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BOLU ABANT İZZET BAYSAL UNIVERSITY
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Department of Chemistry

MONITORING OF SOME BIOCHEMICAL MARKERS IN IRAQI COVID-19 PATIENTS

MASTER OF SCIENCE

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APPROVAL OF THE THESIS

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SURA LUAE ABDULSAHIB AL-QAZZAZ

ABSTRACT

MONITORING OF SOME BIOCHEMICAL MARKERS IN IRAQI COVID-19 PATIENTS.
MSC THESIS
SURA LUAE ABDULSAHIB AL-QAZZAZ
BOLU ABANT IZZET BAYSAL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF CHEMISTRY
(SUPERVISOR: ASSOC. PROF. DR. ERCAN SELÇUK ÜNLÜ)
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BOLU, NOVEMBER 2022
(xiii + 59)

The world has recently witnessed the emergence of the SARS-CoV-2 virus, which has led the world to enter a pandemic. There is an urgent necessity to reach diagnosis and treatment and to limit the spread of SARS-CoV-2, which has become a death-threatening concern for patients with COVID-19. The aim and scope of this study are to monitor and evaluate the role of levels of biochemical parameters for the markers in Iraqi COVID-19 patients and to study the correlation between biochemical parameters. The study comprised 60 people who seemed to be in good health as a control group and 107 COVID-19 patients (64 men and 43 women). The age group with the highest infection rate was 46-59 years, which constituted (39.3%) of the patients. The results of the current study showed that there was a highly significant increase (P value < 0.01) in the levels of biomarkers for coronavirus patients compared to the control group for each of ALT, AST, ALP, Direct bilirubin, GGT, BUN, Urea, Calcium, Chloride, Potassium, Sodium, HDL, LDL, VLDL, Amylase, Lipase, D-Dimer, Ferritin, MG, LDH, IL -1. These results also showed a significant positive correlation (P value <0.05) in total bilirubin level, as well as Non-Significant (NS) in creatinine level. It can be concluded that liver and kidney functions are both affected by COVID-19.

KEYWORDS: COVID-19, Biochemical parameters, D-Dimer, LDH, Ferritin, Liver Functions, Kidney Functions

ÖZET

IRAKLI COVİD 19 HASTALARINDAKİ BAZI BİYOKİMYASAL BELİRTEÇLERİN İZLENMESİ
YÜKSEK LİSANS TEZİ
SURA LUAE ABDULSAHIB AL-QAZZAZ
BOLU ABANT İZZET BAYSAL ÜNİVERSİTESİ
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Dünya, yakın zamanda pandemiye girilmesine neden olan SARS-CoV-2 virüsünün ortaya çıkışına tanıklık etti. COVID-19 hastaları için ölümcül bir tehtid haline gelen SARS-CoV-2'nin tanısı, tedavisine ve virüsün yayılmasının sınırlandırılmasına yönelik bilgilerin eldesine acil bir ihtiyaç vardır. Bu çalışmanın amacı ve kapsamı, Irak COVID-19 hastalarının aşağıdaki belirtilen belirteçler kapsamında bazı biyokimyasal göstergelerin düzeylerinin rolünü izlemek ve değerlendirmek ve bu biyokimyasal parametreler arasındaki korelasyonun incelenmesidir. Çalışma, kontrol grubu olarak sağlıklı görünen 60 kişiden ve 107 COVID-19 hastasından (64 erkek ve 43 kadın) oluşmuştur. Yapılan analizlerde enfeksiyon oranının en yüksek olduğu yaş grubu 46-59 olup hastaların %39,3'ünü oluşturmuştur. Mevcut çalışmanın sonuçları incelendiğinde, koronavirüs COVID-19 biyobelirteçi olarak ALT, AST, ALP, Direkt bilirubin, GGT, BUN, Üre, Kalsiyum, Klorür, Potasyum, Sodyum, HDL, LDL, VLDL, Amilaz, Lipaz, D-Dimer, Ferritin, MG, LDH, IL -1'in her biri için kontrol grubuna kıyasla hasta grubunda oldukça anlamlı bir artış (P değeri < 0.01) olduğunu göstermiştir. Bu sonuçlar aynı zamanda toplam bilirubin düzeyinde önemli bir pozitif korelasyon (P değeri <0.05) olduğunu ancak kreatin düzeyinde anlamlı bir değişim olmadığını göstermiştir. Çalışma genel olarak değerlendirildiğinde karaciğer ve böbrek fonksiyonlarının her ikisinin de COVID-19'dan etkilendiği sonucuna varılmıştır.

ANAHTAR KELİMELER: COVID-19, Biyokimyasal parametreler, D-Dimer, LDH, Ferritin, Karaciğer fonksiyonları, Böbrek fonksiyonları

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LIST OF ABBREVIATIONS AND SYMBOLS

AKI **:** Acute Kidney Injury.

ALT **:** Alanine Transaminase.

AST **:** Aspartate Transaminase.

ATP **:** Adenosine Triphosphate.

CETP **:** Cholesteryl Ester Transfer Protein.

COVID-19 **:** Corona virus disease 2019.

ELISA **:** Enzyme-Linked Immunosorbent Test.

GGT **:** Gamma-glutamyl Transferase.

IL-1 **:** Interleukin-1.

PCR **:** Polymerase Chain Reaction.

R0 **:** Basic Reproductive Number.

RNA **:** Ribonucleic Acid**.**

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1. INTRODUCTION

The coronavirus study group described this virus as SARS-CoV-2, known as (COVID-19) according to the (WHO), which declared a global outbreak and considered it a pandemic in March 2020. It was originally discovered in Wuhan, China [1], and subsequently spread to other countries. SARS-CoV and MERS-CoV have created epidemics with a fatality, over the last two decades. This is the third high-grade outbreak to be discovered and varies by nation. Due to the high transmissibility, there are 4,5678,440 confirmed cases in 219 countries as of 1 November 2020 according to the WHO. Therefore, scientists and governments have taken immediate action to characterize the acute human infection caused by the SARS virus, monitor the epidemic, and carry out etiological research [2]. Personal contact and respiratory droplets are the main ways that the virus is transmitted. Asymptomatic and asymptomatic carriers can transmit the infection [3]. The virus is a member of the Coronaviridae family and the Coronavirinae subfamily of the Nidovirales order [4]. Coronaviruses are enclosed viruses with positive-sense single -stranded RNA genomes that are the biggest of any known RNA viruses, ranging in size from 26 to 32 kb. The term "coronavirus" originates from the appearance of the coronavirus virion, which looks like a crown under the electron microscope due to the protruding proteins from the surface [5].

The binding of the (S) protein covering the virus surface to the cellular ACE2 receptor is dependent on SARS-CoV-2 entrance [6]. SARS-CoV-2 causes an immunological response that generates inflammatory cytokines and weak interferons (IFNs) once it enters respiratory cells [7]. The condition causes a pneumococcal infection, and symptoms can appear as early as three days after onset but must appear before 13 days. Studies have shown that the incubation period averages about five days [8].

Laboratory results are one of the most important steps in identifying and follow-up of COVID-19 patients. Numerous research has been carried out to investigate biomarkers.

Complete blood counts, tests probing the coagulation (D-dimers), and indications related to inflammation are all essential for COVID-19 patients (ESR, CRP, ferritin, and procalcitonin).

Since viruses have the potential to significantly damage many vital organs such as the liver, kidney, and heart, biochemical marker analysis is a suitable method for clinicians to determine the functional processes of these organs [9]. It has also been discovered that those infected with SARS and COVID-19 exhibit comparable inflammatory infection patterns.

Increased pro-inflammatory cytokine levels (e.g., IL-1, IL-6, IFN, MIP1A, and MCP1) are linked to increased lung infection and damage.

1.1 The History and Appearance of COVID-19

Coronaviruses were initially identified in the 1960s as the common cold. According to a Canadian study from 2001, almost 500 people were diagnosed with influenza-like sickness, with 18 of them being diagnosed with coronavirus strains using polymerase chain reaction (PCR) techniques [10]. In 1965, Tyrrell and Bynoe published the first human case of coronavirus. They discovered that they could spread the B814 virus. It has been isolated from the respiratory tract of persons who have flu-like symptoms and discovered in human embryonic tracheal organ cultures [8].

Until 2002, coronavirus was believed to be a mild, non-lethal virus. In 2003, there were several episodes of severe respiratory syndrome that some cases led to death, as it turned out to be caused by corona, which led to the death of more than 1,000 patients. In another report in Hong Kong, 30 people were diagnosed with the coronavirus in 2003.

Multiple cases of pneumococcal infection of unknown origin were discovered, according to the WHO report from China's Ministry of Health [11]. On January 7, 2019, a Chinese throat swab sample containing the new coronavirus nCoV was discovered. [12].

1.2 Classification of Coronavirus-2

SARS-CoV-2 belongs to the family (Coronaviridae) and the order (Nidovirales). It is composed of the Coronavirinae and Torovirinae subfamilies, which are further split into four genera each. The human coronavirus (HCoV) is contained in the alphacoronavirus; the beta coronavirus contains the (MERS-CoV), and (SARS-HCoV); the delta coronavirus contains viruses that infect pigs and birds, and the gamma coronavirus contains viruses that infect whales and birds, the beta coronavirus subfamily includes the coronavirus 2 [13].

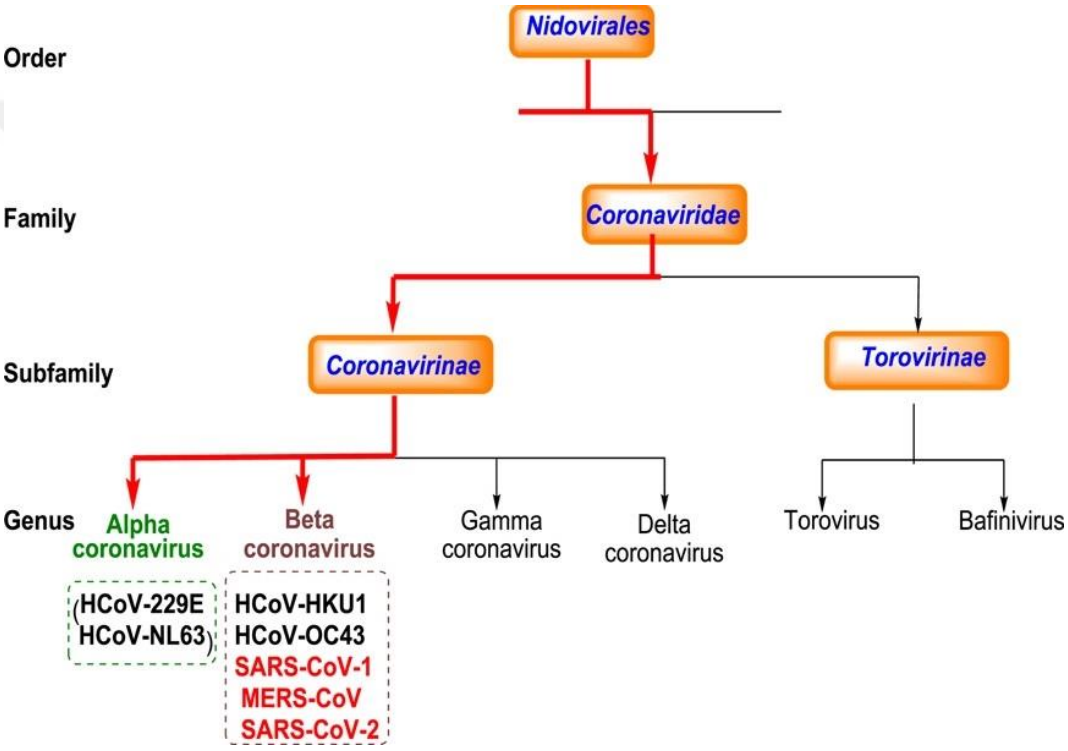


Figure 1.1. Coronaviruses classification [13].

1.3 Transmission of COVID-19

The virus is extremely contagious and spreads mostly through contact, and respiratory droplets [14]. People may often obtain COVID-19 from verified people. Up to 3.000 droplets can be spread by one cough. Anyone who does not wash their hands properly risks spreading various respiratory viruses to anyone they come into contact with, including the flu. However, many smaller particles will linger in the air. Tiny droplets generated from an infected individual's upper

respiratory tract secretions can transmit COVID-19 by direct touch. Such as sneezing, a cold, or coughing that comes from the mouth and nose [12].

For this reason, it is recommended to keep your distance of at least one meter (3 feet) from a sick patient. If the droplets of the virus remain on nearby objects and surfaces, and someone touches their nose, eyes, or mouth after contacting those surfaces with their hands, they may contract COVID-19 [8].

The COVID-priming 19 instances were connected to the animal-to-human transmission. The virus may have been transmitted from person to person earlier [15]. Medical professionals and groups of infected close family members have identified the presence of patient-to-person conduction. After January 1st, fewer than 10% of people had market exposure, and more than 70% had none at all. Since it spreads through respiratory droplets and given that it may survive on surfaces for a long time, droplets can be an important source of spread [16]. Figure 1.2 shows the structure of coronavirus -2 [10].

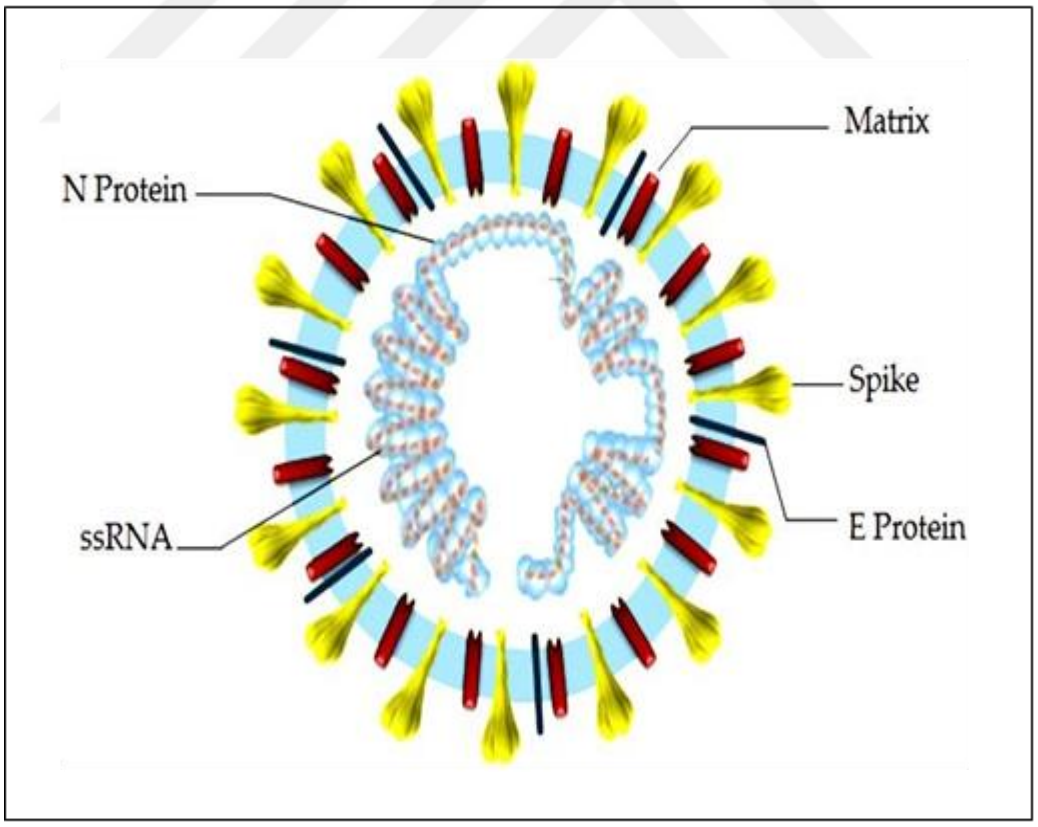
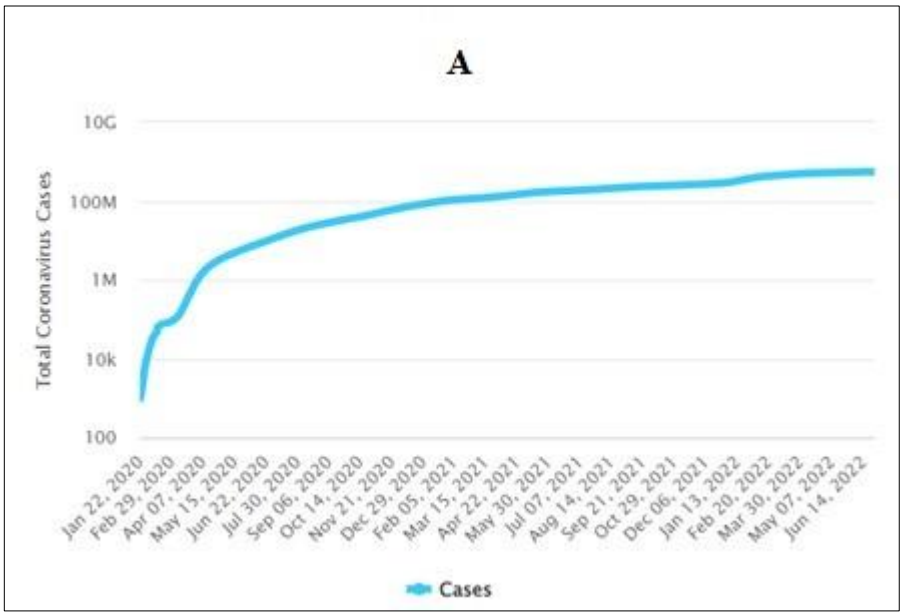


Figure 1.2. The structure of the coronavirus-2.

