T.C.
KIRSEHIR AHI EVRAN UNIVERSITY
HEALTH SCIENCES INSTITUTE
DEPARTMENT OF CHEMISTRY

CORRELATION BETWEEN COVID-19 AND BIOCHEMICAL LABORATORYMARKERS:SERUM FERRITIN, PROCALCITONIN, TROPONINE AND SOME ENZYMES FOR IRAQI PATIENTS

KHALID ZAITER KHALAF

MASTER'S THESIS

KIRŞEHİR / 2023

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ADVISOR
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KIRŞEHİR / 2023

THESIS STATEMENT

I certify that all information in the thesis was obtained and presented within the framework of ethical conduct and academic rules, and in this study prepared according to the rules of dissertation writing, all types of statements that do not belong to me are fully cited as the source of the information.

Khalid Zaiter Khalaf

Based on Articles 9/2 and 22/2 of the Graduate Education and Training Regulations published in the Official Gazette on 20/4/2016; A report was obtained according to the criteria set by the Institute of Science and Technology using the Plagiarism program which Kırşehir Ahi Evran University has subscribed to this postgraduate thesis.

PREFACE

I would like to thank my esteemed advisor Prof. Dr. Aslıhan GÜNEL, from whom I learned how a scientist should work, as well as being an example to me with his calm and patient manner that he has shown since the day I met him during my master's. I sincerely thank Prof. Dr. Aslıhan GÜNEL. I would like to thank my esteemed jury members.

I dedicate my dissertation to especially my family.

January, 2023 **Khalid Zaiter Khalaf**

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LIST of ABBREVIATIONS

Symbols	Description
%	: Percent
°C	: Degrees Celsius
L	: Liter
dL	**:** Deciliter
kg	: Kilogram
ng	**:** Nano grams
mL	: Milliliter
μg	: Micrograms

Abbreviations	Description
AA	**:** Amino acid
ACE-2	**:** Angiotensin converting enzyme-2 receptors
ACS	**:** Acute coronary syndrome
ADP	**:** Infarction adenosine diphosphate
AI	**:** Artificial intelligence
ALP	**:** Alkaline Phosphatase
ALT	**:** Alanine Transaminase
AST	**:** Aspartate transaminase
BMI	**:** Body Mass Index
CAD	**:** Coronary Artery disease
CBC	**:** Complete blood count
CK	**:** Creatine kinsase
COPD	**:** Chronic obstructive pulmonary disease
COVID	**:** Coronaviruses
CRE	**:** Creatinine
CT	**:** Computed tomography
cTnI	**:** Cardiac troponin I
CVD	**:** Cardiovascular disease
DM	**:** Diabetes Mellitus
ELISA	**:** Enzyme Linked Immuno Sorbent Assay
FER	**:** Ferritin
G-3-P	**:** Glycerol 3 phosphate
GOD	**:** Glucose Oxidase
HD	**:** Heart disease
HIV	**:** Human immunodeficiency virus
HL	**:** Hepatic Lipase
HRP	**:** Horseradish peroxidase
hs-cTnI	**:** High-sensitivity cardiac troponin I
hs-cTnT	**:** High-sensitivity cardiac troponin T
ICU	**:** Intensive care unit
IL	**:** Interleukins
LDH	**:** Lactate Dehydrogenase
LFT	**:** Liver function tests
MI	**:** Myocardial infarction
OD	**:** Optical density
PCR	**:** Polymerase chain reaction
PCT	**:** Procalcitonin

RBCs **:** Red blood cells
RFT **:** Renal function test
RNA **:** Ribonucleic acid
SD **:** Standard Deviation
TNF-α **:** Tumor necrosis factor alpha
WHO **:** World Health Organization

ÖZET

YÜKSEK LİSANS TEZİ

COVİD-19 VE BİYOKİMYASAL LABORATUVAR BELİRTEÇLERİ ARASINDAKİ KORELASYON: IRAKLI HASTALAR İÇİN SERUM FERRİTİN, PROKALSİTONİN, TROPONİN VE BAZI ENZİMLER

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Koronavirüsler (COVID), çok çeşitli hayvanları enfekte edebilen ve orta ila şiddetli solunum yolu patojenlerine neden olabilen bir virüs cinsidir. Sırasıyla zoonotik kökenli SARS-CoV ve MERS-CoV yüksek derecede virülan iki tipi canlılarda aniden ortaya çıkmıştır. Bu virüsler akciğer enfeksiyonuna neden olmakta ve yeni bir halk sağlığı sorunu olarak ortaya çıkmaya başlayan COVID-19'u 20. yüzyıla taşımaktadır. Çalışmamız Irak-AL-Anbar Vilayetindeki kovid hastalarında bazı biyokimyasal belirteçlerin COVID-19 enfeksiyonu ile ilişkisini saptamayı amaçlamıştır. Çalışma, Nisan-Aralık 2021 arası çalışma döneminde, Irak, Anbar'daki Al-Ramadi Eğitim Hastanesinde COVID-19 olduğu doğrulanan, yaşları 20 ila 70 arasında değişen (60 COVID-19 hastası ve 40 sağlıklı deneği) toplam 100 hastayı kapsamaktadır. COVID-19 hastalarının tamamında, PCR testi ile virüs pozitif olduğu doğrulandı. Hastalar COVID-19 IgM değerlerine göre dört gruba ayrılmıştır: Hastalığı Hafif şiddette geçirenlerin oluşturduğu grup (IgM 1.0-2.0, N= 25 hasta), hastalığı orta şiddette geçirenlerin oluşturduğu grup (2.0< IgM ≤ 4.0, N=18 hasta) ve hstalığı şiddetli geçirenlerin oluşturduğu grup (IgM >4.0, N= 17 hasta). Çalışılan grup ile yaş açısından da uyumlu IgM <1.0, (N= 40 gönüllü) olarak belirlenen sağlıklık kontrol grubu çalışmaya dahil edilmiştir. Bu araştırmalardaki kontrol grubu, Anbar'daki yerel bir

laboratuvardan alınan. COVID-19 ile enfekte olmamış bireylerden oluşmaktadır. Kişinin adı, yaşı, cinsiyeti, semptomları ve madde bağımlılığı ile ilgili bilgileri bir dosyada kaydedilmiştir. Çalışmadaki deneklerin ortalama yaşı 65 olmakla beraber 55 yaşından büyük hastalarda COVID-19 gelişme riski daha yüksek olmuştur. Bu hastaların %33.5'I yoğun bakım tedavisi gerektiren ileri evere hastalar olup yarısından fazlası daha sonra vefat etmiştir. Enfeksiyona maruz kalan erkek hastaların kadın hastalara oranı ~2,3'tür. Öte yandan, çalışma grupları arasında karşılaştırma yapıldığında, biyokimyasal parametreler çalışması ile COVID-19 şiddeti arasında anlamlı bir ilişki olduğu saptanmıştır.. Sonuç olarak değerlendirilen biyokimya test sonuçları için eğri altında kalan alan (AUC) değerleri test duyarlılık ve özgüllük değerleri dikkate alınarak aşağidaki şekilde özetlenebilir: Ferritin; AUC: %86 duyarlılık ve %100 özgüllük ile 0.969, Prokalsitonin; AUC: %100 duyarlılık ve %89,5 özgüllük ile 0,926, Kardiyak troponin I; AUC: %100 duyarlılık ve %94,7 özgüllük ile 0,988, Alanin transaminaz; AUC: %87.9 duyarlılık ve %100 özgüllük ile 0.957, Aspartat transaminaz; AUC: %81.8 duyarlılık ve %71.4 özgüllük ile 0.764, Alkalin fosfataz; AUC: %69.7 duyarlılık ve %100 özgüllük ile 0.807, Laktat dehidrojenaz AUC: %97 duyarlılık ve %100 özgüllük ile 0.974, Üre AUC: %95 duyarlılık ve %87.5 özgüllük ile 0.958 ve Kreatinin AUC: %95 duyarlılık ve %68.7 özgüllük ile 0.88. Sonuçlar diyabet, astım, hipertansiyon ve kalp hastalığı olan hastaların COVID-19 geliştirme riskinin daha yüksek olduğunu göstermektedir. Ateş ve kuru öksürük gibi COVID-19 semptom ve bulguları olan hastalarda COVID-19 gelişme riski daha yüksek olmuştur (sırasıyla %55 ve %78).

Iraklı hastalarda özellikle 55 yaş ve üzerinde COVID-19 enfeksiyonu ileri yaşlarda arttığı anlaşılmıştır. Komorbiditeler; diyabet, hipertansiyon ve kalp hastalığı olan hastalarda şiddetli, hafif ve orta dereceli vakalar arasında anlamlı bir fark yaratmıştır. Bu fark astım vakalarında oldukça anlamlı görülürken sigara içen hastalarda anlamsızdır. Mevcut prokalsitonin> 0,5 ng/mL ve ferritin> 600 µg/L ile kardiyak troponin I>1600 ng/L, COVID-19'un sürekli olarak izlenmesi ve belki de önlenmesi gereken kritik bir aşamaya ilerlediğini göstermektedir.

Ocak 2023, 58 Sayfa

Anahtar Kelimeler: COVID-19, PCT, FER, cTnI, ALT, AST, ALP, LDH, Üre ve Cre

ABSTRACT

M.Sc. THESIS

CORRELATION BETWEEN COVID-19 AND BIOCHEMICAL LABORATORY MARKERS: SERUM FERRITIN, PROCALCITONIN, TROPONINE AND SOME ENZYMES FOR IRAQI PATIENTS

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Coronaviruses (COVID) are a genus of viruses that really are capable of infecting a wide variety of animals and causing moderate to severe respiratory pathogens. Two highly virulent viral diseases with zoonotic origins, SARS-CoV and MERS-CoV, respectively that suddenly appeared in living beings and causes fatal lung infection, bring starting to emerge COVID-19 into the 20th century as a novel public health issue. Our study aimed at the detection of the relationship of some biochemical markers with COVID-19 infection in AL-Anbar governorate/Iraq. Patients who had COVID-19 confirmed at Al-Ramadi Teaching Hospital in Anbar, Iraq, study period from April to December 2021, The study covers 100 Iraqi patients (60 patients was COVID-19 and 40 normal subject samples with age range 20 to 70 years. All of the patients who had COVID-19 were positive for the virus that was confirmed by polymerase chain reaction test. The patients and healthy subjects were divided into four groups according to COVID-19 IgM values. The mild group (IgM 1.0-2.0, N= 25 patients), moderate group (2.0< IgM ≤ 4.0, N=18 patients) and severe groups (IgM >4.0, N= 17 patients) were comparison with controls (IgM <1.0, N= 40 patients) of matched age. The control group in these investigations was seemingly healthy samples taken from a local lab in Al-Anbar, as well as healthy people (no previous or current infection with COVID-19). The person's name, age, sex, symptoms, and drug history should all be written down in a file. The average age of the subjects in the study

was 65 years old and patients more than 55 years were at higher risk of developing COVID-19. Among of them 17 patients (33.5 %) had extreme to severe stages that required ICU treatment and more than half of them died later. The number of male patients exposed to infection is approximately 70% higher than the number of female patients, who recorded 30%. On the other hand, when comparing between the studies groups, a significant association was reported between biochemical parameters study and severity of COVID-19. The results was finding that Ferritin; AUC: 0.969 with 86% sensitivity and 100% specificity, Procalcitonin; AUC: 0.926 with 100% sensitivity and 89.5% specificity, cardiac troponin I; AUC: 0.988 with 100% sensitivity and 94.7% specificity, Alanine transaminase; AUC: 0.957, with 87.9% sensitivity and 100% specificity, Aspartate transaminase; AUC: 0.764, with 81.8% sensitivity and 71.4% specificity, Alkaline phosphatase; AUC: 0.807, with 69.7% sensitivity and 100% specificity. Lactate dehydrogenase AUC: 0.974, with 97% sensitivity and 100% specificity. Urea AUC: 0.958, with 95% sensitivity and 87.5% specificity and Creatinine AUC: 0.88, with 95% sensitivity and 68.7% specificity. The results demonstrate that patients with diabetes, asthma, hypertension and cardiac disease were at higher risk of developing COVID-19. The patients with COVID-19 symptoms and sign such as fever and dry cough were at higher risk of developing COVID-19 (55% and 78% respectively). Infection with COVID-19 may increase within advanced age especially at 55 years and over in Iraqi patients. Comorbidities showed a significant between severe, mild, and moderate cases in patients with diabetes, hypertension, and cardiac disease. Also highly significant appeared in asthma cases while non-significant may be shown in smoking patients. With current values of procalcitonine > 0.5 ng/mL, and ferritin > 600 μg/L, cardiac troponin I >1600 ng/L indicates that COVID-19 is progressing to a critical stage, which should be continuously monitored and perhaps averted. Monitoring renal function tests, liver and cardiac enzymes, over the course of mild to severe illness is essential because of the possibility of further harm owing to consequences and treatment.

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Keywords: COVID-19, PCT, FER, cTnI, ALT, AST, ALP, LDH, Urea and Cre

1. INTRODUCTION

1.1. Coronavirus Disease

Coronaviruses (COVID) are part of the Coronaviridae family in the Nidovirales order. Because it has crown-like spikes on its outside, it was taken corona infection name because of this (Gupta, P., and Birhman, N., 2020). Coronaviruses were indeed RNA viruses really can make people sick. They belong to a group called the Coronaviruses. There are some diseases which can be potentially lethal in humans that are caused by them. Pneumonia can be caused by these viruses. They usually target the top half lung tissue, having caused moderate and severe illnesses like colds (Guan, W. *et al.*, 2020)**.** In Dec. 2019, a new covid was found in Wuhan, China. It induces pneumonia and is called 2019-CoV by the World Health Organization (WHO). It became known as 2019-CoV (COVID-19) on February 11, 2020, because of its phylogeny, taxonomic status, and identified procedure **(**Liu, Y. *et al.*, 2020).

The COVID-19 caseload is very high. In Wuhan, China, the sickness is softly cased but in other cases has very bad and can be fatal, with a death rate of about 3%. COVID-19 can cause chest pain with difficulty in breathing, cough, fever, extreme tiredness, pain, body or muscle pain, and lately, a decline of taste or smell. All these are signs of COVID-19. It is an infection that damages the whole body, with possible effects on the digestive system, cardiovascular system, kidneys, and pancreatic cell, as well as changes lymphocytes as well as the immune system, because the virus' main entry receptor is found all over the body (Lai, C. C. *et al.*, 2020).

It has also been discovered in some cases where COVID-19 causes liver problems, implying that COVID-19 damages the liver. Bacteria, antigens, and viruses that cause inflammation are constantly in contact with the liver, potentially leading to liver failure (Wang, T. *et al.*, 2021).

Patients with COVID-19 usually experience a respiratory disease, but some have also reported gastrointestinal symptoms such as diarrhea, vomiting, and abdominal discomfort throughout the sickness. In the first case of COVID-19 in the United States (Holshue, M.

L. *et al.*, 2020), the patient had nausea and vomiting for two days before admission to the hospital, followed by diarrhea and flatulence. On the second day of hospitalization, the patients felt pain. On disease day 7, COVID-19 RNA was discovered in the patient's feces using a reverse transcriptase-polymerase chain reaction (Holshue, M. L. *et al.*, 2020).

1.2. Aim of the Study

1. To evaluate the correlation between COVID-19 and biochemical parameters such as ferritin, PCT, liver enzymes, and heart enzymes in Iraqi patients.

