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EVALUATION IMMUNITICALLY OF INTERLEUKIN-6 LEVEL IN VACCINATED AND UNVACCINATED INDIVIDUALS PREVIOUSLY INFECTED WITH COVID-19 AND ITS EFFECT ON P53 PROTEIN

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THAMER FRAYYEH MUTAR MUTAR

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By Thamer Frayyeh Mutar MUTAR

May 2023

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

EVALUATION IMMUNITICALLY OF INTERLEUKIN-6 LEVEL IN VACCINATED AND UNVACCINATED INDIVIDUALS PREVIOUSLY INFECTED WITH COVID-19 AND ITS EFFECT ON P53 PROTEIN

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Master of Science in Biology

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May 2023

Coronavirus 2019 (COVID-19) is a disease epidemic that appeared at the end of 2019, infecting humans and causing many deaths, in addition to its effects on the global economy. The side effects of COVID-19 persist even after recovery and it is called a Post-COVID-19 or long-COVID-19 syndrome. The companies stroke in to produce a vaccine that led to the emergence of the mRNA vaccine which was tested for the first time on humans. The effects of the virus and the vaccine in the medium term after exposure became necessary to study. In this study, interleukin-6 (IL-6) as the most common inflammatory parameter, and protein-53 (P53) associated with cancer in humans, in addition to some biochemical tests were selected. 50 individuals were recruited who infected 3 to 6 months ago as group 1 (G1), and their injury didn't require to use of respiration devices, their ages 25 and 65 years, and 50 individuals were selected who had received the second dose of mRNA vaccine, before 3-6 months ago as group 2 (G2), their ages 25 and 65 years, with 25 healthy people who had not previously shown any symptoms of COVID-19 infection as a control group. While the results of G1 when compared with the control group recorded an important increase for P53 (3960.61±845.00), IgG (26.91±8.28), creatinine (0.75±0.1), TC (200.90±82.14), TG (157.26±61.86), and AST (28.92±8.80), the results were showed a significant decrease for urea (20.6±5.6), UA (4.56±1.1) and ALKP (93.38±30.55). Further, the outcome of this study observed there is no difference between G1 and the control group in terms of IL6 (2.57±1.4), IgM (0.48±0.34), glucose (96.22±12.9), HDL

(47.20±11.54), BiliT (0.80±0.47), ALT (29.10±14.92), Ca (9.00±0.38), Na (139.74±6.01), K (4.30±0.48), LDH (192.68±35.20) and CK (122.48±75.36). While the results of the second group compared with the control group showed an important increase in P53 (3782.25±1104.18), IgG (41.58±59.55), glucose (108.84±46.13), creatinine (0.79±0.2), TC (199.20±57.06) and TG (152.16±81.82), the urea (23.32±7.1), ALKP (61.80±27.21) and Na (138.08±3.25) were showed a significant decrease. Also, there were unimportant differences between G2 and the control group in terms of IL6 (2.61±1.5), IgM (0.48±0.36), UA (4.92±1.6), HDL (45.70±10.70), BiliT (0.59±0.44), ALT (25.18±14.61), AST (23.02±9.12), Ca (8.99±0.44), K (5.56±7.11), LDH (172.16±30.03) and CK (131.12±119.81). While the urea (23.32±7.1), ALKP (61.80±27.21), and Na (138.08±3.25) recorded a significant decrease when compared with the control group. The tests for G1 demonstrated a significant increase for P53, IgG, glucose, TC, TG, ALKP, AST, and Na and a significant decrease for UA and Na when comparing with G2, while no significant difference for IL-6, IgM, HDL, BiliT, ALT, Ca, K, LDH, and CK between G1 and G2.

2023, 79 pages

Keywords: COVID-19, Interleukin-6, Protein-53, mRNA vaccine

ÖZET

DAHA ÖNCE COVİD-19 İLE ENFEKTİF OLAN AŞI VE AŞI OLMAYAN KİŞİLERDE INTERLEUKIN-6 SEVİYESİNİN BAĞIŞIK OLARAK DEĞERLENDİRİLMESİ VE P53 PROTEİN ÜZERİNE ETKİSİ

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Coronavirüs 2019 (COVID-19), küresel ekonomi üzerindeki etkilerinin yanı sıra 2019 yılının sonunda ortaya çıkan, insanları enfekte eden ve çok sayıda ölüme neden olan bir salgın hastalıktır. COVID-19'un yan etkileri iyileştikten sonra bile devam eder ve buna Post-COVID-19 veya uzun COVID-19 sendromu denir. Şirketler, ilk kez insanlar üzerinde test edilen mRNA aşısının ortaya çıkmasına yol açan bir aşı üretmek için harekete geçti. Maruz kaldıktan sonra orta vadede virüs ve aşının etkilerinin araştırılması gerekli hale geldi. Bu çalışmada en yaygın inflamatuar parametre olarak interlökin-6 (IL-6) ve insanda kanserle ilişkili protein-53 (P53) yanı sıra bazı biyokimyasal testler seçilmiştir. Grup 1 (G1) olarak 3 ila 6 ay önce enfekte olan, yaralanması solunum cihazı gerektirmeyen, yaşları 25 ve 65 olan 50 kişi, Grup 2 (G2) olarak 3-6 ay önce 2. doz mRNA aşısı yapılmış, yaşları 25 ve 65 arası 50 kişi, kontrol grubu olarak daha önce herhangi bir COVID-19 enfeksiyonu semptomu göstermemiş 25 sağlıklı kişi seçilmiştir. G1 sonuçları kontrol grubu ile karşılaştırıldığında P53 (3960.61±845.00), IgG (26.91±8.28), kreatinin (0.75±0.1), TC (200.90±82.14), TG (157.26±61.86) ve AST (28.92±8.80) için önemli artış kaydedilirken, sonuçlar üre (20,6±5,6), UA (4,56±1,1) ve ALKP (93,38±30,55) için anlamlı düşüş gösterdi. Ayrıca bu çalışmanın sonucunda G1 ve kontrol grubu arasında IL-6 (2,57±1,4), IgM (0,48±0,34), glukoz (96,22±12,9), HDL (47,20±11,54), BiliT (0,80±0,47), ALT (29,10±14,92), Ca (9,00±0,38), Na (139,74±6,01), K (4,30±0,48), LDH (192,68±35,20) ve CK (122,48±75,36) açısından fark olmadığı görülmüştür. İkinci grup sonuçları ile

kontrol grubu karşılaştırıldığında P53 (3782,25±1104,18), IgG (41,58±59,55), glukoz (108,84±46,13), kreatinin (0,79±0,2), TC (199,20±57,06) ve TG (152,16±81,82) de önemli bir artış gösterirken üre (23,32±7,1), ALKP (61,80±27,21) ve Na (138,08±3,25) önemli azalma göstermektedir. Ayrıca, G2 ile kontrol grubu arasında IL6 (2,61±1,5), IgM (0,48±0,36), UA (4,92±1,6), HDL (45,70±10,70), BiliT (0,59±0,44), ALT (25,18±14,61), AST (23,02±9,12), Ca (8,99±0,44), K (5,56±7,11), LDH (172,16±30,03) ve CK (131,12±119,81) için önemsiz farklılıklar olduğunu gösterilmiştir. G1 için yapılan testler, G2 ile karşılaştırıldığında P53, IgG, glukoz, TC, TG, ALKP, AST ve Na için anlamlı bir artış ve UA ve Na için anlamlı bir düşüş gösterirken IL-6, IgM, HDL, BiliT, ALT, Ca, K, LDH ve CK için önemli bir değişiklik göstermedi.

2023, 79 sayfa

Anahtar Kelimeler: COVID-19, Interleukin-6, Protein-53, mRNA aşı

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Thamer Frayyeh Mutar MUTAR

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LIST OF SYMBOLS

=	Equal
≤	Greater or equal to
<	Greater than
≥	Less or equal to
>	Less than
µL	Microliter
mL	Milliliter
-	Minus
nm	Nanometer
%	Percent
+	Plus
±	Plus-minus

LIST OF ABBREVIATIONS

AIDS	Acquired immune deflciency syndrome
ARDS	Acute respiratory distress syndrome
ALT	Alanine aminotransferase
ALKP	Alkaline phosphatase
ASLA	American society of landscape architects
ACE	Angiotensin converting enzyme
ACE2	Angiotensin converting enzyme 2
ARBs	Angiotensin receptor blockers
AST	Aspartate transaminas
BiliT	Bilirubin total
Ca+	Calcium
CKD	Chronic kidney disease
CD 8	Cluster of differentiation 8
CK	Creative kinase
ELISA	Enzyme-linked immunosorbentassy
FSG	Formula student germany
PLP2	Gene-proteolipid protein 2
TP53	Gene-tumor protein 53
HDL	High density lipoprotein
HKU	Human coronavirus
HIV	Human immunodeflciency virus
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IFN	Interferon
IL6	Interleukin 6
ITCC	International certification council
ICTV	International committee on taxonomy of viruses
LDH	Lactate dehydrogenase
LFT	Liver function test
mRNA	Messenger ribonucleic acid
MERS	Middle east respiratory syndrome
MINOCA	Myocardial infarction with non-obstructive coronary arteries
PBMCs	Peripheral blood mononuclear cell
K	Potassium lons
PD-1	Programmed cell death protein 1
P53	Protein 53
RFT	Renal function test
RAAS	Renin-angiotensin-aldosterone system
SDS	Safety data sheet

Na+	Sodium
TAD	Technical audio devices
ASIA	The autoimmune/inflammatory syndrome induced by adjuvants
NSP3	The multi-domain non-structural protein 3
TC	Total cholesterol
TG	Total triglyceride
TGEV	Transmissible gastroenteritis virus
TNF	Tumor necrosis factor
U.A	Uric acid

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1. INTRODUCTION

In an extraordinary time, intensive care units around the world are battling the effects of COVID-19, viral pneumonia, which can lead to acute respiratory distress syndrome. (McGonagle *et al.* 2020). The primary cause of severe respiratory disease, COVID-19 induces a hyper-inflammatory response associated with a disproportionate cytokine and chemokine that can lead to multiorgan failure and lung damage. This response can also cause death (Darif *et al.* 2021).

There has been a lack of published studies on the effects of IL-6 blockade on maintaining and developing the immune response against COVID-19. In the treatment of COVID-19 patients, several cytokines-based therapies have been used, including tocilizumab, a humanized monoclonal antibody anti-IL-6 receptor (Pelaia *et al.* 2021).

Therefore, effective control of apoptosis in COVID-19, patients is very important. The best-studied apoptosis-marker molecule is the P53 protein which initiates the triggering of mitochondrial apoptosis (Nechiporuk *et al.* 2019). P53 is a target and mediator in signal transmission during mitochondrial apoptosis is being actively studied with the aim of its possible regulation (Gottlieb and Vousden 2010). Pfizer Biotech Company announced a vaccine for “SARS-CoV-2” is built based on mRNA (Pascolo 2021).

The use of ribonucleic acid as a therapeutic protein is considered controversial due to its unstable nature. Although it is sensitive to certain types of ribonucleases, mRNA was first introduced to the market in 1989 as a potential therapeutic (Kowalzik *et al.* 2021). Two years later, it was proposed that mRNA could be used as a vaccine platform (Gottlieb and Vousden 2010). This technology could allow the development of live attenuated vaccines that combine the immunological properties of both the killed and subunit vaccines (Crommelin *et al.* 2021).

1.1 Problem Statement

Nowadays, the Coronavirus incidence has hiked in the world and has increased mortality rates. Thus, researches try to find the linking between some of the cytokines with COVID-19 to evaluate the risk of development of these infection complications in patients (Costela-Ruiz *et al.* 2020). Although most individuals recover from COVID-19 infection in about 12 weeks, some individuals will experience symptoms that last for a longer after their infection. These include COVID-19 syndrome or long COVID. Although the long-term effects of COVID-19 are not related to how people got infected, some people who had mild symptoms during their initial infection still have issues with their health problems (Parkin *et al.* 2021, Flatby *et al.* 2022). Various symptoms can be associated with COVID-19. Some of these include fatigue, shortness of breath, brain fog, difficulty in sleeping, and heart palpitations. These are the main reasons why researchers conducted studies to find out what causes these problems (Nalbandian *et al.* 2021).

The studies have risen about some of the cytokines and showed link between IL-6 and COVID-19 complications (Bonam *et al.* 2021). With the advent of vaccines against this disease, especially the mRNA vaccines, which are among the modern biotechnologies that have not been tried before. With an increased necessity for more research to assess the risks of infection with the SARS-CoV-2 and to evaluate the side effects of vaccines (Ni *et al.* 2021, Brüssow 2022). Therefore, in this study, we will investigate some of the cytokines and several biochemical parameters in patients who had been infected by SARS-CoV-2 who didn't need intensive care units and didn't develop severe respiratory distress syndrome (ARDS), with people who vaccinated by mRNA vaccine compared with the control group (Ni *et al.* 2021).

1.2 Scope of Study

The scope of this study was to investigate the immune response of individuals to COVID-19 infection and mRNA vaccination. Blood samples were collected from three groups of individuals: those who had recovered from COVID-19 infection 2-3 months prior, those who had received the second dose of the Pfizer-BioNTech mRNA vaccine

2-3 months prior, and a control group of healthy individuals. All individuals underwent testing for IgM and IgG antibodies, interleukin 6 (IL-6) levels, and P53 protein levels, as well as 16 biochemical tests.

The investigation of the impact of COVID-19 and the mRNA vaccine on the P53 protein will provide further insight into the potential long-term effects of these treatments on the human body.

Overall, this study holds great potential in providing valuable insights into the immune response to COVID-19 and the mRNA vaccine, as well as their potential long-term effects on human health.

1.3 Objectives

- To estimate the IL-6 in vaccinated and unvaccinated individuals previously infected with COVID-19 and control group.

- To assay IgM and IgG for COVID-19 for all the samples will collect.

- To evaluate the P53 protein in individuals under study and control group.

- To estimate the important biochemical tests for all study samples and control.