Various evaluations of the fundamental reproductive number (R0) have produced varied conclusions. R0 calculates the average number of contagions that one sick person in a community with full sensitivity may cause. R0 was found in studies of prior outbreaks to be in close proportions for both SARS and the 2009 H1N1 influenza pandemic. According to one study, the fundamental reproductive number (R0) was 2.2. R0 was 3.28, however, according to a subsequent study of 12 trials that were still accessible. It is important to take into account the function of super transmitters since R0 reflects an average value and they may be primarily responsible for outbreaks inside big aggregations, but they will not be primarily responsible for controlling the value of R0 [17].

1.4 Epidemiology of COVID-19

Until June 2022, 222 countries around the world have officially registered more than 549 million positive cases, while the global death toll has exceeded 6.35 million. Most cases are registered in the United States of America with over 88.9 million, followed by India with over 43.4 million. Most deaths were recorded in the United States, where about 1.01 million people died.



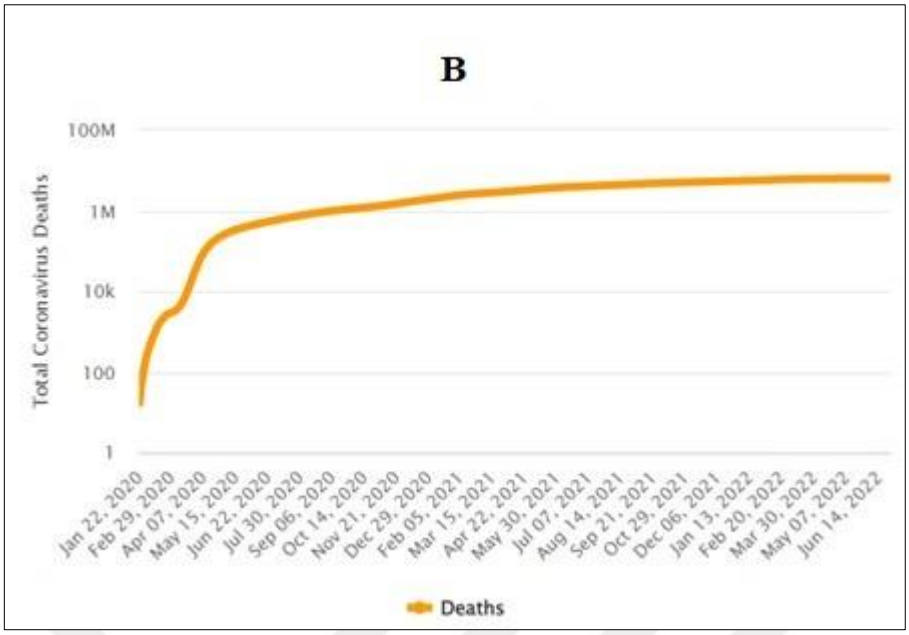
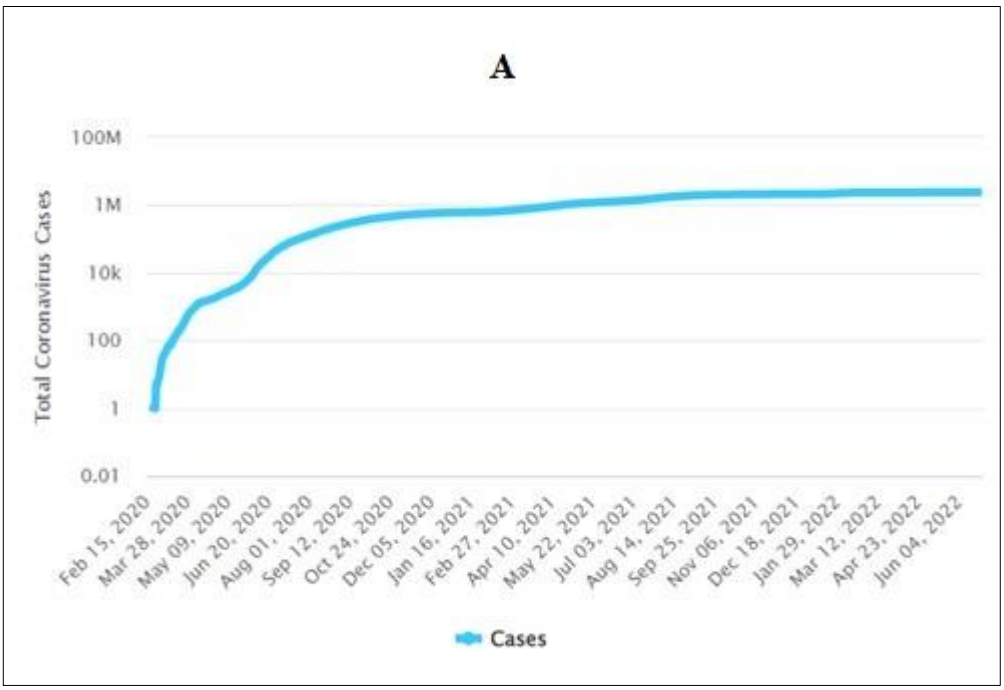


Figure 1.3. Total Coronavirus Cases (A) and Deaths (B) worldwide.

At the same time, more than 2.34 million cases were officially registered as positive cases in Iraq, while the deaths exceeded 25 thousand. Iraq ranked 42nd globally in the rates of injuries and deaths.



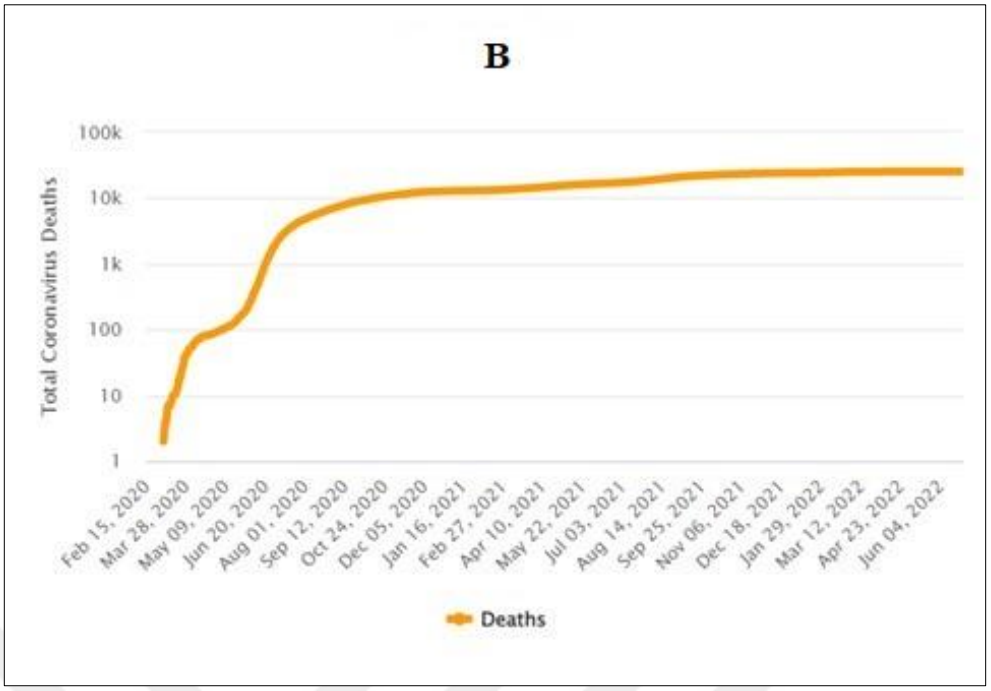
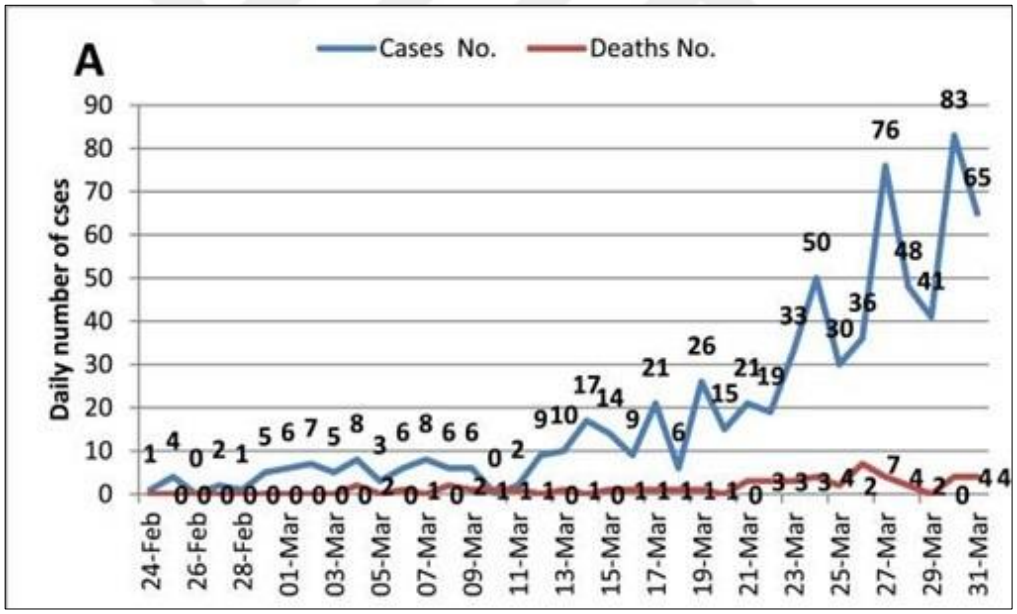


Figure 1.4. Total Coronavirus Cases (A) and Deaths (B) in Iraq.



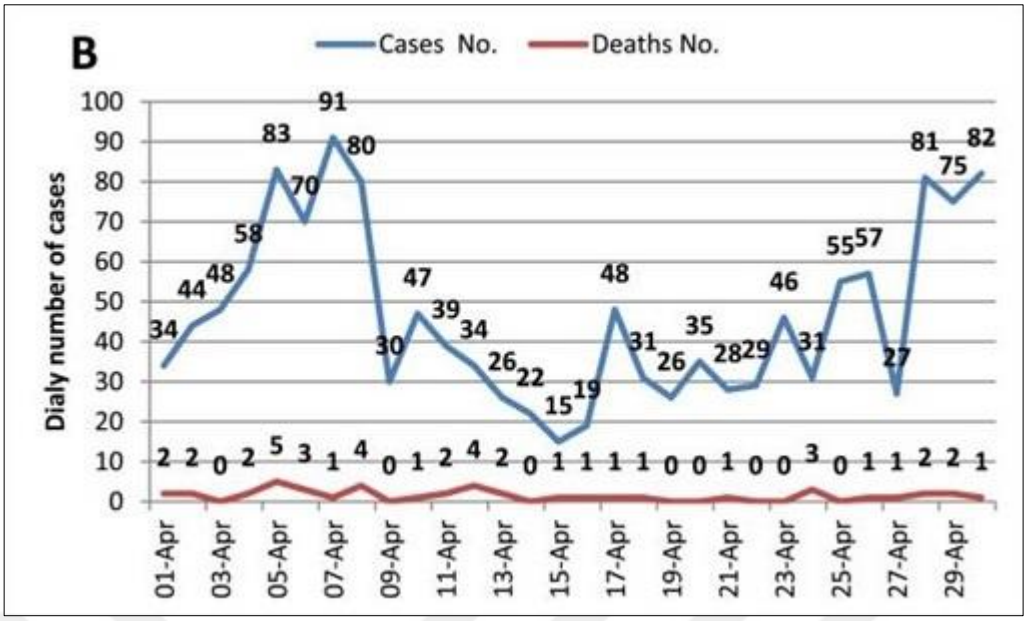


Figure 1.5. COVID-19 situation daily new cases and deaths in Iraq [18].

The severity and start of COVID-19, on the other hand, fluctuate significantly for people of different ages, with symptoms getting worse as people age. Those under 19 have the lowest risk, with death rates between 0 and 0.1 percent, while those between 75 and 84 had mortality rates between 4.3 and 10.5 percent. 85 years of age or older provide the greatest danger, with death rates ranging from 10.4 to 27.3 percent. The mortality rate is also elevated by underlying medical disorders including diabetes, cardiovascular disease, or a weakened immune system [19].

1.5 Pathogenesis Coronavirus-2

The innate immune response is triggered when the coronavirus-2 enters host cells after binding to their receptors. Coronavirus-2 has to be able to block and suppress the host's innate immunological trigger to infect other hosts more frequently. Regardless, it remains incredibly unclear how Coronavirus-2 can suppress the immune response and trigger pathogenesis. Numerous clinical traits of COVID-19 imply that its origins may be comparable to those of SARS-CoV. To prevent the spread of infection, IFN induces the synthesis of ISGs. In order to counteract its antiviral impact, SARS-CoV produces at least eight viral inhibitors that regulate IFN and cytokine production as well as effector (ISG) activity. The

host's immunity against viral contamination, which prompts cellular antiviral actions, is critical in preventing viral generation and transmission.

According to studies, high fever and dry cough are typical signs of pneumococcal infection at the beginning of the disease. More proof points to ACE2 as a possible site of entry for the Coronavirus-2 that comes of the progression of acute respiratory stress syndrome and the severity of the lung damage. ACE2 is widely known to be present in second-type alveolar cells in humans [14].