2. To evaluate relationships between the major risk factors like age and BMI with the severity of COVID-19.

3. To study sample of mild, moderate, and severe to critical cases of COVID-19 and investigate the biochemical marker in the blood of these groups, and if there are any significant differences between them.

4. To study the relationships between COVID-19 and other conditions such as: diabetes, cardiac, liver, and renal disease, also in people with asthma, smoking and hypertension cases in Iraqi patients.

1.3. Importance of the Study

There are few studies in Iraq that reflect the biochemical tests in COVID-19 patients compared to worldwide data. Therefore, the importance of this research lies in adding new information and investigating biochemical changes in Iraqi patients with COVID-19 by dividing the risks of illness cases into different stages of COVID-19 infection (mild, moderate, and severe cases) showing the diagnostic and prognostic relevance in patients.

2. GENERAL PARTS

2.1. Severe Acute Respiratory Syndrome Coronavirus

Coronaviruses are a genus of viruses that can infect a wide range of animals and cause moderate to severe respiratory pathologies; these are the SARS-CoV and MERS-CoV types, two highly virulent zoonotic viral diseases. It strikes living things unexpectedly and causes fatal lung infection (Krishnamoorthy, S. *et al.*, 2020).

SARS-CoV-2, a highly pathogenic disease that emerged in 2019, is a COVID-19 genotype. SARS-CoV-2, as the SARS coronavirus, can be passed from person to person through direct contact with the infected person and their bodily fluids (Chin, A. W. H. *et al.*, 2020). It is a virus with a zoological origin that is genetically similar to bat coronaviruses, implying that it is transmitted by bats (Zhou, P. *et al.*, 2020).

2.2. Epidemiology of COVID-19

Participants in early research reported that 49-66 percent had visited a seafood market that sold live wild animals such as poultry, bats, and pangolins. Wild animals are thought to be to blame for the spread of COVID-19 in Wuhan. COVID-19 was discovered in environmental samples taken from a seafood market in Huanan, China, according to the WHO. According to WHO, as of April 18, 2020, there were 2,164,111 reported cases of COVID-19 worldwide, with 164,198 people dying as a result of the virus (Chakraborty, I. and Maity, P., 2020). From 27 December 2021 to 2 January 2022, the number of infections increased by 71 percent worldwide, with the Americas being the most infected, followed by Southeast Asia at 78 percent and Europe at 65 percent (Taylor, L., 2022). Iraqi officials As of October 9, 2020, there had been 394,566 confirmed COVID-19 cases, with 9,683 deaths (Al-Kuraishy, H. M. *et al.*, 2020).

2.3. Virology and Pathogenesis

Coronaviruses are enclosed, single-stranded RNA viruses that can cause flu-like symptoms as well as more severe lung damage, digestive problems, and neurological issues (Mallah,

S. I. *et al.*, 2021). On February 1- 12, 2020, China CDC researchers gathered 585 specimens from the Huanan fish market in Wuhan, Hubei Province, China, to determine the incidence of COVID-19 infections. They found COVID-19 among 32 cases and determined that it came from animals sold on the market (Hamid, S. *et al.*, 2020). The investigators next performed laboratory testing on 15 patient' pulmonary fluid, serum, and mouth swab samples. The virus-specific genomic RNA sequences there in sample were found to be distinct from that of recognized coronavirus species, from these laboratory testing. COVID-19 is identical to several of the beta- coronaviruses found in bats, according to laboratory findings, and is member of a family of SARS/SARS-like CoVs. (Hamid, S. *et al.*, 2020).

2.4. Clinical Features

The COVID-19 patients show up with many different symptoms, like pharyngeal pain and fever. They also have a lot of different symptoms like fatigue, loss of appetite, headaches, diarrhea, nausea or vomiting, difficulty in breathing, and even moderate to severe respiratory distress syndrome. In the intensive care unit (ICU), there have been a lot of patients who are very sick. As reported by Chen, N. *et al.*, (2020), the total mortality rate for COVID-19 patients ranges from 2% to 5%. This can be higher for the elderly. People in Wuhan, the center of the epidemic, died at a high rate in the early stages. 25% of the cases had WBC counts that were normal or lower. In 66% of cases, lymphopenia could be seen (Saijo, M. *et al.*, 1996). 98% of young residents who had a torax CT scan had both sides of their bodies affected. On admitted to the ICU, a torax CT scan showed that both sides had a lot of lobular and sub-segmental consolidation. Non-ICU patients' torax CT images showed surface opacity on both sides and areas of consolidation in smaller parts of their lungs. As shown in research of 452 patients with COVID-19, fever (92.6%), shortness of breath (50.8%), cough (33.3%), and myalgia or fatigue (21.4%), was the foremost common symptoms, while headache (11.4%), vomiting (9.2%) and Hemoptysis (2.6%) were a rare (Qin, C. *et al.*, 2020; Serte, S. and Demirel, H., 2021).

2.5. Routes of Transmission

The main transmission routes are now thought to be pulmonary droplets and direct touch. COVID-19 has been found in urine as well as feces of lab diagnosed patients, signifying a

potential of fecal matter transmission, according to recent findings (Mukhra, R. *et al.*, 2020). However, it is unknown whether eating virus-infected foods will result in infection or spread. According to new research, COVID-19 cannot be transmitted through the airways to the baby during pregnancy or birth (Rajewska, A. *et al.*, 2020; Schwartz, D. A. and Dhaliwal, A., 2020).

2.6. COVID-19 Structural Proteins

Spike (S) protein is a protein with a large, petal-shaped type I transmembrane domain that attaches to receptor sites on host cellular membranes and causes the membranes to fuse together (Figure 2.1). Spike also is the primary target of COVID-19 neutralisation antibodies because it is important for the virus to spread (Maple, P. A. C., 2021). It is a homotrimer during its stable stage, with each monomer having a large number of structural or functional areas. The COVID-19 virus's globular N-terminal S1 region differs greatly from that of other species, making it easier for the virus to attach to receptors and thus more dangerous and pathogenic (Masters, P. S., 2006). S2, the cell wall proximal stalk, is more stable and has two heptad-repeat segments with a spiral structure that aids in the fusion of viruses and cells after they enter cells. The S1 and S2 sections combine to form a single polypeptide that can be divided in half by host proteolytic enzymes. COVID-19 has a different cleavage threshold. There are a few Alpha CoV spikes that are unbreakable. They can fuse cells together, like the feline infectious peritonitis virus (VanPatten, S. *et al.*, 2020).

A type 3 glycoprotein called membrane protein (M) has 218-263 amino acids (aa) (25-30 kDa) and then it can bind to either N- or O-linked glycan’s on it. This part of protein is called the ectodomain (Mudgal, G., 2014). This has a long C-terminal domain which develops a tight lattice also with endomembrane leaflet, which is why the COVID-19 cell membrane is so thick. M protein is said to play a significant role in viral replication, such as putting epigenetic RNA into nucleocapsids and putting them together (Mudgal, G., 2014). Genome encapsulation, RNA Synthetase, translational, RNA chaperone function, and class I interferon blocker are all functions of the Nucleocapsid protein (Yadav, R. *et al.*, 2021) (Figure 2.1).

Only lineage A of the Beta-CoV genus has the short, spike-like hemagglutinin esterase protein (HE), which is known to interact with sialic acids in MHV. CoV HE proteins and

influenza C virus have 30% similar amino acid sequences. In the absence of spike protein, it may aid in the CoV's initial adsorption to the host cell membrane, but it cannot start an infection (Mudgal, G., 2014).

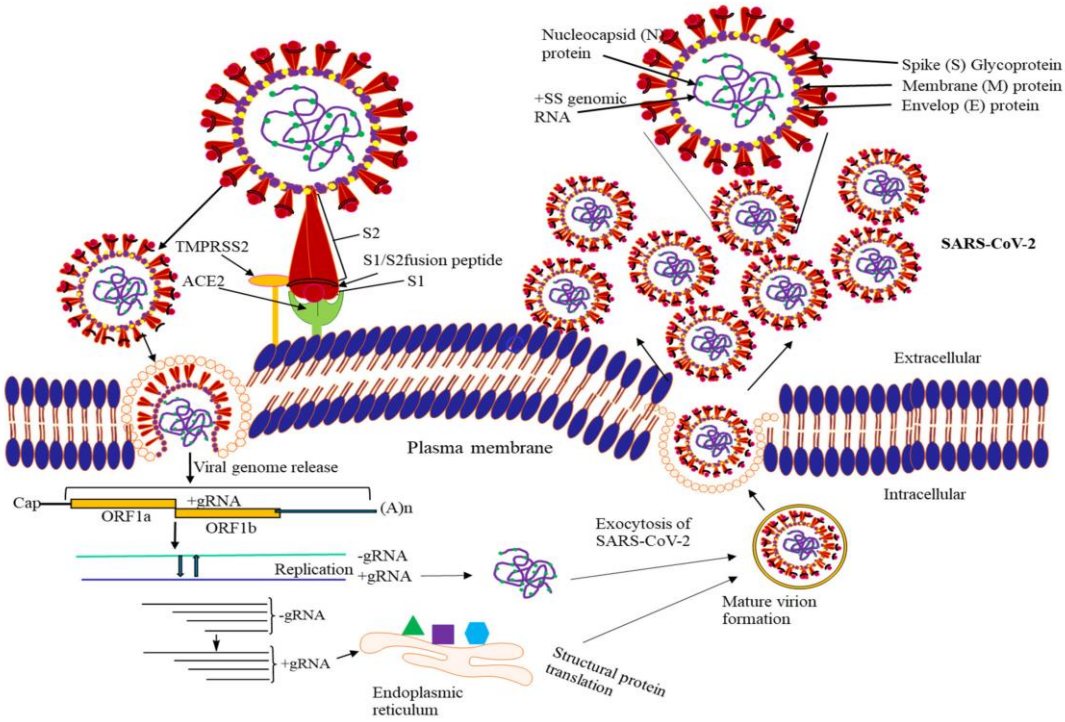


Figure 2.1. The SARS-CoV-2 Structure and its mechanism within the host.

(The activity begins by binding with its S protein (RBD/S1) on host cell receptor, ACE2, driving conformational change in the S2 subunit, and thereby facilitating its fusion with plasma membrane. Immediately after release of +ssRNA, translation leads to formation of non-structural polyproteins pp1a and pp1ab, which undergo proteolytic cleavage and are eventually assembled into functional replicase. The replicase leads to formation of a negative-sense intermediate, which is eventually replicated to form multiple copies of gRNA as well as nested set of sgRNA by discontinuous transcription. These sgRNA are translated into various structural and accessory proteins, which are assembled as virion in the ERGIC, and eventually exit cell via exocytosis (Yadav, R. *et al.*, 2021)).

2.7. Common COVID-19 Markers

The results of a COVID-19 infected person's laboratory tests were "very closely linked to increased morbidity and mortality, even when other health problems such as age group, some other laboratory tests, and some predecessors were taken into account." There may

be ways to identify COVID patients who are at high risk of becoming critically ill and who require more aggressive treatment as soon as possible. Knowing these parameters can be beneficial. These are typical biochemical, serological and coagulation parameters.

2.7.1. D-dimer with the Severity of COVID-19

D_dimers, as degradation products of fibrin, are widely used in the diagnosis (exclusion) of venous thrombosis. In addition, D-dimers have been shown to be of prognostic value in various diseases, including cancer and cardiovascular disease. There is a plethora of data on coagulation disturbances in patients with COVID-19, both clinically and by the use of various laboratory measurements. Since December 2019, the severity of the coronavirus disease2019 (COVID-19) pandemic has been escalating. Coagulopathy is common in critically ill patients with COVID-19. Systemic microvascular thrombosis may occur in most deaths and was corroborated by a recent autopsy. However, less is known about the coagulation parameter D-dimer in the progression of COVID-19 (Connors, J. M. and Levy, J. H., 2020).

In some patients, severe pulmonary and extra-pulmonary complications may lead to respiratory failure and life-threatening events. It has been reported that about 50% of the patients had increased D-dimer levels, and abnormal D-dimer levels are associated with poor prognosis (Mina, A. *et al.*, 2020). Thus, in some stable patients with sudden death, acute organ embolism and infarction should take into consideration. Although the incidence of thrombosis in patients with COVID-19 has not been determined, the incidence of deep vein thrombosis (DVT) and pulmonary embolism (PE) was 20.5% and 11.4% respectively in SARS cases (Iba, T. *et al.*, 2020). In addition, thromboembolism formation was seen in pathologic studies based on autopsies or biopsies, which greatly resemble those seen in SARS and MERS coronavirus infection (Calabrese, F. *et al.*, 2020). Higher D-dimer levels were associated with a greater probability of pulmonary embolism 3, 6, 9, and 12 days after determining D-dimer levels with an OR of 1.7, 2.0, 2.4, and 2.4, respectively in 21 patients from Spain. Similar results were found in 106 French patients, although the D-dimer threshold to exclude PE (2660mg/L) was much higher than usual. A higher threshold was also suggested in 156 COVID-19 patients with asymptomatic deep venous thrombosis (Schutgens, R. E., 2020).

2.7.1.1. D-dimers to Guide Anticoagulation

Anticoagulation therapy was associated with lower mortality in COVID-19 and this was especially true for patients with high D-dimers. As the relationship between D-dimers and the severity of COVID-19 and/or the occurrence of PE is evident and even appears to be dynamic, it is appealing to start an early intervention based on D-dimer levels. Several clinical guidelines already advocate the use of different D-dimer cut-off levels to determine the anticoagulation dose (Mezalek, Z. T. *et al.*, 2020).

However, as a recent review pointed out very clearly, there is a high variety of D-dimer tests with a large variability in the way they report their results. Most importantly, differences in the reported units (either D-Dimer Units [DDU]) or Fibrin Equivalent Units [FEU], the assay cut-off values and the absolute measuring units (mg/L, ng/mL, m/mL) hamper generalizability of the results and the use of a clear cut-off point for decision making. The authors correctly call out for at least fully reporting the necessary variables to ensure study results can be translated to other clinics. Therefore, although the role of D-dimers in guiding treatment of COVID-19 is attractive, clinicians should be aware of the details of their local D-dimer test before implementing standard cut-offs provided by others (Conte, G. *et al.*, 2021).

Elevated baseline D-dimer levels are associated with inflammation in COVID-19 patients and have limited predictive value for thrombosis. In the treatment of COVID-19 patients, the change of D-dimer levels should be observed dynamically. And the abnormal changes of D-dimer and inflammatory factors suggest that anticoagulant therapy might be needed. Also, although the predictive value of VTE score need to be further studied in COVID-19 patients, it might be useful than baseline D-dimer levels for prophylaxis for venous thromboembolism in COVID-19 patients (Yu, B. *et al.*, 2020).

2.7.2. C-Reactive Protein (CRP) with the Severity of COVID-19

C-Reactive Protein is an acute-phase protein that serves as an early marker of inflammation or infection. Generally, the level is much higher in bacterial infections. The protein is synthesized in the liver and is normally found at concentrations of less than 10 mg/L in the blood (Ahnach, M. *et al.*, 2020). During infectious or inflammatory disease states, CRP levels can activate the classical complement cascade of the immune system and modulates the activity of phagocytic cells, supporting the role of CRP in the

opsonization of infectious agents and dead or dying cells. In COVID-19, the exact effect of CRP remains unclear, but it was reported that their level can be used for early diagnosis of pneumonia and assessment of severe pulmonary infectious diseases (Warusevitane, A. *et al.*, 2016).

