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COMPARISON OF IMMUNOLOGICAL, BIOCHEMICAL, HEMATOLOGICAL AND MOLECULAR PARAMETERS ACCORDING TO AGE AND GENDER IN COVID 19 PATIENTS

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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BY

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By Hasan Jawad Kadhim AL RAHAM

March 2023

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ABSTRACT

COMPARISON OF IMMUNOLOGICAL, BIOCHEMICAL, HEMATOLOGICAL AND MOLECULAR PARAMETERS ACCORDING TO AGE AND GENDER IN COVID 19 PATIENTS

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Master of Science in Biology

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March 2023

The coronavirus-19 viral infection has rapidly spread geographically and has become a global pandemic, as declared by the World Health Organization in the first months of 2020. Respiratory infections caused by coronavirus disease 2 (SARS-CoV-2), commonly known as COVID-19, can lead to severe acute respiratory symptoms. The current study aims to detect the COVID-19 virus in patients with respiratory infections in Dhi Qar Province, southern Iraq. The study involved 100 samples collected from patients with COVID-19, of which 58 were male and 42 female; their ages ranged from 14 to 79 years. The samples were collected from the period December 2021 to June 2022 at Al-Hussein Teaching Hospital. The parameters were analyzed using the ELISA technique for IL-6 and TNF; a full automated coulter was used for a complete blood count; and other parameters were analyzed using Cobas. Also, the molecular study involved two genes, S and N, and analysis by both real-time PCR and conventional PCR and sequencing analysis was used for positive samples. In complete blood count, the results of the current study recorded a significant increase in the count of WBC ($p<0.001$), percent of monocytes ($p<0.001$), percent of lymphocytes ($p<0.001$), neutrophils ($p<0.001$), and count of PLT ($p<0.001$) compared with the control group. The count of RBC did not show a significant difference ($p =0.819$), level of Hb ($p=0.118$), and percentage of HCT ($p=0.786$) when compared with the control group. Regarding immunological parameters, the current study showed a significant increase in the concentration of all involved immunological parameters CRP, IgM, IgG, IL-6 and TNF ($p<0.001$) compared with the control group. In terms of biochemical parameters,

the study revealed a significant increase in the concentration of ferritin ($p<0.001$) and D-dimer ($P<0.001$) in COVID-19 patients compared to the control group, while the concentration of vitamin D3 decreased significantly ($p<0.001$). The study also showed a non-significant difference in the concentration of biochemical and hematological parameters according to gender. However, in immunological parameters, the concentration of IgM increased significantly ($p=0.011$) in the female group compared to the male group, while TNF increased significantly in the male group compared to the female group ($p<0.001$). According to age groups, the study noted a non-significant difference in all involved parameters. In a molecular study, the present study noted the ORF1ab gene and N gene of COVID-19 increased significantly in male patients than female patients ($p= 0.048$ and 0.012, respectively), whereas both genes did not score a significant difference according to age groups ($p=0.727$ and 0.592, respectively).

2023, 88 pages

Keywords: Covid 19, Hematological parameters, Immunological parameters, Interleukins -6, Ferritin, D. Dimer, Vitamin D

ÖZET

COVID 19 HASTALARINDA İMMÜNOLOJİK, BİYOKİMYASAL, HEMATOLOJİK VE MOLEKÜLER PARAMETRELERİN YAŞ VE CİNSİYETE GÖRE KARŞILAŞTIRILMASI

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Mart 2023

Koronavirüs-19 viral enfeksiyonu, Dünya Sağlık Örgütü tarafından 2020'nin ilk aylarında ilan edildiği üzere coğrafi olarak hızla yayılarak küresel bir pandemi haline gelmiştir. Yaygın olarak COVID-19 olarak bilinen koronavirüs hastalığı 2'nin (SARS-CoV-2) neden olduğu solunum yolu enfeksiyonları, ciddi akut solunum yolu semptomlarına yol açabilmektedir. Bu çalışma, güney Irak'ta bulunan Dhi Qar İli'ndeki solunum yolu enfeksiyonu olan hastalarda COVID-19 virüsünü tespit etmeyi amaçlamaktadır. Çalışma, yaşları 14 ila 79 arasında değişen, 58'i erkek ve 42'si kadın olmak üzere COVID-19 hastalarından toplanan 100 numuneyi içermektedir. Numuneler Aralık 2021 - Haziran 2022 döneminde Al-Hussein Eğitim Hastanesinde bir araya getirilmiştir. Değişkenler, IL-6 ve TNF için ELISA tekniği kullanılarak analiz edilmiş; tam otomatik bir coulter cihazı kullanılarak tam kan sayımı (Hemogram testi) yapılmış ve diğer değişkenler Cobas kullanılarak analiz edilmiştir. Ayrıca, moleküler çalışma S ve N adlı iki geni içermekte olup pozitif örnekler için hem gerçek zamanlı PCR hem de geleneksel PCR ve dizi analizi kullanılarak analiz edilmiştir. Tam kan sayımında, mevcut çalışmanın sonuçları, WBC sayısında ($p<0,001$), monosit yüzdesinde ($p<0,001$), lenfosit yüzdesinde ($p<0,001$), nötrofillerde ($p<0,001$) ve PLT sayısında ($p<0,001$) kontrol grubuna kıyasla önemli bir artış kaydetmiştir. RBC sayısı ($p=0,819$), Hb düzeyi ($p=0,118$) ve HCT yüzdesi ($p=0,786$) kontrol grubu ile karşılaştırıldığında anlamlı bir fark ortaya koymamıştır. İmmünolojik parametreler açısından, mevcut çalışma CRP, IgM, IgG, IL-6 ve TNF ($p<0,001$) gibi tüm ilgili immünolojik parametrelerin konsantrasyonunda kontrol grubuna kıyasla önemli bir artış olduğunu göstermiştir.

Biyokimyasal parametreler açısından, çalışma COVID-19 hastalarında ferritin ($p<0,001$) ve D-dimer ($p<0,001$) konsantrasyonunda kontrol grubuna kıyasla önemli bir artış ortaya koyarken, D3 vitamini konsantrasyonu önemli ölçüde azalmıştır ($p<0,001$). Çalışma ayrıca biyokimyasal ve hematolojik parametrelerin cinsiyete göre konsantrasyonunda istatistiksel farklılık olmadığını ortaya koymuştur. Bununla birlikte, immünolojik parametrelerde, IgM konsantrasyonunun kadın grubunda erkek grubuna kıyasla önemli ölçüde arttığı ($p=0,011$) ve TNF konsantrasyonunun erkek grubunda kadın grubuna kıyasla önemli ölçüde arttığı ($p<0,001$) belirtilmiştir. Yaş gruplarına göre, çalışma ilgili tüm parametrelerde istatistiksel bir fark olmadığını belirtmiştir. Moleküler bir çalışmada, bu çalışma COVID-19'un ORF1ab geni ve N geninin erkek hastalarda kadın hastalara kıyasla önemli ölçüde arttığını (sırasıyla $p=0,048$ ve 0,012) göstermiştir, ancak her iki gen de yaş gruplarına göre istatistiksel bir fark göstermemiştir (sırasıyla $p= 0,727$ ve 0,592).

2023, 88 sayfa

Anahtar Kelimeler: Covid 19, Hematolojik parametreler, İmmünolojik parametreler, Interleukins -6, Ferritin , D. Dimer, Vitamin D

PREFACE AND ACKNOWLEDGEMENTS

First and foremost, I am deeply grateful to the Almighty Allah, who helped me to complete this thesis. And to the owner of Al-Isra and Al-Miraj, to the master of the messengers, and to the prophet of mercy and light, Our Master Muhammad, may God bless him and grant him peace.

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Thank you to the one who drank the cup empty to give me a drop of love. To the one who harvested the thorns of my path in order to pave the way for me to knowledge. To my dear father, who has a big heart; to my angel in life To the lady of life and the secret of existence To whoever was the object of her prayer, you are the secret of my success, my tender mother. To the sublime heart... To the pure fountain... To the rays of my soul and life... My beloved wife

My best wishes to all the people who have supported me in getting ready. Research this and give any perspective on making this business a success.

Hasan Jawad Kadhim ALRAHAM
Çankırı-2023

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LIST OF SYMBOLS

°C	Celsius degree
Kb	Kilo base
KD	Kilodalton
µL	Microliter
mL	Milliliters
m: s	Minute . second
ng/µL	Nanogram/microliter
pg	Picogram
v/mAmp	Volt / miliamper

LIST OF ABBREVIATIONS

3CLpro	3 chymotrypsin – like proteases
ARDS	Acute respiratory distress syndrome
ACE2	Angiotensin-converting enzyme 2
IBV	Avian ınfectious bronchitis coronavirus
BtCoV	Bat coronavirus CD81
BuCoV	Bulbul coronavirus
CTD	C- terminal domain
COPD	Chronic obstructive pulmonary disease
CRISPR	Clustered regularly ınterspaced short palindromic repeats
CT	Computed tomography scan of the chest
CRP	C-reactive protein
E	Envelope glycoprotein
ER/GIC	ER/golgi intermediate compartment
ExoN	Exoribonuclease
ECMO	Extracorporeal membrane oxygenation
FDA	Food and drug administration
HGF	Hepatocyte growth factor
HRP	Horseradish peroxidase
IP-10	Interferon gamma inducible Protein-10
LKR	Linker region
LAMP	Loop mediated ısothermal amplification
MIP-1	Macrophage ınflammatory protein -1
Mpro	Main protease
MERS-CoV	MERS coronavirus
MERS	Middle east respiratory syndrome
MCP-1	Monocyte chemoattractant protein-1
NTD	N- terminal domain
Nsp	Nonstructural protein
ORF	Open reading frames
OD	Optical density
PLPs	Papain-like proteases
PPE	Personal protective equipment
POCT	Point of care tests
PP1a	Polyprotein
PT	Prothrombin time test
PE	Pulmonary embolism
ROS	Reactive oxygen species
RBD	Receptor-binding domain
RBM	Receptor-binding motif

RANTES	Regulated upon activation, normal T cell expressed and presumably secreted
RLUs	Relative ıllumination units
RT-PCR	Reverse transcription polymerase chain reaction
SR	Serine and arginine
SNP	Single nucleotide polymorphism
TMPRSS2	Transmembrane serine protease 2
TNF	Tumor necrosis factor
UVB	Ultraviolet B
UTR	Un translated region
URIs	Upper respiratory ınfections
VTE	Venous thromboembolism
VTM	Viral transport medium
VNT	Virus neutrralization tests
WHO	World health organization

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1. INTRODUCTION

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic, which was discovered for the first time in Wuhan, China, in December "2019, expanded at an exponential pace around the world, with dramatic and still continuing effects for both health and socioeconomic conditions. SARS-CoV-2, also known as the coronavirus, is a respiratory infection that is highly contagious and is responsible for the sickness that is referred to as (coronavirus 2019). (COVID-19). The clinical experience that we have seen so far demonstrates that COVID-19 is quite diverse. It may range from being asymptomatic and mild to being severe and lethal. Important indicators of the onset and course of illness include host variables such as age, gender, and the presence of concomitant disorders (Chen *et al.* 2021).

The World Health Organization declared the illness resulting from the new virus, COVID-19, a Public Health Emergency of International Concern. By early March 2020, the novel coronavirus—now named SARS-CoV-2—had infected more than 90,000 people worldwide and killed at least 3,100 (Wrapp *et al.* 2020).

Respiratory sickness and a high-grade fever are the most typical clinical symptoms of the condition. Other systemic signs, such as neurologic complaints and renal problems, have been documented in a few cases (Markus and Brainin 2020, Roe 2020, Sellner *et al.* 2020). There are also more reports of unusual clinical problems in the younger group, such as stroke-induced fatalities and big vascular occlusions. These findings suggest that the disease's pathogenesis is more complicated than previously understood. Although scientists have proposed two plausible explanations, namely (i) cytokine storm and (ii) disruption of the angiotensin-converting enzyme (ACE2) (Romero *et al.* 2015), the specific causes of this problem are yet unknown.,

SARS-CoV-2 triggers an innate immune response, resulting in an initial increase in neutrophils and other immune cells, as well as a significant decrease in T lymphocytes (CD4+ and CD8+). However, the decrease in T cells was accompanied by an increase in IL-6 production. and IL-6 has been identified as a distinct feature of SARS-CoV-2

infection (Zhang *et al.* 2020). In individuals with impaired immunity and an inborn deficiency in IFN type I immunity (Zhang *et al.* 2020), Inflammatory cytokines are generated in large quantities,resulting in a cytokine storm.as well as hypercytokinemia Pathological consequences result from the dysregulated and excessive cytokine production severe pneumonia, acute lung damage, and acute respiratory distress syndrome (ARDS) (Mustafa *et al.* 2020).

In the early phase of COVID-19 infection, leukopenia, lymphocytopenia, high level of C-Reactive Protein (CRP), high D-dimer, prolonged Prothrombin Time test (PT), and high levels of fibrinogen have been reported (Cui *et al.* 2020).

Venous thromboembolism (VTE) and cardiac failure, on the other hand, can elicit respiratory distress symptoms identical to COVID-19, VTE, Pulmonary embolism (PE), stroke, and myocardial infarction are all on the rise.in several communities (Wendelboe and Raskob 2016) , One of the approaches is the D-dimer.for detecting thrombotic states (Salomone *et al.* 2003).

Activated vitamin D is implicated at several immune response levels through activating innate immunity and decreasing overactivation of adaptive immunity. Vitamin D treatment may reduce levels of interleukin-6 Vitamin D deficiency, on the other hand, may cause immunological dysregulation and has been linked to an increased risk of intensive care admission and death in patients with severe types of pneumonia (Fiorino *et al.* 2020, Mamani *et al.* 2017).

The study's goal is to diagnose Coronavirus infections and evaluate how blood parameters and immunological factors change in patients infected with the virus and correlate the role of different biomarkers in COVID-19.

2. LITERATURE REVIEW

2.1 COVID 19 Structure

2.1.1 The coronavirus history

Coronaviruses are a group of enveloped, single-stranded, positive-sense RNA viruses that were first identified in the 1960s (Common and Holmes 2003), In immunocompetent hosts, common strains of human coronavirus (HCoV) such as HCV-229E, OC43, HKU1, and NL63 are regarded to be moderate respiratory infections. These strains may be isolated from several individuals with upper respiratory symptoms (Cui *et al.* 2019, Su *et al.* 2016). However, three particularly virulent coronavirus strains have arisen since the turn of the century. In 2002 and 2003, SARS-CoV was first found in Guangdong, China. It then spread to 17 other countries and caused over 8,000 cases (De Wit *et al.* 2016, Zhong *et al.* 2003).

Since 2004, no cases of SARS-CoV infection have been identified. Middle East Respiratory Syndrome (MERS) caused by MERS coronavirus (MERS-CoV) was first identified in the Middle East in 2012–2013, especially in Middle Eastern countries (Zaki *et al.* 2012). In South Korea, a MERS outbreak was also confirmed in 2017 (De Wit *et al.* 2016). MERS is thought to have affected about 2000 cases in total. Animal reservoirs for SARS-CoV and MERS-CoV have been identified in bats and camels, respectively. Even though research into the zoonotic potential of SARS-CoV-2 is still ongoing, it is generally accepted that bats serve as an animal reservoir for the virus (Ahmad *et al.* 2020 , Cui *et al.* 2019). The propensity of these three highly pathogenic coronaviruses to replicate in epithelial cells and pneumocytes in the human lower respiratory tract, which can lead to the development of pneumonia in humans (Cui *et al.* 2019, De Wit *et al.* 2016).