The plasma concentrations of proinflammatory cytokines such as IL1, IL2, IL7, GSCF, and MCP1 are shown to be greater in people with Coronavirus-2 infection than in healthy adults [20]. As previously mentioned, another intriguing discovery was that few incidences of Coronavirus-2 infection in youngsters were found, with older male patients showing a preference for infection.

1.6 Clinical Symptoms of COVID-19

Similar to SARS-CoV, 2019-nCoV's clinical signs include fever, dry cough, dyspnea, chest discomfort, tiredness, and myalgia among other symptoms. Headache, lightheadedness, stomach discomfort, constipation, diarrhea, nausea, and vomiting are less frequent symptoms [20]. About 75% of people tested positive for bilateral pneumococcal infection, indicating that the virus could prefer to invade the lower respiratory tract. Women who are pregnant and those who are not pregnant share comparable traits [21].

Sufferers experienced multiple acute outcomes in a study of 99 subjects, of which 17% developed acute respiratory distress syndrome and 11% died of failure of numerous organs. The median time between the onset of symptoms and ARDS was 8 days. Most virus-infected people will develop the common cold and flu, while a small number will stay asymptomatic. Eighty percent of patients will only have minor sickness symptoms. Adults have the strongest immunity to stave off the infection, but they also have the disadvantage of being more prone to transfer it [10].

1.7 Receptors

The four primary structural proteins of the coronaviral genome are encoded as the (S, M, E, N) proteins. The (S) protein facilitates the attachment of the virus to the host cell's surface receptors, resulting in fusion and subsequent viral entry. The formation of the viral envelope is depended on the conformation of the (M) protein, which is the most prevalent protein. The (E) protein, which plays a role in viral assembly and budding, is the smallest of among the main structural proteins. The (N) protein participates in the assembly and budding of viruses, and it is the only RNA-binding protein. Coronavirus replication starts with attachment and entrance. The (S) protein and its particular receptor interact to cause the virus to attach to the host cell. The virus enters the host cell cytoplasm after attaching to the receptor by cleaving the S protein with a protease enzyme, which is followed by the fusing of the viral and cellular membranes. The replicated gene must subsequently be translated from the virion genomic RNA, followed by the construction of the viral replica complexes. Encapsulation takes place after replication and sub-genomic RNA synthesis, resulting in the development of the mature virus. Virions are released in vesicles through the exocytosis mechanism once they are assembled and ready to be delivered to the cell surface [22].

Using the ACE2 receptors (proteins) on human cells, Coronavirus-2 connects to its target like that of its more seasoned sibling, SARS-CoV. These receptors are widely distributed in the vascular endothelium, small intestine, and lung epithelium of humans.

Chest imaging, (CBC), liver function tests, serum testing IgG and IgM, and polymerase chain reaction on respiratory samples are all used in this grade's diagnosis. The CBC may show low lymphocyte and neutrophil counts without any obvious abnormalities [23].

1.8 Prognosis of COVID-19

Clinical symptoms of the patients progressively get better after a thorough treatment and eventually disappear. However, it should be mentioned that a few variables can affect the prognosis of patients [23].

After receiving appropriate therapy, white blood cell, lymphocyte, D-dimer, and ESR levels in the blood return to normal. However, individuals may have a bad prognosis if these test findings are aberrant [24].

The imaging characteristics and overall computed tomography (CT) scan in a SARS-CoV-2 infection change during the illness, from the time of the first diagnosis until the patient recovers. Ten days following the beginning of symptoms, most of the patients' lung diseases were at their most severe state on a CT scan. Approximately 14 days following the commencement of the initial symptoms, the chest CT findings start to improve. Other significant prognostic markers are variations in the immune systems and vulnerability of the host to infections. Recent findings indicate that coronavirus infections can be more common and cause more severe symptoms because of cardiovascular conditions like hypertension [25].

1.9 Biochemical Markers in COVID-19

Low lymphocytes, a prolonged prothrombin time, and elevated lactate dehydrogenase are all typical laboratory abnormalities in COVID-19 patients. Higher test abnormalities were seen in critical care unit patients than in other hospitalized patients. The majority of people have demonstrated normal serum procalcitonin levels [10].

Individuals with COVID-19 have elevated levels of IL1, IFN-, IP10, and MCP1. Individuals hospitalized in the ICU often have elevated levels of GCSF, IP10, MCP1A, MIP1A, and TNF [14].

The most frequent laboratory abnormalities included hypoalbuminemia, low lymphocytes, a low proportion of lymphocytes and neutrophils, and high LDH. Blurry increased in and range as the sickness got worse, spreading to the center and then covering the whole surface of the lungs. Creatine kinase, myoglobin, cardiac troponin 1, and brain natriuretic peptide are biochemistry indices linked to heart function that may dramatically increase in coronavirus infection [26].

The severe transformation group had significantly longer PT, significantly higher levels of ALT, AST, and LDH, and significantly lower levels of lymphocytes, albumin, and PaO2/FiO2 than the mild group [27].

The most common laboratory findings were decreased albumin (75.7%), high C-reactive protein (58.3%) and high LDH, low lymphocytes (43.1%), high erythrocyte sedimentation rate (41.8%), high bilirubin, and high creatinine kinase [28].

1.9.1 D-dimer

Rising levels of D-dimer show that coagulation and fibrinolysis are being activated as they are produced by the lysis of cross-linked fibrin.

Elevated D-dimer levels are frequently seen in patients. Also, critically ill patients have much higher levels, which can be used as an indicator of mortality [29].

D-dimer has been connected to both COVID-19 mortality and severity. That infection may cause hemostatic system failure, which would lead to a hypercoagulable state, a condition that is typically seen in sepsis [30].

Patients with COVID-19 who are severely unwell have pulmonary small artery occlusion and micro thrombosis, according to recent data from lung pathology dissection [31].

1.9.2 Lactate Dehydrogenase

Lactate dehydrogenase (LDH) and other inflammatory mediators that are released in high quantities in humans have been studied as prognostic and monitoring tools for the onset, activity, and progression of the disease. Despite having no further metabolic function in the extracellular fluid, cytoplasmic cellular enzymes like LDH are nonetheless useful because they serve as markers of cellular integrity problems brought on by the diseased condition. LDH is an enzyme that is found in almost all major organ systems, hence abnormal serum LDH activity is seen in a variety of disturbances [32].

Numerous research sources and studies claim that there are significant differences between animal species and tissue distribution in terms of LDH activity and its isoenzyme patterns. It is important to comprehend the make-up and distribution of each animal's tissue isoenzymes. LDH's extracellular manifestation

is employed to identify cell death or injury. Increased concentrations are observed in several hematological and neoplastic disorders, as well as in cardiac, hepatic, striated muscle, and renal disease. Hemolytic disorders and pernicious anemia have the highest levels of total LDH.

Acute myocardial infarction, pulmonary infarction, and liver disorders such as cirrhosis and liver viral infection all exhibit minor increases of two to three times the upper limit of normal. Increased LDH concentrations are caused by striated muscle disorders and certain leukemias. Most people, especially those with acute lymphoblastic leukemia, have noticeable increase. An elevated total LDH value is a fairly vague finding because there are so many factors that might lead to higher activity. Therefore, if lactate dehydrogenase tests are separated into isoenzyme fractions, they gain more clinical importance [33].

1.9.3 Bilirubin

Bilirubin is an endogenous chemical compound that can be very toxic, specifically in neonates. Unconjugated bilirubin (UCB) has been noted to have a strong antioxidant activity, and modest hyperbilirubinemia may have positive benefits on health. As the final byproduct of hemoglobin breakdown, bilirubin, is used to diagnose problems with the liver and blood. It has a complicated metabolism, which is important in connection to several drug metabolism processes. The heme ring at the carbon bridge is opened to create bilirubin from the heme. The heme oxygenase enzyme facilitates this cleavage, which releases iron and results in the production of carbon monoxide and biliverdin IXa. The latter is converted to bilirubin IXa by the cytosolic enzyme biliverdin-reductase. Mesoporphyrin can temporarily block heme oxygenase, and this suppression results in a low UCB formation. While heme-containing proteins account for about 20% of daily production, bilirubin is derived from hem present in hemoglobin and is freed during the breakdown of senescent erythrocytes. It is created by the monocytic Kupffer cells of the liver, bone marrow, and spleen and is subsequently released into the plasma. A typical adult forms (3.8 mg/kg) , of bilirubin every 24 hours [34].

UCB is found in plasma heavily coupled to albumin since it is very weakly soluble in water. Bilirubin's unconjugated form is yellow and exhibits a distinctive spectrographic peak at 450 nm. Serum samples need to be protected from sunlight

and analyzed as soon as possible because bilirubin is very susceptible to oxidation and light. Since these fluids typically don't include albumin to shield the bilirubin, more extreme precautions are required for the entry of biliary or pigments in urine bile. When handling, only dim or red light should be used, and vitamin C must be given in the amount of 1 to 5 mM as an antioxidant [35].

1.10 Alanine Transaminase and Aspartate Transaminase.

When the hepatic cells are damaged, two enzymes, (ALT) and (AST) are released from the hepatic cells and enter the bloodstream. The ALT is thought to be a more accurate indicator of liver illnesses, while the AST may be increased in diseases of other organs and tissues including the heart or muscle. The ALT and AST levels may rise to the high in cases of severe liver damage, such as acute viral hepatitis. The rise of these enzymes in cirrhosis or chronic liver viral infection may be mild or moderate. A variety of liver diseases may be the cause of a mild or moderate elevation in ALT or AST, which is frequently used to track the progression of a chronic liver viral infection and how well it responds to medications like prednisolone [36].

In comparison to other bodily tissues, the liver has a higher concentration of alanine transaminase than the kidney, heart, or muscle. The transamination process is only catalyzed by ALT in the cytoplasm. Patients with diseases including viral hepatitis, ischemic liver damage (shock liver), and toxin-induced liver injury frequently have substantial increases in ALT values of more than 500 U/L. [37].

Aspartate transaminases catalyze transamination reactions. The mitochondrial and cytoplasmic versions of AST are two separate isoenzyme types that are genetically unique. AST is more abundant in the heart as compared to other bodily tissues such as the liver, striated muscle, and kidney. AST reference serum levels range from 0 to 35U/L. Increased mitochondrial AST is detected in chronic liver diseases, such as liver tissue damage and necrosis, and in myocardial infarction (MI), which causes widespread tissue necrosis. However, the proportion of mitochondrial to overall AST activity has a distinct role in the diagnosis of alcoholic liver virus infection and conditions characterized by liver cell necrosis.

1.11 Ferritin

Ferritin is the basic iron storage protein, and it is significant for iron homeostasis. While protecting lipids, proteins, and DNA from the potentially harmful effects of iron, ferritin supplies iron for essential cellular functions. In clinical practice, ferritin levels are frequently reported to vary, frequently suggesting alterations in iron homeostasis or metabolism. More and more research has shown that ferritin may also act as a trigger for inflammatory response, neural degeneration, and malignant disease [38][39].

Forms of ferritin can bind intracellular or extracellular iron. The form called apoferritin creates a roughly spherical structure that stores ferric iron as the mineral ferrihydrite. There are 24 subunits in the apoferritin shell. The two types of subunits are H and L. Depending on the kind of tissue, these chemical subunit ratios vary widely, and they can also change during inflammatory and infectious situations. H-subunit- and L-subunit-rich tissue ferritins are found largely in the heart and kidney (primarily found in the liver and spleen) [40].

In the plasma, ferritin can also be detected extracellularly and serves as a crucial clinical indication of iron status. Despite being often used in clinical practice, the precise origin of serum ferritin is still unknown. It seems that ferritin L and serum ferritin have an immunological relationship. The hereditary disorder (hyperferritinemia), in which a disorder of the ferritin L gene results in much higher amounts of serum ferritin, cooperates with a relationship between ferritin L and ferritin in serum. H-type ferritin serum concentrations have been found to rise in various pathological situations, such as cancer [41].

A protein in the blood that stores iron for later use by the body is measured by the ferritin test. Dietary iron supplements are among the medications that might raise ferritin levels. Additionally, several illnesses that do not directly impact the body's iron storage can lead to artificially elevated ferritin levels. Infections, aggressive malignancies, lymphomas, and potentially fatal inflammations are some of these abnormalities. Alcoholics frequently have elevated ferritin levels [42].

2. AIM AND SCOPE OF THE STUDY

The aim and scope of this study are:

1. Monitor and evaluate the role of levels of some biochemical indicators (ALT, AST, ALP, Direct bilirubin, Total bilirubin, GGT, BUN, Urea, Creatinine, Calcium, Chloride, Potassium, Sodium, HDL, LDL, VLDL, Amylase, Lipase, D-Dimer, Ferritin, MG, LDH, IL -1) in Iraqi COVID-19 patients.
2. Study of the correlation coefficient between biochemical parameters.

3. MATERIALS AND METHODS

3.1 Equipment and Apparatus

The equipment and apparatus used are shown in the table below.

Table 2.1. The equipment and apparatus used and their origin.

Name	Company	Origin
Atellica	Siemens	**Germany**
STA Compact	Stago	**France**
ELISA Microplate Reader	GENEX	**USA**
Centrifuge NF-400	Nuve	**Turkey**
Deep Freeze	Hitachi	**Japan**
Disposable Syringes	Eppendorf	**China**
Eppendorf Tubes	Eppendorf	**China**
Incubator	Human	**Germany**
Micropipettes	Gilson	**France**
Sodium Citrate Tubes	BD Vacutainer	**USA**

3.2 Chemicals and Kits

Diagnostic kits and chemicals and their manufacturers are shown in the table below.

Table 2.2. Kits and chemicals and their manufacturers.

No	Name	Company	Origin
1	Alkaline Phosphatase Kit	Atellica CH	**USA**
2	Alanine Transaminase Kit	Atellica CH	**USA**
3	Aspartate Aminotransferase Kit	Atellica CH	**USA**
4	Amylase Kit	Atellica CH	**USA**
5	Blood Urea Nitrogen Kit	Atellica CH	**USA**
6	Calcium Kit	Atellica CH	**USA**
7	Chloride Kit	Atellica CH	**USA**
8	Creatinine Kit	Atellica CH	**USA**
9	Direct bilirubin Kit	Atellica CH	**USA**
10	Gamma-glutamyl Transferase Kit	Atellica CH	**USA**
11	HDL Cholesterol Kit	Atellica CH	**USA**
12	LDL Cholesterol Kit	Atellica CH	**USA**
13	Lactic Dehydrogenase Kit	Atellica CH	**USA**
14	Lipase Kit	Atellica CH	**USA**
15	Magnesium Kit	Atellica CH	**USA**
16	Potassium Kit	Atellica CH	**USA**
17	Sodium Kit	Atellica CH	**USA**
18	Total Bilirubin Kit	Atellica CH	**USA**
19	Urea Kit	Atellica CH	**USA**
21	VLDL Cholesterol Kit	Atellica CH	**USA**
22	D-Dimer Kit	Stago	**France**
23	Ferritin Kit	Atellica IM	**USA**
24	Human (IL-1α) ELISA Kit	Elabscience	**USA**

3.3 Study Design

The study comprised 60 people who seemed to be in good health as a control group and 107 COVID-19 patients (64 men and 43 women) who attended Al-Karamah Teaching Hospital in Baghdad. The study's participants' ages varied from (18-81 years). All of the patients in this research had their diagnoses made by specialists, from July 2021 to December 2021, clinical and laboratory testing, particularly PCR, were used to confirm the diagnosis. The study's practical component was completed in Baghdad's Al-Karama Teaching Hospital and Al-Karkh Laboratory Medical Center laboratories.