2.7.3. CBC and the Severity of COVID-19

The complete blood count is a necessary part of the diagnostic evaluation in a broad variety of clinical conditions Similarly, the leukocyte differential count and examination of the blood film, in spite of limitations as a screening test for occult disease, is very important in initial consideration of the differential diagnosis in most ill patients. Although quantitative and qualitative (morphologic) examination of the cells of the blood are considered separately during this chapter, the excellence between these two isn't absolute, and measures once considered “qualitative” become quantitative as technology advances (Riva, G., *et al.*, 2019).The complete blood count (CBC) may be a test that evaluates the cells that circulate in blood. Blood consists of three sorts of cells suspended in fluid called plasma: white blood cells (WBCs), red blood cells (RBCs), and platelets (PLTs). They're produced and mature primarily within the bone marrow and, under normal circumstances, are released into the bloodstream as required.

During asymptomatic forms or the incubation period, patients can manifest a moderate abnormality in the blood count, the potential value of eosinopenia as predictors of early identification of COVID-19. Regarding symptomatic forms, a predominance of lymphopenia (83%), thrombocytopenia (36.2) and neutropenia (33.7%). In severe cases, these abnormalities were more prominent (96.1% versus 80.4% lymphopenia, 57.7% versus 31.6% thrombocytopenia and 61.1% versus 28.1% for leukopenia). Lymphopenia is the most common sign, in fact, the coronavirus attack directly and indirectly the lymphocytes by immune and inflammatory mechanisms . The analysis of the lymphocyte count is therefore a reliable indicator of the severity, which can be really useful in the monitoring and therapeutic adaptation, moreover after clinical improvement the lymphocyte count is corrected (Ahnach, M. *et al.*, 2020).

In summary, the blood count as a routine biological analysis, keeps a prominent role in the early diagnosis and follow-up of COVID-19 infection. The blood cells perturbations are

seen as a prognosis factors, careful analysis and interpretation of lymphocyte and platelet count, allows not only to evaluate the prognosis, but above a clinician to adapt therapeutic care (Yuan, X. *et al.*, 2020).

2.7.4. Lactate Dehydrogenase (LDH) with the Severity of COVID-19

Lactate dehydrogenase is an enzyme among almost all the body's tissues. Increased LDH levels in the blood can be caused by liver illness, anemia, heart attacks, bone fractures, muscle damage, malignancies, and infections such as encephalitis, meningitis, and human immunodeficiency virus (HIV) (Farhana, A. and Lappin, S. L., 2020). Additionally, LDH is a non-specific marker of tissue turnover, a natural metabolic process. Numerous malignancies result in an overall rise in LDH levels or one of its enzymes. As a result, it may be a generic tumor marker that is ineffective in identifying the type of malignancy. Due to the non-specific nature of LDH and the lack of routine isoenzyme measurement in clinical laboratories, LDH measures provide insufficient information and other assays such as muscle creatine kinase (CK), and troponin (cTnI) for heart disease (HD) are required (Farhana, A. and Lappin, S. L., 2020). Hemolysis of the blood sample also affects LDH activity. Hemorrhaging red blood cells (RBCs) increased the LDH protein in RBCs, resulting in false-positive results. To add insult to injury, the widespread distribution of necrosis across tissues makes it difficult to use as a biomarker for more complex diseases (Farhana, A. and Lappin, S. L., 2020).The COVID-19 illness was closely linked to LDH. The fact that LDH is a measure of damage to the lungs and that COVID-19 predominantly infects the lower respiratory tracts may explain the significant link between LDH and COVID-19 sickness (Ferrari, D. *et al.*, 2020).

2.7.5. Ferritin with the Severity of COVID-19

There is a key cytosolic iron - binding protein called ferritin (FER) which is a type of acute inflammatory oxidizing agent that is formed and increased a in a lot of inflammatory disorders, like acute infections. People with hyperferritinemic illnesses, these included macrophage stimulation syndrome, catastrophic antiphospholipid syndrome, adult-onset Still's disease, and septic shock, have a very great level of FER in their bodies (Perricone, C. *et al.*, 2020; Meng, J. *et al.*, 2021). FER is a critical mediator of immunological dysfunction, specifically under severe hyperferritinemia, through acute immune-suppressive and pro-inflammatory activities, leading to a cytokines storms. In generally, elevated FER values predict poor prognostications in hospitalized. It has been shown that

COVID-19-related fatalities are followed with cytokine storm sickness, implying that illness severity is influenced by cytokine storm syndrome (Meng, J. *et al.*, 2021).

Many diabetics have raised FER values, and it's well recognized that they're more likely to develop major COVID-19 problems. On this line, we evaluate the facts supporting the concept suggesting FER levels may be a key determinant of COVID-19 incidence (Vargas-Vargas, M., and Cortés-Rojo, C., 2020). However, FER amount might play role during inflammation after COVID-19 diagnosis starts. If an inflammatory illness found, it is probable for effective FER creation to happen at some point. This may be because immune cells (macrophage), which also contribute to making cytokines as well as form the bulk of immune cells to the lungs parenchyma, could be the source of serum levels of FER. Inflammatory mediator's cytokine, like interleukin 6 (IL-6), can also make FER. COVID-19 people with the disease who already have high IL-6 levels have more severe symptoms (Meng, J. *et al.*, 2021). As a result, although FER may be released first at the site of inflammation, it is possible that FER can do more than just store iron. As a signaling molecule, FER has been linked to the immune response.

Aggregating NF-B signaling by certain inflammatory cytokines (e.g., tumor necrosis factor (TNF) and IL-1) can induce heavy FER subunits (H- FER) transcription. In human hepatoma cells, IL-1 also boosted translational expression of light FER subunits (L- FER) via enhancing L- FER mRNA's interaction with ribosomes. Although iron chelation prevented this impact, it still does not appear to be due to an increase in H- FER transcription or if the IRP system was saturated. On the transcriptional level (primarily H-FER), inflammatory cytokines can influence the expression of FER and the translational level (both H- and L- FER). 4.8 kb upstream of the transcription start site was a cis-acting area by deletion mapping of the H- FER promoter regions (Alkhateeb, A. A. and Connor, J. R., 2013).

2.7.6. Liver Function Tests (LFT)

The liver is a hub of activity, from the synthesis of various enzymes, proteins, and carbohydrates to the removal of toxins. is in the right upper part of the body, underneath the diaphragm, and plays a major role in metabolic activities, too. It decides how so much carbohydrate is stored in the body. It also breaks down blood cells as well as makes hormones (Cai, Q. *et al.*, 2020). In the hepatocytes, the lobes are not the same size and shape. People usually have a liver that equals about 1.5 kg. Laboratory liver function tests

(LFT) could be used to figure out how well a person's liver is working and how to treat them. Using these tests, you can find out existence of liver disease, identify the types of liver diseases, measure the incidence of known liver damage, and track how well your treatment is working (Cai, Q. *et al.*, 2020).

The relationship of LFT with COVID-19 was unclear. Some studies indicate that there is an increase in the Aspartate aminotransferase (AST) level, Alanine aminotransferase (ALT), AST/ALT ratio, Alkaline phosphatase (ALP) and Gamma-glutamyltransferase (GGT) were elevated and a highly (peak) increase with the increase in the severity of the COVID-19 (Hundt, M. A. *et al.*, 2020; Ferrari, D. *et al.*, 2020; Benedé-Ubieto, R. *et al.*, 2021) Other research studies do not find any relationship (Bertolini, A. *et al.*, 2020).

2.7.6.1. Aspartate transaminase (AST)

In the body, AST is a very essential enzyme in the process of breaking down amino acids. It helps to move an amino group back and forth between aspartate and glutamate in a way that can be reversed. They are all made up of AST. They are in the hepatic, the heart, the muscular, the brain, as well as the RBC. To check the integrity of the liver, serum AST as well as ALT levels, and also their ratio (AST/ALT ratio), are invaluable parameters. Sinusoidal cells within the liver are already in charge of getting rid of AST (Wu, J. *et al.*, 2019).

2.7.6.2. Alanine transaminase (ALT)

ALT, like AST, is a transaminase. Both enzymes are pyridoxal dependent. It was named as a glutamic-pyruvic transaminase by Arthur Karmen and other scientists in the 1950s, and they called it that at the time alanine aminotransferase (ALT). It plays a role in liver metabolism, serum AST as well as serum ALT values, and their ratio (AST/ALT), to evaluate liver function. ALT has a half-life about 47 hours in circulation (Chung, J. Y. *et al.*, 2019).

2.7.6.3. Alkaline phosphatase (ALP)

Alkaline phosphatase (ALP) is an 86-kilodalton dimeric protein and enzyme that works together. They all have five cysteine residues; also, it contains 2 zinc atoms, with 1 magnesium atom, which have been important for catalytic reactions. They performThe reaction was performed throghly in alkaline pH environments. There is a lot of ALPs in the

hepatic, biliary duct, renal, skeletal, and fetal membranes (placenta). ALP can be found in all tissues of the body. There are two types of ALP isozymes in the blood: skeletal and hepatic, both of which are abundant in serum (Sharma, U. *et al.*, 2014).

2.7.6.4. Effect of COVID-19 on Liver

During the last epidemic of COVID-19, about 60% of patient populations had liver failure, according to reports. 14–53 % of COVID-19 patients in this pandemic have had problems with their livers, especially those who have had a lot of damage (Jothimani, D. *et al.*, 2020). Mechanism of COVID-19 -induced liver damage remains unknown. Angiotensin converting enzyme II (ACE2) is expressed in cholangiocytes of liver cells, according to research by Jothimani, D. *et al.*, (2020). Another investigation found that SARSCoV2 employs the same ACE2 entering receptors as SARS-Cov (Hoffmann, M. *et al.*, 2020). Aberrant liver enzymes were often noted as an extrapulmonary clinical feature and approximately half of the patients had some degree of hepatotoxicity, so we can investigate this liver injury by monitoring liver enzymes (Hoffmann, M. *et al.*, 2020). Li, L. *et al.*, (2020a) reported significantly higher ALT in ICU patients with COVID-19. Li, L. *et al.*, (2020a) studies also revealed that CRP levels were significantly higher in patients with high ALT levels. They speculated that COVID-19-related hepatic damage could be caused by cytokine storm disorder. In extreme situations, the albumin level drops from roughly 37.25 g/L to 34.26 g/L at the day of the third week of hospitalization (Farshidpour, M. *et al.*, 2020). The proportion of extreme COVID-19 patient populations with liver damage was significantly higher than the proportion of medium COVID19 patient populations. COVID-19 patients who already had elevated hepatic enzyme rates were more likely to develop severe pneumonia, be admitted to the ICU, and die, according to a much larger study (Piano, S. *et al.*, 2020). Mild micro-vesicular steatosis and lobular interaction in the hepatic were also discovered in a recent autopsy case, which could be a sign of either COVID-19 infectious disease or drug-induced liver damage (Chen, N. *et al.*, 2020). This is based on both virological and pathological evidence. People with the disease of COVID-19, who were admitted to a hospital 76.3 percent of the time had elevated liver tests, and 21.5 percent had liver damage, according to study by Hundt, M. A. *et al.*, (2020) and Cai, Q. *et al.*, *(*2020). ALT and AST, as well as other liver enzymes that measure liver function, hepatic cells integrity, and difficulties with the biliary tract (like ALP and GGT) could be used as markers for liver damage (Cai, Q. *et al.*, 2020; Farshidpour, M. *et al.*, 2020).

2.7.7. Troponin (cTn) with the Severity of COVID-19

Cardiac troponin (cTn) is a protein that is very sensitive and selective. It could be set to release when myocardial cells are damaged. As a result, it is used as a favorite diagnostic marker for acute coronary syndrome (ACS) prognosis (Thygesen, K. *et al.*, 2018). High-sensitivity cardiac troponin T (hs-cTnT) and I (hs-cTnI) are two different types of cTn that can be used together the field (Thygesen, K. *et al.*, 2018). The cTn levels that are high in elderly people and used to always be linked to a heart attack (Alcalai, R., *et al.*, 2007). Because elderly people who don't have a heart attack or stroke can have a high proportion of cTn, which can be hard to interpret, it's important to know what the different types of cTn are and what they can do in the body (Januzzi, J. L. *et al.*, 2019). This way, a misdiagnosis could be avoided. Although high cTn levels have been linked to an increased risk of death and illness in the elderly with hyperglycemia, pulmonary hypertension, myocardial infarction (MI), high blood pressure, renal disease, and chronic obstructive pulmonary disease (COPD), people in this group should be wary of any situation that could raise their cTn levels. Older people with stroke, disruptive sleep apnea, and various types of cancer are more likely to have high cTn levels than younger people, but the absence of it in young people raises some concerns (Pavo, N. *et al.*, 2015).

Cardiac troponin is a well-known, prolonged prognostic factor that has been shown to be a good predictor of both short-term and long-term HD risk, even in people who have heavy respiratory distress. Data from the COVID-19 studies that show that more cTn is associated with poor outcomes, in this opinion, are not shocking, but they add to the evidence that cTn is an important predictive marker in a variety of situations (Crewdson, K. *et al.*, 2019).

Recent COVID-19 investigations have reaffirmed a number of key messages. First, there is a clear link between myocardial injury and negative outcomes such as an increased risk of death and arrhythmias. Second, there is a consistent link between cTn levels and consequences, with higher hs-cTn concentrations leading to higher mortality rates. (Farshidpour, M. *et al.*, 2020). Third, people with COVID-19-positive chronic CVD are not only more likely to have an acute MI, but also to die. Finally, the COVID-19 studies emphasize the use of serial measures to detect shifting patterns of cTn and/or NP that aid in distinguishing survivors from non-survivors and identifying individuals who may require additional testing or therapies (Guo, T. *et al.*, 2020).

2.7.8. Procalcitonin (PCT) Relation with the Severity of COVID-19

Blood PCT levels are also linked to the severity of sepsis and organ failure, providing prognostic information and assisting risk categorization algorithms. As a result, PCT is frequently used to assist critically ill patients who are at risk of infection or sepsis (Shim, B. S. *et al.*, 2019). Procalcitonin (PCT) is thought to be a good diagnostic tool and it is used in the diagnosis of patients who have a suspected bacterial infection or sepsis (Meisner, M., 2014). In addition, it is used if subjects need to take antibiotics for respiratory tract infections (Schuetz, P. *et al.*, 2012), and for people who are very sick in intensive care. A biomarker known as PCT is becoming increasingly important in determining the best way to use antibacterial drugs, potentially avoiding problems such as anaphylaxis and the spread of antibiotic-resistant bacteria (Schuetz, P. *et al.*, 2012). Finally, PCT testing aids in the early detection of significant bacterial infections in people hospitalized in the emergency room with such signs of infection and sepsis (Shim, B. S. *et al.*, 2019). Procalcitonin (PCT) is a polypeptide precursor to the hormonal calcitonin that is routinely generated and secreted by thyroid Para follicular C cells. It is being studied as a potential diagnostic for the early presence of infectious-like bacterial pathogenesis. Elevated PCT is common in patients with sepsis and septic shock. In a previous study, researchers discovered that a higher level of PCT was linked to incidence and death in COVID-19 patients (Liu, Z. M. *et al.*, 2020). Similarly, Ou, M. *et al.*, (2020) found that greater PCT concentrations are linked to risk of serious COVID-19 in their meta-analysis.