Furthermore, in the most severe cases, (ARDS) is a key characteristic shared by these three highly infectious coronaviruses. The angiotensin-converting enzyme 2 (ACE2)

serves as a functional cellular receptor for both the SARS coronavirus and the SARS coronavirus 2 strain (Bourgonje *et al.* 2020, Hoffmann *et al.* 2020).

2.1.2 The virus particle (morphology)

SARS-CoV-2 belongs to the genus Betacoronavirus and it belongs to the tribe Coronavirinae (Malabadi *et al.* 2021). Virus particles with a diameter of about 60–140 nm are spherical or pleomorphic (Smereka *et al.* 2020).different protein structures than other coronaviruses. It's non-segmented, single-stranded RNA with no DNA stage. Coronavirus has linear and helical capsids, but the nucleocapsid lies within the virion envelope (Ferner and Aronson 2020).

This virus has 80–160 nM RNA peplomers with a 27–32 kb positive polarity (Sahin *et al.* 2020). Coronaviruses have club-shaped spikes on their surfaces. These spikes resemble solar coronas, thus the name coronavirus. Spike protein (S), membrane protein (M) and 16s are key structural components. N and E are encoded at the viral genome's 30 end (Agrahari *et al.* 2021).

The researchers were able to describe SARS-COV-2 using transmission electron microscopy, the virus that causes COVID-19, isolated from a patient in the United States, showing virus particles emerging from the surface of cells cultured in a laboratory. The spikes on the outer edges of virus particles give coronaviruses their name, like a crown (Zhai *et al.* 2021).

2.1.3 Positive sense RNA genome

The 30 kb, single-stranded, positive-sense RNA genome is the biggest RNA viral genome that has ever been discovered. Infectious, it has a cap at the 5'-end and polyadenylation at the 3'-terminus, and it is capped at the 5'-end. Because of its vastness, the expression of particular genes takes place via a complicated process that involves the production of sets of nested mRNAs, all of which have the identical

sequence at the 5' end. The process of heterologous RNA recombination has the potential to result in extensive rearrangements. There is a possibility that the 5' end of the genome contains an untranslated region (UTR) that ranges in length from 65 to 98 nucleotides. In addition to being located at the 5' ends of all subgenomic mRNAs, this sequence, which is also known as the leader RNA, can be found there. At the 3' end of the RNA genome, there is another part that has not been translated. This part is between 200 and 500 nucleotides long, and it is followed by a poly(A) tail.Both of the RNA sections that are not translated play a crucial role in the regulation of RNA replication and transcription (Figure 2.1) (Burrell *et al.* 2016).

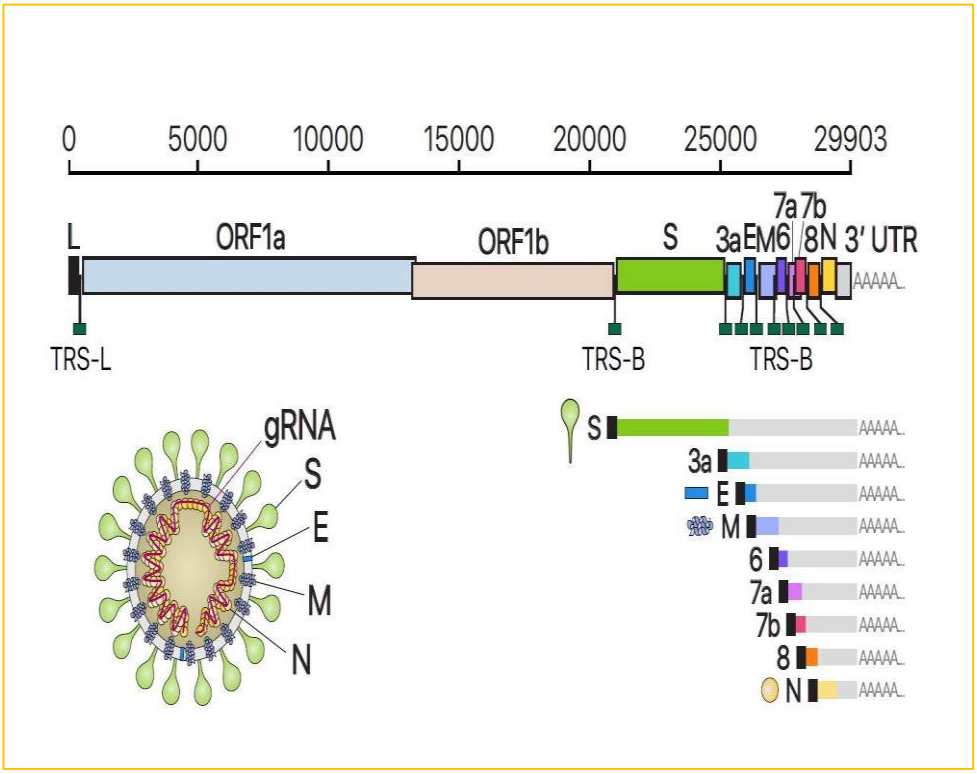

Figure 2.1 SARS-CoV-2 genome organization, subgenomic mRNAs, and virion structure (Kim *et al.* 2020)

2.1.4 Structure of proteins for cornavirus

There are four primary structural proteins that come together to make the viral particle: the spike, the envelope, the membrane, and the nucleocapsid. Viral envelopes consist of

the membrane protein nucleocapsid and the envelope protein envelope, with the spike protein allowing the virus to bind to the host cell membrane (Rota *et al.* 2003).

There are 16 different nonstructural proteins that are generated when the replicase polyprotein cleaves itself (NSP1 to NSP16) (Hartenian *et al.* 2020), which are found in membranes that originated in the endoplasmic reticulum and make up the replication-transcription complex (Hartenian *et al.* 2020). NSP10 is an activation cofactor in a complex with NSP14 to proofread and edit newly produced RNA, and NSP13, NSP14, and NSP16 all play roles in mRNA capping (Ma *et al.* 2015).

Also, during viral genome replication, positive-sense viruses require the nonstructural RNA genome of RNA-dependent RNA polymerase (NSP12) to function (Durmuş and Ülgen 2017). Formation of the complex between NSP10 and NSP16 is required for viral replication, suggesting its importance in the coronavirus life cycle (Mirza and Froeyen 2020).

There are a total of six ORFs that are encoded by the coronavirus genomes and subgenomes (Chan *et al.* 2020)."More than 5" The end is occupied by ORF1a/b, which produce 16 nsps. Two proteins,PP1a and PP1ab, initially from ORF1a/b by a single frame change between ORF1a and ORF1b (Niinemets and Sack 2006). The virus-encoded protease cleaves multiple proteins in individual nsps (the main proteases [Mpro], chymotrypsin-like proteases [3CLpro], and papain-like proteases [PLPs]) (Masters 2006). SARS-CoV-2 also encodes these nsps (Chan *et al.* 2020).

2.2 Coronavirus Origin and Transmission

At least four recent coronavirus zoonotic jump events from animals to people have resulted in epidemics. At least nine animal species, including humans, were infected by the first -coronavirus. To get CoV OC43, this virus had to travel from bovines to people roughly 120 years ago. This virus and H-CoV-229E were discovered in 1967 and 1966. The earliest human coronaviruses were CoV OC43 and H-CoV-229E. For forty years, coronaviruses were considered to be the sole human-infecting viruses (Ye *et al.* 2020).

According to phylogenetic study, the SARS-COV-2 virus has the highest degree of similarity (89.1 percent nucleotide similarity) with a group of SARS-like βcoronaviruses (βCoVs) that were discovered in bats in China, which is where the virus was discovered for the first time (Hu *et al.* 2018). There are two more coronaviruses known as SARS-CoV and MERS-CoV. Both of these viruses are responsible for infections of the respiratory system that are very serious and often fatal (Yin and Wunderink 2018).

The sequence of SARS-genomic CoV-2 is 96.2% comparable to that of bat CoV RaTG13 but only 79.5% identical to SARS-CoV. Evidence from viral genome sequencing and evolutionary research suggests that bats may be the natural hosts of SARSCoV-2, with the virus potentially being transmitted to humans via as-yet-unidentified intermediate hosts. ACE 2 may be a target for SARS-CoV-2, as it was for SARS-CoV (Zhang *et al.* 2020), to infect humans.

Given that bats were not for sale at Wuhan's seafood market and that comparable viral receptor residues were found in several species (Liu *et al.* 2020), other intermediate hosts such as turtles, pangolins, and snakes were recommended (Liu *et al.* 2020, Zhang *et al.* 2019). SARS-CoV and MERS-CoV often infect intermediary animals such as civets or camels before jumping to humans (Cui *et al.* 2019). This suggests that SARS-CoV-2 was most likely transmitted to humans by animals other than bats. According to certain investigations, pangolin-CoV is the closest of SARS-CoV-2 (Liu *et al.* 2019, Zhang *et al.* 2020).

First instances of SARS-CoV-2 sickness were related to Huanan Wholesale Market, suggesting the COVID-2019 virus was spread from animals to humans (Chen *et al.* 2020). genome study shows that the virus originated somewhere before being brought to the market, where it spread fast (Cui *et al.* 2019).

SARS-CoV-2 can be transmitted directly (through droplets and human-to-human transmission) as well as indirectly (by contaminated objects and airborne contagion). Meanwhile, personal security Airborne infections could also come via personal

protective equipment (PPE) (Yuan *et al.* 2020). As previously stated, SARS-CoV-2 is thought to transmit mostly by respiratory droplets, which occur when a patient coughs. Sneezes, talks, or even sings. Droplets can usually only travel so far. more than six feet (almost two meters) and only stay in the air for a short time. In droplets, however, SARS-CoV-2 remains intact and contagious.(less than five microns in diameter) and can float in the air for a long time (Van Doremalen *et al.* 2020), up to three hours As a result, airborne separation and room ventilation are essential. and adequate disinfectant treatment (particularly in restrooms) inhibit viral spread by aerosol (Figure 2.2) (Santarpia *et al.* 2020).COVID-19 can be contracted by touching a contaminated surface. The hands then come into direct touch with SARS-CoV-2. mucous membranes, such as those found in the eyes, nose, and mouth (VDI *et al.* 2020).

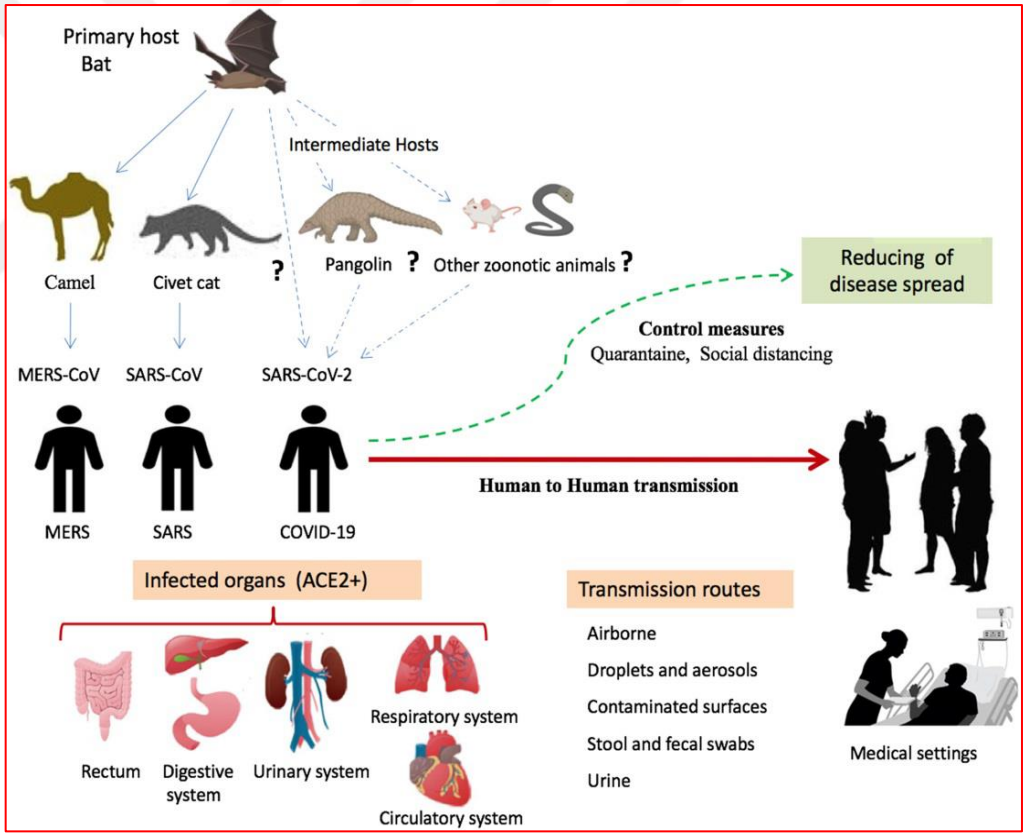

Figure 2.2 Origin of COVID 19 and potential transmission routes to humans (Huang *et al.* 2020)

2.3 Classfication Coronavirus

The coronaviridae, arteriviridae, roniviridae, and mesoniviridae families make up the majority of the viruses that belong to the Nidovirales order, making the covs the biggest group of viruses (Figure 2.3). One of the two subfamilies that are included in the Coronaviridae family is called the Torovirinae, and the other subfamily is called the Coronavirinae. The family Coronavirinae can be further classified into four genera, which are Alpha, Beta, Gamma, and Delta, respectively. At first, the viruses were separated by their antibodies, but now they are put into groups based on phylogenetic and evolutionary (Khan *et al.* 2020).

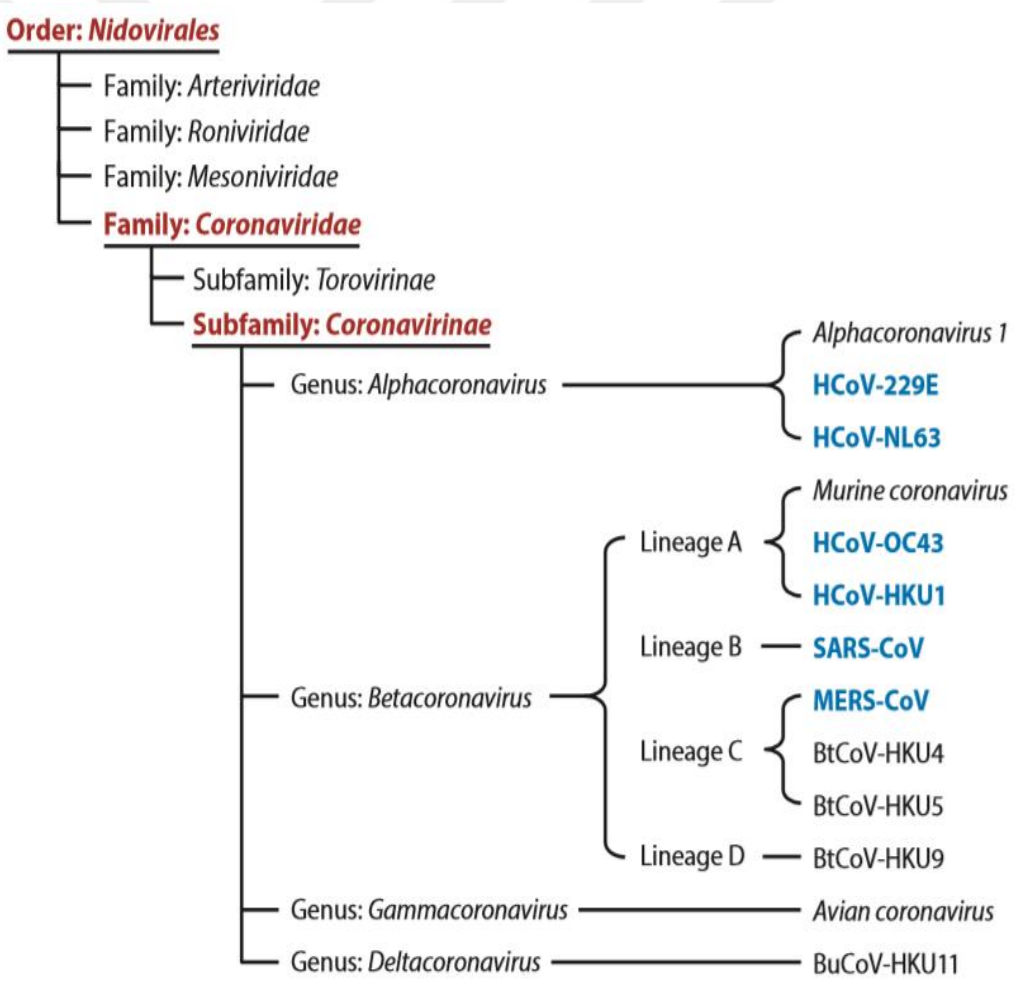

Figure 2.3 Classification human COVID 19 and other coronavirus (Fung and Liu 2019)

2.4 Coronavirus Life Cycle (Entry To Exit)

The life cycle of SARS-CoV begins when its S protein connects to the cellular receptor ACE2 (Dimitrov 2003, Li *et al.* 2003).which is expressed on the primary target cells, pneumocytes and enterocytes in the respiratory system, as well as other susceptible cells, including intestinal mucosal cells, renal tubular epithelial cells, cerebralneurons, and immune cells, (Gu and Korteweg 2007).