3.3.1 Sample Processing and Collection

For each person (patients and controls), 5 mL of blood was obtained, and 1.8 mL of this blood was placed in anticoagulant tubes containing sodium citrate, the sample was then separated by centrifugation at 3000 rpm for 15 minutes. Finally, the serum was isolated and stored at -20 ^{0}C pending analysis.

The remaining samples were put into gel tubes and allowed to coagulate for 30 minutes at room temperature. After 15 minutes of centrifugation at 3000 rpm, the sample was separated, and the serum was extracted and stored at -20 ^{0}C until analysis.

3.4 METHODS

3.4.1 Biochemistry and Ferritin

The Biochemistry and Ferritin samples were measured by Atellica® Solution Immunoassay & Clinical Chemistry Analyzer (Siemens). The device has the ability to construct over 300 different configurations, adaptable, scalable, automation-ready assays, immunological and biochemical tests created to give control and simplicity, bidirectional magnetic sample transfer technology, and a thorough checklist with tested detection methods. The device is an entirely automated analyzer containing 115 predilution Tray dilution cuvettes (five segments of 23 cuvettes) and two trays refrigerated (70 positions each), (0.4 to 5.0 µL) is the volume of the photometric sample for each test (varies with assay).

Details and specifications of the device we used are included in the appendix of this master's thesis.

The values were then automatically calculated by the Atellica Data Manager, and calibration curves were calculated using the calibration devices provided by the manufacturer, compatible with the detection steps. All of the assay steps were performed automatically by the instrument.

The variables tested are provided in Table 2.3.

Table 2.3. Variables tested.

No	Variable	Normal Range
1	ALP	**40-120 (U/L)**
2	ALT	**0-41 (U/L)**
3	Amylase	**25-125 (U/L)**
4	AST	**10-50 (U/L)**
5	BUN	**5.8-22.8 (mg/dL)**
6	Ca	**8.6-10.0 (mg/dL)**
7	Cl	**90-110 (mmol/L)**
8	Creatinine	**0.57-1.25 (mg/dL)**
9	Direct Bilirubin	**0-0.2 (mg/dL)**
10	Ferritin	**22-322 (ng/mL)**
11	GGT	**< 73 (U/L)**
12	HDL	**40-50 (mg/dL)**
13	K	**3-5.2 (mmol/L)**
14	LDH	**135-225 (U/L)**
15	LDL	**0 -129 (mg/dL)**
16	Lipase	**12 -53 (U/L)**
17	MG	**1.8-2.8 (mg/dL)**
18	Na	**136-156 (mmol/L)**
19	Total Bilirubin	**0-12 (mg/dL)**
20	Urea	**15-45 (mg/dL)**
22	VLDL	**2-30 (mg/dL)**

Picture 2.1. Siemens (Atellica® Solution) device.

3.4.2 IL-1 alpha

Human IL-1(Interleukin1 Alpha) ELISA Kit (E-EL-H0088) from Elabscience Biotechnology Company was used to test plasma using an ELISA Microplate Reader automated immunoassay analyzer (GENEX, USA).

Picture 2.2. GENEX (Microplate reader).

3.4.2.1 The principle

The Sandwich-ELISA method was employed, and an antibody that is specific to human IL-1 was coated on the supplied micro-ELISA plate.

By contrasting the (OD) of the samples with the standard curve displayed below, it was possible to determine the amount of Human IL-1α in the samples.

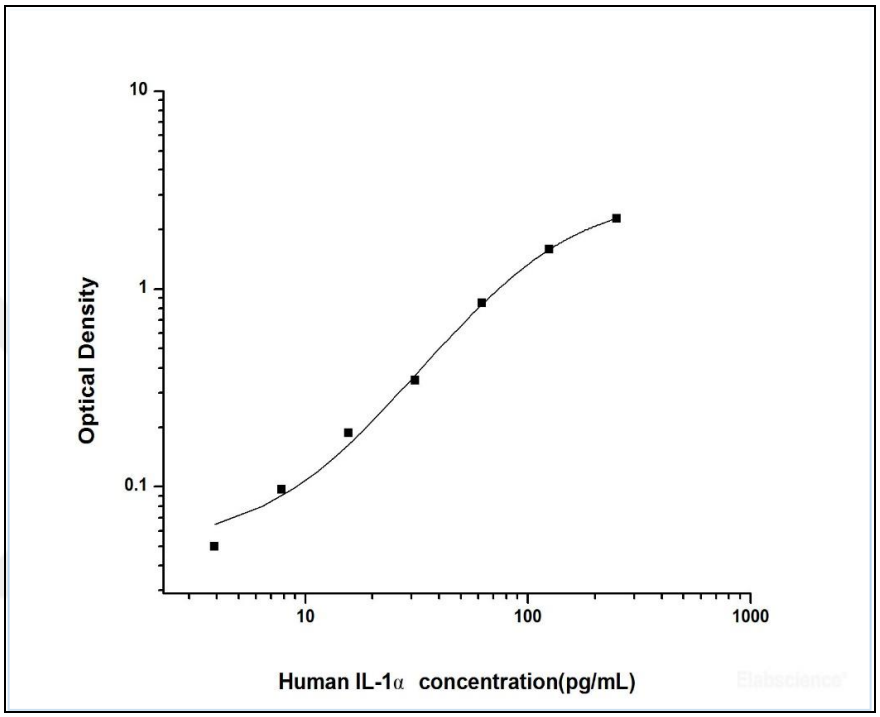
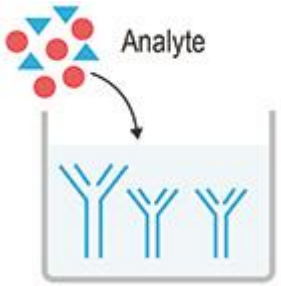


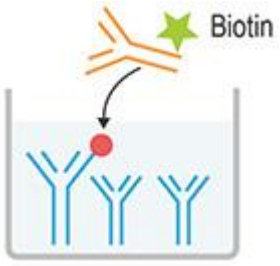
Figure 2.1. Standard curve of the relationship between (OD) value and human IL-1α.

3.4.2.2 Assay Procedures

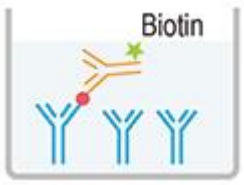
A. Standards or samples were added into the well and waited for 90 minutes at 37°C.



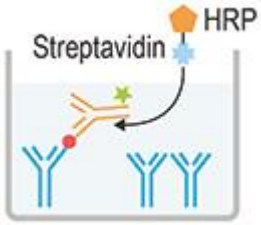
B. Liquid part was removed and working solution (100 µL Biotin) was added and incubated for 60 min at 37 °C.



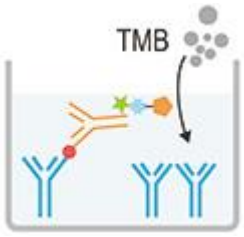
C. The plate was pulled out and washed 3 times.



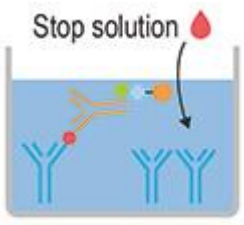
D. The HRP conjugate working solution was added, at 37°C for 30 minutes. Then pulled out the dish and washed it 5 times.



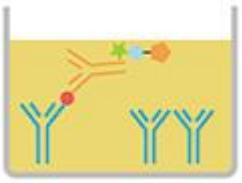
E. 90µL Substrate Reagent was added. Wells were incubated.



F. Stop solution (50µL) was added into the wells



G. The plate was read at 450nm instantly. The results were calculated.



3.4.3 D-Dimer

The D-dimer samples were measured by the instrument STA Compact Max® device. The analysis process is carried out inside the device by withdrawing the cuvette. It was placed in the incubation. The device withdraws from the sample (Plasma) and puts it inside the cuvette. 1 (Buffer) is added to the sample in the cuvette during the incubation period of 214 seconds. The sample is moved from the incubation position to the measurement position after 214 seconds. Liatest 2 (Latex) is placed on the sample while it is in the measurement position. The result is obtained after 144 seconds using the photometric technique.

(Stago) STA Compact Max® device is an entirely automated analyzer built on a highly reliable platform containing 96 sample positions and 45 reagent positions. The values were then automatically calculated by the STA Coag Expert Software. Calibration curves were performed using the calibration devices provided by the manufacturer, consistent with the detection steps. All of the assay steps were performed automatically by the instrument. The normal range for D-Dimer was <5 µg/ml.

Details and specifications of the device we used are included in the appendix of this master's thesis.

Picture 2.3. Stago (STA Compact Max®).

3.5 Ethical Approval

All procedures were performed according to guideline numbered UOT-ASD-02042020, dated April 2, 2020, and approved by the Human Care and Ethics Committee at the Biotechnology Division, Applied Sciences Department, University of Technology, Baghdad, Iraq.

3.6 Statistical Analysis

The SAS program was employed in this study to find the influence of various variables on the study parameters. To compare means substantially, a T-test was applied. Chi-square analysis was used to compare percentages in a meaningful way (0.05 and 0.01 probability), with the use of the GraphPad Prism program in displaying the results charts for comparison between patients and control, to increase the clarification and ensure the statistical analysis of the data.

4. RESULTS AND DISCUSSION

4.1 Distribution of the Study Sample Based on Age and Gender

The statistical distribution of the study groups by age and gender was expressed as the results as shown in Table 4.1. According to this table, the age group with the highest infection rate was (46-59) years, which constituted (39.3%) of the group of patients (Fig. 4.1). The same table also showed that males constituted (59.8%), and females constituted (40.2%) of the patients while the gender distribution in the control group was equal to 50% each.

Table 4.1. Distribution of the study sample based on age and gender.

Items	Sub-groups	COVID-19 Patients (N= 107)		Control Group (N= 60)		Chi-Square (P value)
		Freq.	%	Freq.	%	
Age (Years)	18-31	5	4.7	3	5	**8.025 ** (0.0087)**
	32-45	27	25.2	22	36.7	
	46-59	42	39.3	29	48.3	
	60-73	24	22.4	6	10	
	74-81	9	8.4	0	0.0	
Gender	Males	64	59.8	30	50	**0.052 NS (0.916)**
	Females	43	40.2	30	50	
**** (P≤0.01), NS: Non-Significant.**						

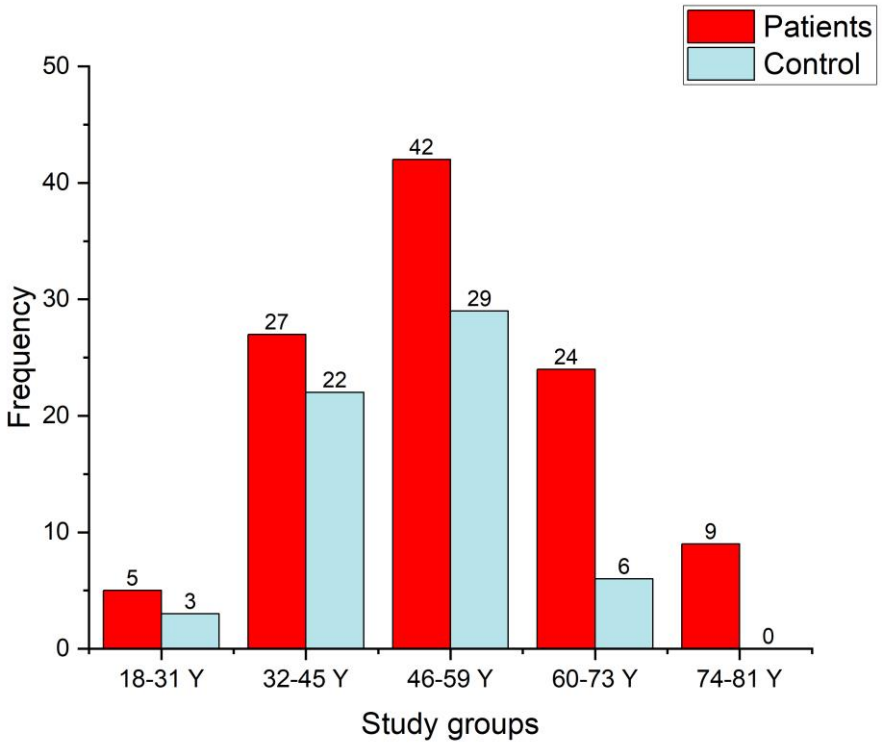


Figure 4.1. Bar chart showing sample study according to age in patients and control groups.

Through the study of (107) patients with COVID-19 (64 men and 43 women) and (60) healthy individuals as a control group, the following results were obtained:

4.2 Liver Functions

P values reveal a significant difference in ALT, AST, ALP (P = 0.0001), total bilirubin (P = 0.0203) and direct bilirubin (P = 0.0001), and GGT (P = 0.0004). It was found through biochemical analyzes conducted on patients infected with COVID-19 to exacerbate liver disease, and consequently worsen clinical outcomes, which calls for monitoring of liver function. Together with the results obtained and atypical pneumonia, they collectively indicate liver dysfunction associated with high mortality. Referring to recently published research on this topic, patients with severe infection showed a twice as high rate of abnormal liver function tests, compared to those with moderate COVID-19, indicating possible liver damage in addition to severe respiratory symptoms [43]. Furthermore, patients with impaired liver function showed higher levels of inflammatory cytokine and serum

chemokines, a feature of the cytokine storm associated with severe COVID-19 infection, than those with normal liver function.

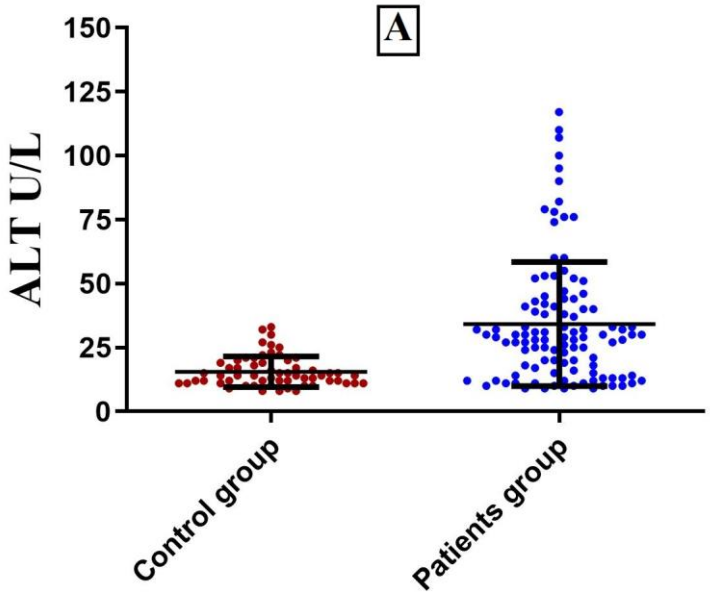
It has been observed that the main entrance of the COVID-19 virus to human cells is through the ACE2, which appears predominantly in the respiratory tract. However, this enzyme is also found in a small percentage of hepatocytes (2.6%) and (59.7%) of bile duct epithelial cells, allowing the virus to infect these cells and impair liver function.

