitals, Baghdad, Iraq, compared with 30 healthy control groups. Samples were checked for COVID-10 antigen by real-time PCR at the hospitals before. The demographical and clinical information of those individuals were also collected. The blood samples were taken to be delivered to the laboratory via a cold chain, and the serum was collected by clotted blood samples' centrifugation and stored at -20°C until tested (as recommended by the World Health Organization). The study's participants were divided into groups (a group without chronic disease, a group with asthma, and a group with heart disease). In this study, the distributions of antibodies (percentage of their IgG and IgM) according to target groups and disease severity/recovery scores were considered.

3.1.2 SARS-COV-2 IgM and IgG ELISA kit

All blood (serum) samples were tested for the presence of SARS-COV-2 IgM and IgG antibodies using a commercial ELISA kit (Sunlong Biotech.Co. Ltd., China).

Antibodies' levels peak after only 3-4 weeks. Therefore, it is recommended that the test be done up to 4 weeks after the last symptoms of infection have passed, in order to increase the chance of detecting the antibodies. If only mild symptoms were present 3-4 weeks ago, there may not be enough antibodies to detect them.

3.2 Method

3.2.1 Sample preparation

All study groups that established were included 30 COVID-19 patients only, 30 COVID-19 patients having asthma and 30 COVID-19 patients with heart disease, and the socio-demographical and clinical information was collected for individuals who had COVID-19 only or including those with chronic diseases (asthma and heart disease). One hundred-twenty blood samples were taken and delivered to the laboratory and then stored at -20°C until work began as all samples were tested for the detection of IgG and IgM using the commercial ELISA kits (Sunlong Biotech.Co.,Ltd. China). Finally, the IgM and IgG antibodies levels for all groups were calculated.

3.2.2 SARS-COV-2 IgM and IgG ELISA

ELISA was performed according to kit's procedure:

1. The ELISA kit was taken out of the refrigerator 20 minutes before, and the test was performed when it was equilibrated to room temperature, then the concentrated wash solution was diluted with double distilled water (1:25) as the manufacturer's instruction; this solution was used to wash the ELISA plate from the undesirable and unattached materials
2. The pre-coated ELISA plate should be washed with the diluted washing buffer (1X) before adding any of the standard dilution series or samples, as per the manufacturer's instructions.
3. One mL of standard diluent buffer was added to a lyophilized standard SARS-CoV-2 Spike Protein S1 IgM and IgG antibodies and rolled for 10 minutes at a roller mixer until all samples were completely dissolved. The standard dilutions series were prepared as per the instructions of the manufacturer (200, 100, 50, 25, 12.5,

6.25, 3.12 units/mL) through preparing 7 clean tubes were taken and numbered from 1 to 7, then 300 μ L of standard sample diluent buffer were added into each tube. A volume of 300 μ L from the diluted standard solution was pipetted to the first tube and mixed well, and then this step was repeated up to the 6th tube. The 7th tube of the standard dilution series was a negative control (standard dilution buffer only).

4. A volume of 50 μ L of each standard dilution series and samples were added to the anti-SARS-CoV-2 IgM and IgG antibodies plates' wells, then the two plates were incubated for 30 minutes at 37 C° after covering with the sticky cover, then the two plates were washed for three times with the diluted washing buffer (1X) to remove all the unwanted and unattached antibodies from the wells.
5. With reference to the required amount, the antibody diluent buffer used to dilute the biotinylated concentrated antibody (1:100) to form the biotin-treated antibody liquid. The preparation was done 30 minutes in advance.
6. A volume of HRP-labeled Antibody that was prepared previously (50 μ L) of each of anti-SARS-COV-2 IgM and IgG antibodies was added to the proper plate and incubated for 30 minutes at 37 C, then the two plates were washed 5 times with the diluted washing buffer (1X).
7. A volume of TMB Substrate (50 μ L) was added to each well for each plate, then covered with the sticky cover and incubated at room temperature for 10 minutes.
8. A volume of stop solution (50 μ L) was added to each well for each plate, the color changed from blue to yellow, then the absorbance of the solutions in the ELISA plate wells was measured using an ELISA reader instrument with a wavelength 450 nm.
9. The results were calculated according to the standard curve of each anti-SARS-COV-2 IgM and IgG antibodies ELISA kit as shown in Figure 3.1 and Figure 3.2.