2.7.9. Relationship of blood urea and creatinine (CRE) with COVID-19

Blood urea and CRE are the final processes of nitrogen metabolism. Since they are small molecules, they were easily filtered from the nephrons. Usually, blood urea, about 30%-40% is reabsorbed from tubules, while CRE is not reabsorbed. Blood urea and CRE are reflecting glomerular filtration function (Ok, F. *et al.*, 2021). Inflammation, such as cytokine storm, has been shown in severe and critically ill COVID-19 patients, causing increased blood urea reabsorption (Ok, F. *et al.*, 2021). According to Xiang, J. *et al.*, (2021), serum urea and CRE concentrations were significantly higher in severe COVID-19 patients than in mild COVID-19 patients. This method can be used to distinguish between severe and mild COVID-19.

2.7.10. Serum glucose with the severity of COVID-19

In COVID-19, hyperglycemia is a significant risk factor for death in both people with and without diabetes. It has been reported that acute hyperglycemia occurs in approximately 50% of COVID-19 patients hospitalized, while the prevalence of diabetes in the same population is approximately 7%. One possible explanation is that "severe acute respiratory syndrome" affects pancreatic b-cells, resulting in decreased insulin secretion. At the same time, the infection causes a surge in the production of cytokines, which may lead to insulin resistance. In hyperglycemia, both decreased insulin secretion and insulin resistance may be present (Ceriello, A., 2020). The significant hyperglycemia that occurs in COVID-19 patients during the acute inflammatory state has been recognized and found to be more pronounced in those with diabetes and prediabetes. For chronic diabetes complications, a bidirectional link between chronic inflammation and hyperglycemia had previously been described. Several changes in the immune system, for example, included changes in specific cytokines and chemokines (Gianchandani, R. *et al.*, 2020).

2.8. Diagnosis

2.8.1. Nucleic Acid Test

Viral detection is one of the most important tools for COVID-19. Diagnostic methods involving virus genome sequencing by Real time-Polymerase chain reaction (RT-PCR) and possibly the next phase involving the construction of next-generation sequencing platforms became available relatively quickly. Many medical researchers have developed RNA detection kits, and the China Food and Drug Administration has certified and sequenced a batch of fluorescence quantitative tests on an expedited basis (Jayamohan, H. *et al.*, 2021). The most important thing to watch out for in nucleic acid testing is false negatives. To address the issue of low recognition efficiency, new and improved viral RNA-generated positives have been created. A nucleic acid check, in particular, has been used to help identify COVID-19 in 3 minutes with the naked eye. As a result, the RT-PCR test for COVID-19 is more sensitive and specific than the chest computed tomography (CT) scan, and the chest CT scan should be considered a complementary diagnostic tool (Waller, J. V. *et al.*, 2020).

2.8.2. Serologic diagnosis

Serological responses have been seen in patients exposed to the virus with COVID-19. As a result of immunochromatography, colloidal gold, and other technological advances, relating identification materials (reagents) were quickly made (Ciotti, M. *et al.*, 2020).

2.8.3. Imaging technology

In the world, a chest X-ray or radiography (CT-scan) is a tool for diagnosing COVID-19. On CT scans, people with COVID-19 had the same symptoms, such as joint patchy darkness and opaque ground glass (Yang, X. *et al.*, 2020). It was demonstrated the value of using a transfer learning method to identify radiological graphics for COVID-19 diagnosis.

A computer program known as Artificial Intelligence (AI) can read CT scans of COVID-19 patients on a 3D CT volume who may have new crowns in 20 seconds and obtain a 96 percent global prediction accuracy with the results obtained, greatly improving diagnostic efficiency. This technique is already in use in hospitals (Serte, S. and Demirel, H., 2021).

3. MATERIAL And METHOD

3.1. Subjects

From April to December 2021, a study was done in the labs of the biochemical department of the AL-Ramadi Teaching Hospital. There were 100 Iraqi patients of all ages, from 20 to 70 years. All participants in this research were screened at the hospital by a senior cardiologist based on medical history, lab results such as physical examination, RT-PCR test, D-dimer test, serological test, and other test results such as chest X-ray or radiography (CT-scan), was investigated to identify COVID-19. RT-PCR tests are more sensitive and specific than chest CT scans and certain other assays. They were divided into the following groups:

3.1.1. Patients' groups

Patients who had COVID-19 confirmed at Al-Ramadi Teaching Hospital in Anbar/ Iraq, as well as (60) blood samples from people who were infected were used in this study to find out more about the disease that included patients who were diagnosed in the hospital as having COVID-19, previously diagnosed with clinical signs (D-dimer) and physical measurements (CTScan, Chest Xray & Respiratory Test) by specialized doctors, and to ensure that the Sending them to the PCR examination, which is universally approved in diagnosing the disease, we have adopted only the positive cases that were confirmed to be infected by PCR Technique. We did not do a D-dimer analysis because it was measured at the stage of diagnosing the infection by doctors, and we did not conduct it because we are not going to follow up on the case, but we did other analyzes such as Troponin as well as LDH as parameters that could give an idea of the work of heart enzymes during injury, liver and kidney functions. IgM levels were then used to group people into groups based on level of high their COVID-19 IgM values (Table 3.1). The person's name, age, sex, symptoms, and drug history should all be written down in a file. Data was gathered through a direct interview with patients as a questioner, and information was gathered from patient subjects on a survey data sheet (Appendix). The total number of patients was separated into groups based on their age and gender. Table 3.2 shows the classification of the group

Table 3.1. Classification of patient groups according to their severity and gender

Group / **Gender**	Mild cases IgM (1-2) N= 25	Moderate cases (2.0< IgM≤ 4.0) N=18	Sever cases IgM(>4) N= 17
Male	18	12	14
Female	7	6	3

Table 3.2. Classification of patient groups according to their age and gender

Age (year)	20-35	36 – 50	More than 50
Male (N)	6	16	22
Female (N)	1	6	9

3.1.2. Healthy Control Group

The control group in this investigation consisted of forty (40) seemingly healthy samples taken from a local lab in Anbar, as well as healthy people (no previous or current infection with COVID-19). The total was separated into three groups based on their age, as shown in Table 3.3.

Table 3.3. Classification of control groups according to their age and gender

Age (year)	20 - 35	36 - 50	More than 50
Male (N)	7	10	12
Female (N)	3	3	5

3.1.3. Blood Samples Collection

Venipuncture samples taken by all patients and normal with the use of a 5 ml plastic syringe, and the sample was then dispensed into a gel tubes and allowed to coagulate for 15minutes before being tested. After that, it was centrifugation at 3500 rpm for 10 minutes to obtain serum, which was then stored in the freezer (-20°C) until it’s used.

3.1.4. Body Mass Index

The person with a BMI is a measurement size that is based primarily on an individual's weight and height. The BMI level of a human is characterized as the body weight divided by the square of their height, with the resultant value always expressed in kilograms per square meter (kg/m^2) by following the formula:

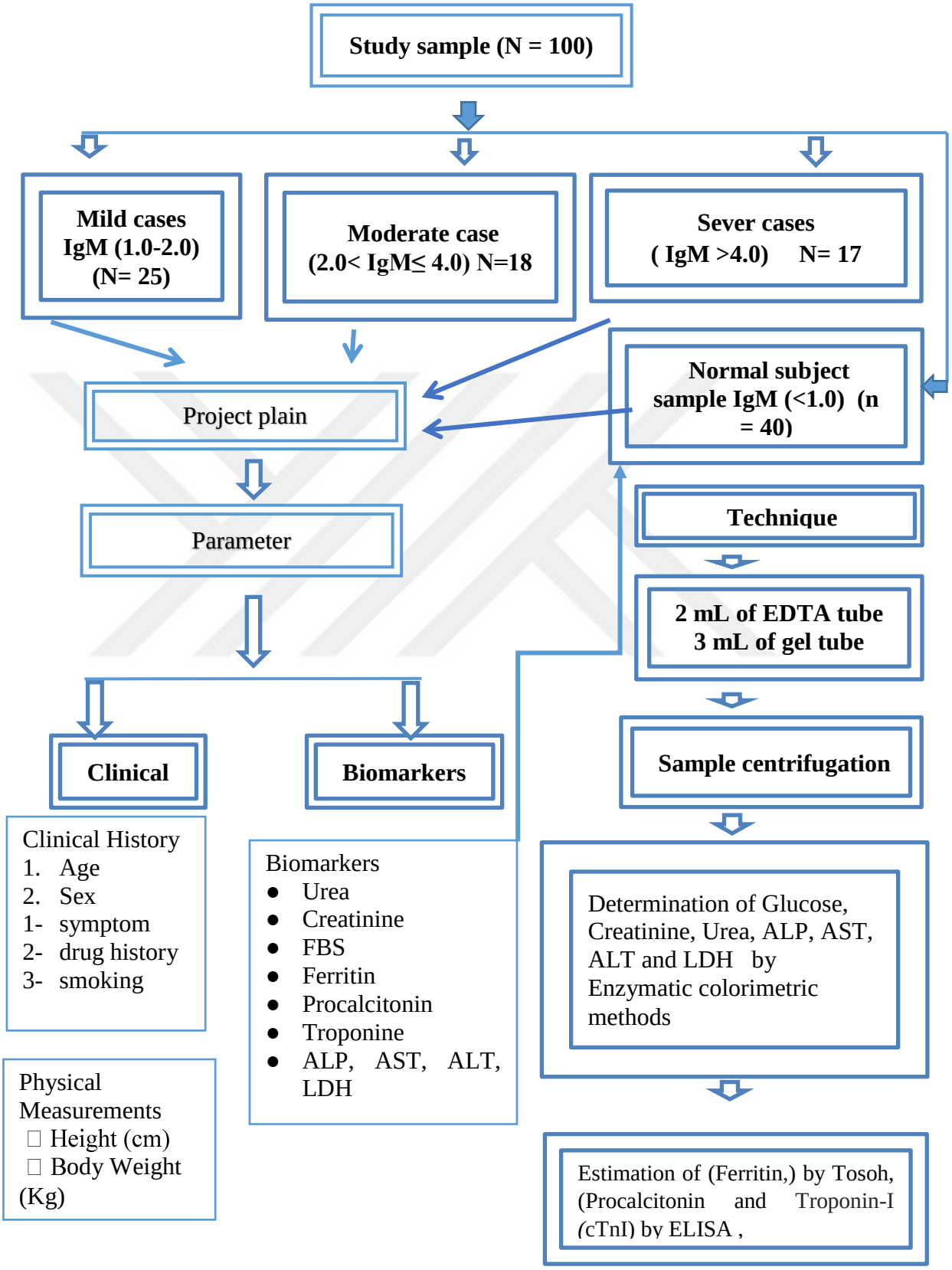
BMI (Kg/m^2) = weight (Kg) / Height (m^2)

Having a BMI ranging from 18.5 to 24.9 indicates that the person is at their ideal weight while being less than 18.5 indicates that the person seems to be underweight, a value ranging from 24.9 to 29.9 may reflect the fact that the individual is overweight, while ranging from 30 and above may indicate that the individual is obese (Weir and Jan 2019).

3.1.5. Excluded Criteria

One of the important criteria that were excluded in this study is that all patients who were taken to the study were without any history of liver dysfunctions, chronic renal failure (CRF), arteriosclerosis, or taking cholesterol-lowering drugs and thyroid dysfunctions. It may affect the course and results of the search.

3.2. Flowchart of study samples



3.3. Equipment and Tools

Following Table 3.4 Many research instruments and equipment were used.

Table 3.4. A list of the study's instrumentation

No.	Instruments	Company	Country
1	Micropipettes (different volumes)	Eppendorf	Germany
2	Multichannelpipette(50-300µL)	Biohit	U.K
3	Absorbance Microplate Reader	Karl Kolb	Germany
4	Capped plastic tubes	Afco-Dispo	Jordan
5	Centrifuge	Kokusan	Germany
6	Ependroff tubes	Sigma	England
7	Deep Freezer (-80 °C)	Angel Antoni	Italy
8	Gel Tubes	Afco	Jordan
9	Incubator	Memmert	Germany
10	Spectrophotometer	Shimadzu	Japan
11	Water bath	Kottermann	Germany
12	Refrigerator	Arjelic	Turky
13	ELISA washer	Seri	Italy
14	ELISA recorder	Seri	Italy
15	Hormone Analyzer	TOSOH 600	Japan

3.4. Kits Used in the Study

Many hormone and biomarker testing kits are included in the Table 3.5.

Table 3.5. Kits for the detection of biomarkers

No	Apparatus	Origin	Company
1	Ferritin by (cTnI) by AIA 600 TOSOH	France	Demeditec Diagnostics GmbH
2	(Procalcitonin) by ELISA	Germany	Human
3	Troponin-I (cTnI) by ELISA	Germany	Human
4	Glucose, Urea, Creatinine, ALP, AST, ALT, LDH	Biosystems	Spain

3.5. Methods

3.5.1. Determination of Biochemical Study in Serum

All biochemical parameters are calculated and identified by using commercial ELISA Microwells kits and by AIA 600 TOSOH., which were used in accordance with the manufacturer guidelines.

3.5.1.1. Human PCT (Procalcitonin) ELISA kit

This ELISA kit is used to test for Human PCT in serum, plasma, and other biological fluids in a lab.

Test Principle

The ELISA kit employs the Sandwich-ELISA principle. This kit includes a micro enzyme Immunoassay plate pre-coated to antibodies that are specific to Human PCT. The micro ELISA plate wells are filled with a specific antibody and a standard or sample. After that, each plate is really treated with biotin - conjugated antibody specificity for Human PCT and an Avidin-Horseradish Peroxidase (HRP) conjugate. The materials that aren't needed are rinsed away. Biotin-conjugated each well receives the substrate. Blue dye will be applied to the 96-well plates Human PCT, biotin-conjugated detecting antibody, and Avidin-HRP coupling. The enzymatic interaction with substrate is halted when stop solution is added, as well as the color turns to yellow. The optical density (OD) may be measured using a spectrophotometer at 450 nm 2 nm wavelength. As the quantity of Human PCT in a sample increases, the optical density (OD) value increases. The quantity of Human PCT in the samples may be assessed by multiplying the OD of samples to that of the standard curve.

3.5.1.2. Human cTnI (Cardiac Troponin-I) ELISA Kit

Principle of the Assay

A sandwich enzyme immune assay was used to make this product. The 96-well plates had previously been coated with the capture antibody. Antibodies that were coated with biotin that employed to monitor the presence of antibodies. In the next step, the wells were washed with wash buffer, and then the biotin-conjugated detection antibody was added. After HRP-Streptavidin was added, the unbound conjugates were washed away with wash

buffer in order to remove them. The HRP enzymatic process was seen when TMB substrates were used. An acidic stop solution made a blue product turn yellow when it was mixed with TMB. Yellowness is a measure of how many samples were taken from the plate. In a micro plate reader, read the O.D. absorbance at 450nm, and then figure out how much you need.

3.5.1.3. Ferritin

The ST AIA-PACK FER is only for use on Tosoh AIA System Analyzers, which are used to measure FER in human serum or heparinized plasma.

The iron used to make hemoglobin, myoglobin, and other iron-containing proteins comes from FER, which is one of the most important soluble iron store proteins. Inside the cells like liver cells, spleen cells, and red cell precursors that are growing in bone marrow with FER, there is high level of it. The amount of FER in extracts from different types of tissue changes. Iso- FER that are more acidic are found a lot in the heart, kidney, and many types of tumors. More simple iso- FER can be found in the liver, spleen, and blood. In most immunoassays, basic iso-f FER that have been crystallized from the human liver or spleen are used to show how much FER there is. Tests on people with iron deficiency and excess disorders say that measuring ferritin concentrations can help people figure out how much iron they have stored in their bodies.