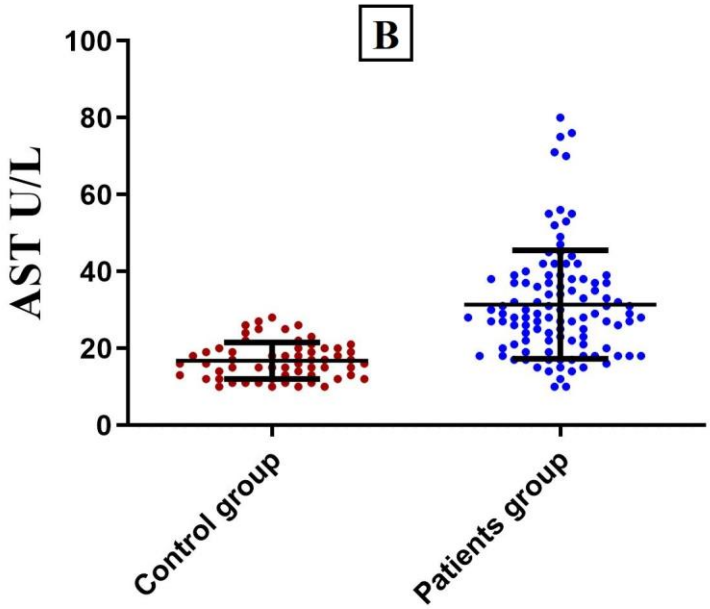
Systemic viral infections are often associated with a temporary elevation of liver biomarkers resulting from immune activation without impaired liver function, a phenomenon known as transient hepatitis [43]. Patients with COVID-19 who already have liver problems may experience liver stress from drugs used to treat the virus to severely exacerbate the liver disease. Acute and chronic liver failure is more likely to occur in COVID-19 -infected patients with cirrhosis. Patients with non-alcoholic fatty liver also often have steatosis associated with comorbid conditions such as diabetes, obesity, and cardiovascular disease, which increase the risk of a severe form of COVID-19 [43].

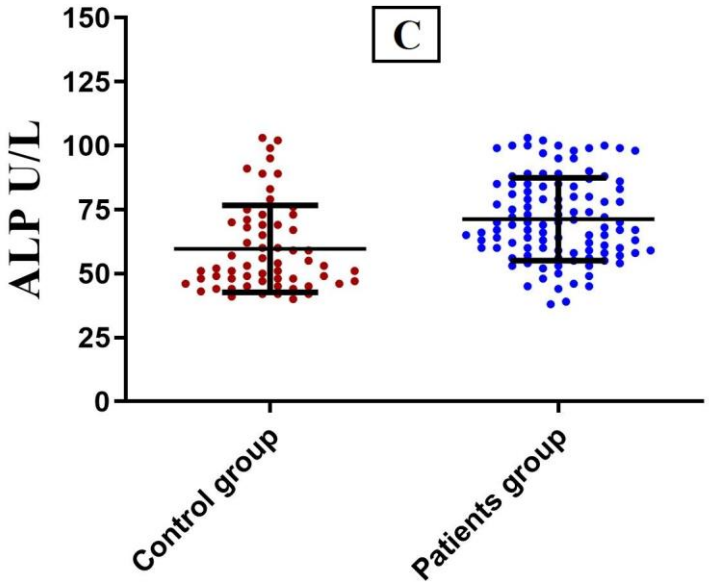
The table below shows a comparison of liver function.

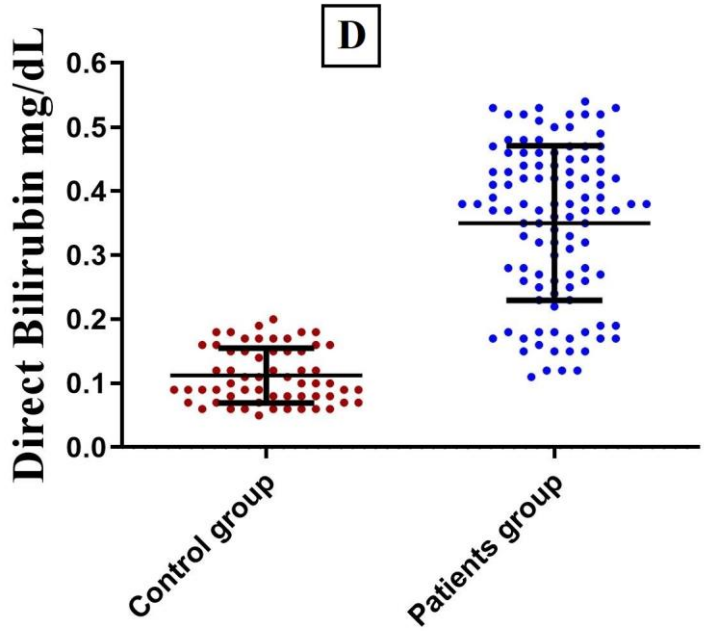
Table 4.2. Comparison in Liver functions.

Group	Mean ± SE					
	ALT (U/L)	AST (U/L)	ALP (U/L)	Direct bilirubin (mg/dL)	Total bilirubin (mg/dL)	GGT (U/L)
Patients	34.19 ±2.34	31.40 ±1.36	71.25 ±1.55	0.349 ±0.01	0.723 ±0.03	37.83 ±1.77
Control	15.57 ±0.76	16.80 ±0.62	59.70 ±2.19	0.112 ±0.01	0.614 ±0.04	27.53 ±2.12
T-test	6.291 **	3.715 **	5.237 **	0.0318 **	0.0918 *	5.637 **
P-value	0.0001	0.0001	0.0001	0.0001	0.0203	0.0004
*** (P≤0.05), ** (P≤0.01).**						


A
ALT U/L
150
125
100
75
50
25
0
Control group
Patients group


B
AST U/L
100
80
60
40
20
0
Control group
Patients group


C
ALP U/L
150
125
100
75
50
25
0
Control group
Patients group


D
Direct Bilirubin mg/dL
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Control group
Patients group

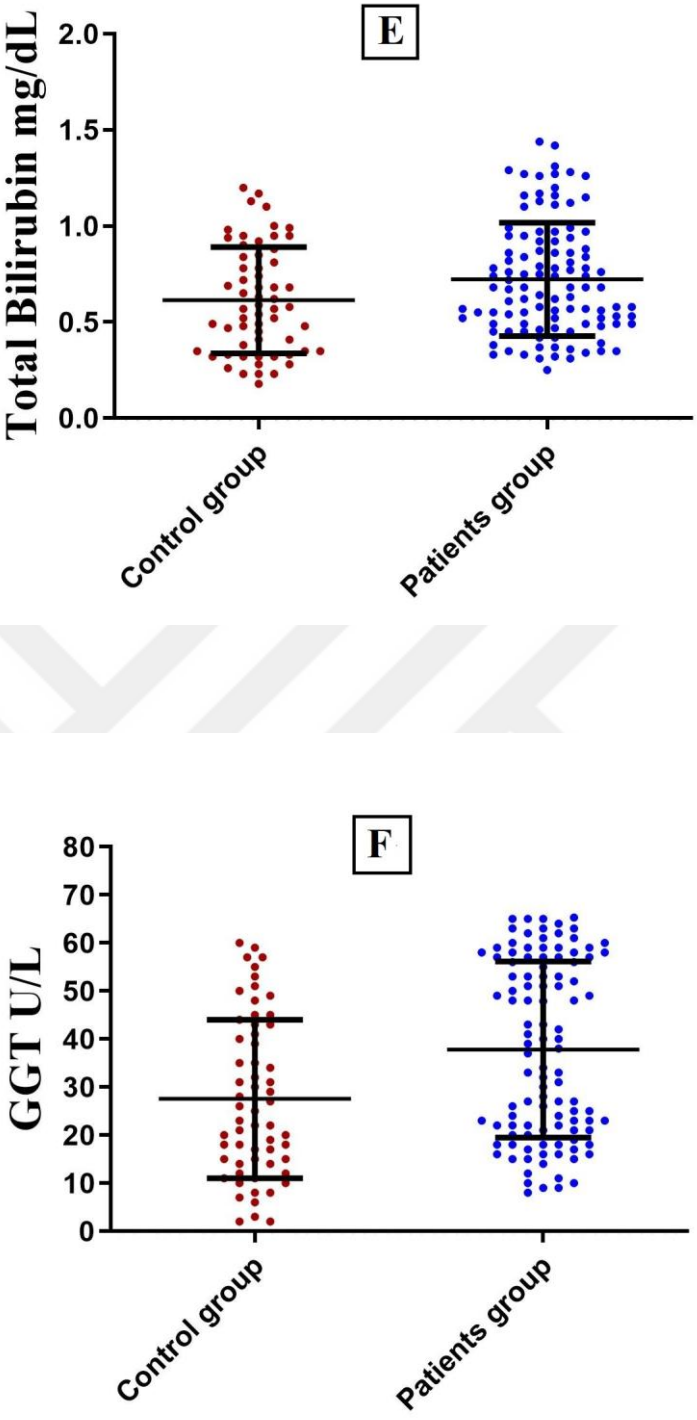


Figure 4.2. Comparison of liver function tests A, B, C, D, E, F.

4.3 Kidney Functions

P values reveal a significant difference in BUN, Urea, Calcium, Chloride, Potassium, and Sodium (P = 0.0001), at the same time, the results indicate that there is no significant difference in the creatinine (P = 0.919), explaining a tiny, small change that can be overlooked in serum creatinine during the first stage of admission in COVID-19 patients [44].

It was found through biochemical analyses conducted on patients infected with COVID-19 to exacerbate kidney disease, consequently worsening clinical outcomes, which calls for monitoring of kidney function. Together with the results obtained and atypical pneumonia, they collectively indicate kidney dysfunction associated with high mortality.

Medical reports did not appear that the Coronavirus caused damage to the kidneys for patients who suffered from mild or moderate symptoms, but those who developed severe symptoms and required the use of a respirator developed kidney problems [45]. Although this virus infects the respiratory system, it damages all organs of the body.

Statistics show that between 20-30% of COVID-19 patients suffer from kidney failure. According to what doctors have found in the last period due to the spread of the new Corona epidemic and the presence of many critical cases in the intensive care rooms, it has been found that this virus leads to blood clotting faster, forming small clots and consequently leading to a blockage in the blood vessels, and this may occur in the small arteries of the kidneys are called “small clots in the kidney tissue”[45].

Then, those who suffer from chronic kidney disease are among the groups most vulnerable to infection with the emerging virus, as it is one of the chronic diseases that may occur due to diabetes, high blood pressure, obesity, and atherosclerosis, as well as aging, which in turn contributes to high mortality rates [45]. Doctors attribute the cause of the effect of the emerging coronavirus on the kidneys to the occurrence of a cytokine storm, this is an immune reaction that occurs in some people infected with the Coronavirus, and it leads to a number of body parts

becoming severely infected. Also, the drugs used to treat the Coronavirus, may itself be a cause of patients' kidney problems as a side effect of these drugs.

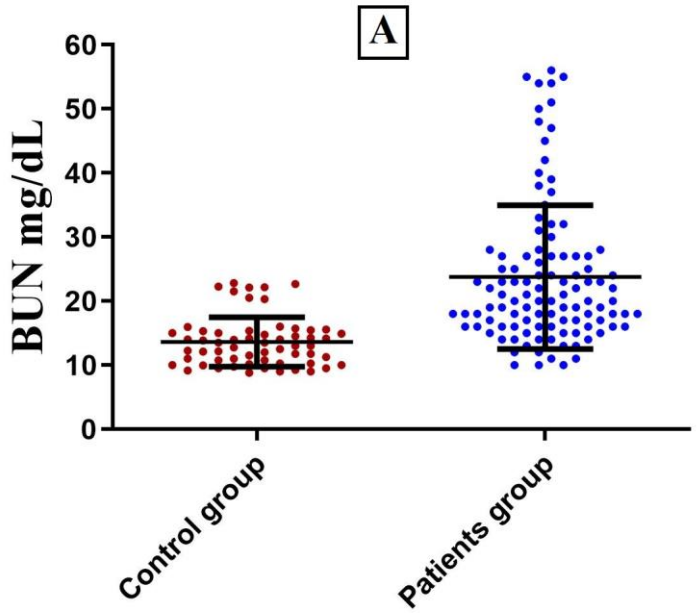
Elevated BUN, Urea, Chloride, and Potassium indicate renal impairment, and according to previous studies, elevated BUN levels were observed in 31% of the total patients which was evident in severe cases [46]. A death rate has also been connected to acute renal failure [47]. AKI has been described in this context as a stand-alone risk element for COVID-19 patients [46].

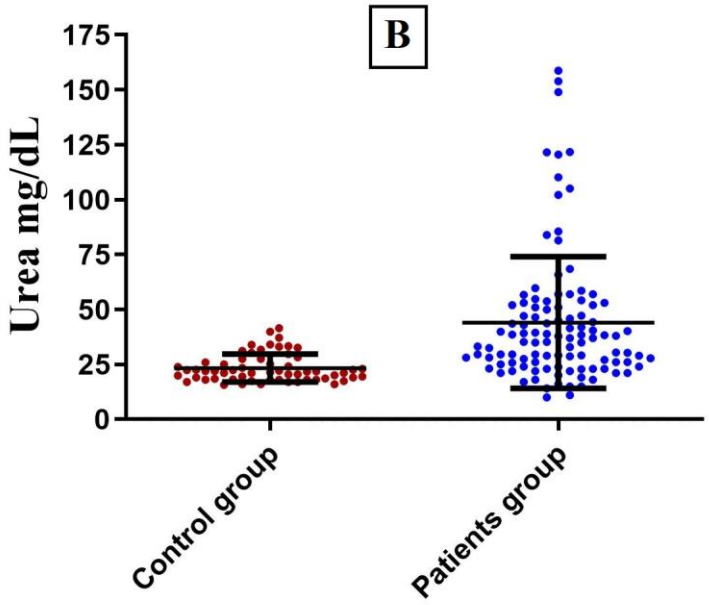
Therefore, in addition to treating pneumonia, the kidneys must be protected from viruses and cellular storms.

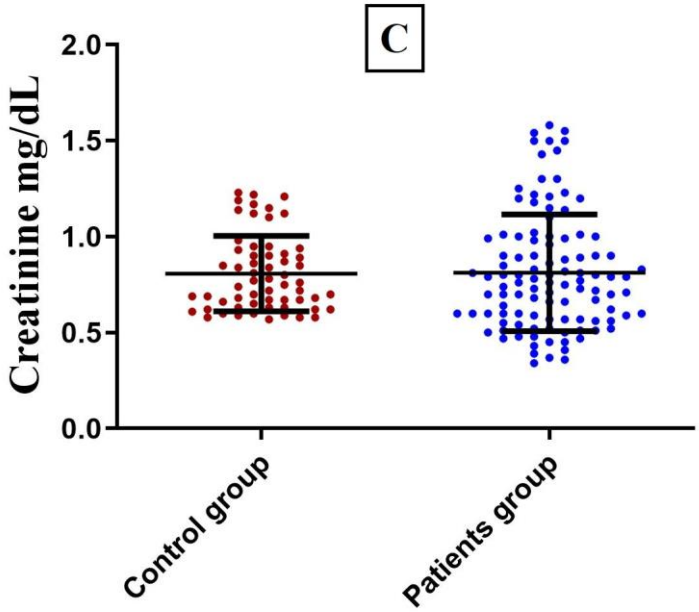
The table below shows a comparison of kidney function.