After penetrating these cells, By changing its shape, the S protein facilitates endosomal fusion of the viral envelope with the cell membrane. During viral replication, the RNA genome is translated into the polypeptides PP1a and PP1b that serve as viral replicase and are later processed by viral proteinases into a variety of small, infectious particles. At the same time, polymerase, which produces a sequence of subgenomic mRNAs via discontinuous transcription, is translated into the correct viral proteins (Figure 2.4). Viruses are released from cells by first assembling their proteins and genomic RNA in the endoplasmic reticulum (ER) and Golgi before being shuttled to the ERGIC lumen. Atypical pneumonia, characterized by rapid respiratory deterioration and failure, results from an abnormally high synthesis and activation of proinflammatory chemokines and cytokines (Jiang *et al.* 2012).

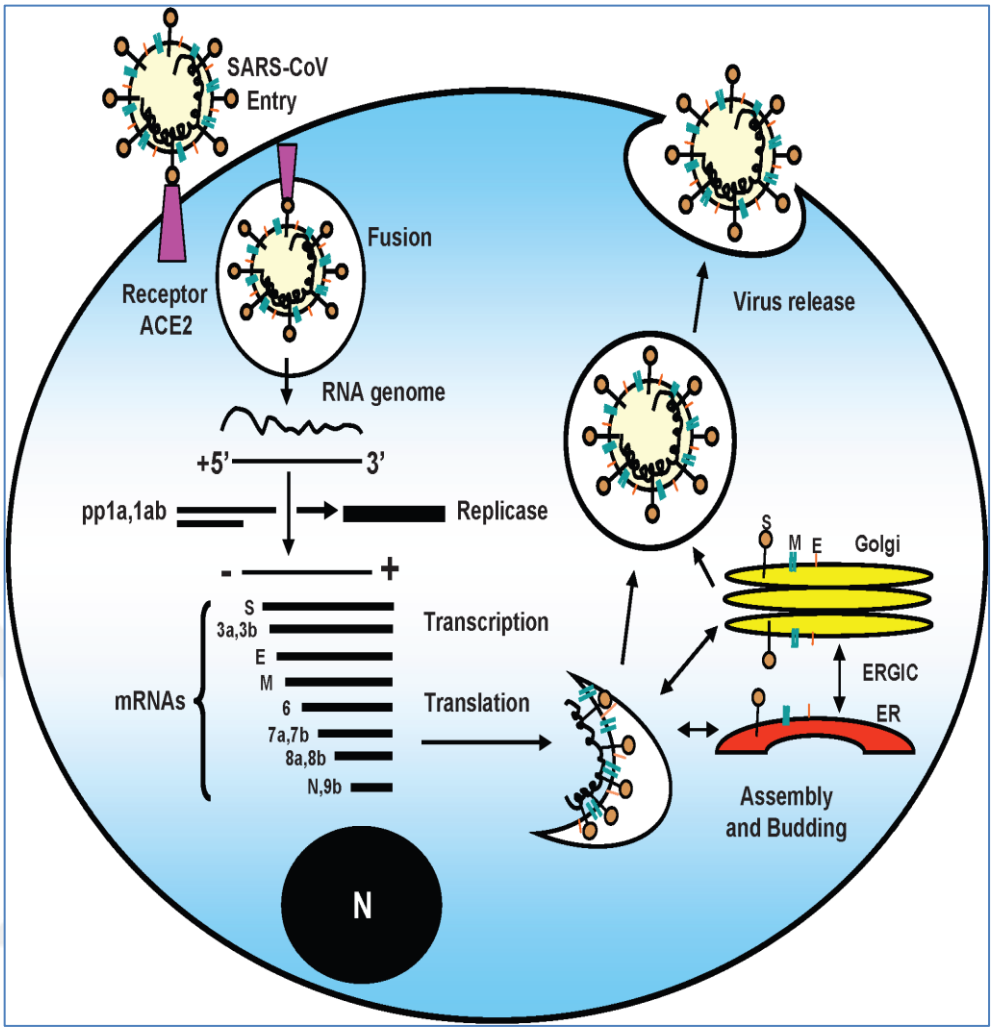

Figure 2.4 Life cycle covid 19 (Jiang *et al.* 2012)

2.5 Immunopathogenesis Covid 19

After the coronavirus has successfully infiltrated the human body, the innate immune system is stimulated. It then identifies the virus and triggers the production of cytokines and chemokines that promote inflammation. After this, adaptive immune system activation takes place, in which activated T cells directly kill virus-infected cells and B cells create antibodies that are unique to the pathogen. (Quan *et al.* 2020).

Few patients acquire (ARDS), sometimes known as breathing problems. ARDS can lead to breathing problems., problems with other organs, and even death (Yang *et al.*

2020). ARDS, cytokine storm, septic shock, metabolic acidosis, and coagulation dysfunction are more likely to happen to older people and people with serious illnesses like hypertension, COPD, diabetes, and heart disease (Bai *et al.* 2020, Chen *et al.* 2020, Lleo *et al.* 2020, Yang *et al.* 2020). Several abnormalities have been seen, such as a lack of immune cells, activation of coagulation, damage to the heart muscle, liver, and kidneys, and a secondary bacterial infection (Chen *et al.* 2020).

In most cases, the white blood cell count is normal or reduced, and lymphocytopenia is seen (KuiLiu *et al.* 2020). However, neutrophil counts, D-dimer levels, blood urea and creatinine levels, and lymphocyte counts are all considerably elevated in very ill individuals. Viral'molecular patterns' (such as doublestranded RNA) are recognized by the innate immune system (Akira *et al.* 2006), and T and B cells that create pathogen-specific antibodies destroy virus-infected cells in the adaptive immune system (Li *et al.* 2020). Tissue healing and an extended adaptive immune response against viruses are all possible outcomes of a well-regulated immune response (Schoeman and Fielding 2019).

2.6 Epidemiology

SARS likely began in November 2002 in live animal markets in Foshan, China and spread globally in 2002-2003. The first large epidemic occurred in Guangzhou, China among medical personnel and their families (Zhong *et al.* 2003). SARS-CoV, a new coronavirus, was then identified from those atypical pneumonia patients (Peiris *et al.* 2003). Air travel was used to propagate the pandemic after March 2003. Before the worldwide epidemic was declared ended in July 2003, (8098) cases and (774) fatalities, "9.6 %" had been documented WHO , Numerous sars cases were detected in 2005 after interaction with palm civets, the suspected source of infection (Wang *et al.* 2005).

In June 2012, MERS-CoV was discovered in a patient who died of a severe respiratory ailment in Jeddah, Saudi Arabia (Zaki and Bestebroer 2020, Zumla *et al.* 2015). MERS then came out at a public hospital in Zarqa, Jordan, affecting 11 individuals, including eight health care professionals. In June 2015, 30 MERS cases were identified in Seoul, South Korea, making it the biggest epidemic outside the Middle East. Nosocomial and

travel-related transmission are increasing (Chan *et al.* 2015). in the MERS -COV outbreaks were recorded in 27 countries with 2494 cases and 722 fatalities (case fatality rate 34%) (Wash *et al.* 2019).

In December 2019, a seafood wholesale market in Wuhan, China, saw many atypical pneumonia cases (Quan *et al.* 2020). SARS-CoV-2, a previously unknown coronavirus, was discovered in these individuals (Zhu *et al.* 2020). It affected males more than women, like MERS-CoV but not SARS-CoV (Channappanavar *et al.* 2017, Yang *et al.* 2020). Globally, almost 8 million confirmed cases and 461,000 fatalities were documented until June 21, 2020. (The case fatality rate is 5.3 percent.).

2.7 Incubation Peroid For Infection Covid 19

The incubation period is the amount of time between exposure to a pathogenic organism and the start of symptoms. During this time, an organism can multiply and reach a critical number, which is needed for symptoms to show up in the host (Acter *et al.* 2020). It is very important to know both the average and the range of the incubation period of a typical infectious disease in order to figure out when an outbreak is most likely to happen (Kirsch *et al.* 2002). At the moment, the time it takes for a new coronavirus to spread is usually between 2 and 14 days (Lombardi *et al.* 2020).

Figured out how long COVID-19 takes to spread from person to person based on 181 confirmed cases outside of China's Hubei province. People who were exposed to SARS-CoV-2 were found to have symptoms within (5-11)days, while the average incubation period was about 5 days, which is the same as with SARS (Lauer *et al.* 2020).

In extreme cases, though, it's thought that longer monitoring periods might be okay. So, a person who has been to a COVID-19-infected area in the last 14 days is at high risk and must follow quarantine rules (Liu *et al.* 2020 , VDI 2020).

2.8 Clinical Features and Symptoms of COVID-19

In symptomatic COVID-19 infections, the clinical presentation might vary from unremarkable to life-threatening situations at any one time. The most significant symptom of a COVID-19 infection is pneumonia, which is an infection of the lower respiratory tract. As determined by studies obtained from the Wuhan population, the most prevalent clinical symptoms at the onset of the illness are fever, weariness, and coughing (Wang *et al.* 2020).

Fever, cough, weariness, sputum production, shortness of breath, sore throat, and headache were shown to be the most prevalent clinical symptoms in a recent research conducted by Prof. Nan-Shan Zhong (Guan *et al.* 2020). Also, several individuals had gastrointestinal problems such as diarrhea and vomiting , The clinical symptoms matched individuals from Hubei province (Chen *et al.* 2020, Huang *et al.* 2020, Wang *et al.* 2020). Upper respiratory and gastrointestinal symptoms were infrequent, indicating changes in viral tropism compared to SARS-CoV (Lee *et al.* 2003), MERSCoV (Assiri *et al.* 2013), and influenza (Wang *et al.* 2016). The elderly and those with underlying diseases (hypertension, COPD, diabetes, and cardiovascular disease) deteriorated swiftly into ARDS, septic shock, metabolic acidosis, and coagulation malfunction, even mortality (Huang *et al.* 2020), As in Figure 2.5.

Most patients had normal or low white blood cell counts, and lymphocytopenia (Guan *et al.* 2020). The neutrophil count, D-dimer, blood urea, and creatinine levels were greater in the severe patients, whereas the lymphocyte counts decreased.but Inflammatory variables (IL-6, IL-10, TNF) rise, suggesting the patients' immunological state. ICU patients exhibited greater levels of IL-6, IL-7, IL-10, GCSF, 10 kD interferongamma-induced protein (IP-10) and TNF (Huang *et al.* 2020).

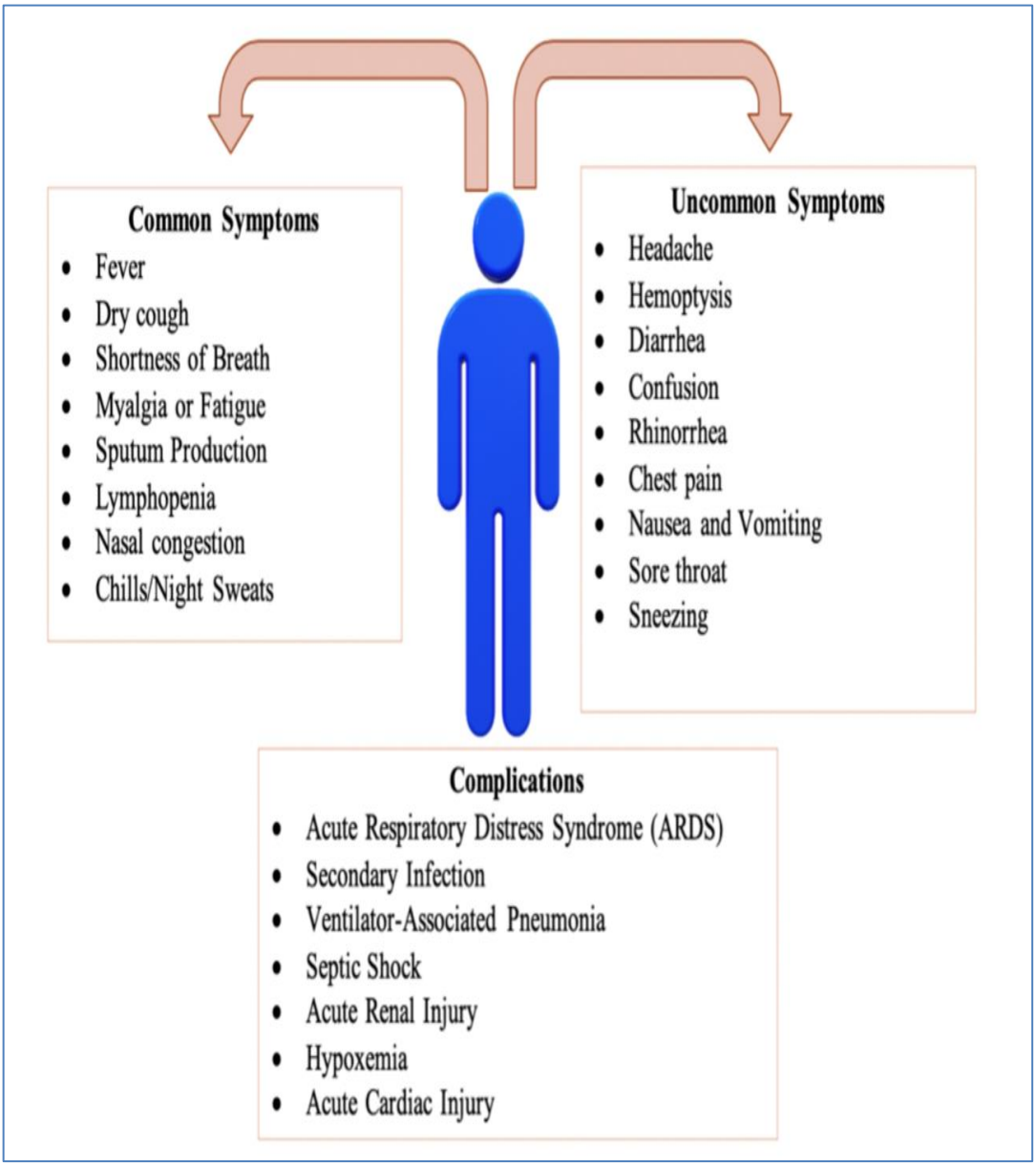

Figure 2.5 Categorization of the common and uncommon symptoms and complications of COVID-19 (Hussain *et al.* 2020)

2.9 Complication of COVID 19

According to existing data, most patients had a favorable outlook, but others were in serious condition, particularly the elderly and those with chronic conditions (Rubio-Pérez *et al.* 2020). ARDS, arrhythmia, shock (Bai *et al.* 2020), acute renal, acute cardiac, liver dysfunction, and secondary infection (Huang *et al.* 2020a) were reported.