2. LITERATURE REVIEW

2.1 Viruses

A virus is an organism that can only replicate in its host. It can infect a wide range of living organisms, such as animals and plants (Mettenleiter 2017). Viruses are so small that a microscope is necessary to visualize them, and they have a simple structure. They are also known to be very diverse. Viruses are a sub-discipline of microbiology (Stres and Kronegger 2019). When a virus infects a cell, it hijacks the cell's machinery to create thousands of copies of itself. However, this process does not protect the cell from harm; in fact, some viruses have a lytic cycle that destroys the host cell. Other viruses have a lysogenic cycle, where the virus integrates its genetic material into the host cell's DNA and replicates along with the cell. In either case, viruses can also exist outside of the host cell as independent particles (Mettenleiter 2017: Brodt *et al.* 2011). A virus does not typically protect a cell; instead, it hijacks the cell's machinery to replicate itself. The components of a virus that are involved in replication and assembly of new virus particles are known as virion components. These include the viral genome, structural proteins that make up the virus particle, and other molecules that help the virus infect new cells. However, some viruses, such as the retrovirus, can integrate their genetic material into the host cell's DNA and provide a protective effect against future infections (Prusinkiewicz and Mymryk 2019).

These viral particles come in a variety of shapes, from simple icosahedral and helical shapes to more complex structures. Most viruses contain virions that are one-hundredth the size of human hair and are too small to be seen with an optical microscope (Gazit and Mitraki 2013). Although it's not exactly clear how they came about, some viruses may have originated from the DNA fragments known as plasmids (Brodt *et al.* 2011). Others may have arisen from bacteria (Koonin 2006). The development of viruses is a crucial part of evolution as they help increase the genetic variety through horizontal gene transfer. Viruses do contain genetic material, but they lack certain traits that are considered essential for life, such as cell structure and the ability to reproduce independently (Mettenleiter 2017). Some biologists argue that viruses should be

considered living forms, as they are capable of evolving and adapting to their environment, and they rely on host cells to carry out their replication process. However, others argue that viruses should not be considered living, as they are unable to carry out metabolic processes on their own and do not have the ability to reproduce without a host cell (Forterre *et al.* 2007). The spread of viruses can be influenced by various factors. One of these is the transmission of diseases by animals and plants. For instance, blood-sucking insects can carry viruses that can infect plants. Influenza viruses can also spread through coughing and sneezing. The faucal-oral route can lead to the transmission of viruses, such as those that cause viral gastroenteritis (Jansen *et al.* 2021). The number of particles needed to cause a human infection from the virus known as norovirus is less than 100. Human exposure to infected blood or sexual fluids can also lead to the transmission of HIV. This means that it can indiscriminately infect a wide variety of organisms (Dąbrowska *et al.* 2021).

Viruses were classified by their infectivity, filter-passing capabilities, and demand for living hosts by the end of the 19th century. The 2 half of the 20th century saw the emergence of the golden age of virus discovery. During this time, most known plant, bacterial and animal viruses were discovered. In 1957, the arterivirus was discovered in horses, and the bovine virus diarrhoea was caused by a pestivirus. In 1963, Blumberg and Temin identified the hepatitis B virus (Murphy *et al.* 1999). Baltimore and Timin introduced the concept of reverse transcriptase, an enzyme used by retroviruses to copy their genetic material. The first known case of HIV was isolated in 1983, at the Pasteur Institute by Montagnier and his team. Two years later, Houghton and his group at the Chiron Corporation identified hepatitis C (Fields 2007).

2.1.1 Classification of viruses

Classification is a process utilized to describe the various types of viruses by identifying them according to their similarities. André Lwoff, Paul Tournier, and Robert Horne were the first to use this approach in 1962. The classification system is a hierarchical system used to organize various types of organisms into phyla, classes, orders, genera, and species based on their characteristics and relationships, rather than simply listing

them as individual type. The characteristics of the viruses were also taken into account to determine their classification (García-López *et al.* 2019). The characteristics of viruses were taken into account to determine their classification, and the ITCC created a new classification system that focuses on specific features of viruses to ensure that family members are not only homogenous. This system aims to provide a better understanding of the viruses for the scientific community (Cuatrimestral 2019). A unified taxonomy has been established for the various types of viruses. However, only a small portion of the virus variety has been studied. The International Committee on Taxonomy of Viruses (ICTV), has recognized various kingdoms, realms, subphyla, classes, subfamilies, and subgenera as of 2020. It has also identified over 9,000 species of viruses (Le Pendu *et al.* 2017, Dodd *et al.* 2018).

2.1.1.1 Baltimore classification system

David Baltimore created the Baltimore classification system, which is used to classify viruses. It is also used to classify ICTV viruses. Classification systems like the Baltimore system classify viruses according to their ability to produce mRNAs. Each type of virus has its way of doing so, and these vary depending on the strain. A single-stranded or double-stranded viral genome can be composed of either DNA or RNA. These types of viruses do not require reverse transcriptase to reproduce (Das *et al.* 2021, Khaitovich *et al.* 2021).

Viruses are divided into seven groups according to this classification (Figure 2.1).

- dsDNA viruses (e.g. Adenoviruses, Herpesviruses, Poxviruses).
- ssDNA viruses (sense or +strand) DNA (e.g. Parvoviruses).
- dsRNA viruses (e.g. Reoviruses).
- (+) ssRNA viruses (sense or +strand) RNA (e.g. Coronaviruses, Picornaviruses, Togaviruses).
- (−) ssRNA viruses (antisense or -strand) RNA (e.g. Orthomyxoviruses, Rhabdoviruses).
- ssRNA-RT viruses (sense or +strand) DNA with RNA intermediate in life-cycle (e.g. Retroviruses).

- dsDNA-RT viruses RNA with DNA intermediate in life-cycle (e.g. Hepadnaviruses) (Buchkovich *et al.* 2008, Baker *et al.* 2013).

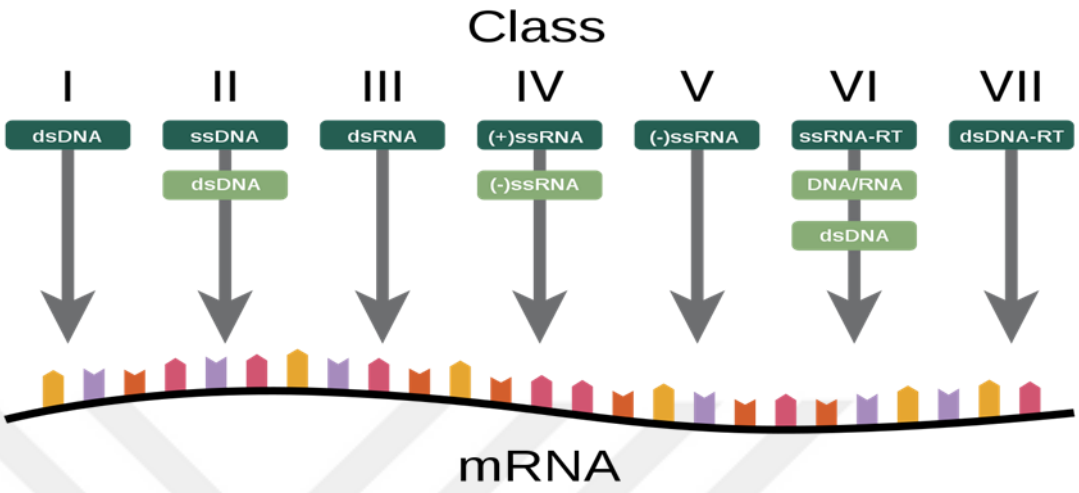

Figure 2.1 Classification of Baltimore viruses is based on viral mRNA, synthesis (Koonin *et al.* 2021)

2.1.2 Role of virus in human disease

A virus can cause various effects when it infects a cell. Some viruses do not cause any harm, while others can replicate inside a cell and attack it. Once the virus is fully developed, it can then enter the body (Eagleman 2020). A lytic infection is a type of viral infection that occurs when the virus enters a host cell and uses the host's machinery to replicate itself until the cell bursts, releasing new virus particles. This type of infection usually occurs when the host's immune system is unable to destroy the virus. In contrast, a lysogenic infection occurs when the virus integrates its genetic material into the host's DNA and remains dormant for a period of time (Darwich and Mateu 2012). During this period, the virus replicates along with the host cell's DNA, and when the conditions are right, the virus can become active and cause a lytic infection. Some viruses can also exist in a latent phase, in which they don't cause any harm to the host cell but can reactivate later under certain conditions. (Doorbar 2018). Although the patient is recovering, the infection can still be transmitted to others. It can also recur once the illness has passed. To spread, a virus must be able to infect the body's immune

system. When the system is compromised, these viruses can cause disease only in some cases. Opportunistic infection is a common issue among people with AIDS. A virus can also infect plants and animals that have a reservoir of nutrients (Darwich and Mateu 2012, Martins *et al.* 2014).

The body's innate immune system is designed to fight infections, but it isn't it is the only system responsible for combating pathogens. It consists of various mechanisms that protect the host from getting sick. This means that the body is still able to fend off viral threats at this time. Upon entering, adaptive immunity kicks in and remembers the encounter with the pathogen. This type of immunity can last for a lifetime. It's referred to as humoral immunity. In this form of immunity, the body produces antibodies against a specific virus (Archer 2015). Some viruses can have a plant or animal reservoir that they can use to infect humans. Two types of antibodies can be used against these viruses (Prince *et al.* 2021). The first type of immune response is known as IgM, which is incredibly powerful at killing viruses. It is produced by cells of the immune system for a few weeks. The second type of response is known as cellular immunity, which includes T cells (Dey *et al.* 2019, Song *et al.* 2020).

2.1.3 Coronaviruses

A coronavirus is a type of virus that has a positive sense single-stranded RNA genome and a helical nucleocapsid. The size of the genome of coronaviruses is approximately 26 to 32 thousand base units (Zehra *et al.* 2020, Rohaim *et al.* 2021).

The name Coronavirus came from the Latin word "corona" which means crown or corona. These particles have surface protrusions that give them the appearance of a king's crown or solar corona (Brake *et al.* 2020, Chorba 2020).

During the 1960s, scientists discovered the first coronaviruses, which were infections caused by the bronchitis virus in chickens. Two other viruses were identified in the nasal cavity of humans (Wang *et al.* 2020). Other coronaviruses that have been

identified as members of this family include the 2004 NL63 human coronavirus, the 2005 HKU1 coronavirus, and the 2012 MERS coronavirus (Schwartz and Graham 2020).

2.1.3.1 Classification of coronaviruses

The Coronaviruses are a subfamily of the Coronaviridae. They are divided into four genera: alphacoronavirus, betacoronavirus, deltacoronavirus, and gammacoronavirus. (Figure 2.2). Both the alphacoronavirus and the betacoronavirus can infect mammals. The other types of coronaviruses, such as the delta and gammacoronaviruses, mainly infect birds (Miłek and Blicharz-Domańska 2018).

Genus: Alphacoronavirus

Species: Alphacoronavirus 1; TGEV, Feline coronavirus, Canine coronavirus, Human coronavirus 229E, Human coronavirus NL63, *Miniopterus* bat coronavirus 1, *Miniopterus* bat coronavirus HKU8, Porcine epidemic diarrhea virus, *Rhinolophus* bat coronavirus HKU2, *Scotophilus* bat coronavirus 512.

Genus: Betacoronavirus

Species: Betacoronavirus 1; Bovine coronavirus, Human coronavirus OC43, Hedgehog coronavirus 1, Human coronavirus HKU1, MERS-related coronavirus, Murine coronavirus, *Pipistrellus* bat coronavirus HKU5, *Rousettus* bat coronavirus HKU9, Severe acute respiratory syndrome (SARS)–related corona virus (COVID-19), *Tylonycteris* bat coronavirus HKU4.

Genus: Gammacoronavirus

Species: Avian coronavirus, Beluga whale coronavirus SW1.

Genus: Deltacoronavirus

Species: Bulbul coronavirus HKU11, Porcine coronavirus HKU15 (Li *et al.* 2019).

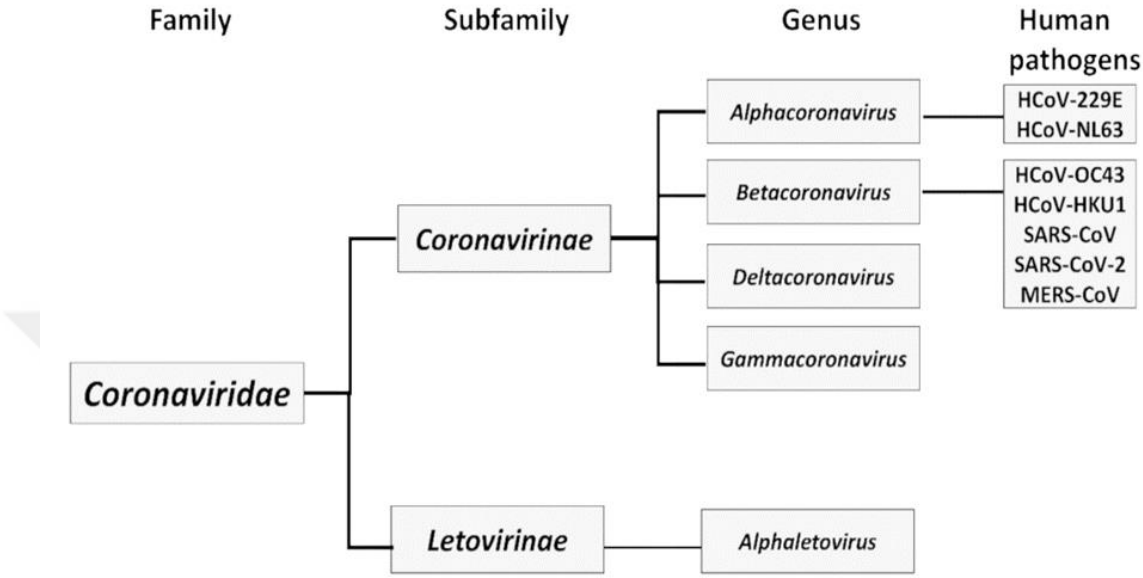

Figure 2.2 Taxonomy of *Coronaviridae* family with an indication of species known to be pathogenic to humans and cause respiratory diseases (Wartecki and Rzymski 2020)

2.1.3.2 Human coronavirus

A type of viral infection known as coronavirus is seen in adults and children. It can cause fever and swollen limbs. Most people infected with the virus develop cold symptoms during the winter season. In 2003, a coronavirus, known as SARS, has been identified. This strain has a unique ability to cause severe illness. It can cause lower respiratory symptoms and upper respiratory symptoms (Kahn and McIntosh 2005, Zimmermann and Curtis 2020).