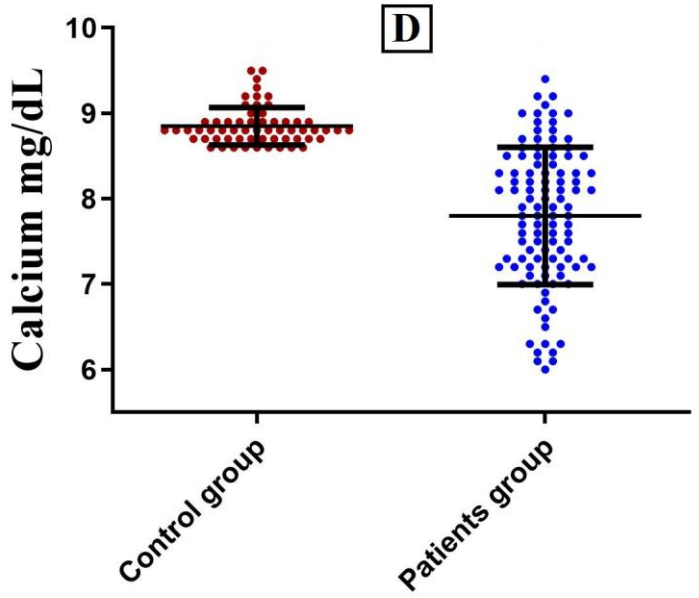
Table 4.3. Comparison in Kidney functions.

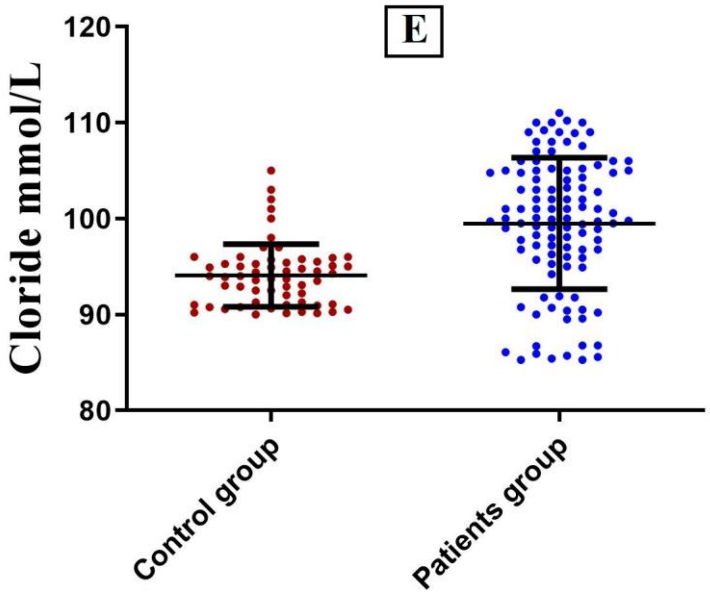
Group	Mean ± SE						
	BUN (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)	Calcium (mg/dL)	Chloride (mmol/L)	Potassium (mmol/L)	Sodium (mmol/L)
Patients	23.75 ±1.08	44.01 ±2.89	0.812 ±0.02	7.79 ±0.08	99.49 ±0.66	3.92 ±0.05	138.42 ±0.81
Control	13.61 ±0.49	23.30 ±0.81	0.807 ±0.02	8.85 ±0.02	94.09 ±0.42	3.55 ±0.06	142.11 ±0.50
T-test	2.955 **	7.74 **	0.086 NS	0.209 **	1.856 **	0.180 **	2.285 **
P-value	0.0001	0.0001	0.919	0.0001	0.0001	0.0001	0.0001
**** (P≤0.01).**							

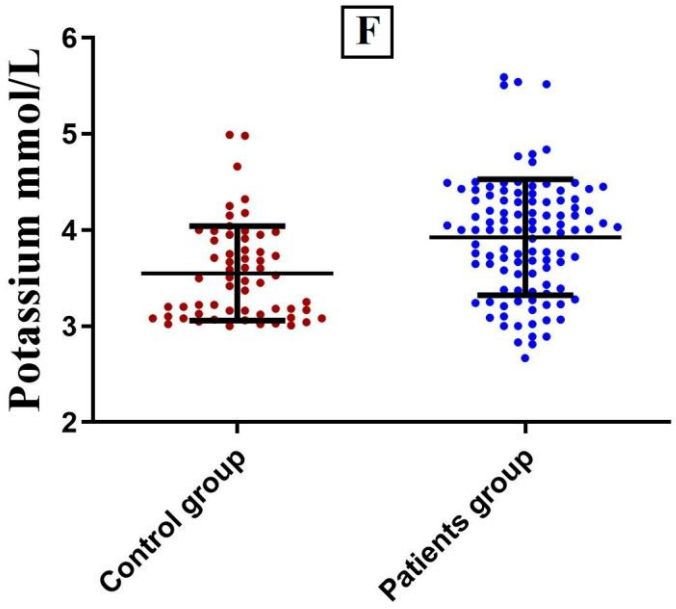

A
BUN mg/dL
60
50
40
30
20
10
0
Control group
Patients group


B
Urea mg/dL
175
150
125
100
75
50
25
0
Control group
Patients group


C
Creatinine mg/dL
2.0
1.5
1.0
0.5
0.0
Control group
Patients group


D
Calcium mg/dL
10
9
8
7
6
Control group
Patients group


E
Cloride mmol/L
120
110
100
90
80
Control group
Patients group


F
Potassium mmol/L
6
5
4
3
2
Control group
Patients group

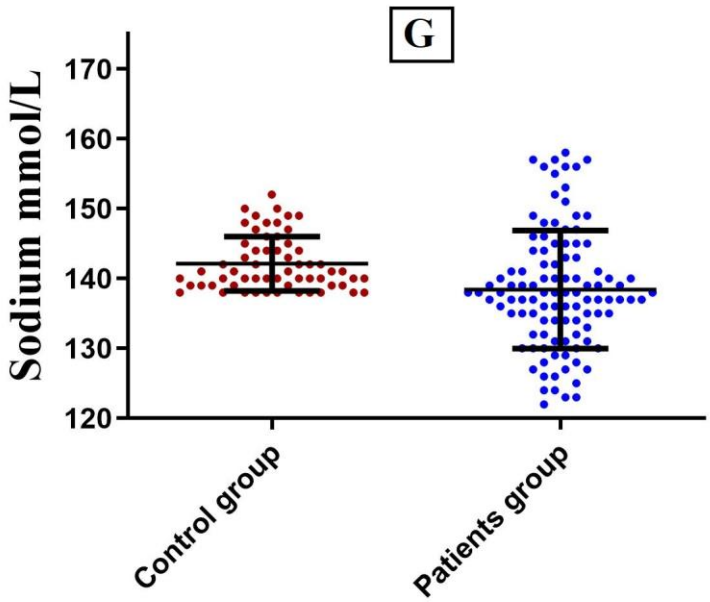


Figure 4.3. Comparison in kidney function tests A, B, C, D, E, F, G.

4.4 Lipid Profile

The level of HDL-c in the studied samples decreased as a result of infection with the Coronavirus, where the results of the statistical analysis as shown in Table 4.4. showed a decrease in the value of HDL with a significant difference (P = 0.0001).

The reason for the low value of HDL-c in groups of patients may be attributed to the rise of chylomicrons and VLDL, which leads to an increase in the level of triglyceride-rich lipoprotein, and this causes a disturbance in the activity of the enzyme CETP that works to transfer the triglycerides from lipoprotein rich in triglycerides to HDL and LDL, and this results in this disorder low levels of high-density lipoprotein cholesterol.

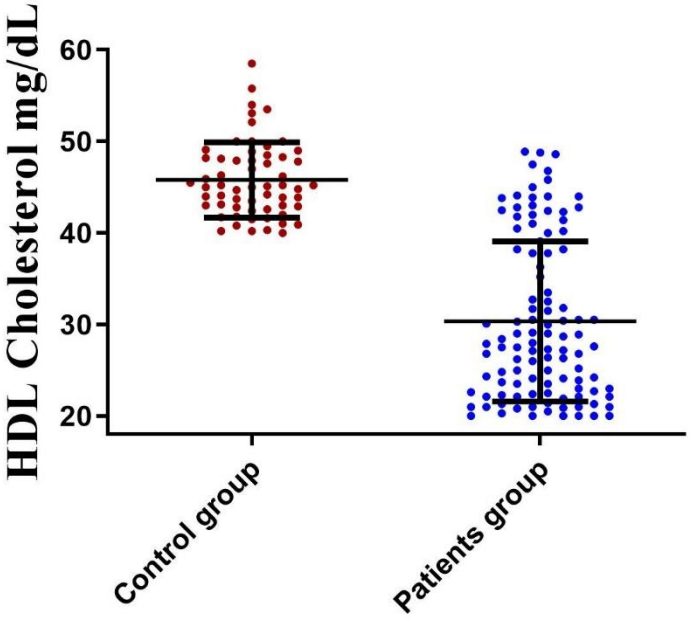
The results also showed that there were significant differences in P= 0.0001 represented by an increase in the level of LDL and VLDL. The concentration of LDL cholesterol increases in large quantities in the blood when its quantity exceeds the number of LDL receptors present in the body.

The study literature states that some studies found that HIV patients' lipid profiles changed due to a decline in HDL-c and an increase in LDL-c values [48]. Patients with dengue fever also have low levels of LDL-c in their blood [49].

Infected people may develop dyslipidemia as a result of viral-induced inflammation, it demonstrates how more cholesterol and lipids from plasma escape into the alveolar space as a result of severe inflammation and worsened vascular permeability. We also proposed the hypothesis that dyslipidemia, whose mechanism is currently being studied, plays a significant role in the pathological development of COVID-19.

Table 4.4. Comparison in Lipid profile.

Group	Mean ± SE		
	HDL Cholesterol (mg/dL)	LDL Cholesterol (mg/dL)	VLDL (mg/dL)
Patients	30.33 ±0.84	91.04 ±4.52	32.88 ±1.40
Control	45.80 ±0.53	47.41 ±2.75	11.75 ±0.70
T-test	2.369 **	12.609 **	3.861 **
P-value	0.0001	0.0001	0.0001
**** (P≤0.01).**			



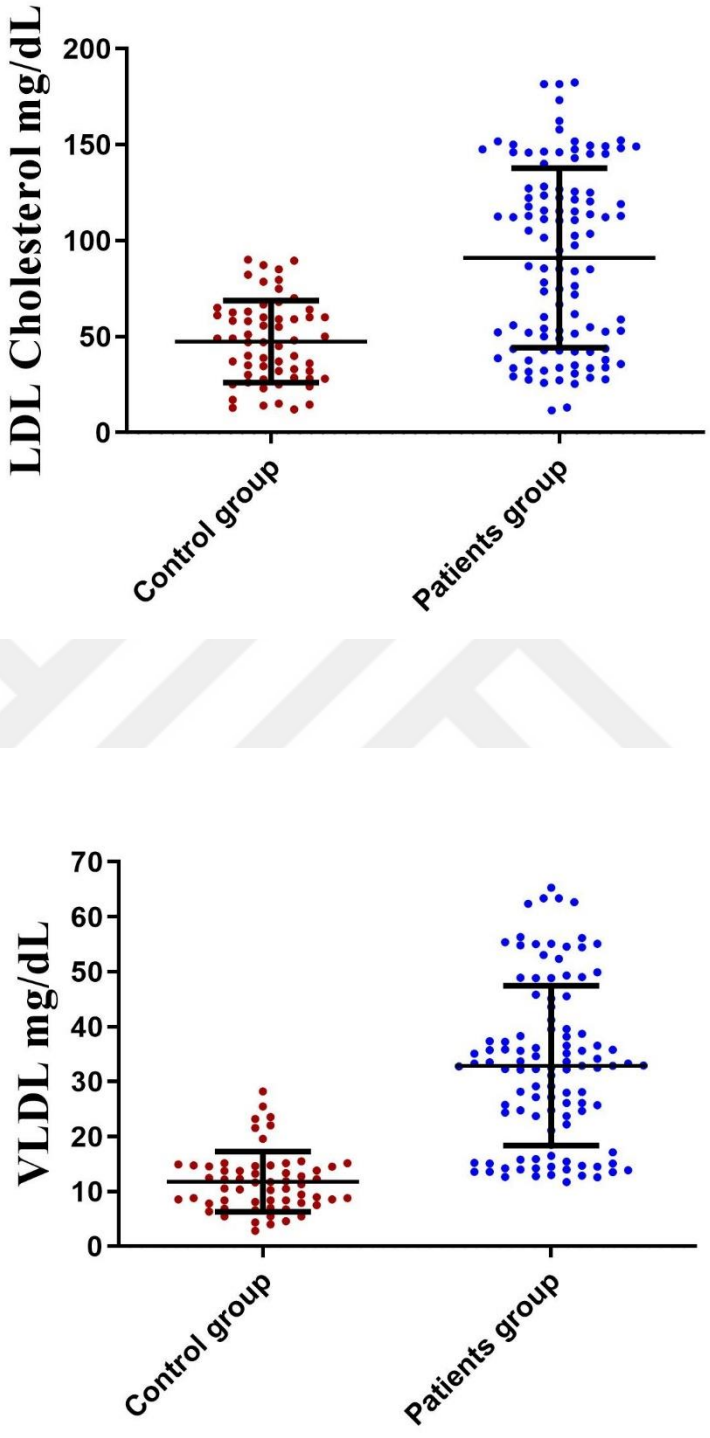


Figure 4.4. Comparison in Lipid profile.

4.5 Pancreatic Enzymes

Through the results and statistical analysis, it was found that there is a significant difference in amylase (P = 0.0002) and lipase (P = 0.0001) activities (Table 4.5), which indicates an increase in the values of pancreatic enzymes in people with coronavirus disease.

The test of amylase and lipase enzyme that was performed helps in the diagnosis and monitoring of pancreatic diseases, and it is worth noting that the analysis of lipase is more specific than the analysis of amylase in the blood. Amylase is an enzyme secreted by the body through the pancreas and salivary glands in the mouth, which in turn works to digest carbohydrates, while lipase is one of the digestive enzymes involved in fat digestion.

It was found through previous research that pancreatitis occurs with diseases that are caused by a viral infection, but with Coronavirus disease, the respiratory system was the main organ that caused the damage.

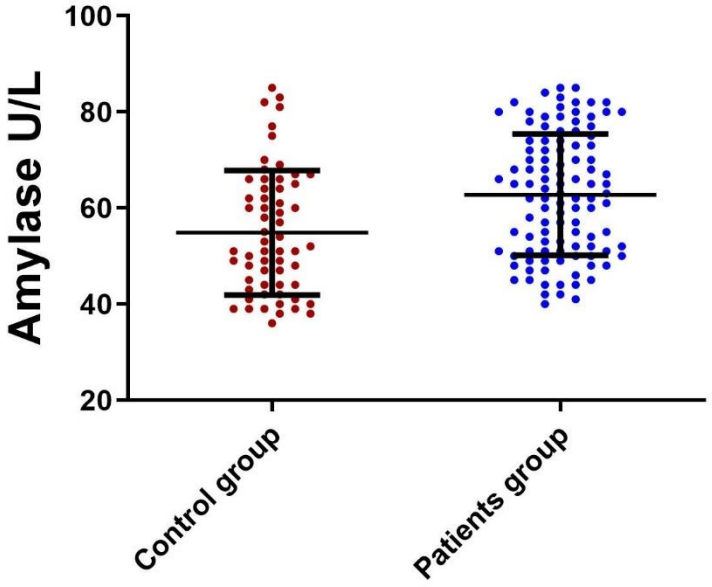
Not only may a pancreatic infection or inflammation induce high blood levels of amylase, but also be a disease of the salivary glands [50]. According to research, 13% of a group of COVID-19 patients revealed positive salivary SARS-CoV-2 RNA. This suggests that hyperamylasemia might result from SARS-CoV-2 infection of the salivary glands [51][52].