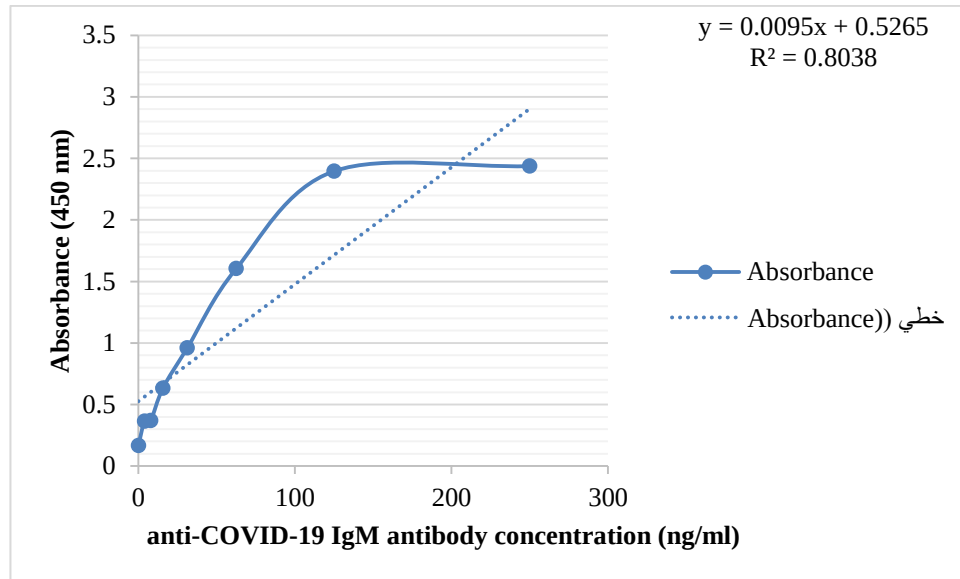


Figure 3.1 Standard cuve of anti-SARS COV-2 IgM antibody

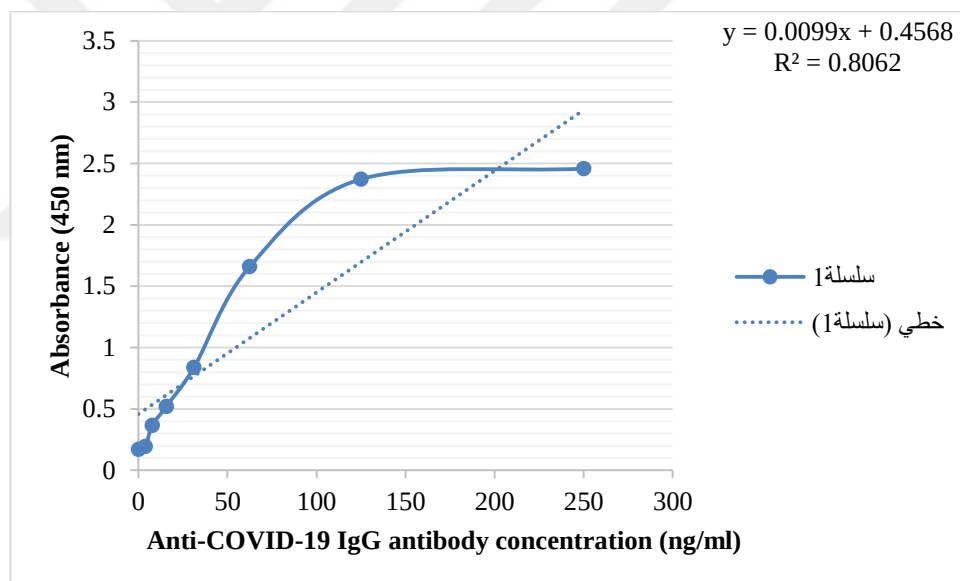


Figure 3.2 Standard curve if anti-SARS COV-2 IgG antibody

4. RESULTS AND DISCUSSION

4.1 Results

The results in Table 4.1 showed the numbers and percentages of samples collected from COVID-19 patients with asthma, heart disease, and without chronic disease (COVID-19 only) compared to the control group, distributed by gender. The results showed that the number of participants was 120, most of whom were females with a percentage of 69 (57.5%) and males with a percentage of 51 (42.5%), each group has 30 patients, and the results of COVID-19 patients having heart disease and COVID-19 patients' groups also showed that the infection rate among males was higher than females by 53.3% and 46.7% respectively. As for COVID-19 patients having asthma, the results showed that females were higher than males by 83.3% and 16.7%, respectively. As for the control group, the percentage of females was 53.3% and 46.7% for males.

Table 4.1 The frequency and frequency percentage of the studied groups according the associated diseases with COVID-19

Groups	Gender frequency (%)			Probability
	Total	Males	Females	
Heart disease infected with COVID-19	30 (25.0)	16 (53.3)	14 (46.7)	P < 0.05
COVID-19 with no chronic disease	30 (25.0)	16 (53.3)	14 (46.7)	
Asthma infected with COVID-19	30 (25.0)	5 (16.7)	25 (83.3)	
Control(Healthy group)	30 (25.0)	14 (46.7)	16 (53.3)	
Total	120 (100.0)	51 (42.5)	69 (57.5)	

Table 4.2 and Figure 4.1 showed the mean and standard deviation of age according to the studied groups, The age mean was 70.17 ± 14.16 for COVID-19 patients having asthma, 66.46 ± 11.54 for COVID-19 patients having heart disease, 54.82 ± 11.32 for COVID-19 patients without chronic diseases compared to 55.18 ± 11.61 of a control group.

Table 4.2 The age means of studied groups

Groups	Age mean $\pm$ SD (Years)
Heart disease infected with COVID-19	66.46 $\pm$ 11.54
COVID-19 with no chronic disease	54.82 $\pm$ 11.32
Asthma infected with COVID-19	70.17 $\pm$ 14.16
Control (Healthy group)	55.18 $\pm$ 11.61

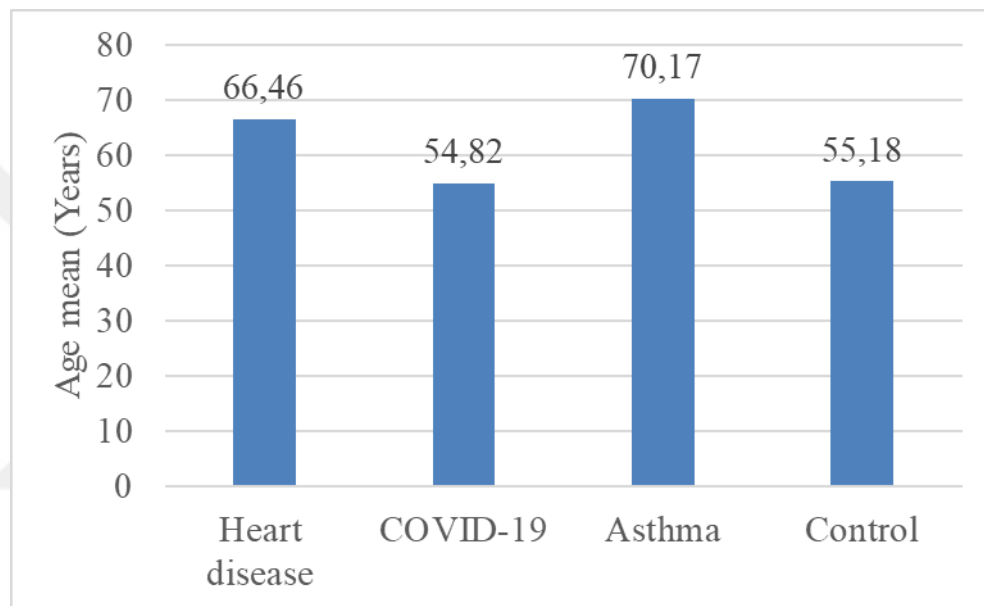


Figure 4.1 The age means of studied groups

The results in Table 4.3 and Figure 4.2 showed the mean and standard deviation of the studied groups' age distributed by gender. In COVID-19 patients having the heart disease group, the age means of males was 60.17 ± 2.14 and 74.0 ± 14.02 years for females, Also in COVID-19 patients having the asthma group, the age means for males was 71.0 ± 12.44 and 70.17 ± 15.83 years for females, while in COVID-19 patients without any chronic disease group, the age means was 60.17 ± 10.09 for males and 48.40 ± 94 for females, whereas the age means in the control group was 54.75 ± 17.26 for males and 55.56 ± 2.92 years for females.