There are seven types of human coronaviruses:

- Human Coronavirus 229E, (HCoV-229E)
- Coronavirus OC43, (HCoV-OC43)

- Associated Coronavirus Human C
- Human Coronavirus NL63, (HCoV-NL63)
- HKU1 Human Coronavirus
- Novel Coronavirus/Novel Coronavirus 2012, (HCoV-EMC).
- Novel coronavirus (COVID-19) commonly referred to as Wuhan coronavirus or Wuhan pneumonia (Liu *et al.* 2021a).

2.1.3.3 COVID-19

COVID-19 is a zoonotic respiratory illness that can be caused by the coronavirus subtype 2, This illness is very similar to the SARS virus (Synowiec *et al.* 2021). The coronavirus pandemic started in 2019 when it was first discovered in Wuhan, China. It has since affected various parts of the world (Chinazzi *et al.* 2020). Common symptoms of COVID-19 are fever, cough, shortness of breath, and muscle aches. Although most infections do not progress to a serious illness, they can still lead to severe illness. Although most people with COVID-19 do not experience severe symptoms, those with this illness can experience septic shock, blood clots, and organ failure (Chen *et al.* 2020a).

The incubation period for the virus can range from two to 14 days. Symptoms usually last for five days. The virus can cause long-term damage to any tissues and organs in the body, such as the heart and lungs. It is also possible that patients who recovered from the initial phase of the illness but then experienced various symptoms for several months after their recovery are still experiencing these symptoms (Lu *et al.* 2020). The virus can spread between people when they cough and sneeze. Small droplets produced by these actions usually fall to the ground or surfaces instead of flying over long distances (Balachandar *et al.* 2020). Some people may get sick after touching contaminated surfaces. The virus is most contagious for three days after the initial symptoms appear. It can also infect people who do not show any symptoms (Shimizu 2020).

For people who might have the virus, a face covering is also helpful. It can be worn by those who are worried about their health and care for them. However, some people are conflicted about using a face covering. For instance, some authorities are against it and others are advising people to use it. The effectiveness of mask-wearing by healthy individuals in preventing the spread of respiratory illnesses is still a topic of debate and research, and there is no clear consensus on its benefits (Howard *et al.* 2021).

2.2 Vaccine

A vaccine is an immunological preparation that can give a person an active resistance to a specific disease (Ogra *et al.* 2001). Usually, the vaccine is consist of various components, such as the toxins or the surface proteins of the pathogen. The purpose of the vaccine is to stimulate the body's immune system to recognize and destroy a specific type of pathogen. This process then keeps a replica of the same germ in the body to trigger the body's response and destroy it (Jin *et al.* 2019, Ma *et al.* 2019). There has been a lot of scientific evidence supporting the efficacy of vaccines. Some of these include the human papillomavirus vaccine and the respiratory illness vaccine. Vaccination is regarded as the initial effective method of preventing infectious diseases. It was also help in the elimination of some of the most common diseases, such as smallpox. In most parts of the world, the spread of immunity from vaccines has helped prevent diseases such as polio, measles, and tetanus. According to the World Health Organization, there are currently over 20 licensed vaccines that can help in the management of various infectious diseases (Plotkin 2005, Principi and Esposito 2019).

Preventive vaccines are intended to prevent a disease from developing. They can also be used to improve the results of treatment (eg, there are prescribed vaccines against cancers) (Melief *et al.* 2015). The origin of the time period and the vaccine for cowpox can be traced back to the variolae vaccine. Edward Jenner first coined the term cowpox when he was a teenager. He used the term cowpox in 1798 to describe the importance of the vaccine in fighting smallpox. Louis Pasteur proposed improvements to the development of vaccines based on new discoveries in the field, building upon the origin of the time period and the vaccine for cowpox traced back to Edward Jenner's variolae

vaccine (Gross and Sepkowitz 1998). Vaccines are usually composed of either harmless or inactivated organisms. Various types of vaccines can be used. They can be done in various ways to minimize the risk of contamination. One of these is utilizing unique techniques to prevent exposure to contaminated materials (Soiza *et al.* 2021).

2.2.1 Types of vaccines

- Attenuated vaccine
- Inactivated vaccine
- Toxoid
- Subunit vaccine
- Conjugate vaccine
- Outer membrane vesicles
- Heterologous vaccine
- Genetic vaccine
- Viral vector vaccine
- DNA vaccine
- RNA vaccine (Fuller and Berglund 2020).

Some of the vaccines being developed to combat the COVID-19 pandemic include those that are designed to use RNA. Some of these have also been approved in other countries. For example, the Pfizer-Biontech and Moderna mRNA vaccines are approved for use in adults. These two vaccines were also approved for teens aged 16 to 17 in the USA (Owusu *et al.* 2022).

There are 8 licensed vaccines for COVID-19. Some of these include Pfizer-Biotech, Sinovac, Moderna, Sputnik, Turkovac, and Johnson & Johnson. Studies on the development of a vaccine against the COVID-19 coronavirus have been useful because they show that the virus can be similar to an emerging coronavirus that's known to use the ACE2 pathway to infect humans (Carneiro *et al.* 2021).

2.3 Cytokines

A protein or biologic that is utilized in signal and intercellular communication is known as a cytokine. B-cells are known to produce cytokines that prompt T-cells to develop. T-cells then produce various kinds of cytokines that continue to boom and prompt other T-cells. The role of cytokines, in the development of the embryonic and immune systems is a major factor in this process, and they are also involved in cell movement. The phrase cytokine refers to the various parts of a protein that are involved in this process. Like other chemical signals, cytokines are used to allow a mobile cellular to communicate with other cells. The members of the cytokine family include various kinds of proteins and glycoproteins. Compared to hormones, cytokines are produced by specialized organs inside the body. They are then transported to the bloodstream. They are also produced using one-of-a-kind cells. The significance of cytokines is also linked to the various infections and diseases they can help prevent. They can also help treat various conditions such as blood poisoning. In addition to being utilized by the immune system, cytokines also play a role in the development of other tissues. For instance, they can help exchange information among multiple cells throughout the embryonic development stage (Lackie 2010, Campuzano *et al.* 2020).

2.3.1 Type of cytokines

There are five types of cytokines: Interleukin, colony-stimulating factor, IFN, chemokine, and TNF.

2.3.1.1 Interleukins

The term interleukin refers to a group of molecules that are part of a cellular messenger system that regulates the behavior of cells. The first known example of this was identified in 1970 (Kim *et al.* 2009).

In 1979, the name "interleukin" was established to replace the multiple names given to different research groups to identify certain cytokines. These include the B-cell differentiation factor, hediquine, and T-cell replacement factor. During a workshop held in Switzerland in 1979, the decision was made to change the name of the lymphokine to reflect the new scientific understanding of its role in the development of immune responses. It was initially believed that the main function of the cytokines was to act on other leukocytes, which is why they were originally referred to as interleukin. However, due to their involvement in the mounting immune responses, they were eventually thought to be only useful as mediators of these responses (Garlet 2010, Kany *et al.* 2019).

It is known that interleukins have an important function, and they can interact with other cells and play various physiological roles. The role of interleukins in the body is much greater than previously thought, as they are present in a diverse group of cells, including white blood cells and certain types of cells of the immune system. These cells are known to expel harmful substances and bacteria from the body (Van Dyken and Locksley 2013). Different types of white blood cells are known to have different types of cytokines. These include the interleukins 1, 2, and 3 (Kubick *et al.* 2021). The body's white blood cells can fight diseases by working together to form a chain reaction. This process involves the activation of certain immune cells. When a wound is infected with bacteria, white blood cells can detect the presence of these organisms at the site of the wound. These white blood cells then release a chemical known as interleukin-1 to trigger the release of other white blood cells, which then attack the bacteria. The chemicals also stimulate other immune system cells to attack the invaders (Baumgart and Carding 2007).

2.3.1.2 Interleukin-6

The human IL-6 is a type of cytokine that controls the development and maintenance of various immune responses. It is a gene that is on chromosome 7p21 and contains over 200 amino acids (Tanaka *et al.* 2014). This type of cytokine has anti-inflammatory and

pro-inflammatory properties. It can also be used to treat various chronic inflammatory diseases, such as Alzheimer's disease and rheumatoid arthritis (Skytthe *et al.* 2020).

The control of the secretion of IL-6 is important during disease. It is known that different types of cells, such as macrophages, T-cells, and monocytes, are involved in the activation of this cytokine. The targets of this cytokine are T-cells, macrophages, B-cells, and monocytes (Turner *et al.* 2014). The function of IL-6 on B-cells is to differentiate them into different types of cells, such as IgM differentiation and B-cell. It also controls the activation of T-cell and leukocyte survival. After an injury, a cytokine storm can cause T and B-cells to develop and differentiate. It can also increase low-grade IL-6 levels in cancer patients. The secretion of IL-6 by B-cells leads to the production of antibodies and the promotion of hypergammaglobulinemia. It also causes chronic inflammation and the activation of CD4 cells, which can lead to Th17 differentiation (Bao and Cao 2014, Wen *et al.* 2020).

2.3.1.3 Relationship between COVID-19 disease and IL-6

IL-6 with high levels is known to be associated with various inflammatory diseases, such as asthma and lung damage. In patients with COVID-19, these levels have also been associated with symptoms such as pneumonia and severe lung damage. The absence of an inhibitor of cytokine-3 signaling also led to the development of negative feedback mechanisms. In COVID-19 patients, the lack of a suppressor of cytokine-3 signaling also led to the development of negative feedback mechanisms. This mechanism is responsible for regulating the levels of IL-6 (Abbasifard and Khorramdelazad 2020). Another study in COVID-19 patients with severe infections revealed that IL-6 levels were higher. This could be a potential marker for the development of severe infection (Galván-Román *et al.* 2021). Due to the presence of the IL-6 receptor, patients with COVID-19 are prone to experiencing a viral cytokine storm. Some studies suggest that the use of a humanized monoclonal antibody known as tocilizumab can be used to treat this condition. A recent study conducted in China revealed that tocilizumab important improves symptoms of COVID-19 in patients (Magro 2020). It also didn't cause any adverse reactions. Unfortunately, for patients

with COVID-19, tocilizumab is only approved for use when the viral load has reached the end of its course. These patients include those with severe respiratory failure, interstitial pneumonia, and high levels of D-dimer, F-ferritin, and IL-6 (Fu *et al.* 2020, Magro 2020).

The protein known as TNF-α is a key mediator of the production of chemokine and cytokines. It plays a role in the development of chronic and acute systemic inflammatory responses. TNF-α levels were elevated in patients infected with COVID-19. This finding suggests that treatment with adalimumab could be beneficial for these patients. A randomized, controlled trial was conducted to evaluate the effects of this drug on the disease severity (Teixeira *et al.* 2021). In patients with COVID-19, immunosuppressants and corticosteroids can be used to suppress the development of a cytokine storm. Recent studies have shown that the use of hydrocortisone can increase the viral load in COVID-19 patients (Hariharan *et al.* 2021).

2.4 TP53 Gene

The TP53 gene is known to play a major role in the development of cancer cells. It can produce P53 which helps in inhibiting the growth of tumors. If the TP53 gene is defective, it can allow damaged cells to survive and eventually develop into cancer cells (Rivlin *et al.* 2011).

TP53 gene is a tumor suppressor that helps prevent the formation of new tumors. Individuals who inherit only one copy of this gene from their parents are at risk of developing cancer. They can also develop several independent tumors throughout their lives. This condition, known as Li-Fraumeni syndrome, is very rare. However, mutations in the TP53 gene can lead to different types of tumors. These mutations contribute to the formation of complex molecular events that are involved in the development of cancer (Achatz and Zambetti 2016).

2.4.1 Protein 53

The protein known as P53 is a tumor suppressor that plays a role in the regulation of the cell cycle. It is also involved in the development and maintenance of cancer prevention and treatment. P53 is also called the "guardian of the genome" and "the guardian of genetic information". It prevents genetic mutations from happening. The molecular mass of P53 is shown on the SDS page as 53 KDA, However, this figure is 43.7 kids. This is because the amino acid residues are calculated using the same method (Reckzeh *et al.* 2019, García-Mato *et al.* 2021).

2.4.2 Structure of P53

Human P53 consists of 393 amino acids and is divided into seven axes:

1. N-terminal: transcription-activation domain (TAD), also known as activation domain 1 that activates transcription factors: residues 1-42.
2. Activation domain 2, is important for apoptotic activity: residues 43-63.
3. A proline-rich region important for the apoptotic activity of P53: residue 64-92.
4. Principal DNA-binding domain. Contains one atom of zinc and several amino acids arginine: residues 100-300.
5. Signal field to identify the nucleus, residues 316-325.
6. Homo-oligomerisation domain: residues 307–355.
7. C-terminal: responsible for reducing the activity of the main DNA binding domain: residues 356-393 (Harms and Chen 2005, Tian *et al.* 2010).