Principle of the Assay

They are used to do a two-site immune enzymometric experiment. The ST AIA-PACK FER test cups help. Monoclonal antibodies on magnetic beads and enzyme-labeled monoclonal antibodies in the test cups bind to FER in the test sample, which is found in the sample. The magnetic beads are rinsed to get rid of any enzyme-labeled monoclonal antibodies that aren't attached to the beads. Then, they're treated with 4-methylumbelliferyl phosphate, which makes flowers bloom (4MUP). FER in the test sample affects how many enzyme-labeled monoclonal antibodies comes into contact with the beads. This is because the amount of FER in the test sample affects how many enzyme-labeled antibodies come into contact with the beads. Unknown sample concentrations are found by looking at a standard curve that has been made.

3.5.1.4. Assay of Liver and Heart Function Tests

3.5.1.4.1. Aspartate Aminotransferase (AST)

Principle of the Assay

This process is called aspartate aminotransferase (AST), and it helps to transfer the amino group from aspartate to oxoglutarate. This enzyme is called aspartate aminotransferase (AST). Malate dehydrogenase (MDH) converts nicotinamide adenine dinucleotide to malate when there is less nicotinamide adenine dinucleotide in the body (NADH). When NADH is oxidized to NAD+, the rate at which the absorbance of the sample goes down is called the rate of change in absorbance, which is a measure of the activity of AST in the sample. This is done kinetically at 340 nm.

Calculations:

AST U/L = ΔA/min x 3333

3.5.1.4.2. Alkaline Phosphatase (ALP)

Principle of the Assay

It is called alkaline phosphatase because it helps break down nitrophenyl phosphate (4-NPP) to make free nitrophenol and inorganic phosphate, with the alkaline buffer acting as an acceptor of phosphate groups from the nitrophenol. Kinetically, the rate at which 4-nitrophenol is made is measured at 405 nm. This is because the activity of ALP in the sample is related to the rate at which 4-nitrophenol is made.

Calculations: ALP U/L = ΔA/min x 2764

3.5.1.4.3. Serum Alanine Aminotransferase (ALT)

Principle of the Assay

It's called Alanine aminotransferase (ALT) and it helps transfer alanine's amino group to oxoglutarate, which makes both glutamate and pyruvate. Lactate dehydrogenase (LDH) converts nicotinamide adenine dinucleotide to lactate when there is less nicotinamide adenine dinucleotide in the body (NADH). The rate at which the absorbance of a sample drops when NADH is oxidized to NAD+ is measured kinetically at 340 nm. This is

because the activity of ALT in the sample is related to how much NADH is being oxidized to NAD+.

Calculations:

ALT U/L = ΔA/min x 3333

3.5.1.4.4. Serum Lactate Dehydrogenase (LDH)

Principle of The Assay

Lactate dehydrogenase (LD/LDH) converts pyruvate to lactate when there is less nicotinamide adenine dinucleotide (NADH) in the body (P-L). At 340 nm, the rate at which the absorbance of a sample drops because of the oxidation of NADH to NAD+, which is a measure of how active LDH is in the sample, is measured.

Calculations:

LDH U/L = ΔA/min x 8095

3.5.1.5. Assay of Renal Function Tests

3.5.1.5.1. Determination of Urea Concentration

Principle of The Test

The urea concentration in serum and urine was estimated by using the diagnostic kit supplied by CAM TECH MEDICAL company and by spectrophotometric method, as urea binds with water to turn into ammonia and carbon dioxide in the presence of the enzyme urease, and thus the ammonia produced is estimated through interaction with sialicylate And Hypochlorite to be 2, 2 - Dicarboxy Indophenol green in color, through which it is sensitive to urea concentration. The colored complex is read at a wavelength of 580 nm (263).

Calculation

Urea (mg / dl) = (Abs. sample / Abs.standard) x standard conc. (mg/dl)

Urea (mmol/L) = 2.9–8.2 mmol/L

3.5.1.5.2. Determination of Creatinine (CRE) Concentration

Principle of The Test

The creatinine concentration is estimated according to the reaction of (Jaffe) in the blood serum and urine by using the diagnostic kit supplied by Biolabo-France company, where CRE reacts with alkaline picrate to give a yellow complex. The absorbance of the colored complex is read at a wavelength of 490-510 nm.

Calculation

Creatinine conc. = (A1-A2) sample / (A1-A2) standard) x standard conc.

3.5.1.6. Estimation of Glucose

Principle of The Test

The colorimetric reaction occurs so when glucose oxidase (GOD) converts glucose to D-gluconate, resulting in the production of peroxide as a byproduct. It has been demonstrated that in the presence of peroxidase (POD), hydrogen peroxide may be used to oxidize a combination of phenol & 4-amino antipyrine (4-AA) to generate an orange quinone imine color with a concentration proportionate to the amount of glucose present.

$$Glucose + O_2 + H_2O \xrightarrow{Glucose\text{-}oxidase} gluconate + H_2O_2$$

$$2H_2O_2 + phenol + 4\text{-}aminoantipyrine \xrightarrow{Peroxidase} red\ chinonimin + 4H_2O$$

Detection range; 70- 105 mg/dl

3.89- 5.83 mmol/L

3.6. Statistical Analysis

The normality, homogeneity, and normally distribution of all data were checked. It was expressed as mean ± SD, The student t-test was used to evaluate the magnitude of the difference between the average scores of any two groups. IBM SPSS version 22.0 deemed *P:* 0.05 significant. While, the cut-off value for biochemical parameters assessment was determined using percentile analysis. The correlation coefficient is used to measure the correlation between a set of variables evaluated and another. Statistical significance was defined when it < 0.05.

4. RESULTS

4.1. Baseline Characteristics of The Study Population

Patients who had COVID-19 were confirmed at Al-Ramadi Teaching Hospital in Anbar/ Iraq, All of the patients who had COVID-19 were positive for the virus that was confirmed by PCR test. IgM levels were then used to group subjects into subgroups based on how high their COVID-19-IgM values to study sample as following:

- Study sample at mild case with beginning of infection (IgM=1.0-2.0) N= 25.
- Study sample with moderate case (2.0< IgM ≤ 4.0), N=18.
- Study sample with severe to critical cases (IgM >4.0), N= 17.
- Healthy subject as control group (IgM <1.0) N= 40

Discussion of present study was in two parts:

1- The first part was the related risk factors that participate in COVID-19.

2- The second part is study of the biochemical marker in the blood of these groups.

4.2. Risk Factors that Related to Participate in COVID-19

4.2.1. Age Distribution

The range of sample age in this study was (20-70) years for patients and healthy control group. The result showed that the healthy control group matches the patients' age group (p <0.01). Mean value ± SD of age for all patients with COVID-19 and controls are listed in Table 4.1 and Figure 4.1. Samples of each major group were divided into three subgroups, as age with (20- 35 years, 36- 50 years, and age more than 50 years) to understanding of the influence of age on the relationship with COVID-19 in this study. People who participated in the study were an average of 65 years old on a range (20-70). The results showed that there were a significant (p =0.05) increase of mean age that is proportional to the increase in the severity of COVID-19.

Table 4.1. The value of age group in COVID-19 patients and control groups

Groups	Age subgroup (Year)	No (%)	Age (Year) mean ± SD (Range)	*P value*
Mild case N= 25	20- 35	4 (22%)	28.68 ± 6.30 (Range 22-33)	0.003
	36- 50	9 (36 %)	36.5 ± 7.05 (Range 35-47)	
	More than 50	12 (42 %)	56.06 ± 11.25 ((Range 35-47)	
	Total	25 (100%)	39±6.92 (Range 20-70)	
Moderate case N=18	20-35	2 (11 %)	33.66 ± 6.82 (Range 20-35)	0.0031
	36- 50	9 (50 %)	40.75± 7.55 (Range 37-50)	
	More than 50	7 (39%)	55.68 ±12.58 (Range 50-70)	
	Total	18(100%)	44±8.33 (Range 20–70)	
Severe case N= 17	20-35	1 (6 %)	29.38 ± 4.48 (Range 30-45)	0.003
	36- 50	3 (18 %)	44.16 ± 9.03 (Range 36-50)	
	More than 50	13(76 %)	63.11 ± 11.30 ((Range 56-70)	
	Total	17 (100 %)	65± 13.1 (Range 20–70)	
Healthy subject N= 40	20-35	21(52%)	25.38 ± 3.18 (Range 20-31)	0.03
	36- 50	10(25%)	48.16 ± 9.03 (Range 36-45)	
	More than 50	9(23%)	59 ± 11.30 ((Range 56-64)	
	Total	40 (100 %)	26.41± 4.11 (Range 53–69)	

(X2 = 38.118, df =13, *p-value* ≤ 0.01)

Among the 60 confirmed COVID-19 patients who were admitted to ICU, 17 patients (33.5 %) had extreme to severe stages that required ICU treatment, and more than half of these patients was died.

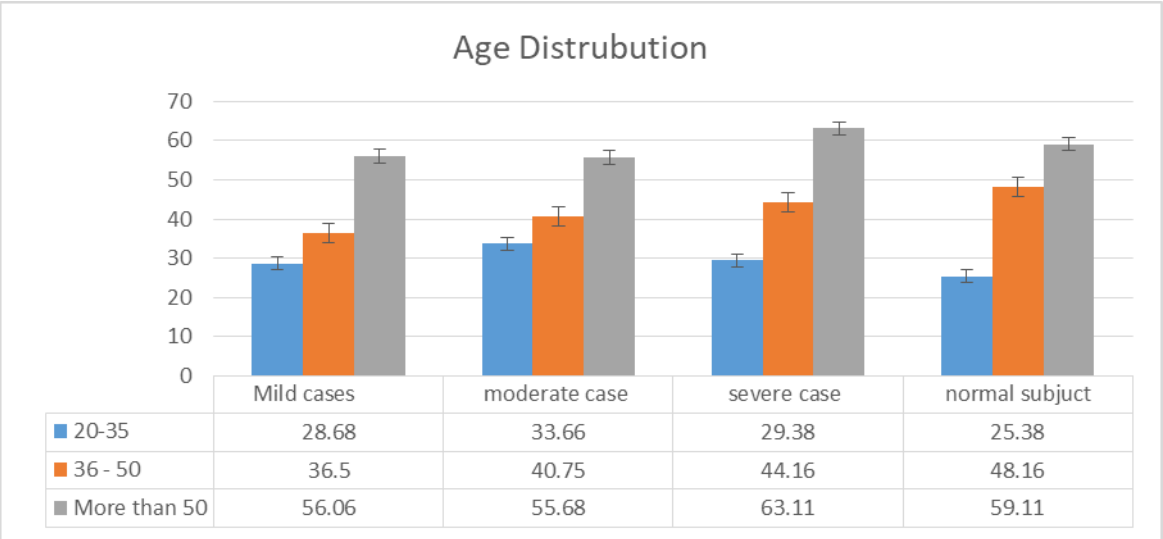


Figure 4.1. The age group in COVID-19 patients and control groups.

In addition, as shown in Table 4.2 and Figure 4.2 the present study revealed that the optimal age cut-off was 55 years.

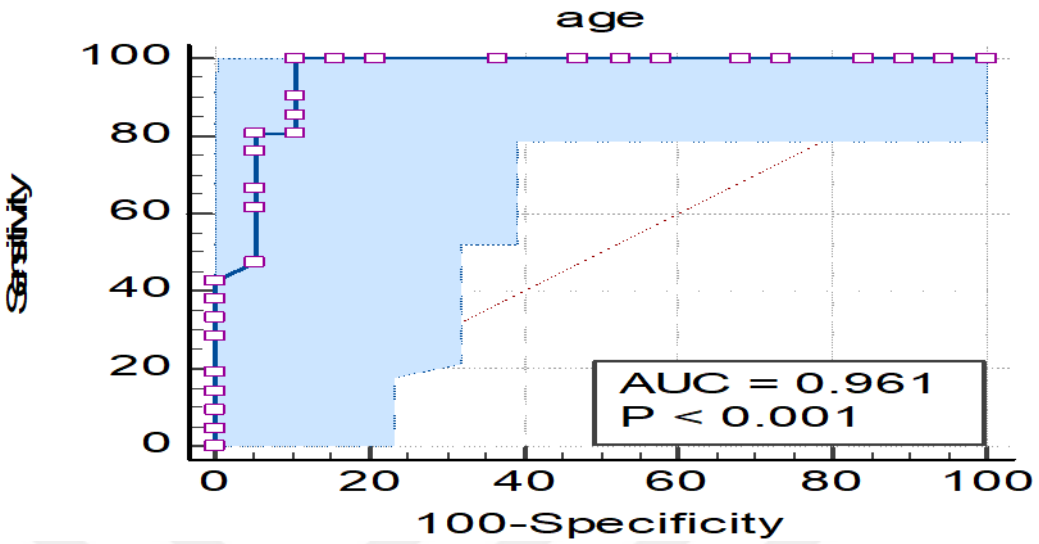


Figure 4.2. The ROC curves of age in patients with COVID-19.

Table 4.2. The cutoff value, sensitivity and specificity of age in COVID-19 patients

Sample size			**Disease prevalence**	**Area under the ROC curve (AUC)**	**SD**	**T -test**	***P value***
Age	Positive	Negative	52.5 %	0.961	0.0313	14.754	<0.001
	21 (52.50%)	19 (7.50%)					
	Associated criterion		Specificity		Sensitivity		
	>55		89.47		100		

4.2.2. Clinical characteristics of the patients at baseline

Demographic and clinical characteristics of patients were illustrated in Table 4.3, the number of male patients exposed to infection is approximately 70% higher than the number of female patients, who recorded 30%

Only 45% of infected male patients have reached critical and severe cases, while 23% of the patients have moderate cases and the rest have mild harm totaling 32%. On the other hand, comorbidities had shown a significant between severe, moderate and mild cases as compared with diabetes (p =0.003) and cardiac disease (p =0.036), but highly significant

appeared in asthma case (p =0.001). Hypertension has a significant (p =0.0027) in severity cases in contrast to another group. While non-significant may be showed in smoking (p =0.05).

COVID-19 signs and symptoms such as fever and dry cough are prevalent (55% and 78%) more than others, such as headache (18%), Chest pain (22%), diarrhea (21%) and vomiting (18%) according to the results of the current study.

Table 4.3. Demographic and clinical characteristics of patients' groups with COVID-19

Characteristics	Total (60 patients)	Mild cases	Moderate cases	Severe cases (Critical case)	*P value*
Gender					
Male	44 (70%)	14 (31.8%)	10 (22.7%)	20 (45.5%)	0.001
Female	16 (30%)	12 (75%)	3 (18.75%)	1 (6.25%)	
Comorbidities					
Smoking	6 (10%)	4(66%)	-	2 (33%)	NS
Hypertension	12(20%)	5 (41.66%)	-	7 (58.33)	0.002
Diabetes	17 (28.3%)	4 (23.5%)	3(17.6%)	10 (58.82%)	0.003
Cardiac disease	15 (25%)	8 (53.3%)	3 (20%)	4 (26.66%)	0.036
Asthma	8 (13%)	4 (50%)	1(12.5 %)	3 (37.5%)	0.001
Signs and symptoms					
Fever	28 (55.5%)	11(40%)	4(14%)	13 (46%)	-
Headache	9 (18%)	7(78%)	-	2 (22%)	-
Dry cough	39 (78%)	19 (49%)	5 (13%)	15 (38%)	-
Chest pain	11 (22%)	-	2 (19%)	9 (81%)	-
Abdominal pain	5 (10%)	1(20%)	-	4(80%)	-
Vomiting	9 (18 %)	7 (78%)	1(11%)	1 (11%)	-
Diarrhea	11 (21%)	-	9 (82%)	2 (18%)	-

4.3. Relation of Biochemical Parameter with COVID-19

4.3.1. Relation of PCT, FER, and cTnI Parameter With COVID-19

Table 4.4 and Figure 4.4 show the results of the studies performed as mean±SD for PCT, FER, and cTnI, with a highly significant (p =0.001) increasing correlation in the severe

cases group. PCT, FER, and cTnI levels were found to differ statistically (p =0.001 and p =0.05) between COVID-19 individuals with mild to moderate disease.