The severity of illness affected the clinical outcome. The illness progresses quicker in the elderly, with a median of fewer days between the onset of symptoms and mortality (Raedler and Schaub 2014, Wang *et al.* 2020). Individuals with comorbidities and ARDS had a greater mortality risk than H7N9 patients (Gao *et al.* 2013). More than 100 children were affected, the youngest at 30 hours after birth (Wang *et al.* 2020). Children and the elderly need extra care owing to their undeveloped immune systems.

2.10 Coronavirus Treatment and Vaccination

2.10.1 Current therapies

Without effective antiviral medication for COVID-19, current therapies concentrate on symptomatic and respiratory support, according to the National Health Commission of the People's Republic of China's Diagnosis and Treatment of Pneumonia Caused by COVID-19 (Ding *et al.* 2021). WHO suggested ECMO for individuals with refractory hypoxemia. According to their circumstances, some patients get rescue therapy with convalescent plasma and immunoglobulin G (Chen *et al.* 2020).

2.10.2 Antiviral treatments

Some therapeutic techniques against coronaviruses may be learned from earlier experience combating SARS and MERS epidemics (Zumla *et al.* 2016). Antivirals and corticosteroids are not effective against COVID-19 and should not be used. Results from in vitro and animal models indicate that Remdesivir (GS-5734) has an antiviral RNA effect and may inhibit NSP12 polymerase even when ExoN assay activity is intact. it may have the potential to treat COVID-19 (Team 2020).

Because the inhibitory concentration that needs to be achieved for effective anti-SARS-CoV-2 activity is significantly higher than the maximum plasma concentration that can be achieved by administering the approved dose of ivermectin, it is possible that ivermectin has limited utility in the management of COVID-19. According to Patr &

Fabbrocini's 2020 research, the antimalarial medication chloroquine also has antiviral properties (Patrì and Fabbrocini 2020).

2.10.3 Vaccination

Utilizing previous SARS and MERS-related research, the experimental vaccine is now being created using mRNA as its genetic basis (Cheng *et al.* 2007 , van Doremalen *et al.* 2014). Among the 120 known SARS-CoV-2 genetic sequences, locating B cell and T cell epitopes that are generated from the spike (S) and nucleocapsid (N) proteins is the foundation of a successful vaccination (Peng *et al.* 2020).Vaccination would play a crucial role in preventing the spread of the virus and eradicating it from the host (Ahmed *et al.* 2020).

2.11 Prevention of Covid-19

Several public health strategies may prevent or delay COVID-19 spread, including patient isolation, contact identification and monitoring, environmental cleaning, and personal protective equipments (PPE) usage (Wei and Ren 2020). For medical workers, suspected cases, and proven cases, psychological therapies are indicated (Adhikari *et al.* 2020).

Use of face masks, covering coughs and sneezes with tissues, washing hands regularly with soap or disinfecting with a hand sanitizer containing 60% alcohol, keeping an appropriate distance, avoiding contact with infected people, and not touching the nose, mouth, and eyes with unwashed hands can be reduced. risk of exposure to the virus (Hageman 2020).

The World Health Organization (WHO) has released new guidelines on the use of face masks in healthcare settings. Healthcare staff are advised to use particulate respirators such as those licensed N95 or FFP2 while conducting aerosol-generating procedures

and to use surgical masks when treating suspected or confirmed cases (Mersha *et al.* 2021).

2.12 Diagnosis Covid 19

2.12.1 Molecular diagnosis covid 19

Nucleic acid amplification tests:

The nucleic acid amplification assays, also known as NAAT, are used to confirm COVID-19 cases on a routine basis. These tests look for distinctive sequences of viral RNA , The method known as real-time reverse transcription polymerase chain reaction (RT-PCR) may be used to carry out this test. In the field of molecular biology, real-time reverse transcription-PCR (RT-PCR) is a well-known method (Freeman *et al.* 1999) for monitoring the amplification of a specific DNA molecule in real time. On the other hand, RT-PCR is a combination of reverse transcription of RNA into DNA and PCR amplification of the DNA, which is then read out using fluorescence (Bidlingmaier *et al.* 2009).

A nasopharyngeal swab is a procedure used to obtain a clinical test sample of secretions from the posterior pharynx and the back of the nose. Samples may be taken either from the lungs or from the blood for the test. The results of the test are typically available anywhere from a few hours to two days after the test has been taken (Khan *et al.* 2020, Organization 2020). The fact that the genetic sequences of RNA strains of the new coronavirus have already been identified and published on a country-by-country basis (Huang *et al.* 2020a, Shao 2020) is a source of optimism.

Quantitative reverse transcription–polymerase chain reaction:

As a preliminary test, reverse transcription quantitative polymerase chain reaction (RT-qPCR) is the method of choice for identifying SARS-CoV-2. (Rauch *et al.* 2021). It is

currently thought to be the "gold standard" test because it is very sensitive, can quickly spot changes, and has a number of other desirable qualities (Böger *et al.* 2021). In addition, the RT-qPCR methodology is the approach that is the most appropriate choice since it enables viral detection as well as quantification. Three crucial phases are included in these tests: The first step is to extract viral RNA from the specimens that have been collected; the second step is to reverse-transcribe the viral RNA into a single-stranded DNA (cDNA) using the enzyme reverse transcriptase; and the third and final step is to amplify the cDNA in conjunction with fluorescent detection *(Chappleboim et al.* 2021).

Loop mediated isothermal amplification (LAMP):

Isothermal amplification eliminates the requirement for a high-precision equipment in RT-qPCR tests (Roosa *et al.* 2020). This approach amplifies DNA and RNA. Its exponential amplification and six target sequences identified by four primers provide it exceptional sensitivity and specificity (Parida *et al.* 2008).

Visually identify and quantitatively quantify a LAMP positive solution reaction using fluorimetry, colorimetry, and turbidity. The insoluble LAMP byproduct (magnesium pyrophosphate) is cloudy (Götzinger *et al.* 2020).The LAMP approach is rapid and doesn't need costly chemicals or equipment, unlike RT-qPCR (Lin *et al.* 2022). RT-LAMP includes a reverse transcriptase step to identify RNA targets. The technology was subsequently refined to allow RNA detection by reverse transcription and has been used to identify H7N9 (Ahn *et al.* 2019), Zika (Tacken *et al.* 2006), MERS-CoV (Lee *et al.* 2017), and SARS-CoV (Hui *et al.* 2022). five-minute SARS-CoV-2 detection (Vashist 2020).

Clustered regularly interspaced short palindromic repeats:

Novel genetic diagnostics made possible by CRISPR/Cas make use of Cas stimuli's activity to quickly identify sensitive, precise, and quick DNA. CRISPR-Cas tests can be run in a single reaction directly on primary clinical material (Esbin *et al.* 2020,

McCarthy 2020). The single-stranded portion of the CRISPR RNA (crRNA), which is complementary to the target DNA sequence, directs the Cas12 or Cas13 proteins to target a particular DNA sequence. The roles of Cas12 and Cas13 are distinct: While Cas13 targets ssRNA, Cas12 targets ssDNA (Feng *et al.* 2020).

2.12.2 Assays for serology and immunology

Serological tests are easy to do and give quick results. They are a high-throughput way to find out if someone has a viral infection. For SARS-CoV-2, the main antigenic targets are the spike (S) glycoprotein and nucleocapsid (N) phosphoprotein (Dutta *et al.* 2020, Walls *et al.* 2020). There was the least level of similarity in the SARS-CoV-2's S1 subunit compared to other coronaviruses. Serological tests for COVID-19 should focus on the S1 and N proteins (Okba *et al.* 2020). To learn how antibodies function and how the immune system reacts, researchers use several antigen detection methods.

Binding antibody detection methods:

The binding antibody detection approaches employ purified SARS-CoV-2 proteins. Compared to molecular detection technologies, antibody detection methods provide faster responses, lower costs, and more mobility. They are less accurate and sometimes need confirmation by molecular techniques like RT-qPCR, which adds time and expense. (Carter *et al.* 2020; Faustini *et al.* 2020; Kilic *et al.* 2020; Shyu *et al.* 2020). Antigen-binding antibody tests may be classified into two types.

1) Point of care tests (POCT)

Antibodies are detected in saliva, plasma, serum, and whole blood. POC tests may be conducted swiftly on fingersticks or nasopharyngeal and oropharyngeal swabs (Prazuck *et al.* 2020).

The FDA has given its approval for the use of the Abbott Alinity I SARS-CoV-2 IgG kit as well as the Access Bio CareStart COVID-19 IgM/IgG kit. The majority of these studies present the final conclusions as colored lines, each accompanied by a control line. Test lines with colored markers indicate the presence of the target antibody. These tests are efficient, low-cost, and simple to study for. These assays require additional validation using RT-qPCR as a result of this reason. (Chilamakuri and Agarwal 2021).

2) Laboratory tests

ELISA, CIA, and LFI are examples of these assays . These tests need specific equipment, reagents, or skilled specialists (Augustine *et al.* 2020). ELISA is a diagnostic process that has a high sensitivity and a high throughput, and it uses multiwell plates that have been coated with viral proteins. Samples of the patient's blood, plasma, or serum are examined in order to detect and capture antibodies. These tests offer an excellent analytic sensitivity and a short detection time, but they require qualified staff and the validation of secondary test results (Weissleder *et al.* 2020).

Neutralizing antibody detection:

When it comes to detecting active antibodies against live viruses, viral neutralization tests (VNT) are considered to be the gold standard. These tests seek for cytopathic effects caused by viruses, which occur when neutralizing antibodies stop the reproduction of viruses and allow cells to divide and multiply. These tests are used for the development of vaccines and drugs because VNT is labor-intensive and requires the use of specialized laboratories (Chilamakuri and Agarwal 2021).

Antigen detection methods:

using a technique that is commonly referred to as "conventional immunocapture." Even while the virus is in the process of actively multiplying, these tests have the potential to identify the SARS-CoV-2 viral antigen n protein. This helps to account for the great

level of specificity achieved by these various types of tests. Other rapid test kits are currently being considered for use in the research and development of antigen detection techniques (Chen *et al.* 2020).

2.12.3 Computed tomography (CT) scan of the chest

CT scans are among the most common diagnostics and have been shown to have a higher level of sensitivity than RT-qPCR (Al-Tawfiq and Memish 2020). Patients infected with SARS-CoV-2 have symptoms that are characteristic of pneumonia during the early stages of illness. These findings include bilateral parenchymal ground-glass opacities and unilateral lung with subpleural lesions(20%) (Imai *et al.* 2020, Li *et al.* 2020, Ye *et al.* 2020).

2.13 The Parameters Used in The Study

2.13.1 Hematological markers

Tests for blood parameters include white blood cells, lymphocytes, neutrophils, and platelets, which are commonly prevalent in viral and bacterial illnesses (wei Sun *et al.* 2020).

2.13.2 Ferritin

During hyperferritinemia, ferritin has both immunosuppressive and proinflammatory effects, which contribute to the cytokine storm. Cytokine storm syndrome is connected to deadly COVID-19 findings; hence, it has been proposed that the cytokine storm is linked to disease severity. Because of elevated blood ferritin levels in diabetics, COVID-19 has a greater potential to do major harm to the body. COVID-19 severity may be influenced by ferritin levels, which have been found in high concentrations in the blood (Vargas-Vargas and Cortés-Rojo 2020).

2.13.3 Interleukin 6

IL-6 affects cell proliferation, differentiation, and immune response. Human IL-6 is a chromosome 7p21 gene with 212 amino acids and a 28-amino-acid signal peptide. IL-6 is a pro-and anti-inflammatory cytokine. Atherosclerosis, Alzheimer's, systemic lupus erythematosus, multiple myeloma, rheumatoid arthritis (RA), autoimmune deficiency syndrome, chronic inflammatory illness, and cancer all produce IL-6. IL-6 release during illness, especially following immune response activation, is crucial (Vatansever and Becer 2020).

2.13.4 TNF

TNF is a homotrimer of 157 amino acids and has a molecular weight of 17 kilodaltons. It is produced by macrophages, T lymphocytes, and natural killer cells. (Robinson *et al.* 2020).Tumor necrosis factor-a (TNF-a) is a pleiotropic modulator of acute and chronic systemic inflammatory reactions. It's engaged in anti-tumor, inflammation, and immune system homeostasis functions (Aggarwal 2003, Croft 2009).Dysregulated TNF-a signaling may induce CRS, one of the most significant pro-inflammatory cytokines of the innate immune response.

2.13.5 C. reactive protein

It is an acute-phase reactant that is raised in infection and inflammation. Higher values suggest COVID-19 illness severity (Chen *et al.* 2020, Liu *et al.* 2020) .CRP is a nonspecific acute phase protein generated by hepatocytes during infection or inflammation, After an inflammatory insult, secretion commences 4–10 h later and peaks at 48 h, with a 19-h half-life. It may rise before vital signs or leukocytes are compromised, CRP's biomarker profile makes it valuable in clinical diagnosis (Pepys and Hirschfield 2003).

2.13.6 D. dimer

The breakdown of fibrin results in the formation of a D-dimer. As a biomarker for thromboembolism and as a prognostic marker for patients in critical care, it has gained widespread recognition in recent years. Due to the fact that COVID-19 is a procoagulant condition, D-dimer has been investigated as a potential biomarker for determining the severity of the illness (Mishra *et al.* 2020).

2.13.7 Vitamin D

Vitamin D, also known as cholecalciferol, is a lipid-soluble vitamin that is a member of the same vitamin family as vitamins D1, D2, and D3. When the skin is exposed to UVB rays from the sun, the body produces vitamin D naturally. You can receive vitamin D through some foods and supplements, both of which can help you maintain healthy levels of the vitamin in your blood. Vitamin D has various key roles. The most important of which are controlling the proportion of calcium and phosphorous and enhancing the function of the immune system (Ross *et al.* 2020).

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipment and apparatus used in this study

Table 3.1 list of Equipment and apparatus used in this study

NO	Equipment and Apparatus	Company	Origin
1	PCR C1000 TOUCH	BIO RAD	China
2	IChroma II Portable Immune-Assay Analyzer	Boditech	China
3	I chroma Incubator	Boditech	China
4	ELISA Microplate reader and washer	Human	Germany
5	Microplate Shakers	IKA.MS3Digital	USA
6	Finecare™ FIA Meter Plus	Wondfo biotech	China
7	Incubator	Jrad	China
8	Coulter (XN 2000 ,3 diff hematology analyzer)	Sysmex	Japane
9	Shaker Machine Laboratory Roller Mixer	Karl kolb	Germany
10	Eppendorf Centrifuge	Hermle	Germany
11	Micropipette /10-100 µL 100-1000 µL	Larksci	China
12	Refrigerator	Beko	Turkey
13	Water distilater	Mikrotest	Turkey
14	Water bath	Labtech	Korea
15	Viral transport medium tube with swab	Bioneer	China
16	Gel tube	Malaga	Spain
17	Plastic disposable Syringes 5 ml	Medeco	UAE
18	EDTA tube	Malaga	Spain
19	Eppendrof tubes	Hermle	Germany
20	Plain tube	Arth Alrafidain	China
21	Medical Cloves	Omega	China
22	Cobas C411	Roche	Germany

3.1.2 Diagnostic kits

Diagnostic kits, The kits utilized in this study are listed in Table 3.2.