2.4.3 The effect of coronavirus on P53

Recent studies have shown that the P53 gene is a direct target of the type 1 IFN pathway. When infected with a virus, this gene is activated by certain cytokines (Brisse and Ly 2019). In innate antiviral immunity, this provides new insight into P53 function (Miciak and Bunz 2016). The development of a tumor suppressor is seen as a consequence of the activation of P53, an essential factor in the development of viral immunity. According to various studies, this protein is also involved in the regulation of

IFN production and apoptosis in an infected cell. The activities of this protein are also known to help prevent the spread of various viruses. It additionally helps to explain the conserved role of this protein in invertebrates, which do not have cancer-related diseases (Yuan *et al.* 2015). According to studies, type 1 IFN can prevent the development of coronaviruses. However, they have also revealed that low levels of type IFNs in the body can contribute to viral replication. This suggests that the immune system is not able to fight the infection (Mantlo *et al.* 2020).

The low-level IFN refers to the active replication of the virus. It suggests that the coronavirus could either inactivate or evade the immune response. The low-level IFN production is also not clear in the molecular mechanism by which it is carried out. It shows that the proteolipid protein 2 (PLP2) decreases the activity of P53 by increasing its nuclear export and ubiquitination (Clementz *et al.* 2010). The PLP2 inhibitor prevents the synthesis of type I IFN and apoptosis by shutting down the P53-mediated production of these two components. A study suggested that P53 contributes to the upregulation of type I IFN genes. It also provides strong evidence that this protein is an important component of the innate immune system (Yuan *et al.* 2015).

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment and chemicals

High-purity chemicals and general laboratory equipment were used in this study. They are listed in Table 3.1 together with their suppliers.

Table 3.1 Chemicals and general laboratory equipment

Chemicals and Equipment	Suppliers
Micropipette 10-50 μL	Eppendrof, Germany
Micropipette 100-1000 μL	Slamed, Germany
Spectrophotometer	Pye-Unicam, Holland
Spectrophotometer	Cecil, CE 1011
Water bath	Kottermann-W, Germany
Centrifuge	Rotina 35, Germany
Centrifuge	Heraeus-Christ, Digfuge
ELISA	Bio-Teke, USA
Reflotron	Roche, Switzerland
IL-6, P53, IgM, IgG kits	Cusabio, USA
Glucose, urea, creatinine, cholesterol, triglyceride, HDL, Na, UA Kits	Biolabo, France
K Kit	Roche Reflotron Plus
LDH, BiliT, ALKP, ALT, AST, CK, Ca^{+2}, kits	Linear Chemicals, Spain

3.1.2 Samples

This study conducted during the period from March 2022 to the end of May 2022. One hundred fifty individuals previously infected with coronavirus and vaccinated with the mRNA coronavirus vaccine. All subjects were chosen from Ramadi city. The subjects in this study classified into three groups.

3.1.3 Group A

Subjects who were infected with coronavirus 2-3 months ago with moderate symptoms: will consist of 50 individuals. Their ages ranged from 25 to 65 with a mean±SD of 51.36±13.04.

3.1.4 Group B

Individuals who were vaccinated with mRNA COVID-19 vaccine before 2-3 months: consist of 50 individuals. Their age ranged 23-65 years with (mean±SD: 51.27±8.43).

3.1.5 Controls

Thirty healthy individuals will include in this study as a controls group. Their age ranged 25-65 years with (mean±SD: 52.13±11.37 years}-.

None of the control would be diabetic, alcoholic, heavy smoker, had a history of coronary heart disease, thyroid disorder, or other metabolic diseases, and none of the females will be pregnant before taking part in this study.

3.1.6 Blood samples

Five mL of blood drew from each person and each control, randomly. The sample would be transferred to a clean tube, at room temperature, the example would be left for 15 min for coagulation, at -29°C and the serum would be separated and kept in a sterile line in the refrigerator until the time of examination.

3.2 Methods

3.2.1 Measurement of fasting serum glucose

Principle: According to Equation (3.1) and Equation (3.2), fasting serum glucose (FSG) levels were determined for all patients and controls by measuring glucose levels according to glucose oxidase to gluconate-dependent oxidation of glucose with hydrogen peroxide release and after a phenol release reaction in the presence of peroxidase with 4-Amino -antipyrine and peroxide to form quinonemine detected by spectrophotometry at 505 nm (Perez *et al.* 2021).

$$Glucose + H_2O + O_2 \rightarrow glycolic\ acid + H_2O \quad (3.1)$$

$$2H_2O_2 + Phenol + 4- aminoantipyrine \rightarrow Quinoimin + 4H_2O \quad (3.2)$$

Procedure:

- The contents of R1 have been added to R2 and the mixed work detector (WR).

- The spectrophotometer is suitable for zeroing with distilled water.

- As follows in Table 3.2 three bulkheads are made.

Table 3.2 Test solutions and quantities of serum glucose

	Blank	**Standard**	**Sample**
Working solution	1000 µL	1000 µL	1000 µL
D.W	10 µL	-	-
Standard	-	10 µL	-
Sample	-	-	10 µL

- The cuvettes were incubated, at 37°C for 10 min.

- According to Equation (3.3) at 505 nm, the glucose concentration and absorbance reading (A) were calculated.

$$Conc.of\ glucose\ in\ sample\ \left(\frac{mg}{dL}\right) = \frac{A\ sample}{A\ standard} \times standard\ conc. \quad (3.3)$$

3.2.2 Determination of serum urea levels

Principle: According to Equation (3.4) and Equation (3.5) carbon dioxide and ammonia via urea is decomposed. In the presence of sodiumnitroprusside as a coupling agent, sodiumsalicylate will react with the generated ammonia and alkaline hypochlorite. The intensity of the color formed is proportional to the urea concentration in the sample to give green rocks (Liang *et al.* 2019).

$$Urea\ +\ 2H_2O\ \rightarrow\ 2NH^{+3}\ +\ CO_2 \quad (3.4)$$

$$NH^{+4}\ +\ Salycilate\ +\ NaClO\ \frac{Nitroprusside}{OH-} \rightarrow Indophenol\ +\ NaCl \quad (3.5)$$

Procedure:

- At a temperature of 23-27°C, Reagents, and samples were left.

- 24 volumes of R2, and one volume of R1, were mixed to prepare the working reagent.

- As shown in Table 3.3, three tubes were prepared.

Table 3.3 Work methods steps of serum urea

Tubes Solutions	Blank	Standard	Sample
Working Reagent	1 mL	1 mL	1 mL
D.W	10 µL	-	-
Standard	-	10 µL	-
Sample	-	-	10 µL
R3	1.0 mL	1.0 mL	1.0 mL

- In a water bath, the tubes were incubated and mixed at 37°C for 5 min.
- To each tube, 1 ml of R3 was added after step 3.

- In a water bath for 5 min, at 37°C the tubes were mixed and incubated.

- At 650 nm, the absorbance (A), was read and the urea concentration was calculated according to Equation (3.6).

$$\frac{Abs\ Sample}{Abs\ Standard} \times C\ standard\ = \frac{mg}{dL} \quad (3.6)$$

3.2.3 Determination of serum creatinine levels

Principle: Using a colorimetric method, serum creatinine was determined and the range of tools available from Biolabo-France, the principle is based on that creatinine in an alkaline solution reacts with picrate to form a color complex amber-yellow solution, then this solution measured photometrical (Peake and Whiting 2006).

Procedure

The test procedure was carried out in the following steps:
- Let specimens and reagents at room temperature (20-25°C).
- The contents of vial R1 were added to vial R2 contents (1 volume/ 1 volume).
- Into the labeled tubes pipetted as mentioned in Table 3.4.

Table 3.4 Test solutions and quantities of serum creatinine

Tubes	Blank	Standard	Sample
Reagent Working	1 mL	1 mL	1 mL
Demineralized water	100 µL	-	-
Standard	-	100 µL	-
Sample	-	-	100 µL
Supernatant	-	-	1 mL

-After ending addition of 100µL from the sample, the tubes were mixed gently for 30 seconds, record the absorbance A1 at 490 nm, and after 2 min read the absorbance A2 at 490 nm, as shown in Equation (3.7).

$$Creatinine\ \left(\frac{mg}{dL}\right) = \frac{Abs\ (A1-A2\ Assay)}{Abs\ (A1-A2\ Standard)} \times standard\ concentration \quad (3.7)$$

3.2.4 Measurement of serum TC

Principle: According to Equation 3.8, Equation 3.9, and Equation 3.10, TC present in the sample produces a colored compound (Artiss and Zak 1997).

$$Cholesterol\ esters + H_2O \rightarrow Cholesterol\ +\ Fatty\ acids \quad (3.8)$$

$$Cholesterol + O_2 \rightarrow 4 - Cholestenone +\ H_2O_2 \quad (3.9)$$

$$2H_2O_2 + phenol + 4 - Aminophenazone \xrightarrow[(complex\ colored)]{peroxidase} Quinoneimine + 4H_2O \quad (3.10)$$

The amount of quinonimide produced by the red dye is proportional to the concentration of cholesterol, the absorbance at 500 nm, and the quinonemine was read.

Procedure:

- To form WR, the contents of R1 were added into R2, mixed gently.
- In the distilled water, the spectrophotometer was set to zero.
- As follows in Table 3.5, three cuvettes are made.

Table 3.5 Work methods steps of total cholesterol

	Blank	**Standard**	**Sample**
WR	1 mL	1 mL	1 mL
D.W	10 µL	-	-
Standard	-	10 µL	-
Sample	-	-	10 µL

- For 5 min, at 37°C the huts were incubated.
- In Equation (3.11), the cholesterol concentration was calculated, absorbance reading of 505 nm.

$$Conc.\,of\ TC\ in\ sample\,\frac{mg}{dL} = \frac{A\ sample}{A\ standard} \times 200 \quad (3.11)$$

3.2.5 Measurement of serum TG

Principle: According to Equation 3.12, Equation 3.13, Equation 3.14, and Equation 3.15, fatty acids and hydrolyzed glycerol, TGs were enzymatically determined (Eberly *et al.* 2003).

$$Triglycerides \xrightarrow{Lipase} glycerol\ +\ free\ fatty\ acids \quad (3.12)$$

$$Glycerol\ +\ ATP \xrightarrow{Glycerol\ kinase} glycerol\ -3-\ phosphate\ +\ ADP \quad (3.13)$$

$$Glycerol-3-phosphate\ +O_2 \xrightarrow{Glycerol\ -3-\ phosphate\ oxidase} dihydroxy\ acetone\ phosphate\ +H_2O_2 \quad (3.14)$$

$$H_2O_2\ +parachlorophenol\ +\ 4-aminophenazone \xrightarrow{Peroxidase} Quinone\ +\ H_2 \quad (3.15)$$

The amount of red dye made up of quinonimide, is proportional to the concentration of triglycerides, at 500 nm, the absorbance of quinonemin was read.

Procedure:
- To form WR, the contents of R1 in R2 were added and gently mixed.
- In the distilled water set to zero, a spectrophotometer was installed.
- As described in Table 3.6, three cuvettes are made.

Table 3.6 Test solutions and quantities of triglycerides

	Blank	Standard	Sample
W.R	1 mL	1 mL	1 mL
D.W	10 µL	-	-
Standard	-	10 µL	-
Sample	-	-	10 µL

- For 5 min, the cuvettes were incubated at 37°C.
- According to Equation (3.16), the TG concentration and absorbance reading at 505 nm, were calculated.

$$Conc.\,of\ triglyceride\ in\ sample\,\frac{mg}{dl} = \frac{A\ sample}{A\ standard} \times 200 \quad (3.16)$$

3.2.6 Measurement of serum high-density lipoprotein-cholesterol

Principle: According to Equation 3.17, Equation 3.18, and Equation 3.19 at pH 6.2, by adding phosphorous-tungstic acid containing magnesium chloride, the VLDL, fraction, the quantitative chylomicron fraction, and LDL, protein are precipitated, after centrifugation, the supernatant contains the cholesterol concentration of the HDL, fraction determined using the cholesterol group. (Hirano *et al.* 2008).

$$Cholesterol\ esters \xrightarrow{Cho.esterase} Cholesterol\ +\ Fatty\ acid \quad (3.17)$$

$$Cholesterol\ +\ 1/2\ O_2 \xrightarrow{Cho.oxidase} Cholestenone\ +\ H_2O_2 \quad (3.18)$$

$$2H_2O_2\ +\ 4-Aminoantipyrine\ +\ DCFS \xrightarrow{Peroxidase} Quinoneimine\ +\ 4H_2O \quad (3.19)$$

Procedure:
- 0.5 mL of R1 was added to 0.5 mL of serum and mixed gently (turbid), then left on for 10 min and centrifuged at 4000 ×g for 15 min.
- The R2 contents were added to the supernatant.

- In the distilled water on zero, a spectrophotometer was installed.
- As shown in Table 3.7, three cuvettes were prepared.

Table 3.7 Test solutions and quantities of HDL

	Blank	**Standard**	**Sample**
Standard	-	10 µL	-
Supernatant	-	-	10 µL
W.R	1 mL	1 mL	1 mL

- The cuvettes were incubated, at 37°C for 5 min.
- According to Equation 3.20, the concentration of HDL-C was calculated, and the absorbance was read at 505 nm.

$$Conc.of\ HDL-C\ in\ sample\ \frac{mg}{dL} = \frac{A\ sample}{A\ standard} \times (standard\ conc.) \quad (3.20)$$

3.2.7 Determination of serum calcium levels

Using a specially prepared laboratory set, calcium was determined. The principle was determined based on the specific binding of cresolftalein, metallochromic 2, and calcium at alkaline pH with the resulting shift in the absorption wavelength of the compound (Takahashi *et al.* 1999).

Procedure:

Working reagent:

As shown in Table 3.8, recap reagents immediately after use, mix 1 volume of R1, + 1 volume of R2. Stable 5 days at 2-8°C.

Table 3.8 Test solutions and their quantities of serum calcium

	Blank	**Sample**	**CAL standard**
Working Reagent	1.0 mL	1.0 mL	1.0 mL
Sample	-------	10 µL	-------
Cal standard	-------	--------	10 µL

At room temperature mix the tubes and leave for 2 min, then measure the absorbance (A) of samples (Sample A), and the standard (A), against the reagent blank at 570 nm, according to Equation (3.21).

$$\frac{A\ Sample}{A\ Standard} \times Conc\ of\ standard\ =\ mg\ /\ dL\ total\ Calcium \quad (3.21)$$

3.2.8 Determination of serum sodium ions levels.

Principle: Sodium was previously identified as the colorimetric method, based on the modified Trindess and Maruna method, both proteins and Na are precipitated by uranyl magnesium acetate as uranyl MgNa, the acetate salt, potassium reacts with excess uranyl to produce a brown color, color intensity is measured at 530 nm (500-546 nm) (Cogswell *et al.* 2016).