Table 4.3 The age means of studied groups according to gender

Groups	Age mean $\pm$ SD (Years)	
	Males	Females
Heart disease infected with COVID-19	60.17 $\pm$ 2.14	74.0 $\pm$ 14.02
COVID-19 with no chronic disease	60.17 $\pm$ 10.09	48.40 $\pm$ 9.94
Asthma infected with COVID-19	71.0 $\pm$ 12.44	70.17 $\pm$ 15.83
Control (Healthy group)	54.75 $\pm$ 17.26	55.56 $\pm$ 2.92

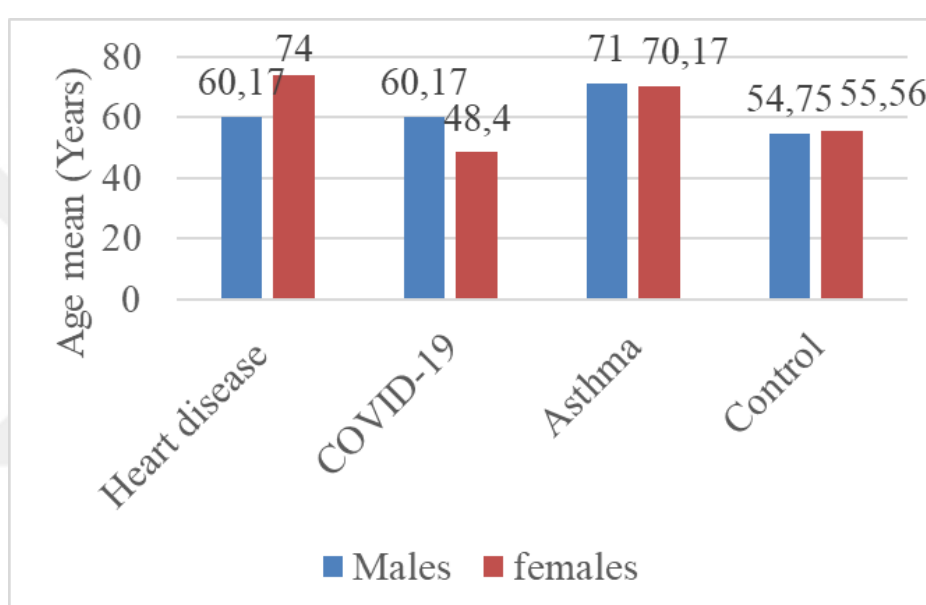


Figure 4.2 The age means of studied groups according to gender

The results in Table 4.4 showed the mean level $\pm$ SD (ng/ mL) of anti-SARS-COV-2 IgM antibodies among the studied groups. The results showed a significantly increased level of anti-SARS-COV-2 IgM antibodies ($P < 0.05$) on the tenth day compared to the twentieth day for all COVID-19 patients group. Such the results of anti-SARS-COV-2 IgM antibodies level mean for both the tenth and the twentieth days showed a significantly increased level ($P < 0.05$) in all COVID-19 patients groups compared to the healthy control group (197.52 $\pm$ 24.49 ng/mL for COVID-19 patients having heart disease group, 175.95 $\pm$ 19.62 ng/mL for COVID-19 patients having asthma group and 157.47 $\pm$ 74.0 ng/mL for COVID-19 patients without any chronic diseases group vs. 8.90 $\pm$ 1.31 ng/mL for a healthy control group during the 10th day and 133.22 $\pm$ 71.56

for ng/mL for COVID-19 patients having heart disease group, 97.15 ± 72.40 ng/mL for COVID-19 patients having asthma group, and 110.29 ± 77.93 ng/mL for COVID-19 patients without any chronic diseases group vs. 8.90 ± 1.31 ng/mL for a healthy control group during the 20th day.

Table 4.4 Sample distribution by level of COVID-19 antibody IgM $\pm$ SD (ng/mL)

Groups	Anti-COVID-19 IgM antibody level mean ± SD (ng/mL)		Probability
	at 10 th day	at 20 th day	
Heart disease infected with COVID-19	197.52 ± 24.49 ^a	133.22 ± 71.56 ^a	P < 0.05
COVID-19 with no chronic disease	157.47 ± 74.0 ^a	110.29 ± 77.93 ^a	P < 0.05
Asthma infected with COVID-19	175.95 ± 19.62 ^a	97.15 ± 72.40 ^a	P < 0.05
Control (Healthy group)	8.90 ± 1.31 ^b		
Significant (P < 0.05) compared to control group			

Also, the results of Table 4.5 showed the mean level $\pm$ SD of anti-SARS-COV-2 IgM antibodies distribution (ng/mL) on both the tenth and twentieth days according to gender. There were various non-significantly differences in the anti-SARS-COV-2 IgM antibodies levels (ng/mL) between the gender of each group. While there was a significantly increased level ($P < 0.05$) between all the male COVID-19 patients' groups and the control group, there was a non-significant increased level ($P > 0.05$) in COVID-19 patients having the asthma group compared to both COVID-19 patients having heart disease and COVID-19 patients without any chronic diseases' groups (204.47 ± 47.22 ng/mL vs. 191.91 ± 32.17 and 167.76 ± 78.03 ng/mL, respectively). In contrast, there was a significantly increased level of anti-SARS-COV-2 IgM antibodies levels (ng/mL) in female COVID-19 patients having heart disease compared to the female COVID-19 patients having asthma (204.24 ± 10.13 vs. 170.24 ± 15.40 ng/mL, respectively), while the level significantly decreased in female COVID-19 patients without any chronic diseases group compared to the female COVID-19 patients having asthma group (158.64 ± 74.20 vs. 170.24 ± 15.40 ng/mL, respectively), and there was a non-significantly increased level between the female COVID-19 patients having heart disease compared to female COVID-19 patients without any chronic diseases (204.24 ± 10.13 vs. 158.64 ± 74.20 , respectively). whereas all the female COVID-19 patients'

groups have a significantly increased level compared to the female healthy control group.

Table 4.5 Gender distribution of COVID-19 IgM antibody level $\pm$ SD (ng/mL) on the 10th day

Groups	Anti-COVID-19 IgM antibody level mean $\pm$ SD (ng/mL) at 10 th day		Probability
	Males	Females	
Heart disease infected with COVID-19	191.91 $\pm$ 32.17 ^a	204.24 $\pm$ 10.13 ^a	P > 0.05
COVID-19 with no chronic disease	167.76 $\pm$ 78.03 ^a	158.64 $\pm$ 74.20 ^a	P > 0.05
Asthma infected with COVID-19	204.47 $\pm$ 47.22 ^a	170.24 $\pm$ 15.40 ^b	P > 0.05
Control (Healthy group)	8.93 $\pm$ 1.17 ^b	8.90 $\pm$ 1.31 ^c	P > 0.05
Duncan test: the similar letters referred to non-significant differences (P > 0.05) compared between the studied groups of the same gender			