The results also showed that there was highly significant in patient groups with COVID-19 in different degrees when compared with that of the control group.

Table 4.4. The value of PCT, FER and cTnI in patients' groups with COVID-19 and control group.

Groups / **Parameter**	**Control**	**Patients with COVID-19**			
	Normal case Without inflammation	**Mild –case (beginning of infection)**	**Moderate-case**	**Severe- case (Critical case)**	***P value***
	Mean±SD	**Mean±SD**	**Mean±SD**	**Mean±SD**	
PCT (ng/ml)	0.02 ±0.0018 (0.01, 0.04)	0.08 ±0.02 (0.04, 0.14)	0.10 ±0.026 (0.09-0.13)	0.571±0.31 (0.14, 0.56)	0.0012
FER (µg/L)	77±25.38 [42–129]	130±61.20 [78–229]	290± 102.92 [42–229]	645.7 ±473.09 [383.8–2289]	0.001
cTnI (ng/L)	8.87±2.59 (0-26)	98.426± 44.61 (36-128)	490± 216.09 (291-786)	1618 ±1023 (871-2543)	0.001

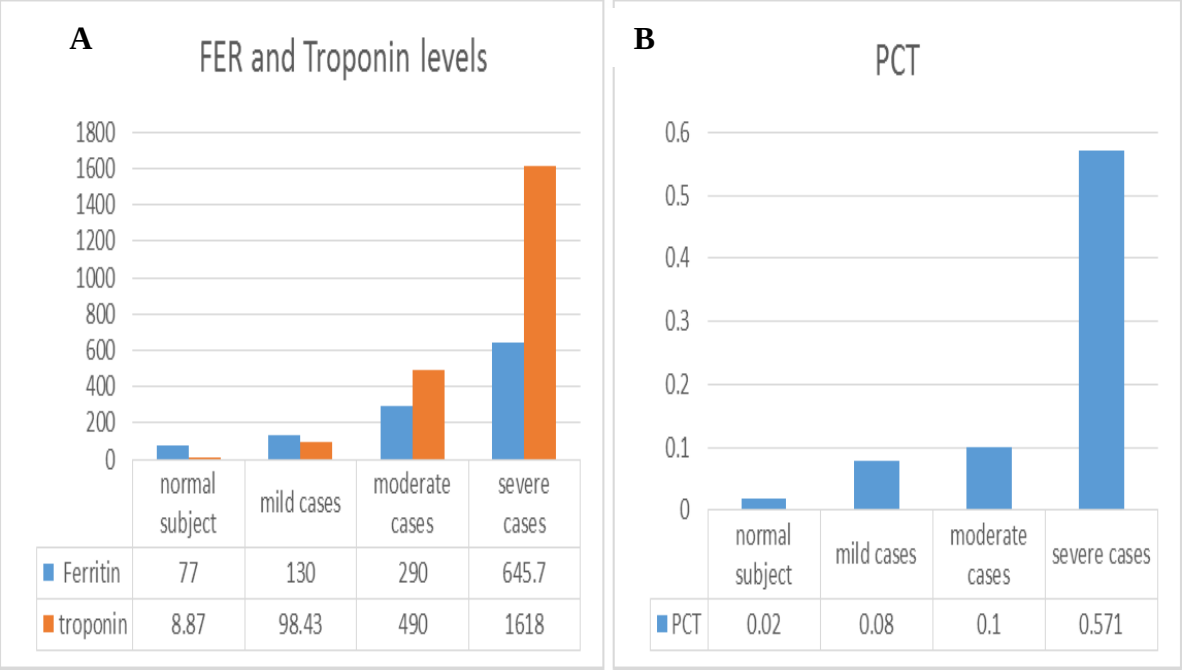


Figure 4.3. (A) The value of FER and cTnI in patients' groups with COVID-19 and control group (B)**:** The Mean ± SD of PCT in patient groups with COVID-19 and control group.

Moreover, our research revealed that the ROC curves of cTnI, PCT and FER were administered, as well as the best cut-off point of as shown in Figure 4.4 and Table 4.5.

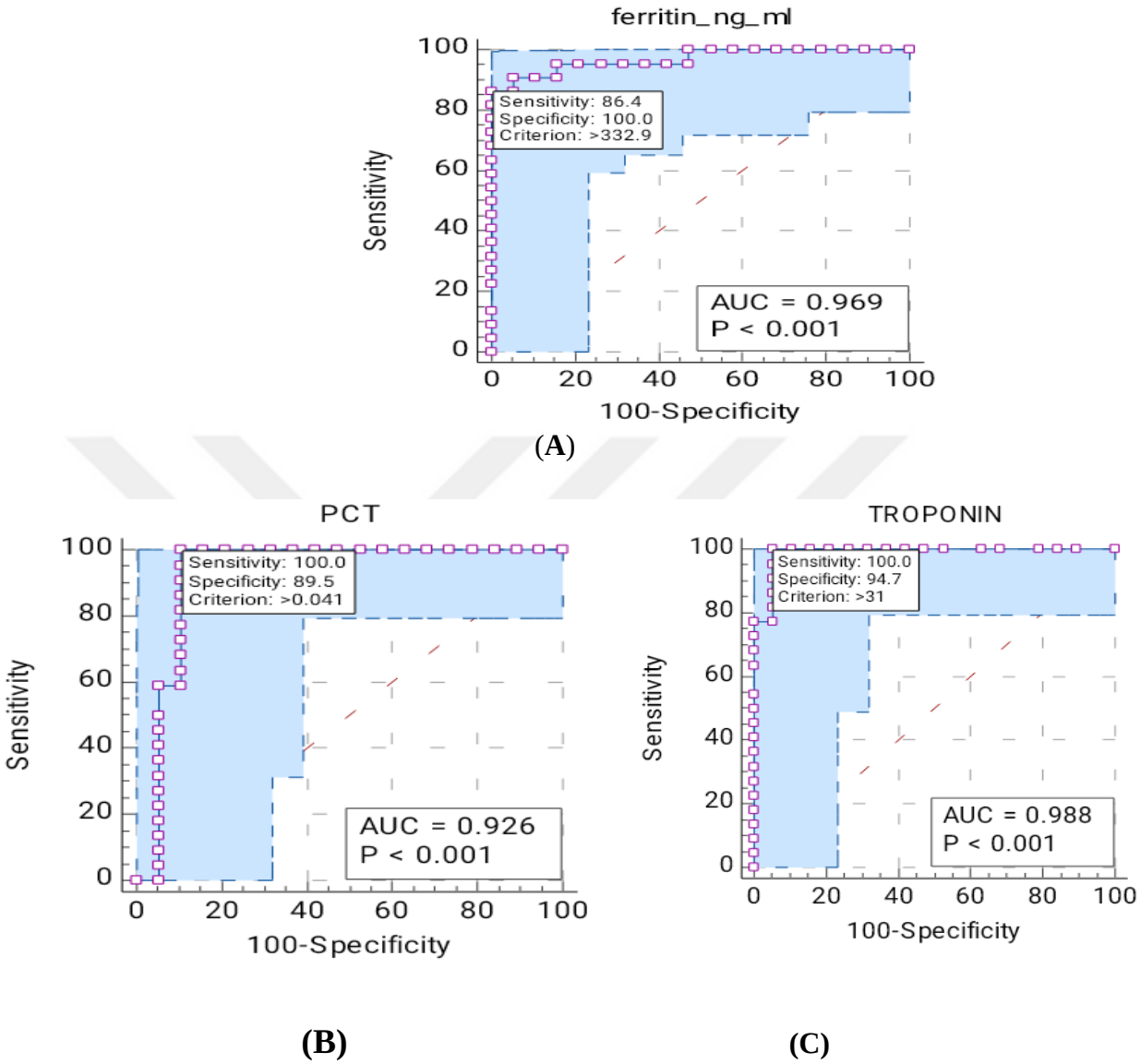


Figure 4.4. The ROC curves of (A) FER, (B) PCT and (C) cTnI in COVID-19 patients.

The results were finding that FER; AUC: 0.969 with 86% sensitivity and 100% specificity, PCT; AUC: 0.926 with 100% sensitivity and 89.5% specificity and cTnI; AUC: 0.988 with 100% sensitivity and 94.7% specificity,

Table 4.5. The cutoff value, sensitivity and specificity of PCT, FER and cTnI in COVID-19 patients

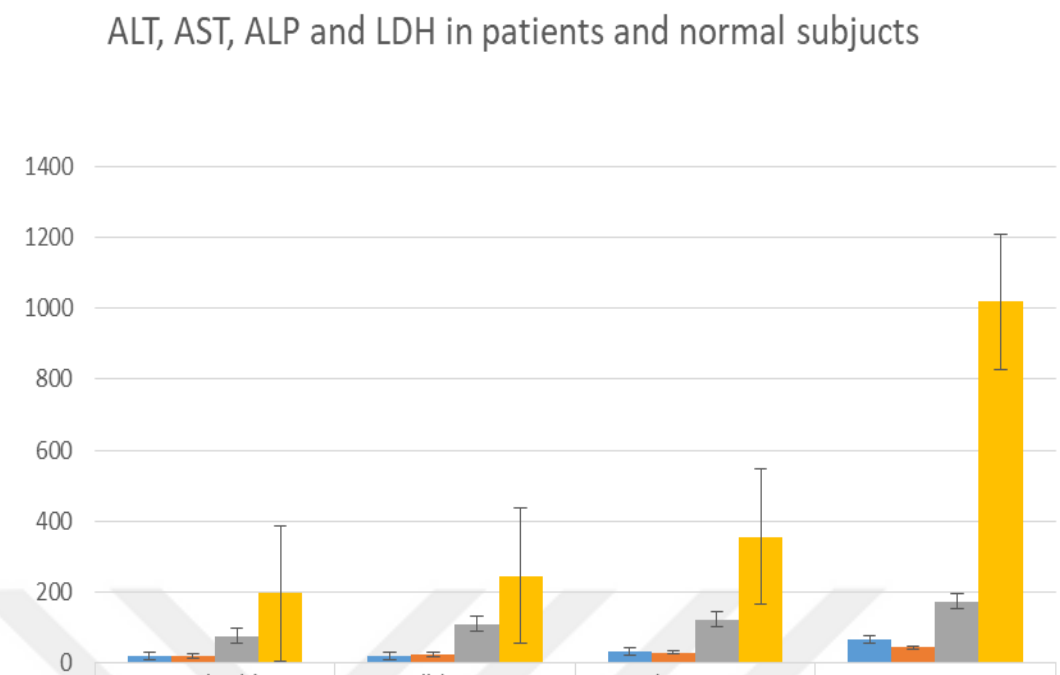
Parameters	Cut-off	Area under the ROC curve (AUC)	SD	T - test	Sensitivity	Specificity	*P value*
FER	>332.9	0.96	0.024	19.13	88.4	100.0	<0.001
PCT	>0.041	0.926	0.054	7.77	100.0	89.5	<0.001
cTnI	>31	0.988	0.013	36.5	100	94.7	<0.001

4.3.2. Relation of liver function and LDH parameters with COVID- 19

The results of serum liver function test and LDH enzyme in Table 4.6 and Figures 4.5 showed that there was a statically significant increase in the levels of ALT (p =0.048), ALP (p =0.039), and LDH (p =0.001) and non-significant with AST (p =0.075) in patients with COVID-19 compared with control group.

Table 4.6. The value of ALT, AST, ALP and LDH in patients' groups with COVID-19 and control group

Group / Parameter	Control	Patients with COVID-19			
	Normal case Without inflammation	Mild case (beginning of infection)	Moderate case	Sever case	*P value*
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
ALT (U/L) N.R (5–40)	19.33±6.62 (4.01-33.99)	22.00± 6.99 (17.00- 47.00)	32.50 ±14.3 (21.00- 48.50)	66.53±18.54 (22.85-161.39)	0.048
AST (U/L) N.R (8–40)	19.87±6.99 (2.39-29.035)	25.50± 7.01 (21.00- 34.75)	30.00±10.72 (20.00- 40.00)	43.84± 13.32 (31.93-66.140)	0.0750
ALP U/L NR (60-170)	77.27±14.05 (56.11-120.0)	111.15±42.61 (77.01-123.21)	123.98± 49.71 (109.01-143.00)	174.64±66.11 (161.00-213.53)	0.039
LDH (U/L) NR (109–245)	198.0 ±29.01 (154.0, 219.0)	245.0±34.19 (188.5, 345.5)	356.7±52.2 (194-385)	1017.6±661.8 (639-1852)	0.001



	normal subjuct	mild cases	moderate cases	severe cases
ALT	19.33	22	32.5	66.53
AST	19.87	25.5	30	43.84
ALP	77.27	111.15	123.98	174.64
LDH	198	245	356.7	1017.6

Figure 4.5. The Mean of ALT, AST, ALP and LDH in patients' groups with COVID-19 and control group.

Moreover, our research revealed that the ROC curves of LFT and LDH were administered, as well as the best cut-off point of as shown in Figure 4.6 and Table 4.7.