Assay procedure: As follows in Table 3.9, 1000 µL of R1, was added to 10 µL of serum and then mixed and left for 5 min, at a temperature between 23-27°C.

Table 3.9 Work methods steps of serum sodium

	Sample	**Standard**
R1	1000 µL	1000 µL
Sodium Standard	-	10 µL
Sample	10 µL	-

- To obtain clear supernatant, the content was centrifuged at 3000 xg for 2 min.
- As described in Table 3.10 three tubes were prepared.

Table 3.10 Test solutions and quantities of serum sodium

Tubes **Solutions**	**Blank**	**Sample**	**Standard**
R2	1000 µL	1000 µL	1000 µL
Supernatant from step2	-	20 µL	20 µL
R1	20 µL	-	-

-At temperatures between 23-27˚C, the tubes were mixed gently and left for 5 min.
-According to Equation (3.22), the concentration of Na ions was calculated and the absorbance was read at 530 nm.

$$\frac{Abs\ of\ blank - Abs\ of\ Sample}{Abs\ of\ blank - Abs\ of\ Standard} \times 150 = Concentration\ of\ sodium\ mmol/L \qquad (3.22)$$

3.2.9 Determination of serum potassium levels

Reflectance additive is an *in vitro* diagnostic tool designed for quantitative measurement using reflectance test strips of clinical chemistry parameters, it works on the principle of reflective photometry and guarantees fast and reliable results with ease of use, several additional components are also available for Reflotron Plus, these are useful items for analysis or allow you to check system performance (Asirvatham *et al.* 2013).

Reflotron tests:

Reflection tests are test strips designed to identify important clinical chemical determinants using undiluted sample material, using whole blood plus serum and plasma. The incorporation of a serum-separating system allows some tests to be performed with pre-diluted urine. Reflection tests have a long shelf life, with only a few exceptions, at room temperature they can be stored. There is a magnetic strip on the back of each test strip containing all test and batch data.

3.2.10 Measurement of serum bilirubin

Principle: By denaturation of sulfanilic acid properties and photometry, bilirubin is converted to azobilirubin colored. Of the two parts of serum bilirubin-glucuronide and free bilirubin bound to albumin, only the first reacts directly, while free albumin reacts after being removed from the protein by an accelerator. The difference between the measurements of direct bilirubin (without accelerator) and total bilirubin (with accelerator), allows the calculation of indirect bilirubin (Kirk 2008).

Procedure:

BiliT:

- As follows in Table 3.11, pipette into tubes labeled:

Table 3.11 Test solutions and their quantities of serum bilirubin

TUBES	Reagent Blank	Sample Blank	Sample	CAL
D.W	100 µL	–	–	–
Sample	–	100 µL	100 µL	–
CAL	–	–	–	100 µL
RT	–	1.0 mL	–	–
Working reagent	1.0 mL	–	1.0 mL	1.0 mL

- At room temperature, mix well and let the tubes stand for 2 min.

- Versus distilled water, read the absorbance of sample voids at 540 nm.

-Versus the reagent vacuum, read the absorbance of the samples at 540 nm. At room temperature, color is stable for 60 min, according to Equation (3.23).

$$\frac{A\ Sample - A\ Sample\ blank}{A\ Cal} \times C_{Cal} = mg/dL\ total\ or\ direct\ bilirubin \quad (3.23)$$

3.2.11 Measurement of serum alkaline phosphatase (ALKP)

Principle:

ALKP catalyzes the hydrolysis of p-nitro-phenyl phosphate, with the formation of p-nitro-phenol and a free inorganic phosphate, acting as an alkaline buffer as the acceptor of the phosphate group. At the rate of p-nitrophenol formation, the reaction is kinetically monitored at 405 nm, in proportion to the ALKP activity present in the sample (Campos-Roldan *et al.* 2018).

Procedure:

- For reaction temperature, incubate samples, controls, and working reagent.

- Absorption with distilled water, set the photometer to zero.
- Pipette 1 mL working reagent and 20 µL sample or control into a cuvette.
- By inversion and mixing gently, insert the cuvette into the cell holder, and start the stopwatch.
- Record the initial absorbance reading, by incubating for 1 min.
- After 1, 2, and 3 min, repeat the absorbance readings.
- Calculate the difference between absorbance.
- To get the average change, calculate the average the results in absorbance per min (ΔA / min) as shown in Equation (3.24).

$$U/L = \Delta A/(min)\ x\ 2764 \quad (3.24)$$

3.2.12 Measurement of serum ALT

Principle: ALT via LDH catalyzes the transfer of the amino group from alanine to oxoglutarate with the formation of pyruvate and glutamate. The former is reduced to lactate, in the presence of nicotinamide adenine dinucleotide (NADH). At 340 nm the reaction is kinetically monitored, due to the oxidation of NADH to NAD+, at a rate of decrease in absorbance, in proportion to the ALT activity present in the sample (Helmke *et al.* 2015).

Procedure:
- For reaction temperature, incubate samples, working reagent, and controls.
- Absorbance with distilled water set the photometer to zero.
- As shown in Table 3.12, pipette into a cuvette:

Table 3.12 Work methods steps of serum ALT

Reaction temperature	**37ºC**	**30ºC**
Working reagent	1.0 mL	1.0 mL
Sample	50 µL	100 µL

- By inversion, mix gently, insert the cuvette into the cell holder, and start the stopwatch.
- Record the first absorbance reading, by incubating for 1 min.
- After 1, 2, and 3 min, repeat the absorption readings.
- Calculate the difference between absorbance.
- To get the average change, calculate the average of the results in absorbance per min (ΔA/min), as shown in Equation (3.25) and Equation (3.26).

$$U/L = \Delta A/(min)\ x\ 3333\ (37ºC) \quad (3.25)$$

$$U/L = \Delta A/(min)\ x\ 1746\ (30ºC) \quad (3.26)$$

3.2.13 Measurement of serum AST

Principle: AST catalyzes the transfer of the amino group of aspartate to oxoglutarate with the formation of oxaloacetate and glutamate. By malate dehydrogenase, the former is reduced to malate, in the presence of NADH. At 340 nm, the reaction is kinetically monitored, due to the oxidation of NADH to NAD+, at a low absorption rate in proportion to the AST activity present in sample 1 (Vella *et al.* 2012).

Procedure:
- For reaction temperature, incubation of samples, working reagent, and controls.
- Absorbance with distilled water set the photometer to zero.
- As described in Table 3.13, pipette into a cuvette:

Table 3.13 Test solutions and quantities of serum AST

Reaction temperature	**37ºC**	**30ºC**
Working reagent	1.0 mL	1.0 mL
Sample	50 µL	100 µL

- By inversion, mix gently, insert the cuvette into the cell holder, and start the stopwatch.
- Record the first absorbance reading, by incubating for 1 min.
- After 1, 2, and 3 min, repeat the absorbance readings.

- Calculate the difference between absorbance.

- To get the average change, calculate the average results in absorbance per min (ΔA/min), according to Equation (3.27) and Equation (3.28).

$$U/L = \Delta A/(min)\ x\ 3333\ (37ºC) \quad (3.27)$$

$$U/L = \Delta A/(min)\ x\ 1746\ (30ºC) \quad (3.28)$$

3.2.14 Determination of serum UA

Principle: Serum UA was determined by using an available kit from (Biolabo-France), UA was oxidized by uricase enzyme to hydrogen peroxide and allantoin. By a chromogen oxygen acceptor is detected, phenolaminophenazone under the catalysis of peroxidase enzyme (Nagaya *et al.* 1999).

Procedure:

The test procedure was carried out in the following steps:

-At room temperature (20-25°C), incubate reagents and samples.

-The contents of the vial were transferred into vial R2 and then mixed gently to complete dissolution for about 2 min.

- As follows in Table 3.14, into the labeled tubes pipetted.

Table 3.14 Test solutions and quantities of serum UA

Tubes	Blank	Standard	Sample
Reagent Working	1.0 mL	1.0 mL	1.0 mL
Standard	-	25 µL	-
Sample	-	-	25 µL
Demineralized water	25 µL	-	-

-After ending addition, the tubes were mixed gently and left for 5 min, at room temperature (20-25°C) then, the absorbance was recorded at 520 nm, against a blank reagent, according to Equation (3.29).

$$Uric\ acid\ (mg/dL) = \frac{Abs\ \ (Assay)}{Abs\ \ (Standard)}\ x\ standard\ concentration \quad (3.29)$$

3.2.15 Measurement of serum LDH

Principle: LDH in the presence of NADH catalyzes the reduction of pyruvate to lactate, at pH 7.5. At 340 nm, the reaction is kinetically monitored, due to the oxidation of NADH to NAD+ with a decrease in absorbance in proportion to the activity of LDH present in the sample (Dong *et al.* 2017).

Procedure:

- For reaction temperature 30-37°C, incubate samples, working reagent, and controls.
- Absorbance with distilled water, set the photometer to zero.
- As shown in Table 3.15, pipette into a cuvette.

Table 3.15 Work methods steps of serum LDH

Reaction temperature	**30-37ºC**
Working reagent	1.0 mL
Sample or control	20 µL

- By overturning, mix gently, Insert the cuvette into the hive holder and start the stopwatch.
- Record the first absorbance reading, by incubating for 30 seconds.
- After 1, 2, and 3 min, repeat the absorbance readings.
- Calculate the difference between absorbance.
- To get the average change, calculate average the results, in absorbance per min (ΔA/min), as shown in Equation (3.30).

$$U/L = \Delta A/(min)\ x\ 8095 \quad (3.30)$$

3.2.16 Measurement of serum CK

Procedure:

- For reaction temperature, incubate samples, working reagent, and controls (El Allaf *et al.* 1986).
- The absorbance with distilled water set the photometer to zero,

- As follows in Table 3.16, pipette into a cuvette.

Table 3.16 Work methods steps of serum CK

Reaction temperature	**25ºC**	**30ºC**	**37ºC**
Working reagent	1.0 mL	1.0 mL	1.0 mL
Sample or control	40 µL	40 µL	20 µL

- By inversion, mix gently, Insert the cuvette into the cell holder, and start a stopwatch.
- Record the first absorbance reading, by incubating for 3 min.
- After 1, 2, and 3 min, repeat the absorbance readings.
- Calculate the difference between absorbance.
- To get the average change, calculate the average results, in absorbance per min, according to the Equation (3.31).

$$\Delta A/min\ x\ 4127\ =\ U/L\ CK\ (25/30ºC) \quad (3.31)$$

3.2.17 Detection of immunity tests by ELISA kit

- The tests are:-

1- Human IL-6

2- Serum P53

3- IgM and IgG

Principle of the assay:

This test uses a qualitative enzyme, immunoassay technology.

In this kit, the microtiter plate provided is pre-coated with RBD COVID-19 glycoprotein. Samples are drawn into wells with IL-6, P53, IgM, and IgG conjugated to horseradish peroxidase (HRP). Any antibodies specific to the antigen present will bind to the pre-coated antigen. A substrate solution is added to the wells to remove any unbound reagent after washing. The color developed will match the amount of human COVID-19 testing and S1 RBD testing in the initial step. Measurement of color intensities well stopped and color progression (Kim *et al.* 1996, Wang *et al.* 2004).

Materials provided Table 3.17.

Table 3.17 Material used in the test of ELISA tests

Reagents	Quantity
Coated assay plate	1 (96 wells)
Negative Control	1 x 800 µL
Positive Control	1 x 800 µL
HRP-conjugate(100 x concentrate)	1 x 120 µL
HRP-conjugate Diluent	1 x 20 mL
Sample Diluent	2 x 20 mL
Wash Buffer (25 x concentrate)	1 x 20 mL
TMB Substrate	1 x 10 mL
Stop Solution	1 x 10 mL
Adhesive Strip (For 96 wells)	4
Instruction manual	1

1. As directed in Table 3.17, prepare all samples and reagents.
2. To determine the number of wells to be used, refer to the assay layout sheet and return any remaining wells and desiccants to the ziploc, seal pouch, and at 4°C, store the unused wells.
3. Place a blank well without any solution.
4. 100 µL was added to each well, for diluted sample, positive control, or negative control.
5. Cover the tape provided, and incubate for 30 min, at 37°C.
6. Pull out and wash each well, and repeat the process 2 for a total of 3 washes. Wash by filling each well with wash buffer (200 µl), using a squirt bottle, multi-channel pipette, manifold dispenser, or auto washer, and let it stand for 2 min, complete removal of the liquid at each step is essential to good performance. After the last wash, remove any remaining wash buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
7. To each well, 100 µL of HRP conjugate (1×) was added. Incubate at 37°C, for 30 min, after covering the microtiter plate, with the adhesive strip.
8. As in step 6, repeat the aspiration, and wash process five times.
9. To each well, 90 µL of TMB substrate was added, at 37°C, incubate for 20 min, and protect from light.

10. Of the stop solution, 50 μL was added, to each well to ensure thorough mixing, gently tap the plate.

For each well, take a blank well as zero, and determine the optical density using a microplate reader set to 450 nm, within 10 min.

3.3 Data Processing and Statistical Analysis

The data processing and statistical analysis would be done by the computer using the available statistical software packages (Microsoft Excel XP). And data would be presented a simple statistical measures of mean and SD.

The following statistical procedures will do: t-test for comparing the importance of the difference of means between the 2 groups while a one-way analysis of variance (ANOVA), test will use for comparing the importance of the difference of the quantitative data of more than two groups, simple linear correlation, and regression will use for the determination of the correlation between two quantitative data for the different groups. A level of 0.05 will use to determine statistical significance Figure 3.1.