The results in Table 4.6 showed the anti-SARS-COV2 IgM antibodies levels mean $\pm$ SD (ng/mL) distribution among the studied groups on the 20th day according to gender. There was a non-significantly decreased level (P > 0.05) in male COVID-19 patients having the heart disease group compared to females in the same group (112.04 $\pm$ 68.22 vs. 158.64 $\pm$ 74.20 ng/mL, respectively). While, there was a significantly decreased level (P < 0.001) in male COVID-19 patients having the asthma group compared to the females of the same group (4.19 $\pm$ 4.19 1.76 vs. 115.74 $\pm$ 62.93, respectively). whereas in the COVID-19 patients' group without any chronic diseases and control groups, the results showed a non-significantly increased level (P > 0.05) in males compared to females (151.19 $\pm$ 72.63 vs. 61.21 $\pm$ 55.49 ng/mL, and 8.93 $\pm$ 1.17 vs. 8.90 $\pm$ 1.31, respectively).

when comparing the anti-SARS-COV-2 IgM antibodies level among the studied groups according to the male gender, the results showed a significantly increased level (P < 0.001) in both COVID-19 patients having heart disease and without any chronic diseases groups compared to both of COVID-19 patients having asthma and control groups (112.04 $\pm$ 68.22 and 151.19 $\pm$ 72.63 vs. 4.19 $\pm$ 4.19 1.76 and 8.93 $\pm$ 1.17 ng/mL, respectively), and there was a significantly decreased level (P < 0.05) in COVID-19

patients having asthma group compared to control group (4.19 ± 4.19 vs. 8.93 ± 1.17 ng/mL, respectively), while the differences were not significant when comparing the COVID-19 patients having heart disease and without any chronic diseases groups (112.04 ± 68.22 vs. 151.19 ± 72.63 ng/mL, respectively).

in addition to the comparison of anti-SARS-COV-2 IgM antibodies levels among the studied groups according to the female gender, the results showed a significantly increased level ($P < 0.001$) among all COVID-19 patients group compared to the control group (158.64 ± 74.20 , 61.21 ± 55.49 and 15.74 ± 62.93 ng/mL vs. 8.90 ± 1.31 ng/mL, respectively). Also, there was a significantly increased level ($P < 0.001$) between the COVID-19 patients having heart disease and asthma groups compared to the COVID-19 patients without any chronic diseases group (158.64 ± 74.20 and 15.74 ± 62.93 vs. 61.21 ± 55.49 ng/mL, respectively). Such the same results appeared when comparing the COVID-19 patients without any chronic disease and the control groups (61.21 ± 55.49 vs. 8.90 ± 1.31 ng/mL, respectively).

Table 4.6 Mean COVID-19 IgM antibody level $\pm$ SD (ng/mL) at day 20

Groups	Anti-COVID-19 IgM antibody level mean $\pm$ SD (ng/mL) at 20 th day		Probability
	Males	Females	
Heart disease infected with COVID-19	112.04 ± 68.22^a	158.64 ± 74.20^a	$P > 0.05$
COVID-19 with no chronic disease	151.19 ± 72.63^a	61.21 ± 55.49^b	$P > 0.05$
Asthma infected with COVID-19	4.19 ± 1.76^c	115.74 ± 62.93^a	$P < 0.001$
Control (Healthy group)	8.93 ± 1.17^b	8.90 ± 1.31^c	$P > 0.05$
Duncan test: the similar letters referred to non-significant differences ($P > 0.05$) compared between the studied groups of the same gender			

The results in Table 4.7 and Figure 4.3, for males group showed the mean level $\pm$ SD of anti-SARS-COV2 IgM antibodies (ng/mL) a significantly increased level ($P < 0.001$) between the tenth and twentieth days for the COVID-19 patients having asthma (191.91 ± 32.17 and 112.04 ± 68.22 ng/mL, respectively), while the increasing levels were not significant ($P > 0.05$) when comparing the tenth and twentieth days for the COVID-19

patients having heart disease and without any chronic diseases (167.76 ± 78.03 and 151.19 ± 72.63 ng/mL, respectively).

Whereas the comparison of the females group appeared a significant increased level of anti-SARS-COV2 IgM antibodies between the tenth and twentieth days of the COVID-19 patients without any chronic diseases (204.24 ± 10.13 and 158.64 ± 74.20 ng/mL, respectively), while the increasing was not significant between the tenth and twentieth days for COVID-19 patients having heart disease and asthma (158.64 ± 74.20 , 61.21 ± 55.49 , 170.24 ± 15.40 and 115.74 ± 62.93 ng/mL, respectively).

Table 4.7 Mean COVID-19 IgM antibody level $\pm$ SD (ng/mL)

Groups	Anti-COVID-19 IgM antibody level mean $\pm$ SD (ng/mL)					
	Males		Probability	Females		Probability
	at 10 th day	at 20 th day		at 10 th day	at 20 th day	
Heart disease infected with COVID-19	191.91 ± 32.17	112.04 ± 68.22	$P > 0.05$	204.24 ± 10.13	158.64 ± 74.20	$P > 0.05$
COVID-19 with no chronic disease	167.76 ± 78.03	151.19 ± 72.63	$P > 0.05$	158.64 ± 74.20	61.21 ± 55.49	$P < 0.05$
Asthma infected with COVID-19	204.47 ± 47.22	4.19 ± 1.76	$P < 0.001$	170.24 ± 15.40	115.74 ± 62.93	$P > 0.05$
Control (Healthy group)	8.93 ± 1.17			8.90 ± 1.31		$P > 0.05$