Table 3.2 Diagnostic kits used in the study

No.	Materials	Company	Origin
1.	C411 (IgG , IgM) COVID19 kit	BT LAB	China
2.	ELISA IL6 kit	BT LAB	China
9.	Vitamin D kit	Boditech	China
10.	Ferritin kit	Boditech	China
11.	CRP kit	Boditech	China
12.	D.dimer kit	Wondfo biotech	China
13.	TNF-a kit	Elabscience	China

3.2 Method

3.2.1 Study design

The total number of samples collected for the purpose of the study was 100 samples for those infected with the Covid-19 virus, and the samples were (58 males and 42 females), and samples were collected in Dhi Qar Governorate from health institutions. Al-Hussein Teaching Hospital and Public Health Laboratory and the control group included 100 people: 50 males and 50 females. They were previously diagnosed by PCR and confirmed to be safe from infection with COVID-19 in Thi-Qar Province.

Blood sample collection:

2.5 ml of blood was taken for a complete blood picture and kept at 2–8°C. 5 ml of blood was also taken and separated from its components to obtain a serum for use in the diagnosis of COVID-19 using ELISA technology as well as PCR technology. In addition to other tests, the serum was stored at-20 °C.

Swab sample collection:

The collection of swab samples necessitated the employment of protective equipment (N95/FFP2 respirator, goggles or face shield, vest, and gloves) because of the presence of aerosols. Swab samples were collected using Dacron swabs, which were introduced into the nostril and rotated evenly until they reached the nasopharynx, where the sample was then acquired by spinning the swab gently for 2-3 seconds. Then, the swab was inserted into a 5 ml tube containing 2 ml of viral transmission medium (VTM).

3.3 Molecular And Genotyping Detection

3.3.1 Kits

The kit of the nucleic acid extraction was used in the current study as shown in the Table 3.3.

Table 3.3 Genotyping kit for nucleic acid extraction and amplification

Kits	Company/ Origin
Wizard Genomic DNA Purification kit, Agarose, Ethidium Bromide Solution (10mg/ml), GoTag Green Master Mix, Nuclease Free Water, TAE 40X, Quantifluor dsDNA System	Promega, USA
Absolute Ethanol, Isopropanol	ROMIL pure chemistry, UK
Primers	Macrogen, Korea

3.3.2 Primers

The used primers in the current study are listed in the Table 3.4.

Table 3.4 primers covid 19 viral gene (Fang *et al.* 2002).

Primer Name	Sequences	Annealing Temp. (°C)	Product size (bp)
Forward primer	CTAGTCAGTGTGTTAATCTTACAACC	58	517
Reverse primer	CCCTGTTTTCCTTCAAGGTCC	58	

3.3.3 Reaction setup and thermal cycling protocol for ORF8

The reverse transcription reaction was as outlined in the Tables 3.5 and 3.6.

Table 3.5 Thermal Cycling set-up and protocol for viral ORF8

PCR Mixture	Volume
Master mix	10µl
F Primer	1µl
R Primer	1µl
Nuclease free water	5µl
cDNA	3µl
Total	20µl

Table 3.6 PCR program of ORF 1ab gene and surface protein S

steps	°C	m: s	Cycle
RT. Enzyme Activation	37	15:00	1
Initial Denaturation	95	05:00	
Denaturation	94	01:00	40
Annealing	56	01:00	
Extension	72	01:00	
Final extension	72	07:00	1

3.3.4 Genotyping method of covid-19

Sample's collections were subjected to the WHO guideline for SARS Cov2 sample collection. Samples were collected from suspected persons who visited Al-Hussein teaching hospital at Al-Nasiriya city. Outpatients with Symptoms included fever,sore through, headache, abdominal pain, and losing or reducing the sense of smell and taste include in this study for sample collection. Naso-oropharyngeal swabs were collected from each person with symptoms using (Biobase viral transport media). All samples were processed within less than two hours from the time of collection at the molecular biology laboratory (Covid-19 testing center) at Al-Hussein teaching hospital.

3.3.5 RNA extraction

All samples were processed for viral RNA extraction using an automated system using magnetic beads RNA extraction system using (Wondfo extraction kit, China) according to the manufacture instruction, 200 ul of viral transport media was mixed andr tansferred to the well (plate deep wells) that contains the lysis buffer and 20 ul of proteinase K. Negative control and positive control are also included in each extraction run for quality control purposes to assure the efficiency of extraction and monitoring contamination during extraction. Extracted RNA was used directly after extraction for RT-PCR detection of SARS-Cov-2 using a Bio-Rad RT-PCR instrument (T. Li *et al.* 2021).

3.3.6 Nano-drop estimations for DNA and RNA samples

The isolated total RNA from all samples under study were analyzed using Nano-drop spectrophotometers. All DNA and RNA were measured at 260 nm. The concentrations of DNA and RNA samples were expressed in terms of ng/µL.

3.3.7 First-strand cDNA synthesis protocol

The retro-transcription step was performed using EasyScriptTM kit (Abm, Canada) according to the instructions of the manufacturer. Firstly, the RNA samples and all reagents should be thawed on ice with thorough mixing for each solution. The following recipe of the reaction mixture shown in Table 3.7 was performed, providing that all handling steps were performed on ice to avoid loss in enzyme activity and maintain stability of the RNA samples.

Table 3.7 Recipe for reaction mixture for first strand cDNA synthesis

Component	**Volume**	**final concentration**
Total RNA or poly(A) + mRNA	Variable	1.0 ng – 2.0 µg/rxn 1.0 pg – 2.0 ng/rxn
Oligo (dT) (10 µM) or Random Primers (10 µM) or Gene-Specific Primer	1.0 µL 1.0 µL Variable	
dNTPs (10 mM each	1.0 µL	500 µM
5X RT Buffer	4.0 µL	1X
RNasin (40 U/µl)	0.5 µL	20 U/rxn
EasyScriptTM	1.0 µL	200U/rxn
Nuclease-free H_2O	Up to 20 µL	-------

rxn: reaction mixture, **U**: units

After carrying out the cDNA first strand synthesis reaction, the components in the tube should be mixed well and collected by pulse centrifugation for 30 seconds. The reaction mixture was incubated at 25°C for 10 minutes for Random Primers, this step of incubation should be omitted if Oligo (dT) or Gene-Specific Primer was used. The cDNA synthesis reaction was conducted by incubating the reaction mixture for 50 minutes at 42°C. Finally, the reaction was stopped by heating it at 85°C for 5 minutes, followed by chilling on ice. The newly synthesized first-strand cDNA is ready for immediate downstream applications, or for long-term storage at -80°C.

3.3.8 RT-PCR reaction

One step Wondfo 2019-nCoV real-Time RT-PCR assay (China) kit was used in this experiment to detect COVID-19 RNA in patient samples. Three fluorophores were included in one reaction (multiplex PCR): FAM-fluorophore (Nucleocapsid (N) gene), VIC-fluorophore (ORF 1ab gene), and CY5-fluorophore for internal control. The cycle condition was programmed according to the manufacture's instruction using a Bio-Rad RT-PCR cycler (USA). The program starts with the reverse transcriptase step which involves converting all RNA s in a sample to cDNA at 50ºCfor 10 min (1 cycle) then enzyme activation steps 95ºCfor 2 min. Cycling (40 cycles) starts with a denaturation process at 95ºC for 5 seconds then annealing and extension process at 60ºC for 32 seconds. In addition, Ct values less than or equal to 38 were considered positive for SARS Cov-2 and more than 38 is Negative. Quality control for each Reverse transcription polymerase chain reaction (RTPCR) run was included which involved non-template negative control (NTC) and positive control to assure the quality of kit materials and for monitoring contamination during extraction and reaction process. Results were valid when PC Ct is less or equal to 33 for all fluorophores. NTC more than 38 for FAM and VIC and less than or equal to 33 for CY5 internal control as in the Figure 3.1(Conte *et al.* 2021).

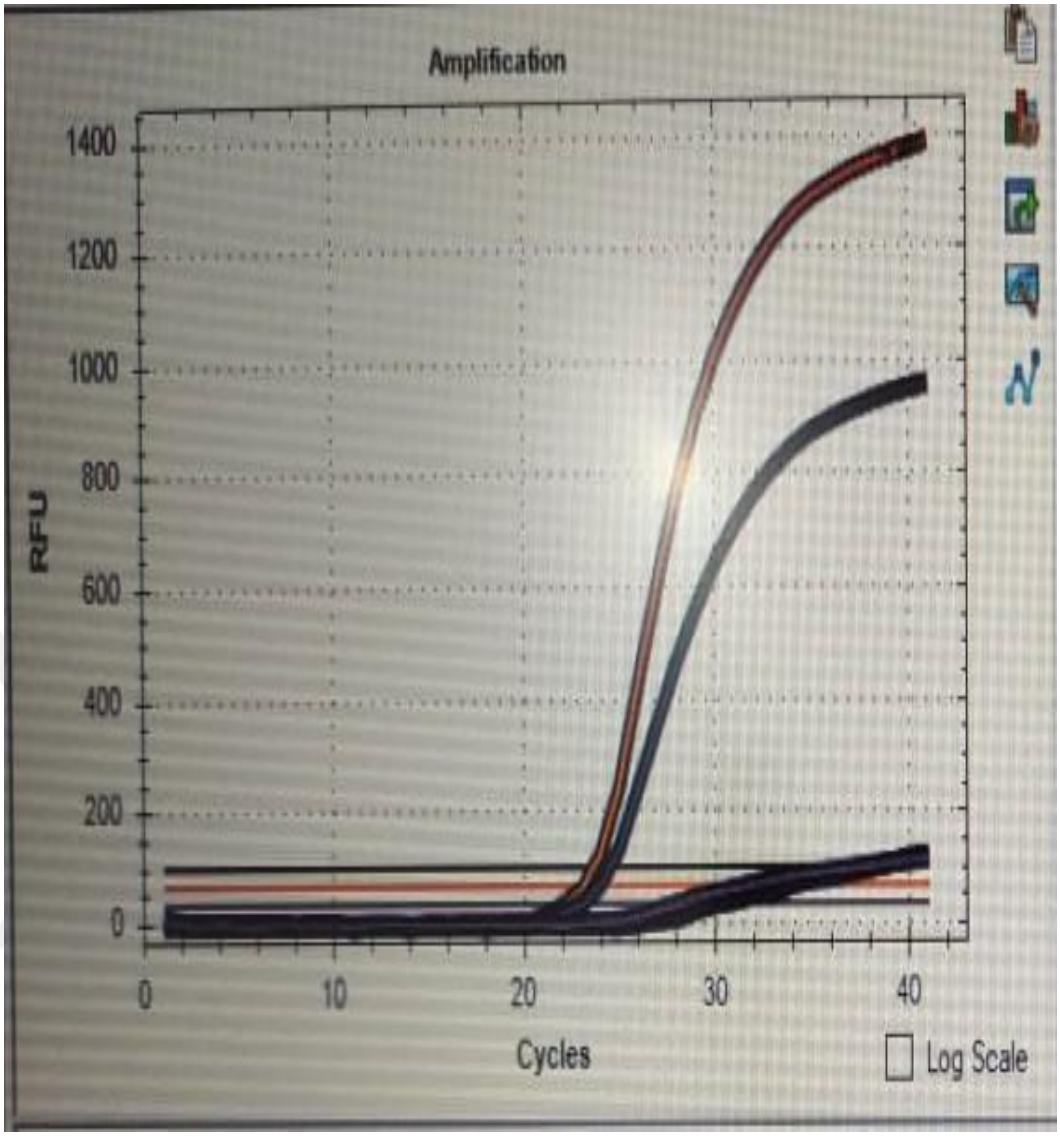

Figure 3.1 RT-qPCR relative fluorescence amplification curve plotted against cycle numbers for COVID 19

3.3.9 Agarose gel electrophoresis

After PCR expansion, amplification was confirmed by running a sample over an agarose gel and electrophoresis. The extracted DNA standards allowed for reliable PCR. The agarose must be prepared in the following way: The final concentration of 1X TAE (10 mg/ml) in the 100 ml of buffered solution was achieved with the addition of 1.5 gm (1.5%) of agarose to the bottle. The fluid was brought to a boil (in the microwave) until all of the gel particles dissolved. After the agarose had been slightly shaken to remove air bubbles, 1 µl (10 mg/ml) of ethidium bromide was added. Casting

the gel onto a horizontal agarose gel cassette occurs when the gel temperature reaches between 50 and 60 degrees Celsius. After screening both borders with cellophane tapes, agarose was poured into the gel container and allowed to set for 30 minutes at room temperature. After carefully taking off the comb, the gel was transferred on the plate. The 1X TAE-electrophoresis buffer was poured onto the plate until about 3-5 mm of buffer remained above the gel. DNA PCR products loaded directly. The PCR product, which required 5 l, was added straight to the wells. The 100v/mAmp electrical current had been on for 75 minutes. More Cathode poles are converted to Anode poles, where DNA is then relocated. In order to see the Ethidium bromide-stained gel bands, a gel imaging equipment was employed.

3.3.10 DNA sequencing

For Sanger sequencing, PCR products were sent by Macrogen Corporation – Korea, using ABI Prism 377, an automated DNA sequencer. The results were then emailed and analyzed with the appropriate genetic software.

3.4 Laboratory Test

3.4.1 Estimation immunological parameters

Estimation of anti-COVID-19 IgG and IgM:

Principle:

The 2019 nipah virus immunoassay for IgG and IgM uses chemiluminescence and indirect chemiluminescence, respectively. One of the cassettes was used for detection, and there were two total. The other is an IgG antibody specific to CoV-2. Patient samples with a CoV-2,IgM antibody that have been hashed. Once both cassettes have been processed individually, a combined report will be generated. IgG and IgM antibody testing. Sample (blood serum in a conventional sample tube or a tube with a

separator gel), buffer, and magnetic microbeads labeled with anti-human IgM monoclonal antibody are combined completely and treated to create immune complexes. After deposition, the supernatant is decanted in a magnetic field and the washing process begins. The complexes are then incubated to create full-length proteins and the manufactured 2019-nCoV antigen is added. Amino-Butyl-Ethyl-Isolubilized Nucleocapsids. After the supernatant has been deposited in a magnetic field, it is drained off and another washing cycle is started. In order to kick off the chemical chain reaction that ends up producing light, a "Starter Buffer" is added. The optical signal is converted by a photomultiplier into relative illumination units (RLUs), which are directly proportional to the amount of IgM present in the sample. The test is performed using a fully automated cobac 411. Series chemiluminescence immunoassay analyzer.

Reagent preparation:

I removed the reagent kit from the packaging and inspected the sealing film and any other components of the reagent kit for signs of leakage. due to the possibility of leakage. The buzzer rings within 2 seconds of preparing the handle of the RFID tag detector, so that one beep indicates successful sensing. Then we keep the detector listed straight down along the path of the empty detector. Noting the detector data displayed on the APP user interface, properly loaded kits with magnetic microbeads that automatically resuspend ensure they are fully suspended and homogenized before use..