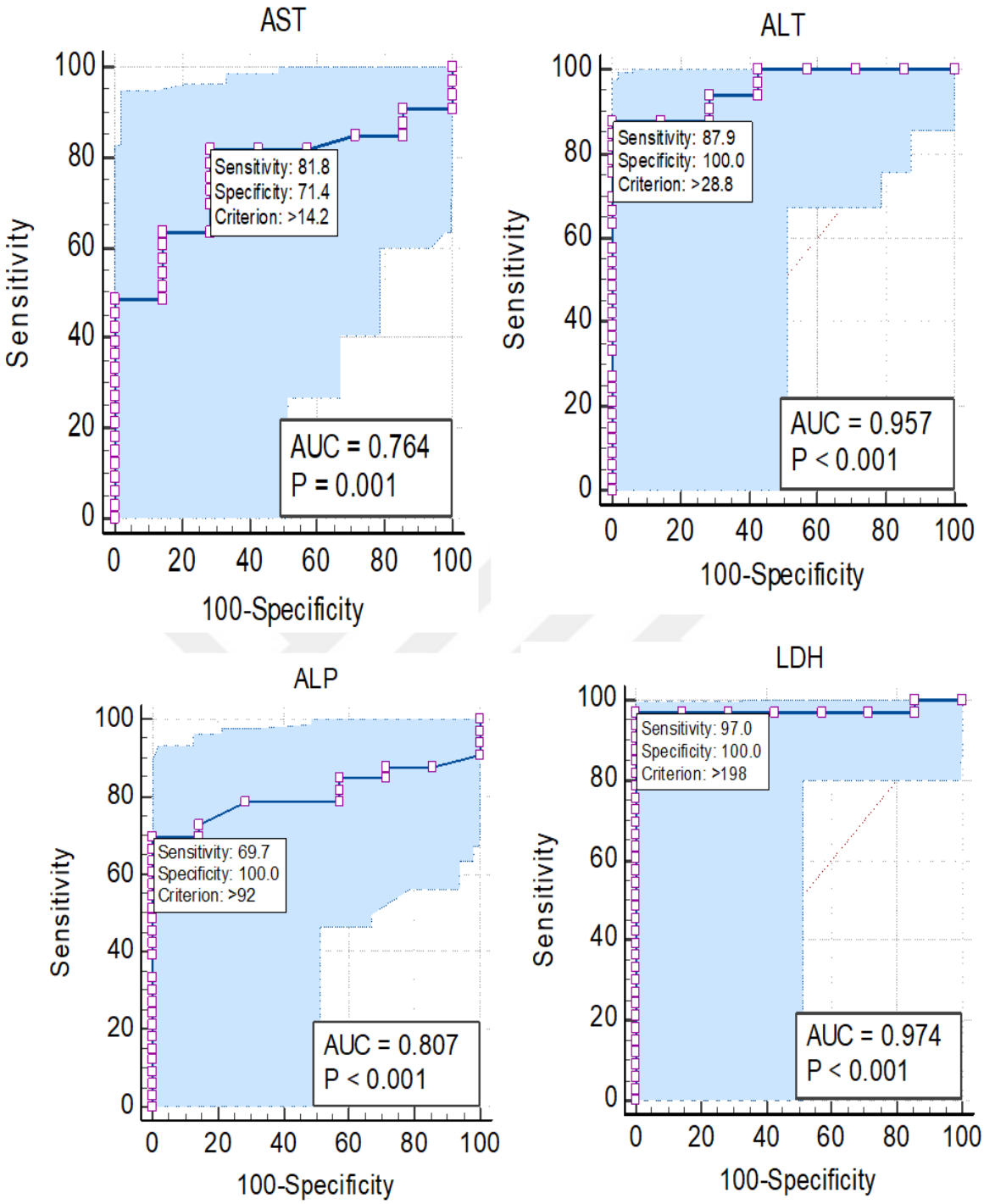


Figure 4.6. The ROC curves of AST, ALT, ALP and LDH in COVID-19 patients.

For ALT, the AUC was 0.957, with 87.9% sensitivity and 100% specificity; for AST, the AUC was 0.764, with 81.8% sensitivity and 71.4% specificity; and for ALP, the AUC was 0.807, with 69.7% sensitivity and 100% specificity. 0.974 for LDH AUC, with 97% sensitivity and 100% specificity.

Table 4.7. The cutoff value, sensitivity and specificity of ALT, AST, ALP and LDH in COVID-19 patients

Parameters	Cut-off	Area under the ROC curve (AUC)	SD	T -test	Sensitivity	Specificity	*P value*
AST (U/L)	>14.2	0.764	0.079	3.332	81.8	71.4	0.001
ALT (U/L)	>28	0.957	0.0307	14.8	87.9	100.0	0.001
ALP (U/L)	>92	0.807	0.066	4.65	69.7	100.0	0.001
LDH (U/L)	>198	0.974	0.026	18.100	97.0	100.0	0.001

4.3.3. Relation of renal function parameters with COVID-19

Urea and CRE were measured in serum of all groups. The mean±SD of urea and CRE for these groups were shown in Table 4.8 and Figure 4.7. Patients with COVID-19 had a significant (p =0.05) increased in urea and CRE levels when compared to the control group, according to the statistical analysis of the results.

Table 4.8. The Level of Urea and CRE in patients' groups with COVID-19 and control group

	Control	Patients with SARS-CoV-19			
Groups	**Normal case Without inflammation**	**Mild –case (beginning of infection)**	**Moderate- case**	**Sever case (Critical case)**	***P value***
Parameter	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**	
CRE (μmol/L) NR (44–133)	53.91±6.31 (40.00, 61.00)	61.81±8.11 (50.00- 111.00)	68.00 ±11.2 (60.10- 91.00)	119.00 ±17.44 (64.00-149.00)	0.011
Urea (mmol/L) NR (2.9–8.2)	3.99±0.88 (2.89-4.50)	4.00±1.01 (3.60-5.10)	4.30 ±1.22 (3.400-6.91)	8.60±1.39 (3.73-10.71)	0.001

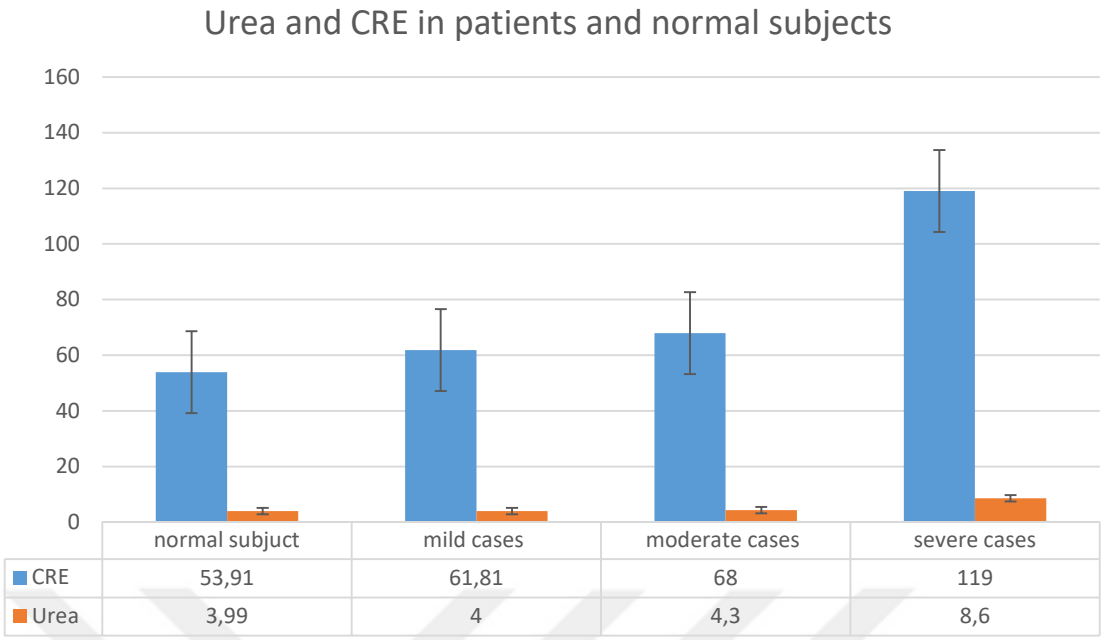


Figure 4.7. The Level of Urea and CRE in patients' groups with COVID-19 and control group.

Moreover, our research revealed that the ROC curves of RFT were administered, as well as the best cut-off point of as shown in Figure 4.8 and Table 4.9.

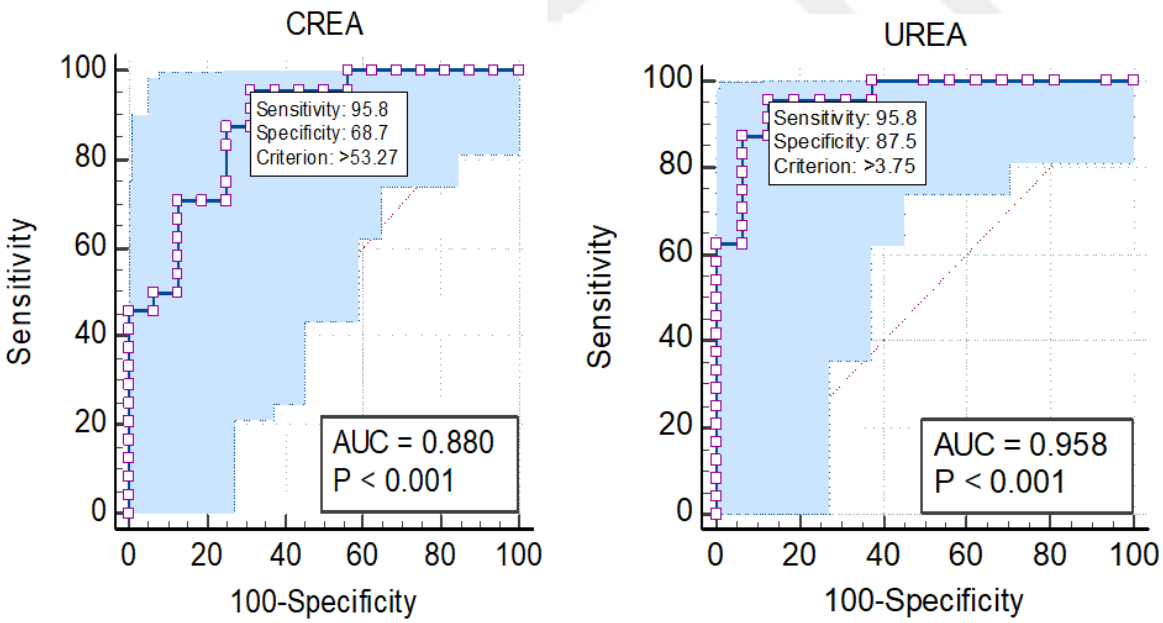


Figure 4.8. The ROC curves of Urea and CRE in COVID-19 patients.

The results were finding that Urea AUC: 0.958, with 95% sensitivity and 87.5% specificity and Creatinine AUC: 0.88, with 95% sensitivity and 68.7% specificity.

Table 4.9. The cutoff value, sensitivity and specificity of Urea and CRE in COVID-19 patients

Parameters	Cut-off	Area under the ROC curve (AUC)	SD	T -test	Sensitivity	Specificity	*P value*
CRE	53	0.880	0.054	6.961	95.8	68.7	0.001
UREA	3.75	0.958	0.029	15.620	95.8	87.5	0.001

4.3.4. Relation of serum glucose levels with COVID-19

The results of serum glucose levels in Table 4.9 and Figures 4.10, showed that there was a statically significantly higher level of glucose in patient with COVID-19 (mild case, Moderate case, and severe cases) compared with healthy subject (control group) and increased levels with severity cases more than others.

Table 4.10. The blood glucose concentrations in patients' groups with COVID-19 and control group

Groups	Control	Patients with COVID-19			
	Normal- case Without inflammation	Mild –case (beginning of infection)	Moderate–case	Severe–case	*P value*
parameter	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Glucose (mmol/L) NR (3.33-6.6)	4.05±1.12 (3.49-5.05)	4.54±1.56 (3.77- 5.8)	5.44 ±1.84 (4.99- 6.6)	9.94 ±2.7 (5.21-9.9)	0.001

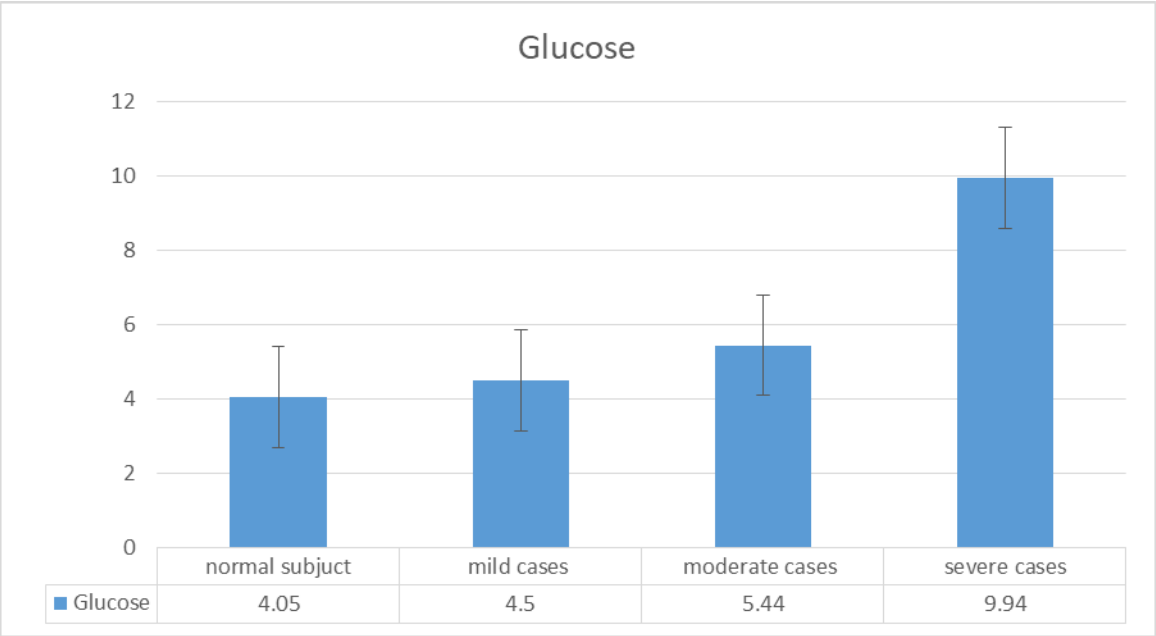


Figure 4.9. The Blood glucose concentrations in patients' groups with COVID-19 and control group.

5. DISCUSSION

COVID-19 is the third found in the previous two decades, following SARS-CoV-1 and MERS-CoV, which have been identified in 2003 and 2012, respectively. SARS-CoV-1 was found in 8.096 people between 2002 and 2003, leading to 774 deaths. There was a modest outbreak of MERS-CoV in the Middle East in 2012. 38 percent of the population was killed in this incident (Soltani, S. *et al.*, 2020; Ali Salih, H. M. *et al.*, 2020).

A total of 60 patients with COVID-19 disease were studied in the administrative region of AL-Anbar. The age range of the patients and control group in this study was 20-70 years.

According to the findings in Table 4.1, there was a significant (P =0.05) increase in mean age that was proportional to the severity of COVID-19. Increasing age, as previously documented by various authors, is a risk factor for developing COVID-19 and worsening the condition. Our study's demographic data supplemented these findings (Ali Salih, H. M. *et al.*, 2020). Another study, by Zhu, P. *et al.*, (2020), discovered that age over 50 was highly correlated with the presence of COVID-19 and that age > 65 was associated with mortality, lending credence to the current study's findings.

The advanced age of a patient was found to have a significant impact on SARS and MERS fatality rates (Yin, Y. and Wunderink, R. G., 2018). Age-related comorbidities (particularly viral infections) because immune response declines with advanced age, and this hypothesis agrees with our study, and this is in agreement with another study by (Lu, L. *et al.*, 2020) who mentioned that the mean age of the patients investigated may play a significant role in chronic conditions of severity cases

Patients with severe disease were also found to be older and to have a greater number of medical issues than those with mild to moderate disease. The findings are consistent with those of Li, W. *et al.*, (2020b) and Hutchins, H. J. *et al.*, (2020), who determined that age >60 years and comorbidity were potential causes of poor outcomes. Furthermore, as shown in Table 4.2 and Figure 4.2, and also in the current study, the optimal age cut-off was 55 years.

According to the current results in Table 4.3, the number of male patients exposed to infection is higher than the number of female patients, is agreement with the findings of Lu, L. *et al.*, (2020). As has been observed by the most researchers, the male gender also has been discovered to have an impact on the incidence of COVID-19 (Peckham, H. *et al.*, 2020). An Iranian study, on the other hand, found that gender might not be a contributing factor to severity (Ali Salih, H. M. *et al.*, 2020).

Comorbidities, on the other hand, showed a significant difference between severe, moderate, and mild cases when compared to diabetes (p =0.003) and cardiac disease (p =0.036), but were highly significant in asthma cases (p =0.001). Hypertension has a significant (p =0.0027) in severity cases in contrast to another group. While non-significant may be showed in smoking (p =0.05).