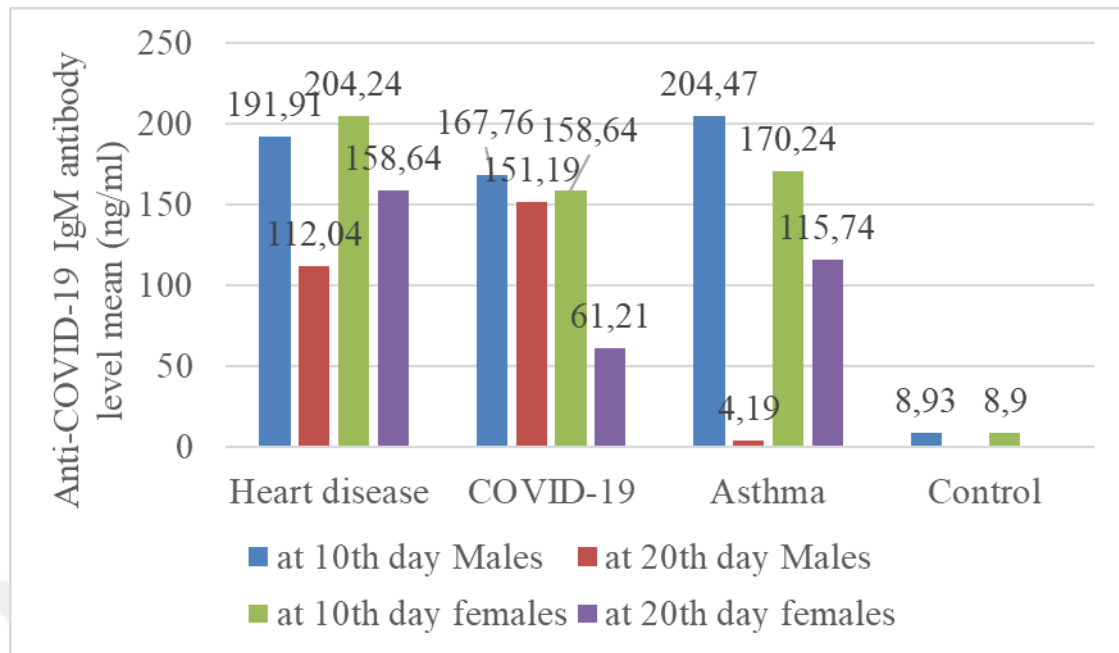
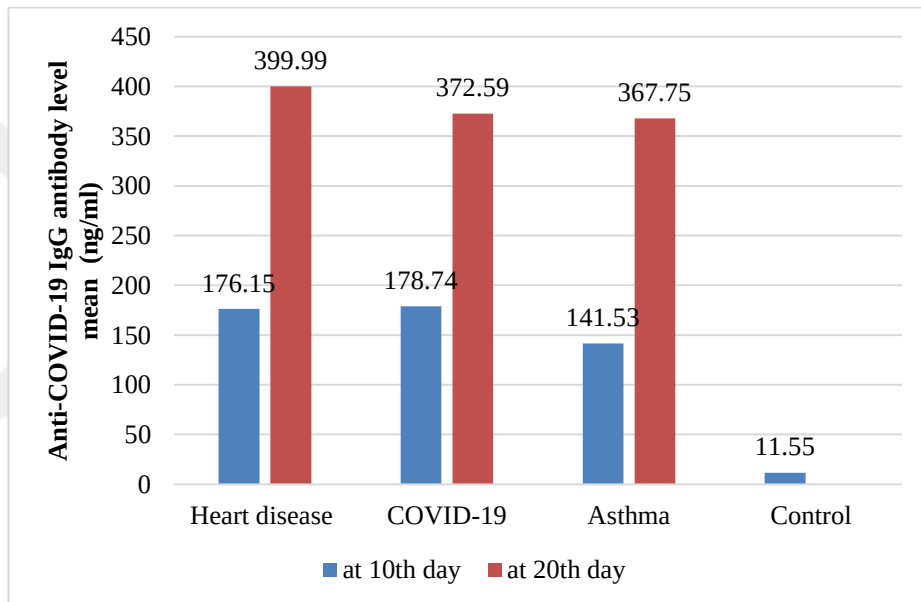


Figure 4.3 Mean COVID-19 IgM antibody level $\pm$ SD (ng/mL)

The results in Table 4.8 and Figure 4.4 showed the mean level of anti-SARS-COV2 IgG antibodies $\pm$ SD (ng/mL) a significant increased level ($P < 0.001$) in the twentieth day comparing to the tenth day in all COVID-19 patients' groups (399.99 ± 37.82 , 176.15 ± 34.03 , 372.59 ± 55.16 , 178.74 ± 50.56 , 367.75 ± 25.96 and 141.53 ± 61.76 ng/mL, respectively). Also, all the COVID-19 patients' groups have a significantly increased level of IgG antibody compared to the control group for the both tenth and twentieth days (176.15 ± 34.03 , 178.74 ± 50.56 , 141.53 ± 61.76 and 11.55 ± 4.82 ng/mL for the tenth day respectively, 399.99 ± 37.82 , 372.59 ± 55.16 , 367.75 ± 25.96 and 11.55 ± 4.82 ng/mL for the twentieth day respectively), while the significance not appeared among the patients' groups. ($P > 0.05$), the highest level was in COVID-19 patients having heart disease group and the lowest level was in COVID-19 patients having asthma group.

Table 4.8 Mean anti-COVID-19 IgG antibody level $\pm$ SD (ng/mL)

Groups	Anti-COVID-19 IgG antibody level mean ± SD (ng/mL)		Probability
	at 10 th day	at 20 th day	
Heart disease infected with COVID-19	176.15 ± 34.03 ^a	399.99 ± 37.82 ^a	P < 0.001
COVID-19 with no chronic disease	178.74 ± 50.56 ^a	372.59 ± 55.16 ^a	P < 0.001
Asthma infected with COVID-19	141.53 ± 61.76 ^a	367.75 ± 25.96 ^a	P < 0.001
Control (Healthy group)	11.55 ± 4.82 ^b		
Significant (P < 0.05) compared to control group			

**Figure 4.4** Mean anti-COVID-19 IgG antibody level $\pm$ SD (ng/mL)

In Table 4.9 and Figure 4.5, the results of anti-SARS-COV2 IgG antibodies levels $\pm$ SD (ng/mL) during the tenth day appeared there was only one significant difference for IgG antibody level (P < 0.05) between the males and females of COVID-19 patients having asthma group (208.20 $\pm$ 49.0 and 128.19 $\pm$ 58.60 ng/mL, respectively). While the other groups have a non-significantly decreased level of IgG antibody between the males and females. Also in males' group, all the COVID-19 patients' groups have a significantly increased level of IgG antibody (P < 0.001) compared to the control group, while the significant disappeared among the COVID-19 patients' groups. The highest level was in

COVID-19 patients having asthma while the lowest level was in COVID-19 patients having heart disease.

Likewise in females' group, the same results appeared among the COVID-19 patients' groups and control group, the highest level of IgG was in COVID-19 patients without any chronic diseases group while the lowest level was in COVID-19 patients having asthma group.

Table 4.9 Mean anti-COVID-19 IgG antibody level $\pm$ SD (ng/mL) on day 10

Groups	Mean anti-COVID-19 IgG antibody level $\pm$ SD (ng/mL) on day 10		Probability
	Males	Females	
Heart disease infected with COVID-19	170.26 $\pm$ 38.94 ^a	183.21 $\pm$ 29.75 ^a	P > 0.05
COVID-19 with no chronic disease	170.74 $\pm$ 32.77 ^a	188.34 $\pm$ 69.55 ^a	P > 0.05
Asthma infected with COVID-19	208.20 $\pm$ 49.0 ^a	128.19 $\pm$ 58.60 ^a	P < 0.05
Control (Healthy group)	11.23 $\pm$ 5.49 ^b	11.83 $\pm$ 4.45 ^b	P > 0.05
Duncan test: the similar letters referred to non-significant differences (P > 0.05) compared between the studied groups of the same gender			