We discovered that in COVID-19 patients, increased blood levels of amylase and lipase do not always indicate pancreatic injury.

Since the presence of a pancreatic infection has not been directly associated with COVID-19 disease, additional studies should be conducted by collecting more data and signs and symptoms to assess this [53].

Table 4.5. Comparison in Pancreatic enzymes.

Group	Mean ± SE	
	Amylase (U/L)	Lipase (U/L)
Patients	62.78 ±1.22	34.63 ±0.86
Control	54.88 ±1.67	28.60 ±1.22
T-test	4.058 **	2.912 **
P-value	0.0002	0.0001
**** (P≤0.01).**		



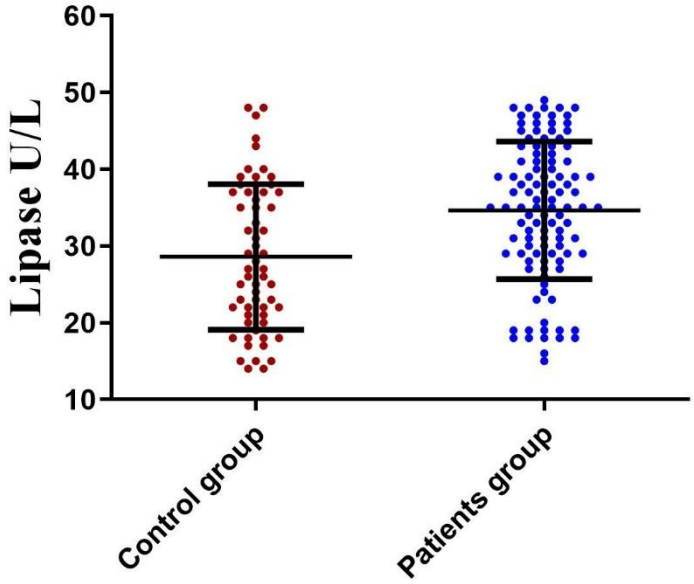


Figure 4.5. Comparison in Pancreatic enzymes.

4.6 D-Dimer and Ferritin

P values reveal a significant difference in D-Dimer and Ferritin (P = 0.0001) as shown in table 4.6. which indicates an increase in the values of people with coronavirus disease.

A d-dimer test is used to see if blood clots may form somewhere within the blood vessels. They are protein molecules that are produced when a blood clot is dissolved or dissolved in the body, and this substance does not appear in the body, except in the case of blood clots forming or in the event of their dissolution.

Several different studies have looked at d-dimer levels to see if d-dimer could be useful in predicting the health status of COVID-19 patients. The current study agreed with previous studies, one of the most common test results in COVID-19 patients requiring hospitalization was said to be increased D-dimer. The test was performed on a group of patients, and non-survivors were found to have significantly greater D-dimer levels than survivors [54].

The severe pneumonia-induced hypoxia and heightened inflammatory response in COVID-19 eventually activate coagulation and fibrinolysis, which is followed by a hypercoagulable condition that results in disseminated intravascular

coagulation and various organ failure. Furthermore, prior research has demonstrated that D-dimer concentrations higher than 2.0 μg/mL at admission can accurately predict in-hospital mortality in COVID-19 patients [55].

Ferritin is a protein that binds to iron and is considered a storage place for iron that facilitates its solubility and reduces its toxicity within the cells of the body.

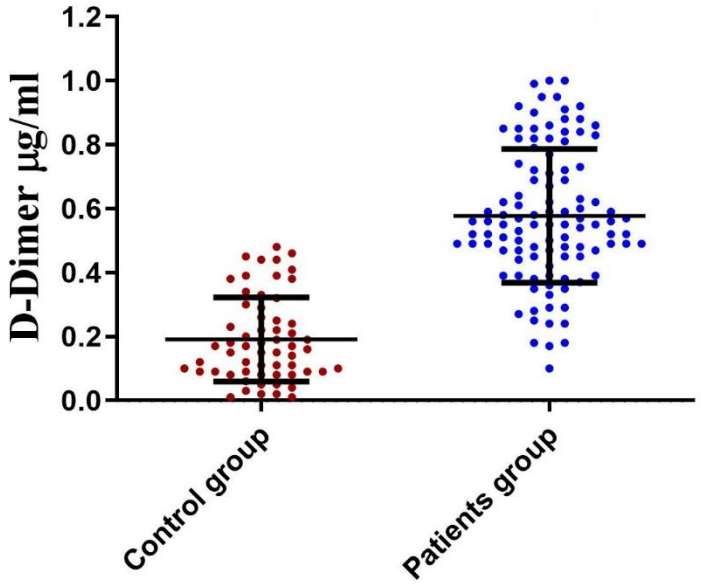
The virus invades the cells on a large scale in the respiratory tract. Among the attacks and surprises, the virus attacks red blood cells, specifically the beta 1 chain, and weakens the ability and production of hemoglobin Hb. After this imbalance will weaken the binding of hemoglobin with oxygen and weaken the oxygen-carrying to the cells, and thus severe hypoxia will occur. Therefore, the body's reaction begins to compensate for the severe lack of oxygen by increasing the production of hemoglobin and thus a massive increase in ferritin. The viscosity of the blood increases and with-it small clots inside the lungs increase. And high levels of ferritin, in turn, will increase the effectiveness of macrophage cells, which are one of the cells of the immune system responsible for defense and resistance against the Coronavirus and others.

When the phagocytic cells become more effective, the levels of cytokines will increase and they will be released in random numbers, and thus a deadly cytokine storm will occur because it causes damage to various organs in the body such as the kidneys, heart, and brain and it may lead to death. So, we conclude that the increase in ferritin in laboratory analysis is an indication that the case of Corona is very severe or dangerous.

Concerning serum ferritin, there was a significant increase in COVID-19 patients, they showed that serum ferritin is severely elevated in patients compared to control healthy individuals. It can be considered a predictive biological marker that contributes to diagnosis and treatment [39]. Although the results of these studies show that the D-Dimer and Ferritin test, in addition to the analysis of other biological markers, can help during the treatment of patients with corona, more studies are required to confirm the role of the D-Dimer and Ferritin analysis that it can contribute to determining the treatment of the virus corona.

Table 4.6. Comparison in D-Dimer and Ferritin test.

Group	Mean ± SE	
	D-Dimer (µg/ml)	Endocrine Ferritin (ng/mL)
Patients	0.577 ±0.02	781.59 ±45.38
Control	0.191 ±0.01	148.44 ±13.64
T-test	0.058 **	121.50 **
P-value	0.0001	0.0001
**** (P≤0.01).**		



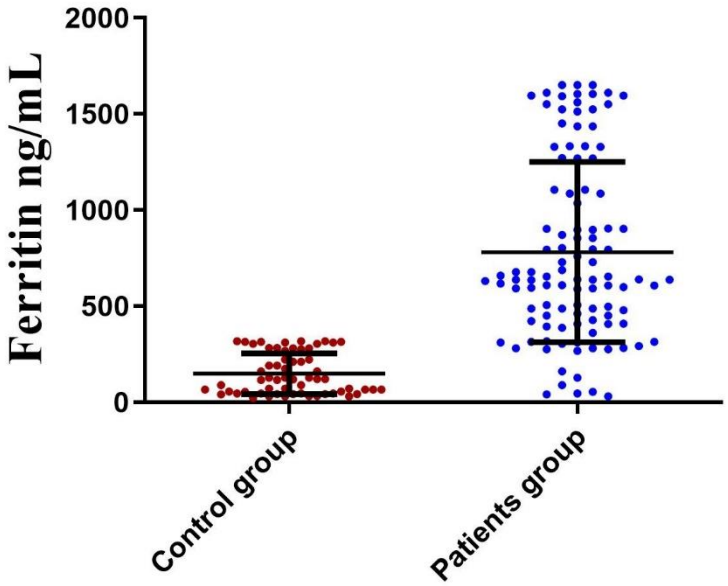


Figure 4.6. Comparison in D-Dimer and Ferritin.

4.7 MG, LDH and IL-1

P values reveal a significant difference in MG, LDH, and IL-1(P = 0.0001) as shown in table 4.7. LDH and IL-1 values increased, and magnesium decreased in people with coronavirus disease.

The blood magnesium analysis is a test that measures the amount of magnesium in the blood. Magnesium is an essential mineral in the body. Magnesium helps maintain bone strength, helps regulate blood pressure and heart rate, and is essential for energy production and nerve function. A low level of magnesium in the blood negatively affects, and a high level of magnesium in the blood may cause problems.

An analysis reading that is less than the normal reading indicates that an individual has low magnesium, and there are many reasons that lead to a low reading, including not getting enough magnesium eating, many diseases or treatments used. Proton pump inhibitors, which are often used, and thiazides that disrupt magnesium balance are associated with serious health problems [56].

Magnesium supplementation also reveals a significant benefit in managing pandemic stress as well as PTSD in COVID-19 survivors, health professionals, and the general public who have to face important changes in their habits and lifestyle. Needless to say, more basic, translational, and clinical research is needed to support potential relationships between Mg status and COVID-19.

Serum magnesium testing is needed in all patients at different stages of the disease as well as further research to determine the causes of hypomagnesemia during this severe disease and its negative impact on the outcome. However, our results highlight a high incidence of hypomagnesemia, which may have an impact on the development of COVID-19, including vascular and inflammatory problems. In order to identify hypomagnesemia and ultimately develop a better treatment plan to halt changes in magnesium balance, it should be advised that serum magnesium monitoring and magnesium supplementation may help them manage PTSD and pandemic stress [57].

Regarding LDH, the LDH test is done to measure the level of lactate dehydrogenase in the blood and other body fluids; In order to help diagnose a number of cases, and its high rate may indicate a health problem that requires additional tests to be diagnosed definitively, and there is a relationship between LDH analysis and corona; It is believed to cause its levels to rise.

The enzyme “lactate dehydrogenase” is one of the enzymes found in human blood that works to stimulate biochemical processes in the body. LDH analysis, which measures the extent of damage in the various tissues of the body. The result of the examination is higher than the percentage of normal LDH analysis, which means that there is damage to some tissue in the body.

Through the results, we noticed an increase in the result of the LDH test in Corona patients. The result of the LDH enzyme test is higher than the normal LDH analysis percentage, which means that there is some damage to the body’s tissues.

Recent studies have been conducted that have linked high levels of LDH and advanced infection with the coronavirus, as high levels of the enzyme are linked to the tissue disintegration that occurs in many pathological conditions, including

pulmonary disorders such as pneumonia, which occurs when infection with the Corona virus.

They demonstrated that as compared to healthy controls, LDH was higher in COVID-19 patients [26]. Revealed that (73%) of patients had elevated LDH levels [14].

Despite having no further metabolic function in the extracellular fluid, cytoplasmic cellular enzymes like LDH are nonetheless useful because they serve as markers of cellular integrity problems brought on by the diseased condition. Since LDH is an enzyme that is found in almost all major organ systems, abnormal serum LDH activity is seen in a wide range of disorders [32].

Oxidative phosphorylation prevents ATP generation when cells are subjected to anaerobic or hypoxic environments. Cells are prompted by this mechanism to use alternate metabolism to create energy. As a result, LDH is increased in this instance to satisfy the demand for energy generation. However, the anaerobic conversion of glucose leads to the failure of lactate metabolism. Other than the liver, no other tissue can further process it. As a result, lactate is released into the blood and carried to the liver, where it is converted to pyruvate by LDH through the Cori cycle's reverse reaction [58].

Reduced oxygenation can cause abnormal LDH levels, which can harm many organs by upregulating the glycolytic pathway. Lactate causes damage through the activity of metalloproteinases and increased macrophage-mediated angiogenesis [54].

We demonstrate that serum LDH may be used to identify lung infections early in their development and that severe COVID-19 cases linked to serum LDH are dynamically correlated with illness severity.

The results showed a significant difference in the level of IL-1 ($P = 0.0001$) and an increase in the values when comparing the results with the control group.

One of the previous studies agreed with our current study, which aimed to identify cytokines and their relationship to COVID-19. Which showed an increase in IL-1α ratios through a study conducted on a group of 108 patients with COVID-

19, whose results were compared with 28 healthy controls, that confirmed the association of IL-1α in patients, and thus IL-1α is considered a biomarker of mortality [59].

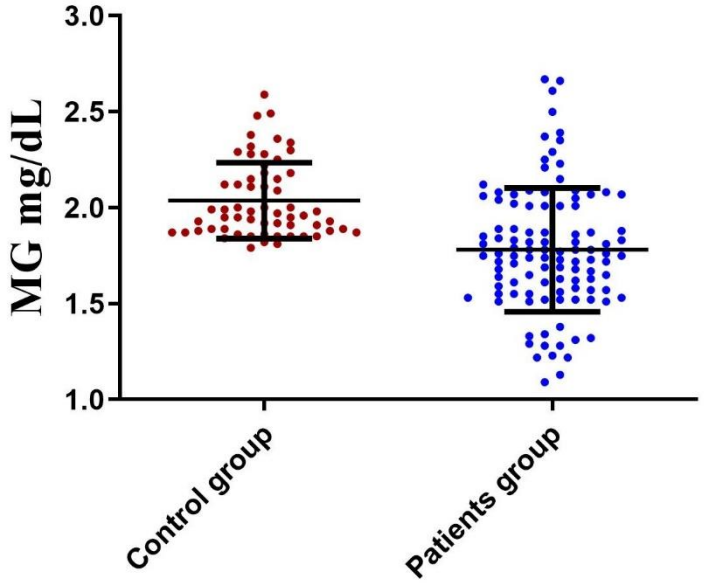
Interleukins are a group of cytokines (secreted signaling proteins/molecules) first seen in white blood cells. Stimulates the body's immune system to resist congestion and disease. Interleukins are formed in a wide range of cells in the body, including white blood cells, also called leukocytes. These cells expel or destroy bacteria and other harmful substances that enter the body, different types of interleukins are found in the different types of white blood cells.

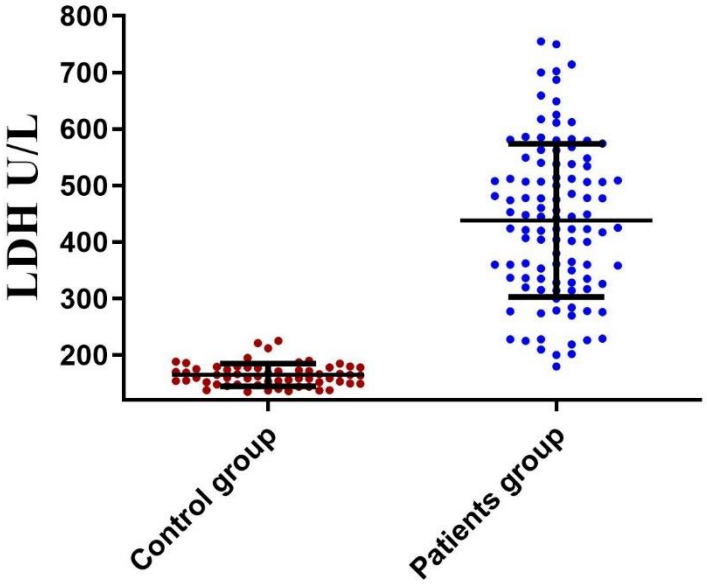
These interleukins work together and trigger a series of reactions that arm the body's white blood cells against disease, one of which we discussed in this study is interleukin 1.