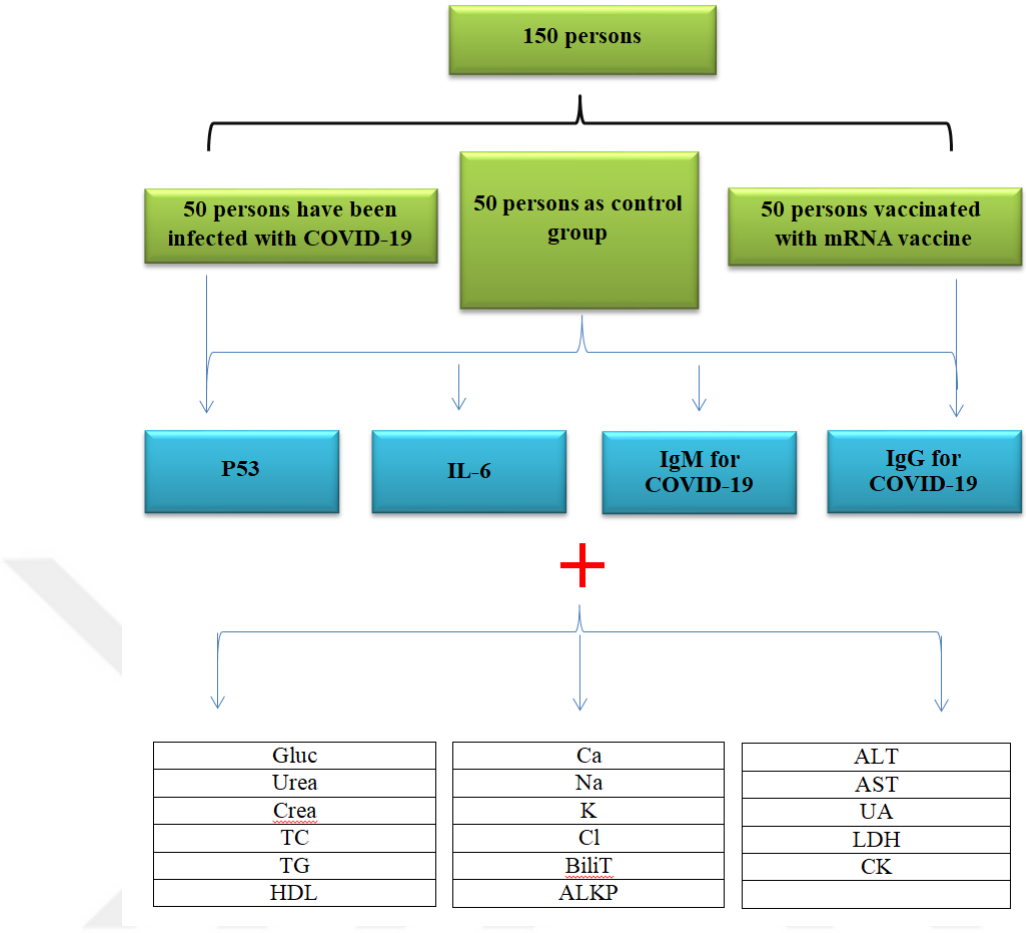

Figure 3.1 Flowchart procedure of this research

4. RESULTS AND DISCUSSION

4.1 Immunological Study

1.1.1 Serum IL-6

Our results indicated that the change within groups wasn't significant for IL-6 levels for individuals who were previously vaccinated (2.61±1.5), previously infected with COVID-19 (2.57±1.4) and control group (3.08±1.6) (P>0.05).

The use of IL-6 antagonists in combination with other drugs to treat COVID-19 was associated with a lower all-cause mortality rate. These agents were also associated with a lower rate of progression to death. The kynurenine pathway is a critical immunomodulatory that contributes to the development of T-cells, suppression, and the maintenance of dendritic cells. It also activates arginine, which can help prevent COVID-19 from entering the body. These results are in agreement with those (Thomas *et al.* 2020, Shankar-Hari *et al.* 2021).

The current study did not examine the effects of IL-6 levels on characteristic deamination and purine breakdown. The study was conducted on individuals who had previously been infected with COVID-19. Since the virus had not been found in the human body after the patient had recovered, we did not find a link between infection and the levels of IL-6.

The goal of vaccines is to stimulate the body's natural immunological response, which then produces antibodies against a specific antigen. In an autoimmune population, vaccinations reduce the burden of infection. To enhance the effectiveness of the vaccine, adjuvants are often added to strengthen their effect on the adaptive and innate immune systems. Although vaccines are generally effective and safe in stimulating the body's natural immune response and reducing the burden of infection in autoimmune

populations, researchers constantly seek ways to enhance their effectiveness through the use of adjuvants (Otani *et al.* 2021, Ng *et al.* 2021).

This process can be triggered by the first dose. A strong reaction to the vaccine at the injection site can lead to a higher level of immunity against the COVID-19 virus. The second dose of the vaccine significantly increased the number of patients experiencing an immunological reaction at the injection site. This suggests that the second dose may help boost immunity against COVID-19.

1.1.2 Serum P53

The mean value of serum P53 in individuals who were previously vaccinated (3782.25±1104.18), and in those previously infected with COVID-19 (3960.61±845.00) found a significant increase compared with a control group (2092.02±1476.62) ($P<0.001$). Previously infected individuals have significantly higher serum P53 levels compared to previously vaccinated individuals ($P<0.05$).

COVID-19 infection has been reported to induce a variety of cellular responses, including the production of the tumor suppressor protein p53. While p53 is commonly associated with its role in promoting cell cycle arrest and apoptosis, recent studies have suggested that p53 may also play a role in regulating the host immune response to viral infections (Levine 2020).

A previous study revealed that P53 can suppress the replication of coronavirus by inhibiting the activity of the antiviral effector IFN. In addition to impairing P53, the COVID-19 viruses also introduce molecular programs that can lead to DNA damage and cell destruction. This could result in massive and rapid apoptosis. It's possible that the nsp3 protein switches off its P53 function during infection, which prevents the virus from developing an innate immune response. When the production of the virus and its replication declines, P53 can be activated by various cell stress response pathways (Cardozo and Hainaut 2021, Zhang *et al.* 2021).

Studies have shown that p53 may be upregulated in response to viral infections, such as influenza and HIV, as a means of activating an antiviral response. In the case of COVID-19, it has been suggested that p53 may also play a role in regulating the inflammatory response, which is a key component of the host immune response to the virus (Rivas *et al.* 2010).

One study examined the expression of p53 in lung tissue samples from patients with severe COVID-19 and found that p53 was significantly upregulated compared to healthy controls. The authors suggested that this upregulation of p53 may be a response to the damage caused by the virus and may contribute to the regulation of the inflammatory response (Nienhold *et al.* 2020).

Another study found that p53 was upregulated in peripheral blood mononuclear cells (PBMCs) from COVID-19 patients, particularly in those with severe disease. The authors suggested that this upregulation of p53 may be a response to the virus-induced cellular stress and may contribute to the dysregulated immune response seen in severe cases (Xiong *et al.*2020).

While the exact mechanisms by which p53 regulates the host immune response to COVID-19 are not fully understood, these studies suggest that p53 may play a role in modulating the inflammatory response and promoting an antiviral state (Lowe *et al.* 2013). Further studies are needed to fully elucidate the role of p53 in COVID-19 infection and to determine whether p53-targeted therapies may have a role in the treatment of severe COVID-19.

In pre-vaccinated individuals, the expression of p53 was found to be significantly increased upon SARS-CoV-2 infection compared to non-vaccinated individuals. This suggests that pre-existing immunity due to vaccination may enhance the cellular response to the virus, resulting in increased p53 expression (Hanan *et al.*2021).

Interestingly, studies have shown that p53 may play a role in the regulation of the immune response to viral infections. Specifically, p53 has been shown to enhance the production of type I interferons, which are crucial for the host defense against viral infections (Rivas *et al.* 2010). Thus, the upregulation of p53 in pre-vaccinated individuals may contribute to the enhanced immune response to SARS-CoV-2 infection.

However, further studies are needed to fully understand the mechanisms underlying the upregulation of p53 in response to SARS-CoV-2 infection in pre-vaccinated and pre-infected individuals. In addition, it will be important to determine whether the upregulation of p53 is associated with better clinical outcomes in pre-infected individuals.

4.1.3 Serum IgM and IgG

Our results indicated that the change wasn't significant ($P>0.05$) for levels of IgM in individuals who were previously vaccinated (0.48±0.36), infected with COVID-19 (0.48±0.34), and control group (0.59±0.20).

Correspondingly the outcome of serum IgG in individuals who were previously vaccinated (41.58±59.55), and previously infected with COVID-19 (26.91±8.28) reported a significant increase compared with a control group (0.59±0.20) ($P<0.01$ and $P<0.001$, respectively).

The results inducted a significant increase in IgG levels in individuals who were previously vaccinated compared with individuals who were infected with COVID-19 ($P<0.05$). But the results indicated that a non-significant difference when comparing individuals who were previously vaccinated and infected with COVID-19 for the levels of serum IgM ($P>0.05$).

In the case of IgM, the body's natural antibodies do not produce resistance to COVID-19. However, in the case of a vaccine, the body's immune system can be triggered by

two mechanisms. One of these is molecular mimicry, which occurs when the combination of an immunological event and the cross-reactivity of an antigen leads to the activation of the acquired and innate immune systems (Chamarti *et al.* 2021). In addition to molecular mimicry, the second mechanism by which a vaccine triggers the immune system is through the use of adjuvants. Adjuvants enhance the effectiveness of the vaccine by activating the innate immune system and stimulating the production of cytokines and chemokines, which attract immune cells to the site of the vaccine injection. This leads to the activation of the acquired immune system and the production of antibodies against the antigen of interest (Principi and Esposito 2019).

As for IgG, the body's natural antibodies are known to be capable of producing a resistance to COVID-19 which is constant with the researchers (Li *et al.* 2022) who reported in the case of a vaccine, the body's immune system can be triggered by the combination of an immunological event and the cross-reactivity of an antigen.

1.2 Biochemical Parameters

1.2.1 Serum glucose measurement

The mean values of glucose concentration in previously vaccinated individuals under this study (108.84±46.13) showed a significant increase compared with the glucose concentration in the healthy group (91.4±11.59) ($P<0.05$). The outcome for the individuals who were infected with COVID-19 (96.22±12.9) indicated a non-significant difference when compared to a control group ($P>0.05$). Also, the mean values of glucose concentration in previously vaccinated individuals observed a significant increase compared with those infected with COVID-19 ($P<0.05$).

Individuals with diabetes are more prone to getting infected due to the defects in their immune system's innate immunity, which include neutrophil chemotaxis, cell-mediated immunity, and phagocytosis. In individuals subject to this study the infection of COVID-19 was not severe, and it did not require hospitalization or artificial respiration.

Because the COVID-19 virus was no longer present in the human body after the infection, it did not affect blood sugar levels (Chen *et al.* 2020b).

It is widely believed that the presence of a vaccine can cause sudden increases in blood glucose levels. However, this phenomenon has not been thoroughly studied. In one case, a hyperosmolar hyperglycemic state was reported following the vaccination for COVID-19. That says the cause of hyperglycemia in some patients is likely to be triggered by the response of the immune system to the vaccine (Mishra *et al.* 2021).

1.2.2 Renal function test

Renal function tests include blood urea, creatinine, and UA test that indicate kidney function.

The mean serum urea level was significantly lower in individuals previously infected with COVID-19 (20.6±5.6) or previously vaccinated (23.32±7.1) compared to the control group (32.6±7.3) ($P<0.001$).

Similarly, serum creatinine levels were significantly decreased in individuals previously vaccinated (0.79±0.2) and previously infected with COVID-19 (0.75±0.1) compared to the control group (0.9±0.2) ($P<0.05$ and $P<0.001$, respectively).

Our results indicated that the change wasn't significant for levels of UA in individuals who were previously vaccinated (4.92±1.6), compared with the control group (5.49±1.1) ($P>0.05$), while levels of UA in the individuals who were infected with COVID-19 (4.56±1.1) the outcome recorded a significant decrease compared with a control group ($P<0.01$).

The results inducted a significant increase in serum urea levels in individuals who were previously vaccinated, compared with individuals who were infected with COVID-19 ($P<0.05$). But the results indicated that the change was insignificant difference when

compared between individuals who were previously vaccinated and individuals who were infected with COVID-19 for the levels of serum creatinine and serum UA ($P>0.05$).

The presence of coronavirus in kidney cells can be caused by the activation of the ACE2-dependent pathway. In addition, the increase in RAAS activity in patients with CKD can lead to the development of an easier infection of COVID-19 cells. Another reason why kidney damage is caused by the coronavirus is that it can exert its effects on kidney tissue. One of the mechanisms that could explain the kidney injury that can occur during COVID-19 infection is the activation of a type of immune response known as a cytokine storm. This response can be triggered by inflammation (Uribarri *et al.* 2020).

In the case of those who were vaccinated with both doses of the vaccine, the spike protein might have caused the increase in their serum renal function levels. This is because the vaccine's outer shell contains a spike protein (Folegatti *et al.* 2020). Another theory suggests that the COVID-19 vaccine could affect kidney function by weakening the T-cell responses. However, this didn't happen in the case of the UA vaccine. The study found that the UA vaccine didn't affect the levels of UA in the subjects (Martin *et al.* 2022).

1.2.3 Lipid profile

Body lipid function tests include blood TC, TG, and HDL that indicate lipid function.

The mean value of serum TC, in individuals who were previously vaccinated (199.20±57.06) and previously infected with COVID-19 (200.90±82.14) was a significant increase compared with the control group (129.28±45.60) ($P<0.001$).

Correspondingly serum TG reported a significant increase in individuals who were previously vaccinated (152.16±81.82) and previously infected with COVID-19 (157.26±61.86) compared with a control group (100.16±26.75) ($P<0.001$).