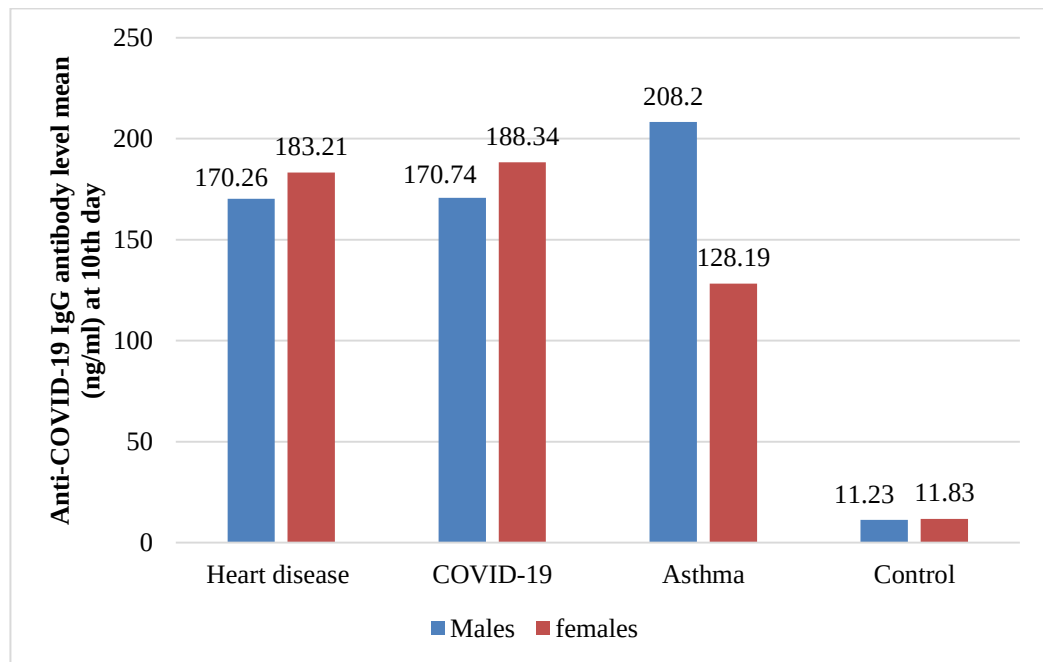


Figure 4.5 Mean anti-COVID-19 IgG antibody level $\pm$ SD (ng/mL) on day 10

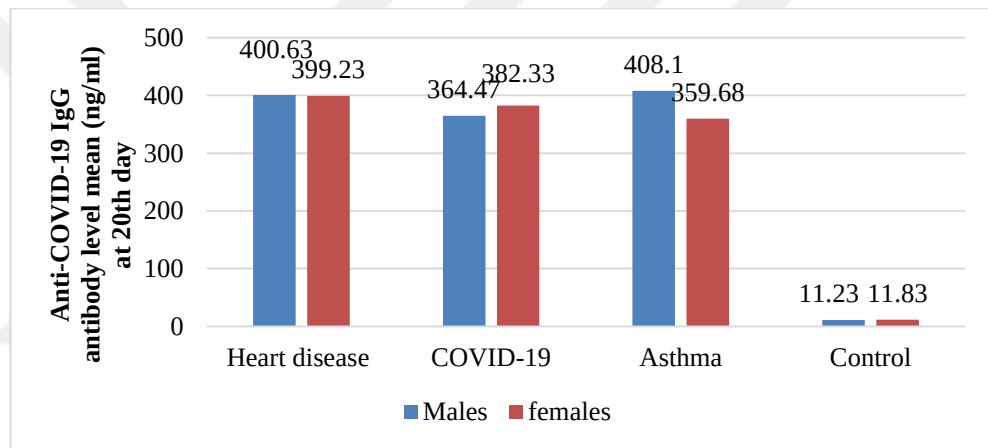
The results of anti-SARS-COV2 IgG antibodies mean levels $\pm$ SD (ng/mL) for the twentieth day appeared in Table 4.10 and Figure 4.6, there was a significantly increased level of anti-IgG antibody in males compared to females for the COVID-19 patients having asthma group (408.10 ± 35.98 and 359.68 ± 18.82 ng/mL, respectively), while in COVID-19 patients having heart disease group showed a non-significantly increased level of anti-IgG antibody in males compared to females (400.63 ± 51.48 and 399.23 ± 16.18 ng/mL, respectively), whereas in COVID-19 patients without any chronic diseases group showed a non-significantly decreased level of anti-IgG antibody level in males compared to females (364.47 ± 73.70 vs. 382.33 ± 24.50 ng/mL, respectively).

In males' group, there was a significant increased level of anti-IgG antibody in all COVID-19 patients' groups compared to control group, while the significant differences didn't appear among the COVID-19 patients' groups, the highest level was in COVID-19 patients having asthma while the lowest level was in COVID-19 patients without any chronic diseases group.

Also in females' group, there was a significant increased level of anti-IgG antibody level in both COVID-19 patients having heart disease and without any chronic diseases compared to COVID-19 patients having asthma and control groups respectively (399.23 ± 16.18 and 382.33 ± 24.50 - 359.68 ± 18.82 and 11.83 ± 4.45 ng/mL, respectively), in addition, the significant difference appeared between the COVID-19 patients having asthma group compared to the control group (359.68 ± 18.82 - 11.83 ± 4.45 ng/mL, respectively). The highest level was in COVID-19 patients having heart disease group while the lowest level was in COVID-19 patients without any chronic diseases group.