COVID-19 signs and symptoms such as fever and dry cough are prevalent (55% and 78%) more than others, such as headache (18%), chest pain (22%), diarrhea (21%) and vomiting (18%) according to the results of the current study. Table (4.3) which are consistent with the findings of other studies by (Qin, C. *et al.*, 2020).

At least one-fifth of COVID-19 patients required care and support in a professional ICU, which is particularly scarce in most developing countries, emphasizing the disease's link to chronic illness. Despite the best supportive measures, there are still high rates of patient mortality, reaching 6.4% in the over-60-year-old group (Li, W. *et al.*, 2020b; Kantri, A. *et al.*, 2021). Those who have hyperglycemia (DM) are more likely to have problems with COVID-19. Diabetics are more likely to experience severe symptoms when they get a virus (Bornstein, S. R. *et al.*, 2020).

If hyperglycemia is well-managed, the incidence of COVID-19 should be reduced (Fleming, N. *et al.*, 2021). People who have heart disease or other problems in addition to diabetes are more likely to become critically ill from COVID-19 and other common pathogens, because having more than one disease makes it difficult for the body to fight the infection. In diabetics, viral infections can cause inflammation. This could also be caused by elevated blood glucose levels, and the resulting inflammation can have far-reaching consequences. An analysis of data from 7,162 people in the United States revealed that 10.9% of them had diabetes (Fleming, N. *et al.*, 2021).

High blood pressure and extreme obesity are prevalent complications in diabetic patients (Hussain, A. *et al.*, 2020). It's uncertain if DM alone has a role in the elevated risk of COVID-19-related incidence and death. Poor glycemic management has been linked to poorer consequences in diabetic patients.

COVID-19 activates in diabetics in several ways: decreased viral elimination, decreased T cell activity, increased susceptibility to excessive inflammatory and cytokine storm, and increased virus entry efficiency (Ng, K. E. and Rickard, J. P., 2020).

This study found no linked with smoking and the severity of the COVID-19, which is consistent with the findings of other studies (Gülsen, A. *et al.*, 2020).

Table 4.4 and Figure 4.4 were indicated that PCT, FER and cTnI of studied patients group was a highly significant (p=0.001) increase correlation in severe cases group as compared with another group.

The findings of study by Ou, M. *et al.*, (2020) show the higher PCT levels were correlated with COVID-19 mortality. Immune reactions in the hepatic can lead to liver failure from hepatotropic viruses such as hepatitis viruses (Omrani-Nava, V. *et al.*, 2020), as well as chronic respiratory disease like infection such as Epstein-Barr-Virus, influenza, and COVID-19 (Chen, T. *et al.*, 2021).

From Table 4.4, the present study clearly showed that the highest rate of the infected patients with increased levels of serum FER, and the result was highly significant, this was matched to a study performed at al Iraq, Indonesia, and Wuhan/China, where they detected high levels of serum FER in patients with COVID-19 (Khudair, K. and khudair AL-Hadraawy, M., 2020; Taneri, P. E. *et al.*, 2020).

Ferritin has a significant role in immune dysregulation, particularly when their levels are high in blood; they have impact on the suppression of the immune system and on proinflammatory action that take in part of the cytokine storming. Elevated serum FER levels are indirectly connected to acute respiratory distress syndrome and severe COVID-19 infection. This might have led to the concept of the existence of secondary hemophagocytic lymph histiocytosis in COVID-19. This is a state of profuse inflammation in which a cytokine storming is a clear characteristic that may lead to death (Rosário, C. *et al.*, 2013).

Our findings show that it inflammatory process is more apparent in the most severe and dying patients, as PCT, and FER were all greater in these patient populations than in other groups. Additional infectious diseases (secondray bactrial infection) could also be a cause of increased neutrophil and PCT in advanced stages of the disease (Yuan, X. *et al.*, 2020).

Considering myocarditis is described as an immune disorder affecting the heart, characterized by inflammatory infiltrates with cardiac damage in the absence of an ischemic etiology, the cTnI level was significantly higher in severe cases than other diagnostic categories (mild and moderate). However, the precise physiopathology and processes of COVID-19-associated myocarditis are unknown. According to present understanding, it is a mixture of inflammatory process caused by an immune cell storm (cytokines storms), cardiac injury was due to by the patient's immune reaction, and direct viral injury to the myocardium (Ahmadian *et al.*, 2021).

COVID-19 infection causes acute cardiac harm and can result in myocarditis, an extremely rare diagnostic disorder characterized by high mortality rates and characterized by a sudden heart inflammatory response and necrosis that eventually leads to cardiac failure, cancerous arrhythmias that can lead to heart failure and eventually death, and cancerous arrhythmias that can lead to heart failure and eventually death (Babapoor-Farrokhran, S. *et al.*, 2020; Kawakami, R. *et al.*, 2021).

Table 4.6 and Figure 4.5 indicated that there was a statically significant increase in the levels of ALT, ALP and LDH and non-significant with AST in patients with COVID-19 compared with the control group.

Liver function marker such as ALP, as well as LDH concentrations, were increased over the maximum limit of ordinary in persons with COVID-19, and there was a strong positive connection among LDH values as well as the AST/ALT ratios and illness outcome, according to Benedé-Ubieto, R. *et al.*, (2021).

According to Benedé-Ubieto, R. *et al.*, (2021), COVID-19 infection typically causes mild changes in blood indicators of liver injury. In many recent studies, LDH levels and AST/ALT ratios were considered a diagnostic hallmark for COVID-19. The findings of study by Lu, L. *et al.*, (2020), show the higher LDH levels were correlated with COVID-19 mortality.

Serum aminotransferase quantities can rise sharply from liver damage or other non-hepatic illnesses, such as thyroid abnormalities, heart, and myocardial infarction. The increased LFT levels in patients with COVID-19 were also reported to be caused by the increased use of (lopinavir/ritonavir) treatment (Fan, Z. *et al.*, 2020), and other drugs, such as remdesivir, which has adverse reactions that cause hepatotoxicity and thus raises the LFT levels (Wong, C. K *et al.*, 2022).

Furthermore, several infections have been linked to cytokine-mediated tissue damage and LDH release. Because LDH izozyme 3 is found in lung tissue, patients with severe COVID-19 infections can be expected to release large amounts of LDH into the circulation, as the disease is characterized by a severe form of interstitial pneumonia. LDH can be released during tissue damage and is involved in a variety of pathophysiological processes, as well as serving as a non-specific indicator of cellular death in a variety of diseases. As a result, this is one of the most common causes of increasing LDH enzyme value (Henry, B. M. *et al.*, 2020a). Similarly, Henry, B. M. *et al.*, (2020b) reported that elevated LDH and PCT levels in people with mild COVID-19 suggest a possibility of heart injury, emphasizing the importance of monitoring cardiac biomarkers in hospitalized patients.

Our findings are also consistent with recent investigations (Ferrari, D. *et al.*, 2020) in which 207 individuals with COVID-19 symptoms were tested for RT-PCR after being brought to the emergency room of (San Raffaele) hospital in (Italy). 105 of them tested positive, while the other 102 were negative. The plasma levels of AST, ALT, ALP, and LDH in the two groups showed statistically significant differences, suggesting that they might be used to distinguish between COVID-19 positive and negative individuals.

Liver damage in COVID-19 subjects was found to be common and minor in nature. Rather than cholestasis, the majority of the liver damage was caused by hepatic cellular damage. This discovery is intriguing because the spikes protein in COVID-19 has a high affinity for angiotensin-converting enzyme receptors in biliary cells. Hepatitis has been linked to the collateral damage caused by virus-specific effector cells produced by pulmonary viral illness in other lung viral infections (Bianco, C. *et al.*, 2021).

Urea and CRE were measured in serum of all groups. The mean±SD of urea and CRE for these groups was shown in Table 4.8 and Figure 4.7. Patients with COVID-19 had a significant (p =0.05) increase in urea and CRE levels when compared to the control group,

according to the statistical analysis of the results. The findings of study by Lu, L. *et al.*, (2020) show the higher urea levels were correlated with COVID-19 mortality. As previously mentioned, COVID-19 can cause respiratory problems, but it can also have an impact on other systems like the kidneys; heart; intestines; blood; and nervous system (Al Nemer, A., 2020). COVID-19 enters the nervous system across nerve cells, so it causes harmful to the respiratory center for most patient populations as well as confusion, tiredness, dizziness, loss of taste and smell sensations in the majority of patients, as well as other symptoms associated with brain disorder in some patients (Karuppan, M. K. M. *et al.*, 2021).

It is possible for COVID-19 to lead to acute renal damage and other abnormalities in renal function. As a result of the holding of nitrogenous waste products and the resulting reduction in GFR, acute renal failure increases blood urea and CRE levels, while also disrupting electrolytes balance and extracellular density (Yalameha, B. *et al.*, 2020; Bagga, A. and Srivastava, R. N., 2016).

Moreover, scientists are still trying to figure out exactly how COVID-19 affects the kidneys, but they do think that COVID-19, directly and indirectly, influences them. Due to the presence of the viral COVID-19 within the urine and the timing of the virus's appearance in the urine occurring during the same time as the commencement of acute kidney injury, it has been hypothesized that this virus has a direct impact on the organ's function (Yarijani, Z. M. and Najafi, H., 2021).

The COVID-19 is attached to membrane-bound angiotensin converting enzyme-2 (ACE-2) receptors in renal podocytes and renal tubules apical membranes to penetrate the kidneys cell; it disrupts the renin-angiotensin ways of balancing in addition to harming the kidney epithelium (Zanza, C. *et al.*, 2021). A primary role for renin in the conversion of angiotensinogen to angiotensin-I and ultimately angiotensin-II is played by the ACE. Additionally, the ACE2 receptors convert angiotensin-II to angiotensin 1, which expands blood vessels. Vascular constriction, renal dysfunction, inflammation, and fibrous necrosis can occur when COVID-19 occupy ACE2 receptors. Acute kidney injury was occurring in 25% of severity COVID-19 patients (Patel, S. K. *et al.*, 2020; Zanza, C. *et al.*, 2021).

The kidneys are damaged by COVID-19, which activates inflammatory processes including cytokine storms, activates clotting pathways, damages renal endothelium, sepsis, hemodynamic instability, and cerebral hypoxia, among other things. By attracting immune

cells to the endothelium, a viral infection can cause a decrease in the generation of vasodilators, including nitric oxide. Vasoconstrictors diminish vasodilatation, which leads to acute renal disease. Oxidative stress created by the inflammatory process may also lead to kidney damage.The result in Table 4.10 and Figure 4.9 showed that there was a statically significantly higher blood glucose level in patient with COVID-19 compared with healthy subject (control group) and increased levels with severity cases more than others.

Serious sepsis, toxic shock syndrome, including traumatic brain damage is all possible causes of hyperglycemia. Some cytokines are elevated and blood glucose levels are also elevated as a first response to these situations (hyperglycemia). Patients who have high blood glucose levels are more likely to be hospitalized or die as a result of their condition (Andersen, S. K. *et al.*, 2004). The breakdown of glycogen, the creation of adrenocorticotropic hormone and glucagon hormone, and insulin resistance all contribute to the elevation in blood sugar during the pro-inflammatory phase in critical (severe) situations of these illnesses (Van Cromphaut, S. J., 2009).

In our study, patients with severe diabetes had greater blood glucose, and that there was a significantly difference in serum glucose among all these groups. These findings are consistent with those reported in investigations assessing health risks for mortality rates (Fleming, N. *et al.*, 2021; Wang, Q. *et al.*, 2020b).

Our findings are also in agreement with Wang, D. *et al.*, (2020a) findings. Prognostic factors for COVID-19 include the quantity of fasting hyperglycemia in the serum at the time of admission. Serum glucose levels rose significantly faster in people with severe acute COVID-19 than in those with milder COVID-19.

6. CONCLUSION And RECOMMENDATIONS

6.1. Conclusion

The findings of this study can lead to the following conclusions.A significant association was reported between advanced age and severity of COVID-19 especially more than 55 years. These results demonstrate that older patients were at higher risk of developing COVID-19.

The number of male patients exposed to infection is approximately 70% higher than the number of female patients, who recorded 30%.

Comorbidities showed significant between severe, mild and moderate cases as compared with diabetes, hypertension and cardiac disease. Also, highly significant appeared in asthma cases while non-significant may be showed in smoking patients

With current values of PCT > 0.5 ng/mL, and ferritin > 600 μg/L, Troponin >1600 ng/L indicates that COVID-19 is progressing to a critical stage, which should be continuously monitored and perhaps averted.

The frequency of abnormal liver biochemical on admission in COVID-19 subjects is substantial. Monitoring liver enzymes over the course of mild to severe illness is essential because of the possibility of further harm owing to consequences and treatment.

The study also indicated that there are significant high levels in glucose, urea and CRE levels, especially in severe cases of COVID-19 patients.

Recommendations

From the findings of the present study, the following can be recommended:

1- Determining the levels of nvarious minerals and vitamins such as serum vitamin D, vitamin C, calcium, and zinc, in COVID-19-patients may be sufficient to induce an immunological response against the disease.

2- To use another test for coagulation caused by SARS-CoVID-19,specifically related to plasmin and coagulation factors
3- Estimation of ACE especially in asthma and other lungs related cases.
4- Estimation of some immunological factor who affected by hematological change such as CD4+, CD8+, especially in severe cases.
5- Utilizing a molecular technique for growth analysis, for example, (constant PCR, Microarray) to reach more sensitivity.

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Appendixes

Questionnaire

1) **Patient name:** …….. …… …….. ……
2) **Living:**……..
3) **Occupation:**……
4) **Age?** □□years
5) **Infection period?** □□days
6) **Fasting?** Yes □ No□
7) **Weight** □□ **Height** □□**BMI** □
8) **IgM values?** □□
9) **Severity of infection?** Mild □ Moderate □ Severe □
10) **Diagnosed with following disease**? Yes □ No□

If yes? Please indicate.

-Cardiovascular disease Yes□ No□
-Diabetes Yes□ No□
- Blood pressure Yes□ No□
- Asthma Yes □ No□
- Kidney disease Yes □ No□
- Liver disease Yes □ No□
-Thyroid disease Yes □ No□
- Cancer Yes □ No□
- Others ……

11) **Symptoms and sign?**

-Fever Yes □ No□
-Dry cough Yes □ No□
-Headache Yes □ No□
-Chest pain Yes □ No□
-Abdominal pain Yes □ No□
-Diarrhea Yes □ No□
-Vomiting Yes □ No□

12) **Smoking history** Yes □ No□

13) **Treatment takes?**

-For COVID-19 Yes □ No□
-For Cardiovascular disease Yes □ No□
-For Diabetics lowering drugs Yes □ No□
-For Lipid lowering drugs Yes □ No□
-For Blood pressure lowering drugs Yes □ No□
-Others …….

14) **Blood tests**

PCT …… ng/ml

FER ……. µg/l

cTnI …….ng/L

ALT……. U/L

AST …….U/L

ALP…….. ...U/L

LDH …… U/L

CRE ……µmol/L

Urea…… mmol/L

Glucose ……mmol\l

CURRICULUM VITAE

Personal Information	
Name and surname	Khalid Zaiter KHALAF
Place of birth	
Date of birth	
Nationality	❒ T.C. ☑ Other :

Education Information	
Undergraduate	
University	University Anbar university
College	Faculty Faculty of Science
Department	Department of Chemistry
Graduation Year	1994

Master's Degree	
University	Kirsehir Ahi Evran University
Name of the Institute	Graduate School of Natural and Applied Sciences
Department	Department of Chemistry
Program	
Graduation Year	2020- Currently