Our results indicated that the change wasn't significant for levels of HDL in the individuals who were infected with COVID-19 (47.20±11.54), and previously vaccinated (45.70±10.70), compared with the control group (44.00±4.56) ($P>0.05$).

For TC and TG levels, previously vaccinated individuals showed a significant increase compared with individuals who were infected with COVID-19 ($P<0.05$). For the levels of serum HDL, the results indicated that the insignificant difference between individuals who were previously vaccinated and infected with COVID-19 ($P>0.05$).

Patients with chronic inflammation are prone to experiencing dyslipidemia, which is caused by the presence of a viral infection. The metabolism of lipid molecules plays a vital role in the development and maintenance of the viral life cycle (Mahat *et al.* 2021). High levels of HDL cholesterol and triglycerides in patients who are hospitalized for COVID-19 should be considered early warning signs of the disease.

On the other hand, those with a pre-infection lipid profile that is low or high in triglycerides and HDL cholesterol are more prone to experiencing worse outcomes (Masana *et al.* 2021).

Inhalation reactions are known to be fatal and cause severe fear and anxiety in the general population. It is believed that the inactive ingredients in vaccines can trigger an unwanted immune response (Gao *et al.* 2021). As for the vaccine in the case of HDL, it is because of the effectiveness of the vaccine-induced T-cell response (Lombardi *et al.* 2021).

1.2.4 Liver function test

Liver function tests include blood BiliT, ALKP, ALT, and AST test that indicates liver function.

Our results indicated that the change wasn't significant for levels of BiliT in the individuals who were infected with COVID-19 (0.80±0.47), and individuals who previously vaccinated (0.59±0.44), compared with a control group (0.70±0.31) ($P>0.05$).

Correspondingly the outcome of serum ALKP in previously infected with COVID-19 (93.38±30.55) and in individuals who were previously vaccinated (61.80±27.21) reported a significant decrease compared with a control group (114.68±39.09) ($P<0.01$ and $P<0.001$, respectively).

Whereas the results of the current study indicated that the change wasn't significant for levels of ALT in the individuals who were infected with COVID-19 (29.10±14.92) and to individuals who were previously vaccinated (25.18±14.61) compared with the control group (28.48±12.48) ($P>0.05$).

The mean values of AST concentration in individuals who were infected with COVID-19 (28.92±8.80), showed a significant increase compared with AST concentration in the control group (23.36±7.87) ($P<0.01$), while the outcome for the previously vaccinated individuals under this study (23.02±9.12) indicated a non-significant difference compared with a control group ($P>0.05$).

The results induced a significant increase in ALKP and AST levels in individuals who were previously vaccinated compared with individuals who were infected with COVID-19 ($P<0.05$). But the results showed that a non-significant difference compared between individuals who were previously vaccinated and individuals who were infected with COVID-19 for the levels of serum BiliT and ALT ($P>0.05$).

A recent study revealed that COVID-19 can cause liver damage by binding to cholangiocytes that are ACE2-positive. This could explain the link between COVID-19 infection and liver test dysfunction. The use of ARBs and ACE inhibitors may affect liver tests for AST and ALKP. For instance, COVID-19 does not directly bind to cholangiocytes that are ACE2-positive. This could explain why the infection rate is low (Cai *et al.* 2020).

In the case of ALKP, the vaccine can trigger a self-inflicted immune response that leads to liver inflammation. This is why the COVID-19 vaccine is used. The immune system can still attack the liver even without an antigen present. A study shows that systemic viral infections can cause hepatic injury. The study revealed that the activation of T-lymphocytes in response to a viral infection can cause severe damage to the hepatocytes. This suggests that the development of systemic inflammatory states could lead to the direct destruction of the liver (Rafa *et al.* 2022). In the case of AST, ALKP, BiliT, and ALT, it is believed that the use of certain vaccines' adjuvants can trigger an immunological response that can lead to autoimmune or inflammatory syndrome (ASIA) (Malayala *et al.* 2021).

1.2.5 Serum Ca, Na, and K

The results indicated that the change wasn't significant Ca levels in individuals who were previously vaccinated (8.99±0.44) and in previously infected with COVID-19 (9.00±0.38) compared with the control group (8.94±0.32) ($P>0.05$).

Our results indicated that the change wasn't significant for levels of Na in the individuals who were infected with COVID-19 (139.74±6.01) compared with the control group (140.32±2.84) ($P>0.05$), while the outcome for the previously vaccinated individuals under this study (138.08±3.25) showed a significant decrease compared with a control group ($P<0.05$).

While the results indicated that the change wasn't significant in serum K levels in individuals who were previously vaccinated (5.56±7.11) and previously infected with COVID-19 (4.30±0.48) compared with the control group (4.19±0.36) (P>0.05).

The results of the current study inducted showed a significant decrease in Na levels in previously vaccinated individuals compared with those infected with COVID-19 (P<0.05). But the results showed a non-significant difference in the levels of Ca and K between previously vaccinated and infected with COVID-19 (P>0.05).

The role of pH imbalance and all-around electrolyte imbalance in COVID-19 mortality has been understudied. In earlier studies, the effects of the opposite extremities were only averaged out based on laboratory measurements (Nahkuri *et al.* 2021). The presence of Ca in the host cells helps the coronavirus enter the cells. It can also be speculated that the virus' consumption of Ca contributes to the development of COVID-19. To better understand the effects of COVID-19 on the development of the disease, it is important to establish baseline electrolytes (Ozdemir *et al.* 2022).

In the case of the COVID-19 vaccine the various T-cell epitopes can trigger cross-reactive immunity (Nahkuri *et al.* 2021). For Na preparations, the majority of effector cells, including memory B-cells, are produced after vaccination.

However, these cells are not commonly found in lymphoid tissues, such as lymph nodes. There are also only a few mature B-cells, circulating in the circulation. Thus, the number of effector B-cells in PBMCs may not reflect the full spectrum of humoral immunity (Liu *et al.* 2021b).

1.2.6 LDH and CK

Results of our study indicated that the change wasn't significant for levels of LDH in infected with COVID-19 (192.68±35.20) and previously vaccinated (172.16±30.03) compared with the control group (185.80±35.90) (P>0.05).

The results indicated that the change wasn't significant for levels of CK in the individuals who were infected with COVID-19 (122.48±75.36) and previously vaccinated (131.12±119.81) compared with the control group (103.48±40.17) ($P>0.05$).

The results showed no significant difference in LDH and CK levels in individuals who were previously vaccinated and infected with COVID-19 ($P>0.05$).

The level of LDH in serum can increase significantly when the body experiences inflammation or hypoxia. COVID-19 is a type of infection that mainly affects the lungs and other organs. It can cause various chronic conditions such as thrombosis and organ injury. High levels of serum LDH are known to be an important laboratory indicator of COVID-19 infection that is constant with the researchers (Li *et al.* 2020). However, since the study was conducted on previously infected individuals, we did not find a link between the level of LDH and the severity of COVID-19 disease. In conclusion, this study shows that the presence of elevated serum LDH at admission can be used to evaluate the in-hospital mortality and disease severity of patients with COVID-19.

Although the safety of certain vaccines is generally considered to be compromised when compared to other live vaccines, certain types of immune-based vaccines are known to be safe when compared to other vaccines. These include those that are designed to stimulate the T-cell response. There have been many studies that show that these types of vaccines can provide a significant boost to the development of T-cell responses (Fierabracci *et al.* 2020).

There are two possible reasons why myocardial insult could occur in CK. One of these is due to a cytokine storm, which could cause a decrease in the expression of ACE2 in the cardiac myocytes. The other is because of a direct effect on the myocardium by increasing the levels of LDH and IL-6. The ACE2 protein is known to reduce the risk of lung and myocardial injury. It is also believed that this protein can protect against the development of fibrosis and blood pressure (Dalia *et al.* 2021).

Although there have been no reports of infarct-like cases of autoimmune myocarditis following the vaccination, the constellation of non-ischemic MINOCA is based on multi-parametric images. Currently, the COVID-19 vaccines are designed to selectively produce the spike protein (Chamling *et al.* 2021).

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

1- The results of this study evaluated the effect of post-infection by COVID-19, and two doses of mRNA vaccine on IL-6 and IgM levels, compared with the control group. We found the effects of the previous infection and two doses of mRNA vaccine non significant on IL6 and IgM levels.

2- The effect of post-injury by COVID-19 and after the second dose of mRNA vaccine on P53 and IgG levels were studied. We found a significant increase in P53 and IgG levels in the individuals infected with COVID-19 and in individuals after taking the second dose of the mRNA vaccine.

3- The study showed that some parameters increased after infection with COVID-19, which are urea, creatinine, TG, cholesterol, and AST, while some parameters decreased, which are uric acid and ALKP, and the the other parameters were not affected, which is glucose, BiliT, HDL, ALT, Ca, Na, K, LDH, and CK.

4- This study estimated the levels of some parameters after taking the second dose of the mRNA vaccine, and found that some of these parameters increased, which areglucose, urea, creatinine, cholesterol, and TG, and some of them decreased, which are ALKP and Na, but others were not affected as, uric acid, HDL, BiliT, ALT, AST, Ca, K, LDH, and CK.

5.2 Recommendations

1- Study of IL-6 and P53 for previously severely injured patients who have long-COVID-19 symptoms.

2- Evaluation of IL-6, P53, and biochemical test levels in patients with chronic diseases such as diabetes or renal disease, due to complications of COVID-19 infection and comparing the results with chronic diseases without the influence of COVID-19.

3- Assessment of the IL-6 and P53 levels for individuals who received the mRNA vaccine and developed some complications after that.

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APPENDICES

APPENDIX 1. Results of statistical analysis

APPENDIX 2. Form of patients selected

APPENDIX 1. Results of statistical analysis

Descriptives									
		N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
Glu	Control	25.00	91.40	11.59	2.32	86.61	96.19	70.00	117.00
	Pre-infected	50.00	96.22	12.91	1.83	92.55	99.89	70.00	131.00
	vaccinators	50.00	108.84	46.13	6.52	95.73	121.95	67.00	260.00
	Total	125.00	100.30	31.38	2.81	94.75	105.86	67.00	260.00
Urea	Control	25.00	32.60	7.34	1.47	29.57	35.63	20.00	45.00
	Pre-infected	50.00	20.64	5.67	0.80	19.03	22.25	11.00	34.00
	vaccinators	50.00	23.32	7.10	1.00	21.30	25.34	8.00	40.00
	Total	125.00	24.10	7.92	0.71	22.70	25.51	8.00	45.00
Crea	Control	25.00	0.90	0.20	0.04	0.82	0.99	0.54	1.26
	Pre-infected	50.00	0.75	0.12	0.02	0.71	0.78	0.53	0.94
	vaccinators	50.00	0.79	0.22	0.03	0.73	0.86	0.46	1.36
	Total	125.00	0.80	0.19	0.02	0.76	0.83	0.46	1.36
TC	Control	25.00	129.28	45.60	9.12	110.46	148.10	70.00	200.00
	Pre-infected	50.00	200.90	82.14	11.62	177.56	224.24	119.00	477.00
	vaccinators	50.00	199.20	57.06	8.07	182.98	215.42	98.00	348.00
	Total	125.00	185.90	71.86	6.43	173.17	198.62	70.00	477.00
TG	Control	25.00	100.16	26.75	5.35	89.12	111.20	60.00	149.00
	Pre-infected	50.00	157.26	61.86	8.75	139.68	174.84	61.00	326.00
	vaccinators	50.00	152.16	81.82	11.57	128.91	175.41	30.00	380.00
	Total	125.00	143.80	69.15	6.18	131.56	156.04	30.00	380.00
HDL	Control	25.00	44.00	4.56	0.91	42.12	45.88	38.00	55.00
	Pre-infected	50.00	47.20	11.54	1.63	43.92	50.48	27.00	65.00
	vaccinators	50.00	45.70	10.70	1.51	42.66	48.74	33.00	81.00
	Total	125.00	45.96	10.16	0.91	44.16	47.76	27.00	81.00
Ca	Control	25.00	8.94	0.32	0.06	8.80	9.07	8.40	9.50
	Pre-infected	50.00	9.00	0.38	0.05	8.89	9.10	8.20	9.80
	vaccinators	50.00	8.99	0.44	0.06	8.87	9.12	8.10	9.90

	Total	125.00	8.98	0.39	0.04	8.91	9.05	8.10	9.90
Na	Control	25.00	140.32	2.84	0.57	139.15	141.49	136.00	145.00
	Pre-infected	50.00	139.74	6.01	0.85	138.03	141.45	124.00	160.00
	vaccinators	50.00	138.08	3.25	0.46	137.16	139.00	128.00	144.00
	Total	125.00	139.19	4.57	0.41	138.38	140.00	124.00	160.00
K	Control	25.00	4.19	0.36	0.07	4.04	4.34	3.50	4.90
	Pre-infected	50.00	4.30	0.48	0.07	4.16	4.43	3.50	5.50
	vaccinators	50.00	5.56	7.11	1.00	3.54	7.58	3.60	40.00
	Total	125.00	4.78	4.53	0.40	3.98	5.58	3.50	40.00
Cl	Control	25.00	101.76	2.92	0.58	100.55	102.97	98.00	107.00
	Pre-infected	50.00	101.66	2.99	0.42	100.81	102.51	97.00	107.00
	vaccinators	50.00	101.30	2.61	0.37	100.56	102.04	95.00	106.00
	Total	125.00	101.54	2.81	0.25	101.04	102.03	95.00	107.00
Bili T	Control	25.00	0.70	0.31	0.06	0.58	0.83	0.20	1.20
	Pre-infected	50.00	0.80	0.47	0.07	0.66	0.93	0.30	1.90
	vaccinators	50.00	0.59	0.44	0.06	0.46	0.71	0.20	2.30
	Total	125.00	0.69	0.44	0.04	0.62	0.77	0.20	2.30
ALKP	Control	25.00	114.68	39.09	7.82	98.54	130.82	50.00	170.00
	Pre-infected	50.00	93.38	30.55	4.32	84.70	102.06	34.00	175.00
	vaccinators	50.00	61.80	27.21	3.85	54.07	69.53	32.00	153.00
	Total	125.00	85.01	37.15	3.32	78.43	91.58	32.00	175.00
ALT	Control	25.00	28.48	12.48	2.50	23.33	33.63	10.00	54.00
	Pre-infected	50.00	29.10	14.92	2.11	24.86	33.34	6.00	72.00
	vaccinators	50.00	25.18	14.61	2.07	21.03	29.33	8.00	66.00
	Total	125.00	27.41	14.35	1.28	24.87	29.95	6.00	72.00
AST	Control	25.00	23.36	7.87	1.57	20.11	26.61	9.00	33.00
	Pre-infected	50.00	28.92	8.80	1.24	26.42	31.42	14.00	49.00
	vaccinators	50.00	23.02	9.12	1.29	20.43	25.61	10.00	54.00
	Total	125.00	25.45	9.14	0.82	23.83	27.07	9.00	54.00
UA	Control	25.00	5.49	1.13	0.23	5.03	5.96	3.50	7.10
	Pre-infected	50.00	4.56	1.16	0.16	4.23	4.89	2.50	6.90
	vaccinators	50.00	4.92	1.66	0.23	4.45	5.39	1.20	8.30