Activated macrophages, neutrophils, epithelial cells, and endothelial cells are the principal producers of IL-1, primarily in charge of causing inflammation, additionally to encouraging fever and sepsis [60].

Table 4.7. Comparison in MG, LDH, IL-1.

Group	Mean ± SE		
	MG (mg/dL)	LDH (U/L)	IL-1 (pg/mL)
Patients	1.78 ±0.03	438.16 ±13.06	126.97 ±5.52
Control	2.04 ±0.02	164.76 ±2.56	8.33 ±0.45
T-test	0.0905 **	34.709 **	14.609 **
P-value	0.0001	0.0001	0.0001
**** (P≤0.01).**			


MG mg/dL
3.0
2.5
2.0
1.5
1.0
Control group
Patients group


LDH U/L
800
700
600
500
400
300
200
Control group
Patients group

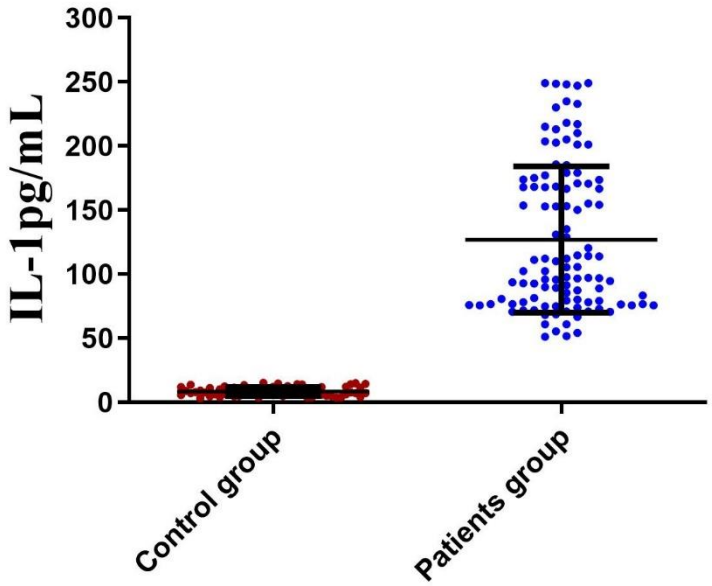


Figure 4.7. Comparison in MG, LDH, and IL-1.

4.8 Correlation Between Parameters Study

Table 4.8. was about the correlation between biomarkers through Pearson correlation coefficients (r) included in the study. As shown in the table that there was a significant positive correlation ($P \leq 0.01$) between each of the following levels.

First, D-dimer and the following biomarkers: ALT (r= 0.91); AST (r= 0.94); LDH (r= 0.98); Ferritin (r= 0.98) and Total bilirubin (r= 0.98). Between the levels of ALT and each of AST (r= 0.98); LDH (r= 0.93); Ferritin (r= 0.92), and Total bilirubin (r= 0.95). Between the levels of AST and each of LDH (r= 0.95); Ferritin (r= 0.92), and Total bilirubin (r= 0.96). Between the levels of LDH and each of Ferritin (r= 0.96) and Total bilirubin (r= 0.98). Highly significant positive Ferritin and Total bilirubin (r= 0.98). One of the previous studies agreed with our current study, indicating higher ALT was independently linked to higher white blood cell count and D-dimer levels. Both systemic inflammation and microvascular thrombosis may contribute to liver failure in the injured. [61].

Table 4.8. The correlation coefficient between parameters study.

Parameters	Correlation coefficient-r					
	D-Dimer	ALT	AST	LDH	Endocrine (Ferritin)	Total bilirubin
D-Dimer	--					
ALT	0.91 **	--				
AST	0.94 **	0.98 **	--			
LDH	0.98 **	0.93 **	0.95 **	--		
Endocrine (Ferritin)	0.98 **	0.92 **	0.92 **	0.96 **	--	
Total bilirubin	0.98 **	0.95 **	0.96 **	0.98 **	0.98 **	--
**** (P≤0.01).**						

Note: Significant (P≤0.05); Highly Significant (P≤0.01); NS: Non-Significant.

5. CONCLUSIONS AND RECOMMENDATIONS

- We can draw the following conclusions from the current study's findings:

1. There was an increase in the serum levels of patients in the following biochemical indicators (ALT, AST, ALP, Direct bilirubin, Total bilirubin, GGT, BUN, Urea, Chloride, Potassium, LDL, VLDL, Amylase, Lipase, D-Dimer, Ferritin, LDH, IL-1).
2. There was a decrease in the serum levels of patients in the following biochemical indicators (Calcium, Sodium, HDL, MG).
3. There was no effect on the serum levels of creatinine in patients.
4. The age group with the highest infection rate was (46-59) years, which constituted (39.3%) of the (107) patients whose ages ranged from (18-81 years).
5. There was a significant positive correlation ($P \leq 0.01$) between the levels of D-dimer and ALT; AST; LDH; Ferriin and Total bilirubin. Between the levels of ALT and each of AST; LDH; Ferritin, and Total bilirubin. Between the levels of AST and each of LDH; Ferritin and Total bilirubin. Btween the levels of LDH and each of Ferritin and Total bilirubin. Finally, there was a highly significant positive correlation between ferritin and total bilirubin.

- Recommendations:

1. Conducting further studies on the biochemical indicators of patients infected and comparing them with the biochemical indicators after full recovery.
2. In future studies, the levels and extent of creatinine effect in patients with COVID-19 should be investigated.
3. Conduct further studies on the biochemical indicators of people who have been provided with the COVID-19 vaccine.

6. REFERENCES

"Vancouver citation system was used in this thesis."

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7. APPENDICES

Appendix 1

(Siemens) Atellica® Solution device.

Test Menu

Atellica Solution

For use outside the U.S.

Flexible, scalable, automation-ready immunoassay and clinical chemistry analyzers engineered to deliver control and simplicity so you can drive better outcomes

Experience the power of the Atellica® Solution, featuring patented bidirectional magnetic sample transport technology, the flexibility to create over 300 customizable configurations, and a broad assay menu with proven detection technologies.

Chemistry

Diabetes
Fructosamine
Hemoglobin A1c (Enzymatic)

Drugs of Abuse/Toxicology
6-Acetylmorphine (6-AM)*
Acetaminophen
Amphetamines
Barbiturates, Urine
Barbiturates, Serum*
Benzodiazepines, Urine
Benzodiazepines, Serum*
Buprenorphine*
Cannabinoids (THC)
Cocaine Metabolite
Ecstasy
Ethyl Alcohol
Ethyl Glucuronide (EtG)†
Fentanyl†
Lysergic Acid Diethylamide (LSD)*
Methadone
Methadone Metabolite (EDDP)†
Methaqualone*
Opiates
Oxycodone
Phencyclidine
Propoxyphene
Salicylate
Tramadol†
Tricyclic Antidepressants*

General Chemistry
Alanine Aminotransferase (ALT)
Alanine Aminotransferase (P5P)
Albumin (BCG)
Albumin (BCP)
Aldolase†
Alkaline Phosphatase
Ammonia
Amylase
Angiotensin-converting enzyme (ACE)†
Aspartate Aminotransferase (AST)
Aspartate Aminotransferase (P5P)
Bilirubin, Direct
Bilirubin, Total
Calcium (Arsenazo)
Calcium (CPC)
Carbon Dioxide
Chloride
Cholesterol
Cholinesterase
CKMB (Activity)†
Copper†
Creatine Kinase
Creatinine
Creatinine (Enzymatic)
G6P-DH†
Gamma-Glutamyl Transferase (GGT)
Glucose (Hexokinase)
Glucose (Oxidase)
HDL Cholesterol
Iron
Lactate
Lactate, CSF
Lactate Dehydrogenase (L-P)
LDL Cholesterol
Lipase
Magnesium
Microalbumin
Pancreatic Amylase
Phosphorus
Potassium
Sodium
Total Bile Acids†
Total Iron Binding Capacity (TIBC)
Total Protein
Total Protein, Urine/CSF
Triglycerides
Urea Nitrogen (BUN)
Uric Acid
Zinc†

Immunosuppressant Drugs
Mycophenolic Acid
Tacrolimus

Specific Proteins
α1-Acid Glycoprotein (AAG)
α1-Antitrypsin (AAT)
Antistreptolysin O (ASO)
Apolipoprotein A-1
Apolipoprotein B
β2-Microglobulin
Ceruloplasmin†
Complement C3
Complement C4
CRP
CRP, High Sensitivity
CRP, Wide Range
Cystatin C
Free Light Chain kappa*
Free Light Chain lambda*
Haptoglobin
Immunoglobulin A (IgA)
Immunoglobulin G (IgG)
Immunoglobulin M (IgM)
Lipoprotein(a)
Prealbumin
Rheumatoid Factor (RF)
Soluble Transferrin Receptor (sTfR)†
Transferrin

Specimen Validity Testing
Creatinine*
Nitrite*
Oxidant*
pH*
Specific Gravity*

Therapeutic Drug Monitoring
Amikacin*
Caffeine*
Carbamazepine
Digoxin
Gentamicin
Lamotrigine†
Levetiracetam†
Lidocaine
Lithium
Methotrexate†
N-Acetylprocainamide (NAPA)
Phenobarbital
Phenytoin
Procainamide
Theophylline
Tobramycin
Valproic Acid
Vancomycin

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**Alliance application manufactured and distributed by Siemens Healthcare Diagnostics Inc.*
†Alliance application manufactured by third party, distributed by Siemens Healthcare Diagnostics Inc.
‡Under development. Not commercially available. Future availability cannot be guaranteed.

SIEMENS Healthineers

Atellica Portfolio of Laboratory Products

Engineered by Siemens Healthineers to deliver control and simplicity so you can drive better outcomes.

Tighter control of your lab, simplified workflow, and more time to focus on driving better business and clinical outcomes—that's the promise of our Atellica® portfolio of laboratory products.

Control. Simplicity. Better Outcomes.

For use outside the U.S.

Atellica Solution Test Menu

Immunoassay

Anemia
- Active-B12
- EPO
- Ferritin
- Folate
- RBC Folate
- Vitamin B12

Autoimmune
- Anti-CCP IgG

Bone Metabolism
- CTx‡
- Intact PTH
- Vitamin D Total

Cardiac
- BNP
- CKMB (Mass)
- High-Sensitivity Troponin I
- Myoglobin
- NT-proBNP

Diabetes
- C-Peptide
- Insulin

Growth
- Growth Hormone (hGH)‡
- IGF-1‡
- IGFBP-3‡

Hepatitis
- Anti-HBe
- Anti-HBs 2
- HAV IgM
- HAV Total
- HBc IgM
- HBc Total II
- HBeAg
- HBsAg II
- HBsAg II Quant
- HBsAg Confirmatory
- HCV

HIV
- HIV 1/O/2 Enhanced (EHIV)
- HIV Ag/Ab Combo (CHIV)

Immunosuppressant Drugs
- Cyclosporine
- Everolimus‡
- Sirolimus‡
- Tacrolimus‡

Inflammation
- IgE Total
- IL2R‡
- IL-6
- LBP
- TNFα‡

Liver Fibrosis
- Enhanced Liver Fibrosis (ELF™) Test
- HA (ELF Test)
- PIIINP (ELF Test)
- TIMP-1 (ELF Test)

Metabolic
- ACTH‡
- Cortisol
- Homocysteine

Neurology
- β-Amyloid 1-42 (AB42)‡
- Serum Neurofilament light chain (sNfL)‡
- Total-Tau (TTAU)‡

Oncology
- AFP
- BR 27.29
- CA 125II™§
- CA 15-3™§
- CA 19-9™§
- Calcitonin
- CEA
- Complexed PSA
- CYFRA 21-1‡
- Free PSA
- Neuron Specific Enolase (NSE)‡
- PSA
- Serum HER-2/neu
- Squamous Cell Carcinoma Antigen (SCC)‡

Reproductive Endocrinology
- Androstenedione
- Anti-Mullerian Hormone (AMH)‡
- DHEA-SO4
- Enhanced Estradiol
- Free ß-hCG
- FSH
- hCG
- LH
- PAPP-A
- PIGF
- Progesterone
- Prolactin
- sFLT-1
- SHBG
- Testosterone II

Sepsis
- Procalcitonin (PCT)

Special ID
- EBV-EBNA IgG‡
- EBV-VCA IgG‡
- EBV-VCA IgM‡
- SARS-CoV-2 Ag (CoVAg)**
- SARS-CoV-2 IgG (sCOVG)††
- SARS-CoV-2 Total (COV2T)††
- Syphilis
- Zika Test

Therapeutic Drug Monitoring
- Digitoxin
- Digoxin

Thyroid
- Anti-Thyroglobulin (aTgII)
- Anti-TPO
- Free T3
- Free T4
- Thyroglobulin (Tg)
- Total T3
- Total T4
- TSH3-Ultra
- T Uptake

TORCH
- CMV IgG
- CMV IgM
- Herpes-1 IgG‡
- Herpes-2 IgG‡
- Rubella IgG
- Rubella IgG II
- Rubella IgM
- Toxoplasma IgG
- Toxoplasma IgM

Product availability may vary from country to country and is subject to varying regulatory requirements. Please contact your local representative for availability.

‡Under development. Not commercially available. Future availability cannot be guaranteed.

§CA 125II, CA 15-3, and CA 19-9 are trademarks of Fujirebio Diagnostics, Inc.

***Under FDA review. Not available for sale in the U.S.*

††This test has not been FDA cleared or approved. This test has been authorized by FDA under an EUA for use by authorized laboratories. This test has been authorized only for detecting the presence of antibodies against SARS-CoV-2, not for any other viruses or pathogens. This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner. Product availability may vary by country and is subject to varying regulatory requirements.

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Appendix 2

(Stago) STA Compact Max® device.

STA Compact Max®

Excellence Born from Expertise

The STA Compact Max is a fully automated benchtop analyzer built on the most reliable platform in the industry. With an expansive test menu, the Compact Max is a robust, high-efficiency analyzer with enhanced throughput making it the perfect system offering for mid-sized laboratories. The system's unique method of sample management offers moderate throughput and rapid processing of STAT samples with no impact to the instrument's time to result. With 96 sample positions and 45 reagent positions, the Compact Max to easily handle the workload with minimal intervention from the operator. Integrated STA Coag Expert Software provides full auto-verification, repeat/reflex testing, a comprehensive QC package, accreditation tools, automated maintenance logs, TAT monitoring and maintains 5 years of patient archives onboard. The Compact Max is available with an optional cap-piercing system to reduce bio-hazard exposure risks.

Measurement	Clotting, chromogenic, immunologic
Methodologies	80 user-definable tests
Samples onboard	96
Continuous loading	Yes
True STAT management	Yes
Reagents Onboard	45
Consumables	Unitary cuvettes (1 test = 1 cuvette)

Dimensions	Height: 27.75in. Width: 38.1in. Depth: 28.73in. Weight: 309lbs.
TLA Capable	No
Cap piercing option	Yes
Test Menu	PT, aPTT, Fibrinogen, Thrombin Time, Extrinsic pathway factors, Intrinsic pathway factors, Anti-Xa (UFH, LMWH, Rivaroxaban*, Apixaban*, Edoxaban*), Anti-IIa*, D-Dimers, Fibrin Monomers*, Antithrombin Activity, Protein C Activity, Protein S Activity, Lupus Anticoagulant, VWF Antigen, Microparticles*, Plasminogen, Antiplasmin and TAFI*, Calibrators, Quality Control