Table 4.10 Mean anti-COVID-19 IgG antibody level $\pm$ SD (ng/mL) at day 20

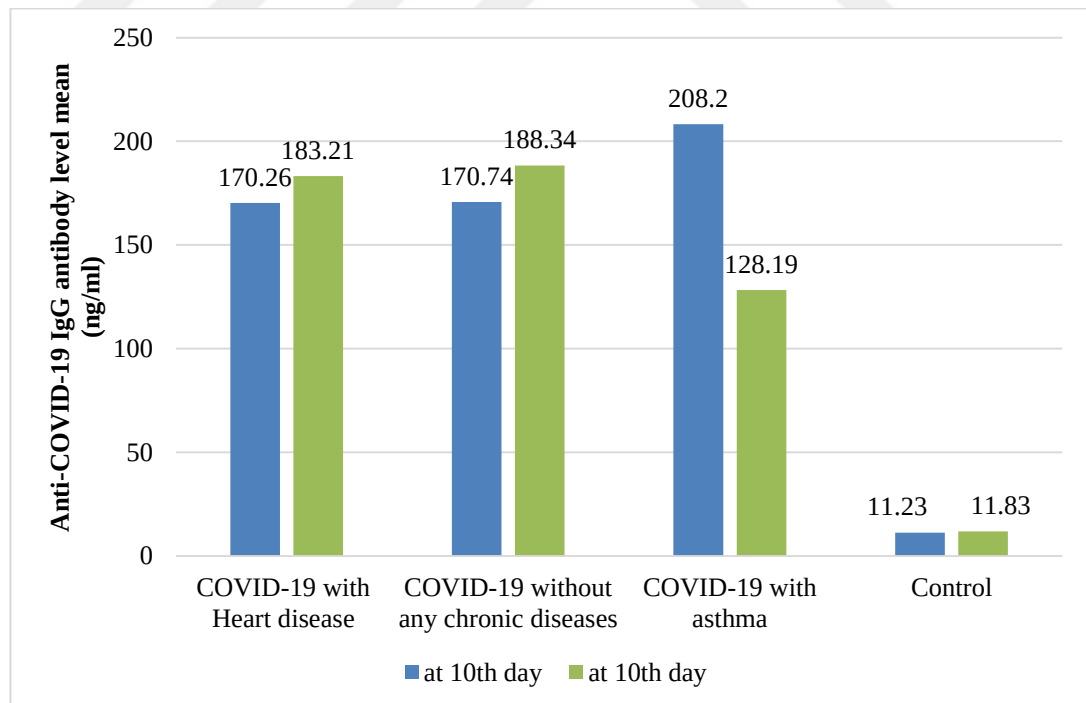
Groups	Anti-COVID-19 IgG antibody level mean $\pm$ SD (ng/mL) at 20 th day		Probability
	Males	Females	
Heart disease infected with COVID-19	400.63 $\pm$ 51.48 ^a	399.23 $\pm$ 16.18 ^a	P > 0.05
COVID-19 with no chronic disease	364.47 $\pm$ 73.70 ^a	382.33 $\pm$ 24.50 ^a	P > 0.05
Asthma infected with COVID-19	408.10 $\pm$ 35.98 ^a	359.68 $\pm$ 18.82 ^b	P < 0.001
Control (Healthy group)	11.23 $\pm$ 5.49 ^b	11.83 $\pm$ 4.45 ^c	P > 0.05
Duncan test: the similar letters referred to non-significant differences (P > 0.05) compared between the studied groups of the same gender			

**Figure 4.6** Mean anti-COVID-19 IgG antibody level $\pm$ SD (ng/mL) at day 20

The comparison of anti-IgG antibody level between the tenth and twentieth days according to the gender demonstrated in Table 4.11, Figure 4.7 and Figure 4.8, there was a significantly decreased level of anti-IgG antibody level in the tenth day compared to the twentieth day for all COVID-10 patients' groups in the males group. The same results were appeared in the twentieth day.

Table 4.11 Mean anti-COVID-19 IgG antibody level $\pm$ SD (ng/mL)

Groups	Anti-COVID-19 IgG antibody level mean $\pm$ SD (ng/mL)					
	Males		Probability	Females		Probability
	at 10 th day	at 20 th day		at 10 th day	at 20 th day	
Heart disease infected with COVID-19	170.26 $\pm$ 38.94	400.63 $\pm$ 51.48	P < 0.05	183.21 $\pm$ 29.75	399.23 $\pm$ 16.18	P < 0.001
COVID-19 with no chronic disease	170.74 $\pm$ 32.77	364.47 $\pm$ 73.70	P < 0.05	188.34 $\pm$ 69.55	382.33 $\pm$ 24.50	P < 0.001
Asthma infected with COVID-19	208.20 $\pm$ 49.0	408.10 $\pm$ 35.98	P < 0.001	128.19 $\pm$ 58.60	359.68 $\pm$ 18.82	P < 0.001
Control (Healthy group)	11.23 $\pm$ 5.49			11.83 $\pm$ 4.45		P > 0.05

**Figure 4.7** Mean anti-COVID-19 IgG antibody level $\pm$ SD (ng/mL) on the 10th day

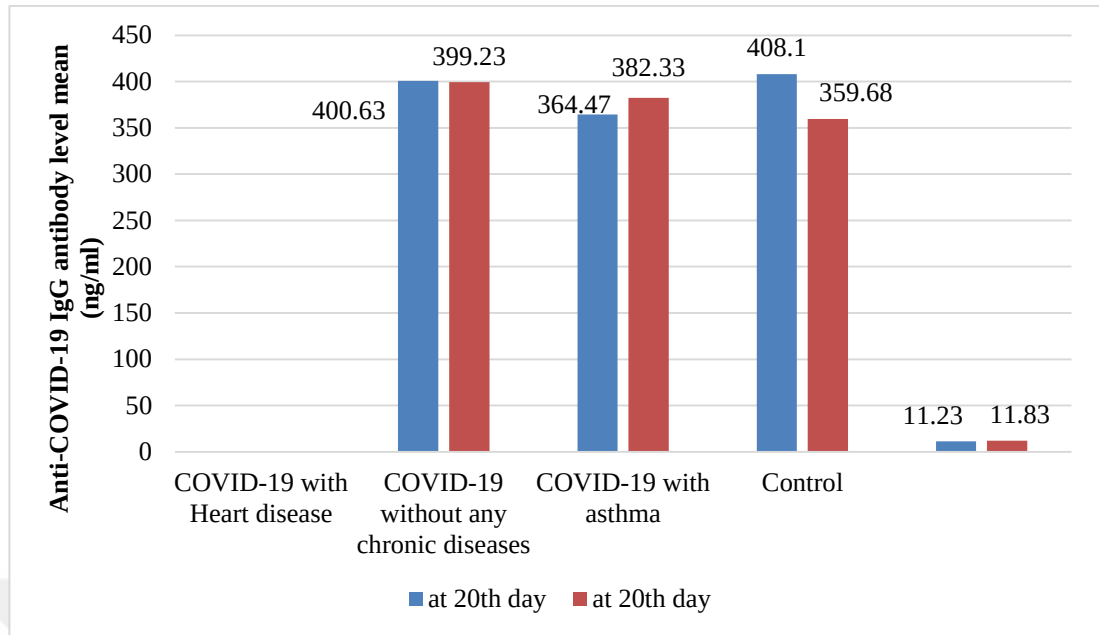


Figure 4.8 Mean anti-COVID-19 IgG antibody level $\pm$ SD (ng/mL) on the 20th day

4.2 Discussion

The results of this study included 120 participants, 90 participants were infected with COVID-19, divided into three groups according to the presence of chronic diseases or not, from the present results, the COVID-19 with heart disease group had the highest level of anti-IgM antibody followed by COVID-19 with asthma group in comparison with the COVID-19 without any chronic diseases group in both the tenth and twentieth days (Table 4.4). Also, the males of COVID-19 with asthma have the highest level of anti-IgM antibodies followed by COVID-19 with the heart disease group on the tenth day (Table 4.5). While the results showed that the COVID-19 without any chronic diseases group had the highest level of anti-IgM antibody on the twentieth day in comparison with the COVID-19 with heart disease and asthma groups (Table 4.6). whereas in females, the COVID-19 with heart disease group have the highest level of anti-IgM antibody followed by COVID-19 with asthma group in comparison with COVID-19 without any chronic diseases group in both the tenth and twentieth days (Tables 4.5 and 4.6). The present results of anti-IgM antibody level are similar to

previous studies results that indicated to increase in the anti-IgM antibody level gradually until 4 weeks and then gradually decreasing after the 4th week after infection (Bai *et al.* 2022, Barton *et al.* 2020, Chen *et al.* 2020, Guo *et al.* 2020, Long *et al.* 2020, Sun *et al.* 2020, Zhao *et al.* 2020, Yang *et al.* 2020, Woo *et al.* 2004).