	Total	125.00	4.89	1.41	0.13	4.64	5.14	1.20	8.30
LDH	Control	25.00	185.80	35.90	7.18	170.98	200.62	125.00	250.00
	Pre-infected	50.00	192.68	35.20	4.98	182.68	202.68	120.00	280.00
	vaccinators	50.00	172.16	30.03	4.25	163.63	180.69	133.00	267.00
	Total	125.00	183.10	34.38	3.08	177.01	189.18	120.00	280.00
CK	Control	25.00	103.48	40.17	8.03	86.90	120.06	45.00	165.00
	Pre-infected	50.00	122.48	75.36	10.66	101.06	143.90	47.00	290.00
	vaccinators	50.00	131.12	119.81	16.94	97.07	165.17	31.00	535.00
	Total	125.00	122.14	91.28	8.16	105.98	138.29	31.00	535.00
IL-6	Control	25.00	3.08	1.69	0.34	2.39	3.78	1.00	6.00
	Pre-infected	50.00	2.57	1.45	0.21	2.16	2.99	1.50	6.18
	vaccinators	50.00	2.61	1.54	0.22	2.17	3.05	1.50	6.47
	Total	125.00	2.69	1.54	0.14	2.42	2.96	1.00	6.47
P53	Control	25.00	2092.02	1476.62	295.32	1482.50	2701.54	548.73	5567.28
	Pre-infected	50.00	3960.61	845.00	119.50	3720.46	4200.76	2815.77	7511.24
	vaccinators	50.00	3782.25	1104.18	156.16	3468.44	4096.05	1889.23	6425.18
	Total	125.00	3515.55	1305.02	116.72	3284.52	3746.58	548.73	7511.24
IgM	Control	25.00	0.59	0.20	0.04	0.51	0.67	0.20	0.90
	Pre-infected	50.00	0.48	0.34	0.05	0.38	0.57	0.12	1.52
	vaccinators	50.00	0.48	0.36	0.05	0.38	0.59	0.14	1.62
	Total	125.00	0.50	0.33	0.03	0.44	0.56	0.12	1.62
IgG	Control	25.00	0.59	0.20	0.04	0.51	0.67	0.20	0.90
	Pre-infected	50.00	26.91	8.28	1.17	24.56	29.26	13.60	44.10
	vaccinators	50.00	41.58	59.55	8.42	24.65	58.50	15.70	327.00
	Total	125.00	27.51	40.67	3.64	20.31	34.71	0.20	327.00

Multiple Comparisons									
LSD									
Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval			
						Lower Bound	Upper Bound		
Glu	Control	Pre-infected	-4.82	7.54	0.52	-19.75	10.11		

		vaccinators	-17.4400*	7.54	0.02	-32.37	-2.51		
	Pre-infected	Control	4.82	7.54	0.52	-10.11	19.75		
		vaccinators	-12.6200*	6.16	0.04	-24.81	-0.43		
	vaccinators	Control	17.4400*	7.54	0.02	2.51	32.37		
		Pre-infected	12.6200*	6.16	0.04	0.43	24.81		
Urea	Control	Pre-infected	11.9600*	1.62	0.00	8.75	15.17		
		vaccinators	9.2800*	1.62	0.00	6.07	12.49		
	Pre-infected	Control	-11.9600*	1.62	0.00	-15.17	-8.75		
		vaccinators	-2.6800*	1.32	0.04	-5.30	-0.06		
	vaccinators	Control	-9.2800*	1.62	0.00	-12.49	-6.07		
		Pre-infected	2.6800*	1.32	0.04	0.06	5.30		
Crea	Control	Pre-infected	.15680*	0.04	0.00	0.07	0.25		
		vaccinators	.11000*	0.04	0.02	0.02	0.20		
	Pre-infected	Control	-.15680*	0.04	0.00	-0.25	-0.07		
		vaccinators	-0.05	0.04	0.20	-0.12	0.03		
	vaccinators	Control	-.11000*	0.04	0.02	-0.20	-0.02		
		Pre-infected	0.05	0.04	0.20	-0.03	0.12		
TC	Control	Pre-infected	-71.6200*	16.30	0.00	-103.88	-39.36		
		vaccinators	-69.9200*	16.30	0.00	-102.18	-37.66		
	Pre-infected	Control	71.6200*	16.30	0.00	39.36	103.88		
		vaccinators	1.70	13.31	0.90	-24.64	28.04		
	vaccinators	Control	69.9200*	16.30	0.00	37.66	102.18		
		Pre-infected	-1.70	13.31	0.90	-28.04	24.64		
TG	Control	Pre-infected	-57.1000*	16.19	0.00	-89.14	-25.06		
		vaccinators	-52.0000*	16.19	0.00	-84.04	-19.96		
	Pre-infected	Control	57.1000*	16.19	0.00	25.06	89.14		
		vaccinators	5.10	13.22	0.70	-21.06	31.26		
	vaccinators	Control	52.0000*	16.19	0.00	19.96	84.04		
		Pre-infected	-5.10	13.22	0.70	-31.26	21.06		
HDL	Control	Pre-infected	-3.20	2.49	0.20	-8.13	1.73		
		vaccinators	-1.70	2.49	0.50	-6.63	3.23		
	Pre-infected	Control	3.20	2.49	0.20	-1.73	8.13		
		vaccinato	1.50	2.03	0.46	-2.53	5.53		

		rs							
	vaccinators	Control	1.70	2.49	0.50	-3.23	6.63		
		Pre-infected	-1.50	2.03	0.46	-5.53	2.53		
Ca	Control	Pre-infected	-0.06	0.10	0.52	-0.25	0.13		
		vaccinators	-0.06	0.10	0.56	-0.25	0.14		
	Pre-infected	Control	0.06	0.10	0.52	-0.13	0.25		
		vaccinators	0.01	0.08	0.94	-0.15	0.16		
	vaccinators	Control	0.06	0.10	0.56	-0.14	0.25		
		Pre-infected	-0.01	0.08	0.94	-0.16	0.15		
Na	Control	Pre-infected	0.58	1.10	0.60	-1.61	2.77		
		vaccinators	2.2400*	1.10	0.04	0.05	4.43		
	Pre-infected	Control	-0.58	1.10	0.60	-2.77	1.61		
		vaccinators	1.66	0.90	0.07	-0.12	3.44		
	vaccinators	Control	-2.2400*	1.10	0.04	-4.43	-0.05		
		Pre-infected	-1.66	0.90	0.07	-3.44	0.12		
K	Control	Pre-infected	-0.11	1.11	0.92	-2.30	2.08		
		vaccinators	-1.38	1.11	0.22	-3.57	0.81		
	Pre-infected	Control	0.11	1.11	0.92	-2.08	2.30		
		vaccinators	-1.27	0.90	0.16	-3.06	0.52		
	vaccinators	Control	1.38	1.11	0.22	-0.81	3.57		
		Pre-infected	1.27	0.90	0.16	-0.52	3.06		
Bili T	Control	Pre-infected	-0.09	0.11	0.38	-0.30	0.12		
		vaccinators	0.12	0.11	0.26	-0.09	0.33		
	Pre-infected	Control	0.09	0.11	0.38	-0.12	0.30		
		vaccinators	.21200*	0.09	0.02	0.04	0.38		
	vaccinators	Control	-0.12	0.11	0.26	-0.33	0.09		
		Pre-infected	-.21200*	0.09	0.02	-0.38	-0.04		
ALKP	Control	Pre-infected	21.3000*	7.64	0.01	6.18	36.42		
		vaccinators	52.8800*	7.64	0.00	37.76	68.00		
	Pre-infected	Control	-21.3000*	7.64	0.01	-36.42	-6.18		
		vaccinators	31.5800*	6.24	0.00	19.23	43.93		
	vaccinators	Control	-52.8800*	7.64	0.00	-68.00	-37.76		
		Pre-infected	-31.5800*	6.24	0.00	-43.93	-19.23		

ALT	Control	Pre-infected	-0.62	3.51	0.86	-7.58	6.34		
		vaccinators	3.30	3.51	0.35	-3.66	10.26		
	Pre-infected	Control	0.62	3.51	0.86	-6.34	7.58		
		vaccinators	3.92	2.87	0.17	-1.76	9.60		
	vaccinators	Control	-3.30	3.51	0.35	-10.26	3.66		
		Pre-infected	-3.92	2.87	0.17	-9.60	1.76		
AST	Control	Pre-infected	-5.5600*	2.15	0.01	-9.81	-1.31		
		vaccinators	0.34	2.15	0.87	-3.91	4.59		
	Pre-infected	Control	5.5600*	2.15	0.01	1.31	9.81		
		vaccinators	5.9000*	1.75	0.00	2.43	9.37		
	vaccinators	Control	-0.34	2.15	0.87	-4.59	3.91		
		Pre-infected	-5.9000*	1.75	0.00	-9.37	-2.43		
UA	Control	Pre-infected	.9300*	0.34	0.01	0.26	1.60		
		vaccinators	0.58	0.34	0.09	-0.09	1.24		
	Pre-infected	Control	-.9300*	0.34	0.01	-1.60	-0.26		
		vaccinators	-0.35	0.27	0.20	-0.90	0.19		
	vaccinators	Control	-0.58	0.34	0.09	-1.24	0.09		
		Pre-infected	0.35	0.27	0.20	-0.19	0.90		
LDH	Control	Pre-infected	-6.88	8.17	0.40	-23.06	9.30		
		vaccinators	13.64	8.17	0.10	-2.54	29.82		
	Pre-infected	Control	6.88	8.17	0.40	-9.30	23.06		
		vaccinators	20.5200*	6.67	0.00	7.31	33.73		
	vaccinators	Control	-13.64	8.17	0.10	-29.82	2.54		
		Pre-infected	-20.5200*	6.67	0.00	-33.73	-7.31		
CK	Control	Pre-infected	-19.00	22.40	0.40	-63.34	25.34		
		vaccinators	-27.64	22.40	0.22	-71.98	16.70		
	Pre-infected	Control	19.00	22.40	0.40	-25.34	63.34		
		vaccinators	-8.64	18.29	0.64	-44.85	27.57		
	vaccinators	Control	27.64	22.40	0.22	-16.70	71.98		
		Pre-infected	8.64	18.29	0.64	-27.57	44.85		
IL-6	Control	Pre-infected	0.51	0.38	0.18	-0.23	1.26		
		vaccinators	0.47	0.38	0.21	-0.27	1.22		

	Pre-infected	Control	-0.51	0.38	0.18	-1.26	0.23		
		vaccinators	-0.04	0.31	0.90	-0.65	0.57		
	vaccinators	Control	-0.47	0.38	0.21	-1.22	0.27		
		Pre-infected	0.04	0.31	0.90	-0.57	0.65		
P53	Control	Pre-infected	-1868.588440*	268.93	0.00	-2400.96	-1336.21		
		vaccinators	-1690.227340*	268.93	0.00	-2222.60	-1157.85		
	Pre-infected	Control	1868.588440*	268.93	0.00	1336.21	2400.96		
		vaccinators	178.36	219.58	0.42	-256.32	613.04		
	vaccinators	Control	1690.227340*	268.93	0.00	1157.85	2222.60		
		Pre-infected	-178.36	219.58	0.42	-613.04	256.32		
IgM	Control	Pre-infected	0.11	0.08	0.16	-0.04	0.27		
		vaccinators	0.11	0.08	0.19	-0.05	0.26		
	Pre-infected	Control	-0.11	0.08	0.16	-0.27	0.04		
		vaccinators	-0.01	0.07	0.91	-0.14	0.12		
	vaccinators	Control	-0.11	0.08	0.19	-0.26	0.05		
		Pre-infected	0.01	0.07	0.91	-0.12	0.14		
IgG	Control	Pre-infected	-26.32260*	9.33	0.01	-44.80	-7.85		
		vaccinators	-40.98800*	9.33	0.00	-59.46	-22.51		
	Pre-infected	Control	26.32260*	9.33	0.01	7.85	44.80		
		vaccinators	-14.67	7.62	0.06	-29.75	0.42		
	vaccinators	Control	40.98800*	9.33	0.00	22.51	59.46		
		Pre-infected	14.67	7.62	0.06	-0.42	29.75		

APPENDIX 2. Form of patients selected

Age		weight		Length		Married	
chronic diseases				Smoked			
Person status							Normal value
Number of sample			Results				
GluC							70 - 120
Urea							20 - 45
CreaC							0.72 – 1.25
Chol							70 – 199
Trig							60 – 149
UHDL							38 – 580
CaC							8.4 – 9.6
Na-C							136 – 145
K-C							3.5 – 5.1
BiliT							0.2 – 1.2
AlkP							40 – 170
ALT							9 – 55
AST							8 – 34
UA							3.5 – 7.2
LDH							125 – 250
CK							
IL-6							33.07 – 43.11
P53							
IgM							
IgG							