Procedure:

Quantitative tests for anti-COVID-19 were measured using a method that was totally automated. using the Auto Analyzer C411 (Cobas) from the Automatic Analyzer Group. Anti COVID - 19 in human serum was measured using a specific equipment. employing a technique based on electrochemical luminescence.

Estimation of human IL-6:

Principle:

The Sandwich-ELISA method is implemented in this ELISA kit. This kit includes a micro-ELISA plate that has already been coated with an antibody targeting human interleukin-6 receptor. Separately, a biotinylated detection antibody and an Avidin-Horseradish Peroxidase (HRP) conjugate are added to each microplate well, and then the plates are incubated. At a wavelength of 450 nm, a spectrophotometer measures the optical density (OD). This was recently shown by a group of researchers (Song *et al.* 2019).

Assay procedure:

1. 100 µl of sample was added to the wells. And incubated for 90 minutes at 37°C. 2. Dispose of the liquid, and immediately 100 µl of Biodetection Ab solution was added to each well. And incubated for 60 minutes at 37°C.
2. The board was pulled out and washed 3 times.
3. A total of 100 µl of HRP conjugate working solution was included in the experiment. Thirty minutes of incubation at 37 degrees Celsius were used. Five times, the board was removed and washed.
4. Then 90 µl of substrate reagent was added. And incubated for 15 minutes at 37°C.
5. 50 µl of stop solution was added and the plate was read at 450 nm immediately. And calculate the results.

Estimation of human TNF- A:

Principle:

The microtiter strips we give have been coated with a monoclonal antibody against TNF alpha. The samples and the antibody are incubated together for the first time. Once the antibody has been washed, the enzyme StreptavidinHRP is added to bind the biotinylated antibody. Adding a TMB substrate solution activates the bound enzyme,

leading to a colorful reaction product. The amount of TNF-Alpha in the samples determines the brightness of the colored product.

Assay procedure:

All reagents are thoroughly combined just before use, with the utmost care to prevent the formation of foam in the vials. One hundred microliters of the sample and the standard are combined, After adding 50 µl l of 1X anti-TNF-alpha to each well, the plates were covered and incubated for 3 hours at room temperature (18-25°C). Three times, using 0.3 mL of 1X Wash Buffer in each well, we empty the plate and wash it. After incubating at room temperature for 30 minutes, 100 µl l of a 1X Streptavidin-HRP solution was added. After three washes of the same volume (3 * 0.3 ml), 100 µl of chromogen TMB substrate solution is added to each well, and the plate is incubated in the dark for 12-15 minutes at room temperature. Because of this, we've taken the precaution of covering the plate with aluminum foil to shield it from the sun. After the allotted time had passed, 100 µl of suspension was poured into each well. The absorbance at 450 nm (the fundamental wavelength) and 620 nm (610 nm), if used, is then read off by a spectrophotometer.

Estimation of C.R. protein:

Principle:

Detector antibodies bind sample antigens; the resulting antigen-antibody complexes migrate onto the nitrocellulose matrix, where they are caught by the other immobilized antibodies on the ichromaTM test strip using a sandwich immunodetection technique. During CRP testing, the device processes a brighter fluorescence signal produced by detection antibodies thanks to complex formation in the buffer.

Assay procedure:

We add 10 µl of blood sample to the detection buffer tube, then shake the tube 10 or more times until the sample comes out by reflection. Then we remove the cap from the top of the collector tube. We discard two drops of the reagent on the paper before applying it to the cartridge. We put only two drops of the sample mixture on the cartridge. Then we leave the cartridge at room temperature for 3 minutes. After the incubation period ends, we wipe the loaded sample cartridge with a chroma device, and the result will appear on the device screen.

3.4.2 Estimation complete blood count by coulter

The Sysmex XN2000 (Sysmex, Kobe, Japan) uses laser light scattering (forward and light scatter) and side fluorescent light to evaluate complete blood counts. It has white precursor cell channels, allowing it to count immature granulocytes and atypical lymphocytes. Fluorescence and side light scatter are used to count the nuclei of NRBCs. Measurement of reticulocyte hemoglobin equivalents and immature reticulocyte fractions is possible in the reticulocyte mode (IRF). The XN2000 can measure 44 diagnostic parameters, with 16 of them being optional. In all modes, the throughput is up to 200 tests per hour, and the specimen aspiration volume is 88 mg/lL.

3.4.3 Estimation biochemical parameters

Estimation of human vitamin D:

Principle:

The ichromaTM test uses a sandwich immunodetection method, in which detector antibodies in the buffer bind to antigens in the sample and are subsequently captured by the other immobilized antibodies on the nitrocellulose matrix.

Assay procedure:

1- 50 µL of plasma was added using a pipette to a tube containing 50 µL of the released release reagent and mixed well by a pipette 10 times, then the tube was left in the inlet tube block at 35 °C. It is stored for 5 minutes.
2- 100 µL of the diluent was added to the reagent tube containing the buffer mixture and the standing sample, mixed well (10 times), and left the tube again at 35 °C for 15 min.
3- 75 µl of the incubation mixture was transferred and the sample was well loaded onto the test cartridge. Then we push the test cartridge into the entire i-Chamber slot. and incubate for 8 minutes. The loaded sample cartridge was checked after the incubation period had ended. Read it with ichroma™ device.

Estimation of human D. dimer:

Principle

The FinecareTM D-Dimer Rapid Quantitative Test employs a sandwich immunodetection technique and is based on fluorescence immunoassay technology. The membrane-bound fluorescence-labeled detector anti-D-dimer attaches to the D-dimer antigen in the whole blood/plasma specimen when the sample is added to the sample well. The concentration of D-dimer in a sample can be viewed on the Finecare FIA Meter based on the fluorescence signal strength of the detector antibody. The system has a detection limit and operational range of 0.1–10mg/L.

Assay procedure:

Once everything was set up and we knew the ID chip was properly placed, 15 µl of whole blood were transferred to the buffer tube with a transfer pipette. After loading 75 µl of the sample combination, we tapped or inverted the tube for 1 minute to ensure thorough mixing of the sample with the buffer. Once the sample has been prepared, it is placed in the test cartridge's well. and the test cartridge is inserted into the test cartridge holder and clicked "Test." After 5 minutes, the result appears on the screen.

Estimation of human ferritin:

Principle

This examination makes use of the sandwich immunodetection method. Initially, complexes are formed when a detector recombinant protein in the buffer binds to an antibody in the sample. The complexes then move onto a nitrocellulose matrix, where they are caught by a different immobilized-antigen on the test strip. The device manipulates it so that an AFIAS ferritin test may be performed.

Assay procedure:

100 µl from the sample was taken using a pipette and the sample was well distributed on the cartridge. Then the cartridge is inserted into the cartridge holder and one of its ends is inserted into the periphery of the cartridge. Then we press the "Start" icon on the screen. After 10 minutes, the test result will be shown on the screen.

3.5 Statistical Analysis

The current study's data after collected were indexed by Excel as gender, age and then was analyzed with Statistical Package for Social Science version 26 (SPSS) by using the following statistical laws as one-way,ANOVA for mean variation, least,significant difference, dependent sample t test, and person for correlation between variance.

4. RESULTS AND DISCUSSION

4.1 Results

4.1.1 Hematological parameters in covid-19 patients and control

The present study noted a significant increase in count of WBC, percent of monocyte, lymphocyte and count of PLT in Covid-19 patients than control group. In contrast the results showed a non-significant difference in other hematological parameters at *P* value < 0.05 as show in Table 4.1.

Table 4.1 Hematological parameters in Covid-19 patients and control

Hematological Parameters	Mean + SD		*P*. value of t test
	Patients No. 100	Control No. 100	
WBC*10^3	10.9 ± 3.33	5.10 ± 0.92	< 0.001
Lymphocyte %	1.92 ± 0.58	1.72 ± 0.53	0.299
MIX%	0.68 ± 0.22	0.40 ± 0.16	< 0.001
Neutrophil %	7.60 ± 2.83	2.98 ± 0.78	< 0.001
RBC*10^6	4.63 ± 0.46	4.60 ± 0.60	0.819
Hb gm/dl	12.1 ± 1.90	12.8 ± 1.98	0.118
HCT %	38.6 ± 4.78	38.2 ± 8.37	0.786
PLT *10^3	310.9 ± 79.2	210.3 ± 54.9	< 0.001

4.1.2 Immunological parameters in covid-19 patients and control

The present study noted a significant increase in concentration of all immunological parameters CRP, IgM, IgG, IL-6 and TNF in Covid-19 patients than control group at *P* value < 0.05 as show in Table 4.2.

Table 4.2 Immunological parameters in Covid-19 patients and control

Immunological Parameters	Mean + SD		*P*. value of t test
	Patients No. 100	Control No. 100	
CRP mg/dl	88.2 ± 16.0	2.97 ± 0.38	< 0.001
IgM	17.4 ± 4.57	0.47 ± 0.22	< 0.001
IgG	1.39 ± 0.53	0.44 ± 0.14	< 0.001
IL-6 ng/L	200.3 ± 57.6	19.3 ± 5.91	< 0.001
TNF ng/L	294.4 ± 68.2	10.0 ± 2.64	< 0.001

4.1.3 Biochemical parameters in covid-19 patients and control

In this research, we looked into why Covid-19 patients had significantly higher concentrations of ferritin and D-Dimer than the control group. But as shown in Table 4.3, the concentration of VitD3 in Covid-19 patients was significantly lower than in the control group at a *P* value of < 0.05.

Table 4.3 Biochemical parameters in Covid-19 patients and control

Biochemical Parameters	Mean + SD		*P*. value of t test
	Patients No. 100	Control No. 100	
Vit-D_3	15.0 ± 4.43	35.8 ± 11.1	< 0.001
Ferritin	896.7 ± 218.9	205.8 ± 51.7	< 0.001
D-Dimer	711.9 ± 123.2	223.7 ± 68.4	< 0.001

4.1.4 Gender differences in hematological parameters among covid-19 patients

The present study noted a non-significant difference in count of WBC, percent of lymphocyte, monocyte, neutrophil, count of RBC, level of Hb and percent of HCT in groups Covid-19 patients. In contrast the results showed a significant increase in count of PLT in male than female at *P* value < 0.05 as show in Table 4.4.

Table 4.4 Hematological parameters in Covid-19 patients according to gender

Hematological Parameters	Mean + SD		*P*. value of t test
	Patients Female No. 42	Patients Male No. 58	
WBC*10^3	10.9 ± 3.42	11.0 ± 3.32	0.922
Lymphocyte %	1.84 ± 0.55	1.99 ± 0.63	0.613
MIX%	0.61 ± 0.24	0.73 ± 0.20	0.056
Neutrophil %	7.60 ± 2.89	7.60 ± 2.83	0.999
RBC*10^6	4.64 ± 0.54	4.63 ± 0.39	0.942
Hb gm/dl	12.4 ± 1.94	11.9 ± 1.88	0.376
HCT %	38.9 ± 5.71	38.4 ± 4.00	0.716
PLT *10^3	278.9 ± 59.7	336.1 ± 84.4	0.010

4.1.5 Immunological parameters in covid-19 patients according to gender

The present study noted a significant increase in concentration of IgM in female than male, while the TNF increased in male than female. On the other hand, the other immunological parameters not recorded a significant difference according to gender at *P* value < 0.05 as show in Table 4.5.

Table 4.5 Immunological parameters in Covid-19 patients according to gender

Immunological Parameters	Mean + SD		*P*. value of t test
	Patients Female No. 42	Patients Male No. 58	
CRP mg/dl	91.5 ± 13.1	85.5 ± 17.7	0.195
IgM	19.2 ± 4.77	16.0 ± 3.92	0.011
IgG	1.36 ± 0.53	1.42 ± 0.54	0.710
IL-6 ng/L	208.5 ± 50.4	193.9 ± 60.1	0.505
TNF ng/L	210.0 ± 29.1	360.8 ± 49.3	< 0.001

4.1.6 Biochemical parameters in covid-19 patients according to gender

The present study noted a non-significant difference in concentration of all biochemical parameters in Covid-19 patents according to gender at *P* value < 0.05 as show in Table 4.6.

Table 4.6 Biochemical parameters in Covid-19 patients according to gender

Biochemical Parameters	Mean + SD		*P*. value of t test
	Patients Female No. 42	Patients Male No. 58	
Vit-D_3	14.9 ± 4.13	15.1 ± 4.73	0.904
Ferritin	907 ± 235.5	888.4 ± 211.1	0.838
D-Dimer	713.7 ± 124.0	710.4 ± 124.9	0.928

4.1.7 Evaluation of WBC and their differential in covid-19 patients according to age groups

The present study noted a non-significant difference in count of WBC, percentage of lymphocyte, monocyte and neutrophil in Covid-19 infected patients according to age groups at *P* value < 0.05 as show in Table 4.7.

Table 4.7 WBC and their differential in Covid-19 patients according to age groups

Hematological Parameters	Mean + SD			
	WBC	Lymphocyte	MIX	Neutrophil
14-29 years	9.94 ± 3.20	2.18 ± 0.64	0.68 ± 0.19	6.76 ± 2.65
30-44 years	9.86 ± 2.75	1.92 ± 0.67	0.73 ± 0.21	6.72 ± 2.02
45-59 years	11.5 ± 2.94	1.83 ± 0.73	0.65 ± 0.18	8.15 ± 2.46
More than	11.7 ± 3.99	1.84 ± 0.66	0.67 ± 0.24	8.19 ± 2.58
***P*. value of ANOVA**	0.343	0.812	0.870	0.394
LSD	**Non-Sig**	**Non-Sig**	**Non-Sig**	**Non-Sig**

4.1.8 Evaluation of RBC, Hb, HCT and PLT in patients in covid-19 by age groups

The present study noted a non-significant difference in count of RBC, level of Hb, percentage of HCT and count of PLT in Covid-19 infected patients according to age groups at *P* value < 0.05 as show in Table 4.8.

Table 4.8 RBC, Hb, HCT and PLT in Covid-19 patients according to age groups

Hematological Parameters	Mean + SD			
	RBC	**Hb**	**HCT**	**PLT**
14-29 years	4.79 ± 0.43	12.5 ± 1.78	38.8 ± 4.87	302.3 ± 52.9
30-44 years	4.83 ± 0.36	12.5 ± 1.14	39.2 ± 3.78	341.7 ± 83.0
45-59 years	4.50 ± 0.53	11.7 ± 2.31	38.5 ± 4.58	289.5 ± 82.4
More than	4.53 ± 0.42	12.1 ± 1.98	38.1 ± 5.76	320.2 ± 89.5
***P*. value of ANOVA**	0.180	0.699	0.950	0.436
LSD	**Non-Sig**	**Non-Sig**	**Non-Sig**	**Non-Sig**

4.1.9 Immunological parameters in covid-19 patients according to age groups

The present study noted a non-significant difference in concentration of CRP, IgM, IgG, IL-6 and TNF in Covid-19 infected patients according to age groups at *P* value < 0.05 as show in Table 4.9.

Table 4.9 Immunological parameters in Covid-19 patients according to age groups

Immunological Parameters	Mean + SD				
	CRP	**IgM**	**IgG**	**IL-6**	**TNF**
14-29 years	87.3 ± 10.7	15.6 ± 2.60	1.32 ± 0.33	195.4 ± 66.2	318.1 ± 72.8
30-44 years	80.5 ± 20.2	17.8 ± 3.77	1.61 ± 0.57	199.3 ± 59.9	295.1 ± 94.4
45-59 years	90.8 ± 11.9	18.0 ± 4.81	1.20 ± 0.40	196.8 ± 55.7	278.0 ± 78.1
More than	90.7 ± 19.6	17.9 ± 5.79	1.51 ± 0.45	208.2 ± 53.3	293.1 ± 69.8
***P*. value of ANOVA**	0.420	0.524	0.238	0.973	0.723
LSD	**Non-Sig**	**Non-Sig**	**Non-Sig**	**Non-Sig**	**Non-Sig**

4.1.10 Biochemical parameters in covid-19 patients according to age groups

The present study noted a non-significant difference in concentration of $VitD_3$, ferritin and D-dimer in Covid-19 infected patients according to age groups at P value < 0.05 as show in Table 4.10.