In addition, the results of IgM antibody levels showed different significance in increasing anti-IgM levels when compared the level on the tenth day with the twentieth day for both males and females groups (Table 4.7 and figure 4.3), these results are in agreement with the results reported by Liu *et al.*, they referred that the titer of the anti-IgM antibody was decreases gradually over time and its presence in COVID-19 patients is considered to be in the intermediate and late stages of infection, this is consistent with the study of (Wang *et al.*, 2021, Xiao *et al.* 2021, Li *et al.* 2020, Liu *et al.* 2020, Long *et al.* 2020, Tian *et al.* 2020).

There is also a significant increasing level of anti-IgM antibody for males with heart disease than females (Table 4.4). This study is consistent with the study of Jameel (2021), who reported that patients with heart disease had an increase in anti-IgM antibodies levels in COVID-19 patients with heart disease. While the results showed that the males of COVID-19 having asthma and COVID-19 without any chronic diseases groups had the higher anti-IgM antibody levels than females (Table 4.4), these results are agreed with the results of previous literatures, that showed the males with asthma and COVID-19 groups had higher anti-IgM antibodies levels than females with asthma and COVID-19 (Wiersinga *et al.* 2020, Ali *et al.* 2021, Cervia *et al.* 2022).

In this study, the anti-IgM antibodies levels were non-significantly increased on the tenth day compared to the twentieth day in the COVID-19 having heart disease for both males and females groups (Tables 4.5 and 4.6). These results are consistent with the results of (Pinto *et al.* 2020, Gregorio *et al.* 2020) that they assessed the anti-IgM antibody levels in cardiac patients and appeared it was non-significantly elevated in females.

In COVID-19 patients without any chronic diseases group, there is a non-significant increased level of anti-IgM antibodies in males compared to females at both collecting days (Tables 4.5 and 4.6), this result is consistent with the results of (Jameel 2021), who reported that the prevalence of anti-IgM antibodies levels to coronavirus in males was higher than in females in COVID-19 patients.

As for females COVID-19 with asthma group, they have a significantly increased level of anti-IgM antibody on the twentieth day and more than levels in males group at the same collecting day (Table 4.7). In addition, the level of anti-IgM antibody levels was more in asthmatic COVID-19 patients than the non-asthmatics COVID-19 groups. these results consistent with (Smith-Norowitz 2021, Cervia *et al.* 2022) studies, reported that there was a difference between the levels of anti-IgM antibodies levels in adults with asthma, that was slightly higher than that of non-asthmatics.

There are several mechanisms assist in explain increasing the level of IgM antibody in heart disease group compared to the other COVID-19 groups, it may be SARS-COV-2 affect and damage directly the cardiac cells. While the indirect pathological mechanisms of SARS-COV-2 including hypoxia-induced myocardial injury because hypoxic respiratory failure and hypoxemia (Chung *et al.* 2021).

While the results of the anti-IgG antibody showed that the COVID-19 without any chronic diseases group have the highest level followed by COVID-19 with heart disease in comparison with the COVID-19 with asthma for the tenth day (Table 4.8). Whereas on the twentieth day, the COVID-19 with heart disease group have the highest level of anti-IgG antibody followed by COVID-19 without any chronic diseases group in comparison with the COVID-19 with asthma group (Table 4.8). According to gender, the COVID-19 with asthma males group has the highest level of anti-IgG antibody at both the tenth and twentieth days (Tables 4.9 and 4.10), whereas in females, the COVID-19 without any chronic diseases group have the highest level, whilst at both the tenth and twentieth days (Tables 4.9 and 4.10). These results are in agreement with the results reported by (Liu *et al.* 2020), they referred that the titer of the anti-IgG antibody was increased gradually after the 3rd week of infection (Liu *et al.* 2020).

In comparison the level of anti-IgG antibody at the tenth and twentieth days, the results showed a significant increasing level at the twentieth day compared to the tenth day for all COVID-19 groups (Table 4.8), these results were compatible with other previous studies reported that the level of anti-IgG antibody significantly increased on the 3rd week compared to the first and second week of infection (Chen *et al.* 2020, Iyer *et al.* 2020, Qu *et al.* 2020, Wolfel *et al.* 2020, Wang *et al.* 2020, ZumLa *et al.* 2020). Also, the present results of anti-IgM and IgG antibodies similar to the results of Ishaq *et al.* and colleagues, they reported a significant association between the COVID-19 infection and the level of anti-IgM and IgG antibodies especially in females (Ishaq *et al.* 2021).

In addition, the present results appeared a significant increased level of anti-IgG antibodies levels in males, compared to females on the twentieth day in the all COVID-19 patients' with chronic diseases (Table 4.11), these results are similar to the results of previous studies (Marchi *et al.* 2021, Amanat *et al.* 2020, Wajnberg *et al.* 2020) who showed a significant increasing in the level of anti-IgG antibodies in males compared to females on the 20th day.

5. CONCLUSIONS AND RECOMMENDATION

The results of this study showed:

- A total of 90 individuals had COVID-19, the older patients with asthma had high levels of anti-IgM and anti-IgG antibodies, and the youngest were the COVID-19 patients who also had high levels of anti-IgM and anti-IgG antibodies.
- There were various statistically significant differences of anti-IgM antibody level between the tenth and the twentieth day.
- For anti-IgM antibody level, it was higher in males' groups compared to females in COVID-19 patients without any chronic diseases and asthma groups, while it appeared different in COVID-19 patients with heart disease.
- According to gender, the results appeared various significant differences in each group of COVID-19 groups.
- For anti-IgG antibody, there was a significant increased level of anti-IgG antibody on the twentieth day compared to the tenth day.
- The results showed various significant differences of anti-IgG antibody levels in males compared to females on both tenth and twentieth days.

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