Table 4.10 Biochemical in Covid-19 patients according to age groups

Biochemical Parameters	Mean + SD		
	Vitamin D_3	**Ferritin**	**D-Dimer**
14-29 years	15.3 ± 3.72	920.2 ± 264.5	693.7 ± 112.0
30-44 years	13.4 ± 3.19	998.8 ± 267.4	725.6 ± 126.6
45-59 years	13.8 ± 3.27	876.7 ± 291.1	696.9 ± 144.0
More than	17.0 ± 3.11	838.2 ± 288.9	732.0 ± 115.1
***P*. value of ANOVA**	0.138	0.684	0.818
LSD	Non-Sig	Non-Sig	Non-Sig

4.1.11 Molecular study

The current study involved evaluation of ORFI gene and N gene in Covid-19 patients in general as show in Table 4.11.

Evaluate viral load of ORF1 gene and N gene:

The current study recorded the gene expression of N gene was higher than ORF1 gene in Covid-19 patients.

Table 4.11 Evaluate viral load of ORF1 gene and N gene in Covid-19 patients

Gene	Mean ± SD	Range	Min	Max
ORF1ab Gene	863,868.2 ± 201,953.9	9,905,495.3	1,389.1	9,906,884.4
N Gene	1,851,203.9 ± 3,333,699. 1	14,808,807.6	491.1	14,809,298.8

Evaluate viral load of ORF1 gene and N gene according to gender:

The present study investigated the mean of ORF1 gene and N gene in male than female of Covid-19 patients at *P* value < 0.05 as shown in Table 4.12.

Table 4.12 Viral load of ORF1 gene and N gene according to gender

Gene	gender	Mean ± SD	*P*. value
ORF1ab Gene	**Male No. 58**	1,296,947.5 ± 2,407,333.3	**0.048**
	Female No. 42	45,829.6 ± 62,992.2	
N Gene	**Male No. 58**	2,771,949.6 ± 3,840,497.3	**0.012**
	Female No. 42	112,017.5 ± 163,166.3	

Evaluate viral load of ORF1 gene and N gene according to age groups:

The present study investigated a non-significant difference in t mean of ORF1 gene and N gene in of Covid-19 patients according to age groups at *P* value < 0.05 as shown in Table 4.13.

Table 4.13 Viral load of ORF1 gene and N gene according to age

Age	ORF1ab Gene	N Gene
14-29 years	14,521.4 ± 12265.3	272,105.4 ± 466,784.3
30-44 years	605,049.2 ± 633470.2	2,732,538.1 ± 2,732,538.1
45-59 years	1,399,363.6 ± 3454929.2	2,915,087.3 ± 5,335,277.0
More than	996,033.6 ± 1,305,901.6	1,216,877.0 ± 1,326,791.6
***P*. value**	0.727	0.592

Agarose gel electrophoresis:

After staining with 0.5 mg/ml Ethidium bromide, PCR products were seen on 1.5% agarose gels using a gel documentation system. When the base pairs in the DNA bands of the samples matched those of the desired product, the findings were considered positive. In the end, the Biometra gel documentation system was used to take photographs of the gel Figure 4.1.

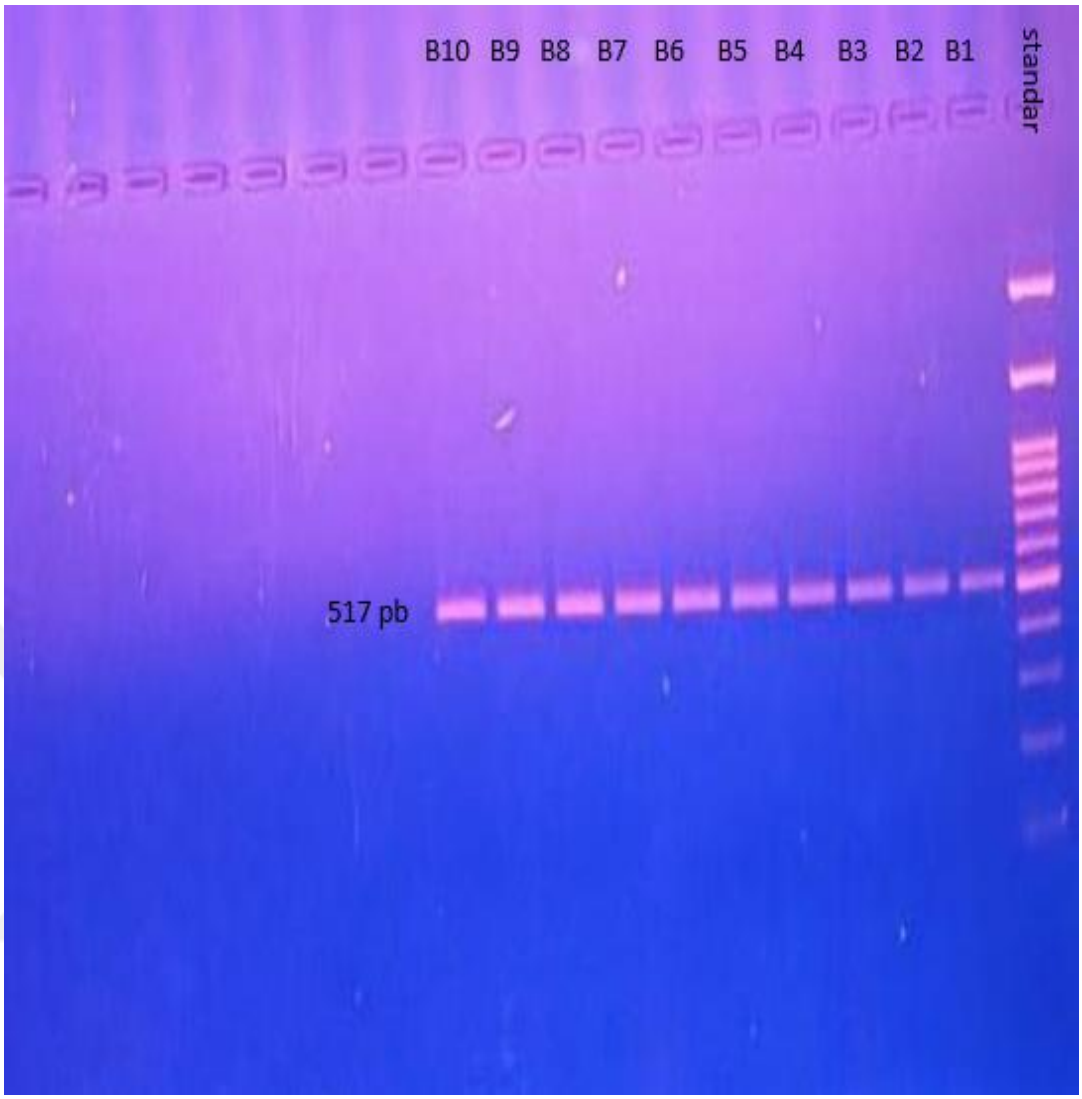

Figure 4.1 5% agarose gel electrophoresis showing PCR amplification of the surface protein F(517pb) from representative samples. Lane M: DNA ladder

Analysis of DNA sequences for seven loci housekeeping genes:

Obtained DNA sequences were analyzed using Bioedit software version 7.5. The sequences were cleaned to trim the overlapped, uncertain nucleotides from the entire sequence. The trimmed, clean DNA sequences were analyzed using the Blastn NCBI database online server, where each DNA sequence was deposited along with the DNA sequences of this locus. After conducting the multiple sequence alignment on the online server of the NCBI database, Where 10 samples were sent and recorded in the genetic bank according to the following symbols shown in the Table 4.14.

Table 4.14 Recorded of COVID 19 strains in GenBank.

ISOLATES CODE	ACCESSION NO. OF NEW STRAIN	SEQUENCE ID WITH COMPARE
B1 Iraq	OQ457464	OQ332345.1
B2 Iraq	OQ457298	OQ332344.1
B3 Iraq	OQ457312	OM739132.1
B4 Iraq	OQ457302	OM739128.1
B5 Iraq	OQ457313	OM739050.1
B6 Iraq	OQ457314	OM897799.1
B7 Iraq	OQ457315	MZ281970.1
B8 Iraq	OQ457461	OM143709.1
B9 Iraq	OQ457462	OL901822.1
B10 Iraq	OQ457463	MZ281970.1

Sample 1 lcl|Query_49529

Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/IRQ / Nasiriyah1/2022 surface glycoprotein (S) gene, partial cds, Sequence ID: OQ457464.1 Length: 390Number of Matches: 1

Range 1: 1 to 390

Score	Expect	Identities	Gaps	Strand
721 bits(390)	0.0	390/390(100%)	0/390(0%)	Plus/Plus

```
Query  1    GTTCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GTTCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCAA  60

Query  61   TGGTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   TGGTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTC  120

Query  121  CACTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGAC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CACTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGAC  180

Query  181  CCAGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CCAGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCA  240
```

```
Query  241  ATTTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  ATTTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGA  300

Query  301  AAGTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTAAGCC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  AAGTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTAAGCC  360

Query  361  TTTTCTTATGGACCTTGAAGGAAAACAGGG  390
            |||||||||||||||||||||||||||||
Sbjct  361  TTTTCTTATGGACCTTGAAGGAAAACAGGG  390
```

Sample 2 cl|Query_62497

Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/ IRQ / Nasiriyah2/2022 surface glycoprotein (S) gene, partial cds ,Sequence ID: OQ457298 .1 Length: 388Number of Matches: 1

Range 1: 1 to 388

Score	Expect	Identities	Gaps	Strand
717 bits(388)	0.0	388/388(100%)	0/388(0%)	Plus/Plus

```
Query  1    TCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCAATG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCAATG  60

Query  61   GTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTCCA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTCCA  120

Query  121  CTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGACCC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGACCC  180

Query  181  AGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCAAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  AGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCAAT  240

Query  241  TTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGAAA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  TTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGAAA  300

Query  301  GTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTAAGCCTT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  GTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTAAGCCTT  360

Query  361  TTCTTATGGACCTTGAAGGAAAACAGGG  388
            |||||||||||||||||||||||||||
Sbjct  361  TTCTTATGGACCTTGAAGGAAAACAGGG  388
```

Sample 3 lcl|Query_338823

Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/IRQ/ Nasiriyah3/ 2022 surface glycoprotein (S) gene, partial cds, Sequence ID: OQ457312.1 Length: 388Number of Matches: 1

Range 1: 1 to 388

Score	Expect	Identities	Gaps	Strand
717 bits(388)	0.0	388/388(100%)	0/388(0%)	Plus/Plus

```
Query  1    TCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCAATG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCAATG  60

Query  61   GTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTCCA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTCCA  120

Query  121  CTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGACCC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGACCC  180

Query  181  AGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCAAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  AGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCAAT  240

Query  241  TTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGAAA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  TTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGAAA  300

Query  301  GTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCCTT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  GTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCCTT  360

Query  361  TTCTTATGGACCTTGAAGGAATACAGGG  388
            ||||||||||||||||||||||||||||
Sbjct  361  TTCTTATGGACCTTGAAGGAATACAGGG  388
```

Sample 4 lcl|Query_60219

Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/IRQ/Nasiriyah4/ 2022 surface glycoprotein (S) gene, partial cds, Sequence ID: OQ457302.1 Length: 388Number of Matches: 1

Range 1: 1 to 388

Score	Expect	Identities	Gaps	Strand
717 bits(388)	0.0	388/388(100%)	0/388(0%)	Plus/Plus

```
Query  1    TCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCAATG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCAATG  60

Query  61   GTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTCCA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTCCA  120

Query  121  CTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGACCC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGACCC  180

Query  181  AGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCAAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  AGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCAAT  240
```

```
Query  241  TTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGAAA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  TTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGAAA  300

Query  301  GTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCCTT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  GTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCCTT  360

Query  361  TTCTTATGGACCTTGAAGGAATACAGGG  388
            |||||||||||||||||||||||||||
Sbjct  361  TTCTTATGGACCTTGAAGGAATACAGGG  388
```

Sample 5 lcl|Query_155447

Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/IRQ/Nasiriyah5/ 2022 surface glycoprotein (S) gene, partial cds, Sequence ID: OQ457313.1 Length: 391Number of Matches: 1

Range 1: 1 to 391

Score	Expect	Identities	Gaps	Strand
723 bits(391)	**0.0**	**391/391(100%)**	**0/391(0%)**	**Plus/Plus**

```
Query  1    TGTTCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TGTTCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCA  60

Query  61   ATGGTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   ATGGTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTT  120

Query  121  CCACTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CCACTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGA  180

Query  181  CCCAGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CCCAGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTC  240

Query  241  AATTTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  AATTTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGG  300

Query  301  AAAGTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  AAAGTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGC  360

Query  361  CTTTTCTTATGGACCTTGAAGGAATACAGGG  391
            |||||||||||||||||||||||||||||||
Sbjct  361  CTTTTCTTATGGACCTTGAAGGAATACAGGG  391
```

Sample 6 lcl|Query_6147

Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/IRQ/Nasiriyah6/ 2022 surface glycoprotein (S) gene, partial cds, Sequence ID: OQ457314.1 Length: 395Number of Matches: 1

Range 1: 1 to 395

Score	Expect	Identities	Gaps	Strand
730 bits(395)	0.0	395/395(100%)	0/395(0%)	Plus/Plus

```
Query  1    GTTCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GTTCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCAA  60

Query  61   TGGTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   TGGTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTC  120

Query  121  CACTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGAC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CACTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGAC  180

Query  181  CCAGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CCAGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCA  240

Query  241  ATTTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  ATTTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGA  300

Query  301  AAGTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  AAGTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCC  360

Query  361  TTTTCTTATGGACCTTGAAGGAAAACAGGGTAGTT  395
            |||||||||||||||||||||||||||||||||||
Sbjct  361  TTTTCTTATGGACCTTGAAGGAAAACAGGGTAGTT  395
```

Sample 7 lcl|Query_32757

Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/IRQ/Nasiriyah7/ 2022 surface glycoprotein (S) gene, partial cds, Sequence ID: OQ457315.1 Length: 386Number of Matches: 1

Range 1: 1 to 386

Score	Expect	Identities	Gaps	Strand
713 bits(386)	0.0	386/386(100%)	0/386(0%)	Plus/Plus

```
Query  1    GTTCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GTTCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCAA  60

Query  61   TGGTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   TGGTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTC  120

Query  121  CACTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGAC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CACTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGAC  180

Query  181  CCAGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CCAGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCA  240
```

```
Query  241  ATTTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  ATTTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGA  300

Query  301  AAGTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  AAGTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCC  360

Query  361  TTTTCTTATGGACCTTGAAGGAAAAA  386
            |||||||||||||||||||||||||
Sbjct  361  TTTTCTTATGGACCTTGAAGGAAAAA  386
```

Sample 8 lcl|Query_22893

Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/IRQ/ Nasiriyah8/ 2022 surface glycoprotein (S) gene, partial cds, Sequence ID: OQ457461.1 Length: 390Number of Matches: 1

Range 1: 1 to 390

Score	Expect	Identities	Gaps	Strand
721 bits(390)	0.0	390/390(100%)	0/390(0%)	Plus/Plus

```
Query  1    GTTCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GTTCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCAA  60

Query  61   TGGTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   TGGTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTC  120

Query  121  CACTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGAC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CACTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGAC  180

Query  181  CCAGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CCAGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCA  240

Query  241  ATTTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  ATTTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGA  300

Query  301  AAGTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  AAGTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCC  360

Query  361  TTTTCTTATGGACCTTGAAGGAAAACCGGG  390
            |||||||||||||||||||||||||||||
Sbjct  361  TTTTCTTATGGACCTTGAAGGAAAACCGGG  390
```

Sample 9 lcl|Query_64523

Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/IRQ/ Nasiriyah9/ 2022 surface glycoprotein (S) gene, partial cds, Sequence ID: OQ457462.1 Length: 390Number of Matches: 1

Range 1: 1 to 390

Score	Expect	Identities	Gaps	Strand
721 bits(390)	0.0	390/390(100%)	0/390(0%)	Plus/Plus

```
Query  1    GTTCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GTTCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCAA  60

Query  61   TGGTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   TGGTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTC  120

Query  121  CACTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGAC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CACTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGAC  180

Query  181  CCAGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CCAGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCA  240

Query  241  ATTTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  ATTTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGA  300

Query  301  AAGTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  AAGTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCC  360

Query  361  TTTTCTTATGGACCTTGAAGGAAAACCGGG  390
            ||||||||||||||||||||||||||||||
Sbjct  361  TTTTCTTATGGACCTTGAAGGAAAACCGGG  390
```

Sample 10 lcl|Query_551091

Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/IRQ/Nasiriyah10/ 2022 surface glycoprotein (S) gene, partial cds, Sequence ID: OQ457463.1 Length: 386Number of Matches: 1

Range 1: 1 to 386

Score	Expect	Identities	Gaps	Strand
713 bits(386)	0.0	386/386(100%)	0/386(0%)	Plus/Plus

```
Query  1    GTTCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GTTCTTACCTTTCTTTTCCAATGTTACTTGGTTCCATGCTATACATGTCTCTGGGACCAA  60

Query  61   TGGTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   TGGTACTAAGAGGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTTATTTTGCTTC  120

Query  121  CACTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGAC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CACTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTCGAAGAC  180

Query  181  CCAGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CCAGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCA  240
```

```
Query  241  ATTTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  ATTTTGTAATGATCCATTTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGA  300

Query  301  AAGTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  AAGTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGCC  360

Query  361  TTTTCTTATGGACCTTGAAGGAAAAA  386
            |||||||||||||||||||||||||
Sbjct  361  TTTTCTTATGGACCTTGAAGGAAAAA  386
```

4.2 Discussion

4.2.1 Hematological parameters in covid-19 patients and control

The present study noted a significant increase in count of WBC, percent of monocyte, lymphocyte and count of PLT in Covid-19 patients than control group. According to gender the study noted a non-significant difference in count of WBC, percent of lymphocyte, monocyte, neutrophil, count of RBC, level of Hb and percent of HCT in groups Covid-19 patients, while the PLT increased significantly in male than female of Covid-19 patients. According to age groups of patients, the study showed a non-significant difference in hematological parameters.

The study of (Szklanna *et al.* 2021) their study revealed a relationship between hematological parameters and their relationship to the severity of infection, and the study noted the count of WBC were increased at the onset of infection, after that the patients suffered from leucopenia , also noted the count of PLT 108.83 × 10^3 platelets/ml are indicative the infection of Covid-19 severity. The study of (Taj *et al.* 2021), investigated the severity of infection not effect on Hb level; Count of WBC was increased in mild and critical status of infection, the PLT increased with severity of infection. Hematological complications of SARS-Cov-2 disease are associated with a poor prognosis (Shah *et al.*2020). Als the study of (Urbano *et al.*2022), recorded a non-significant difference in hematological parameters according to age groups, also, noted the count of WBC and PLT increased in patients at intensive care unit, while lymphocyte, Hb and neutrophil decrease in patients at intensive care unit. Also, noted a non-significant difference according to gender of patients in Covid-19 patients.

In contrast a Turkish study performed by (Usul *et al.* 2020), disagreed with current study, their study investigated the Count of WBC, lymphocyte and PLT were decrease significantly, while neutrophil increase in Covid-19 patients than control. These results may also be-affected by anther causes, such as the presence of anemia or comorbidities, habits such as smoking, or other causes such as bacterial co-infections that lead to an elevated level of white blood cells and their differential.

the higher leukocyte count, higher neutrophil percentage, lower lymphocyte percentage or increased platelet count during a hospital stay are more likely to end up in the ICU. The same is observed if there is a decrease in red blood cells, hemoglobin and average body size. Elevated LDH on admission to the hospital may be an indication for final admission to the intensive care unit (Liu *et al.* 2020).

4.2.2 Immunological parameters in covid-19 patients and control

The present study investigated a significant increase in concentration of all immunological parameters CRP, IgM, IgG, IL-6 and TNF in Covid-19 patients than control group, according to gender the study recorded a significant increase in concentration of IgM in female than male, while the TNF increased in male than female, while other parameters not recorded a significant difference, according to age groups the study revealed a non-significant difference in concentration of all immunological parameters.

The current study agreed with study of (Sun *et al.* 2020), their study recorded the concentration of IgG was decreased with increase CRP concentration, and the patients in intensive care unite have lowest concentration of IgG and highest concentration of CRP compares with non-ICU patients, while IgM concentration has a positive correlation with CRP concentration. Also, the current study agreed with study of (Devreese *et al.* 2020), their study classified levels of IgG and IgM according to complication of Covid-19 patients, and investigated the Covid-19 patients without chronic disease have low level of IgM and high level of IgG, and patients with clotting marabous tissue outside the body circuit, followed by patients with Cardiovascular

thrombosis, while level of IgG increased in patients with, but all patients recorded high level compared with control group. The study of (Maine *et al.* 2020), involved to investigate whether IgG and/or IgM levels correlate with disease severity, we assessed disease severity in a subset of patients based on their oxygen requirements and whether patients were intubated, and noted there was no clear correlation between the IgG index value and the severity of COVID-19, in patients who were severely ill or in critical condition, the IgM level appeared to be higher 3 to 4 weeks after the onset of symptoms than in patients with mild to moderate disease. Despite the difference in the CoV-2 IgM test that has recently become available and compare each form with the Abbott IgG test. The Infectious Diseases Society of America (IDSA) recommends the use of serological testing to diagnose symptomatic patients with significant clinical suspicion and frequently negative molecular test results (Hanson *et al.* 2020). This finding indicates that early switching from IgM to IgG may help predict a better outcome for COVID-19 disease.

TNF-alpha is one of the endogenous pyrogens that induces fever and raises the core temperature of the body. It also has frictional, caloric, and painful qualities. TNF-alpha is responsible for activating and amplifying acute inflammatory responses. Large quantities of the cytokine TNF may be harmful to organ systems. The contraction of the myocardium may be inhibited by TNF-, which can also result in a drop in blood pressure and shock. TNF- is responsible for the activation of tissue factor, a factor that is involved in the blood coagulation cascade (Kalliolias and Ivashkiv 2016). IL-6 is a pro-inflammatory cytokine that has a function in the acute inflammatory response. IL-6 enhances leukocyte migration to the site of inflammation, which in turn increases antibody production by B cell proliferation. Additionally, IL-6 plays a role in the differentiation of Th17 cells into T cells. The intracellular sequence of Janus kinases and signal transducers and activators of transcription (also known as Jak/STAT) is activated when IL-6 is present. In non-immune cells, the inflammatory cascade of IL-6 that is mediated by STAT3 creates a positive feedback loop that may be referred to as an IL-6 activator or IL-6 modulator (AMP). Because of this, NF-B becomes overactive, which leads to the production of a huge number of cytokines, a phenomenon that is sometimes referred to as a "cytokine storm." It is well established that lethal

complications such as acute respiratory distress syndrome, severe pneumonia, multiple organ failure, and thrombosis may be brought on by a cytokine storm in COVID-19 patients (Merza *et al.* 2021).

The IL-6 was increased 10-fold than control group and TNF was increased twenty-nine-fold than control, the increased pro-inflammatory indicates activation of the immune response against Covid-19 infection and this study agreed with finding study of (Han *et al.* 2020), their study revealed IL-6 and CRP showed increased expression with disease severity but not TNF-α.. The study of (Wei *et al.* 2020), showed the decrease concentration of VitD3 can increase disease severity and the VitD3 has a negative correlation with severity of disease by increasing the production of cytokines. The study of (Halim *et al.* 2022), reported the IL-6 has a positive correlation with disease severity, while non-significant difference in TNF concentration according severity of Covid-19 patients.

When the individual's measurements were plotted, this tendency was able to be observed much more clearly. Although substantial differences may be shown between the critical and moderate groups when analyzed by both IL-6, this may be because there was a small number of participants in the study. Some individuals, especially the elderly and the ill, may have a dysfunctional immune system that fails to keep the response to specific microorganisms in control for reasons that aren't totally understood. This may be the case because these people are more likely to be sick. This may result in an immune response that is not under control, which can lead to an excessive generation of immune cells and the signaling molecules produced by those cells, as well as the cytokine storm that is often associated with an influx of immune cells into the lung. SARS-CoV-2 induces excessive and inadequate host immune responses, which are linked with severe lung illness, which ultimately leads to mortality. This is similar to what SARS-CoV and MERS-CoV do (Huang *et al.* 2020b, Zumla and Hui 2019)

4.2.3 Biochemical parameters in covid-19 patients and control

According to the findings of the research, the concentration of ferritin and D-Dimer were substantially higher in the Covid-19 patients than in the control group, although the concentration of VitD3 was significantly lower in the Covid-19 patients. The study found no statistically significant differences between the groups with regard to the gender of the patients. In addition, the research did not find a statistically significant difference across age groups.

The overproduction of pro-inflammatory cytokines in Covid-19 patients is the mechanism that drives the cytokine storm, which ultimately results in acute lung injury and failure of multiple organs. Patients diagnosed with Covid-19 have been shown to have increased levels of serum ferritin and D-dimer. Their findings recorded that both ferritin and D-dimer was higher in Covid-19 patients than control group, and non-difference between gender of patients, also, their study also showed that the high concentration predicts the progression of the severity of the disease. The finding of study obtained by (Qeadan *et al.* 2021), was similar to study finding. Additionally, the research conducted by (Deng *et al.* 2021), shown that the concentration of ferritin was considerably greater in the critical group as compared to the moderate and severe groups. The median ferritin concentration in the group that passed away was roughly three times higher than the concentration found in the group that lived. In addition, a number of studies, such as (Galland *et al.* 2021, Pérez-García *et al.* 2022), found that patients infected with Covid-19 have a higher concentration of ferritin and D-dimer, and that the concentrations of these two proteins have a positive correlation with the prognosis of the severity of the disease. The invasion of many immune cells, such as Th1 cells, CD14+CD16+ monocytes, macrophages, and neutrophils, helps to bring about the immunological and inflammatory response that is caused by SARS-Cov-2 after it has evaded the respiratory system. This reaction is caused by SARS-Cov-2. This results in the emergence of a cytokine storm, which includes the highly expressed IL-6, IL-8, IL-10, TNF-, monocyte chemoattractant protein-1, CRP, and ferritin. Additionally, this causes an increase in the number of monocytes that produce ferritin (Yan *et al.* 2022). In addition to its function as a biomarker for determining the amount

of iron in the body, serum ferritin is also a sign of whether or not a patient will survive some severe conditions, such as hemodialysis and sepsis (Shoji *et al.* 2017).

Patients who already had low initial ferritin and D-dimer values and rising recurring values that subsequently diverge have a higher risk of developing cytokine storm syndrome (CSS), which can lead to the need for invasive ventilation and death in the hospital. Patients who have already had low initial ferritin and D-dimer values are more likely to develop CSS. However, there is a little probability that those whose levels of ferritin and D-dimer were high or low at an early stage and remained steady may develop CSS, need ventilation, or fade away. Because genetic susceptibility factors may play a role in the development of CSS, this may suggest that certain groups of sensitive individuals have distinct biochemical and physiological response mechanisms during SARS-CoV-2 infection (Chen *et al.* 2021, Zietz *et al.* 2020).

In a research that was carried out in the United States on healthy women, it was discovered that there was a strong negative association between blood levels of 25(OH)D and TNF-alpha (Weir *et al.* 2020). In a separate piece of research, it was observed that individuals who lacked vitamin D had elevated levels of the protein IL6 (Giannis *et al.* 2020). Vit-D3 has been demonstrated to downregulate the production of inflammatory cytokines, such as TNF-alpha and IL6, while simultaneously raising the production of inhibitory cytokines in a number of animal studies as well as in vitro cell models. According to the findings of these research, it is possible that maintaining healthy levels of vitamin D might lessen the likelihood of cytokine storms occurring in Covid-19 patients (Rhodes *et al.* 2021). According to the findings of the research, persons who are older than 65 and those who already have many health conditions are at an increased risk for Covid-19. Vit-D3 has been shown to be a risk factor for Covid-19, particularly in instances that are severe or serious. Although further research is required, there is a possibility that taking a vitamin D3 supplement might help prevent or cure the condition known as Covid-19.

4.2.4 Molecular study

The current study recorded the gene expression of N gene was higher than ORF1 gene in Covid-19 patients. According to gender of patients the study investigated the mean of ORF1 gene and N gene were higher in male than female of Covid-19 patients.

There have been reports of a variety of different primer-probe combinations that can detect SARS-CoV-2 using an RT-qPCR test. However, it is possible that the sensitivity of these tests is not high enough to confirm individuals who are thought to have Covid-19 infection at an early stage of the disease. 17,18 In this investigation, we used particular probe sets that had been published before to conduct RT-qPCR analysis. These probe sets targeted the N region and the RdRp/Orf1 region of SARS-CoV-2. The NIID 2019-nCOV N and ORF1ab among-probe sets provided by WHO indicate a suggested performance of RT-qPCR. These sets were developed by the Japan NIID and the China CDC, respectively.

Molecular diagnostic examination of Covid-19 without the use of non-specific amplification or the cross-reactivity of RNA from MERS-CoV, hCoV-229E, or OC43. There was no significant problem with the sensitivity of the primer-probe sets that were advised, despite the fact that certain heterojunctions were discovered at the binding sites of the primer-probe sets in question. It will be necessary to create primer-probe sets for target genes that are more precise by comparing the numerous genomes of SARS-CoV-2 (Nalla *et al.* 2020, Vogels *et al.* 2020).

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

The study was concluded the following:

1. It was shown that, with the exception of neutrophils, all blood parameters were higher in Covid-19 patients compared to the control group.2. The immune response in Covid-19 was significantly higher.
2. The over production of ferritin and D-dimer and Vit-D3 were have positive correlate with disease severity.
3. The immune response in female was higher than male group.
4. The age not effect on level of involved parameters.
5. The gene expression in male was higher than female.

5.2 Recommendation

The study recommended the following:

1. Compare the involved parameters according to disease severity.
2. A correlation study between gene expression and concentration of pro-inflammatory cytokines.
3. Study of vitamin D supplementation and its effect on disease severity
4. Better understanding of the Iraqi variations and their impact on the vaccination campaign requires next-generation sequencing (NGS) of SARS-CoV-2 from Thi-Qar stains.

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APPENDICES

APPENDIX 1. The decision of the search committee in Arabic
APPENDIX 2. The decision of the search committee in Turkish
APPENDIX 3. Facilitate the task