Republic of Iraq
Ministry of Higher Education and Scientific Research
University of Babylon
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Role of Some Genetic Markers in The Prediction of Coronavirus Disease 2019

A Thesis

Submitted to the Council of the College of Medicine / University of Babylon in Partial Fulfillment of The Requirements for The Degree of a Doctorate of Philosophy of Science in Medical Microbiology

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ
دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ۞

صَدَقَ اللهُ العَظِيم

سورة المجادلة الآية (١١)

Supervision Certification

We certify that this thesis, entitled **"Role of Some Genetic Markers in The Prediction of Coronavirus Disease 2019"** has been prepared under our supervision by **"Ali Talib Abdulhamza Mohammed"** at the Department of Microbiology/ College of Medicine/ University of Babylon, as a partial requirement for the degree of a **Doctorate of Philosophy of Science in Medical Microbiology.**

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We, the examination committee, certify that we have read the thesis entitled **"Role of Some Genetic Markers in The Prediction of Coronavirus Disease 2019"** and have examined the student **"Ali Talib Abdulhamza Mohammed"** in its contents, and that in our opinion it is accepted as a thesis for **Degree of Doctorate of Philosophy of Science** in **Medical Microbiology** with **Excellent** estimation.

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Dedication

To my father, *who is the symbol, taught me to be standing in the face of difficulties and paved the way. You will remain the sanctuary and the bond whom I be relied.*

To my mother, *who have overwhelmed me with her love and kindness, the main reason behind my success.*

To my lovely wife, *who is the part that makes me completed to be able to overtake the difficulties, with him my life has been easier and better. Gave me confidence, support, love and kindness.*

To my daughter Rose, my son Rayan *who are the endless love and the secret of my existence.*

To my brothers and my sisters, *who share me my hope and supported my effort.*

To everyone *helped and supported me.*

Ali (2023)

Acknowledgements

First of all, I wish to thank "Allah" the most gracious and most merciful who helped me in achieving this goal

I would like to express my special thanks to the Dean of the College of Medicine ***Prof. Dr. Mohend Abbass Al-Shalah*** *and the Head of Microbiology Department* ***Prof. Dr. Hayam Khalis Al-Masoudi*** *for their assistance.*

I would like to express fully gratefulness to my supervisors ***Prof. Dr. Ifad Kerim Al-Shibly*** *and* ***Assistant Prof. Dr. Mohammed Hasan Al-Hamdani*** *for their guidance and support throughout my study.*

Deep sincere thanks to ***Staff of Microbiology Department*** *for their helping and supporting me throughout my study.*

Special thanks to ***Dr. Hassan Hachim Naser*** *at college of veterinary/ University of AL-Qadisiyah, for helping me during the course of laboratory work.*

Also, I extended my thanks to ***Dr. Thair Wali Ali*** *at college of Medicine/ University of Al-Qadisiyah, for helping me in statistical analysis of this study.*

I express my thanks to ***all patients and people*** *who were included in my study.*

Ali (2023)

Summary

Coronavirus disease 2019 (COVID-19) is a highly contagious viral illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Researchers are still learning about the role of genetic markers in the prediction of COVID-19, but several studies have identified associations between specific genetic variants and an increased risk of severe COVID-19. This study aims to provide an overview of the potential link between COVID-19 and selected biological markers including, micro ribonucleic acid (miRNAs), angiotensin converting enzyme 2 (ACE2) and dipeptidyl peptidase 4 (DPP4), and to evaluate the association of *DPP4* (*rs3788979* A/G) gene polymorphisms and human leukocyte antigen (HLA) alleles genotyping with the susceptibility and severity to SARS-CoV-2 infection, and to evaluate levels of serum ACE2 and serum DPP4 among patients in Babylon province.

The frequency distribution of patients with COVID-19 according to age group revealed that the disease is most frequently seen in the age group from 40 to 49 years (24 %) and is less frequently encountered in the age group between 30 to 39 years (4 %). Males were more frequently seen in severe group in comparison with mild group, 72 % versus 48 %, respectively, so there was no significant difference in the frequency distribution of subjects between patients group and control group. The patients group included 60 (60.0 %) males and 40 (40.0 %) females, making the male to female ratio at 1.5:1.

With respect to residency, patients with COVID-19 were categorized into 72 (72.0 %) and 28 (28.0 %) as belonging to urban areas and rural areas, respectively. Control group included 36 (72.0 %) subjects from urban areas and 14 (28.0 %) subjects from rural areas. There was no significant difference

between patients group and control group in the frequency distribution of participants based on residency.

Essential hypertension was seen only in patients group accosting for 34 % and therefore, statistically the difference was significant. The patients with COVID-19 who have diabetes mellitus (DM) was only seen in patients group when contrasted to control group, 8 % versus 0 %, respectively; however, the difference was statistically not significant.

The rate of vaccination in the patients group was 4 %, whereas, the control group included 52 % rate of vaccination thus the difference was statistically significant.

Comparison of biochemical, serological, and hematological characteristics of patients with COVID-19 according to severity of disease showed that the mean Hemoglobin A1c, random blood sugar, level of serum D-dimer, serum C-reactive protein (CRP), and the white blood cell count were significantly higher in severe group compared to mild group. While, Lymphopenia was seen higher in mild group compared to severe group.

The comparison of miR-423 and miR-195 expression level between patients and control groups was significantly higher in patients with COVID-19 in comparison with control group. While, the level of miR-23a was significantly lower in patients with COVID-19 in comparison with control group. The miRNA expression was no significant difference in the levels of miR-423, miR-195 and miR-23a between mild group and severe group.

Comparison of *DPP4* (*rs3788979* A/G) genotypes between patients group and control group. The genotype AA was considered as reference because it was the most frequently encountered genotype in all cases as well as in control group. Genotype AG was most frequently seen in patients group in comparison with control group, 52 % versus 28 %, respectively and the difference was significant;

the odds ratio was 2.79, thus this genotype can be considered as a risk factor. Genotype GG was less frequently seen in patients group in comparison with control group, 0 % versus 20 %, respectively and the difference was significant; the odds ratio was 0.03, thus this genotype can be considered as a protective factor. The level of DPP4 was significantly lower in patients with COVID-19 in comparison with control group.

In this study, HLA distribution study revealed that *HLA-B*07:03* and *DRB1*12:02* were significantly associated with COVID-19 and can be considered as risk factors. *HLA-B*46:01* and *HLA-DRB1*03:01* were not significantly associated with the disease. The frequency rates of *HLA-B*07:03* was significantly less in severe group in comparison with mild group. The expression of *HLA-B*46:01* was significantly more in severe group in comparison with mild group. There was no significant difference in rates of *HLA-DRB1*03:01* and *DRB1*12:02* genes expression between severe group and mild group. Finally, the level of ACE2 was significantly higher in patients with COVID-19 in comparison with control group.

In conclusion, these genetic markers could potentially be used to develop genetic tests to identify people at high risk of the COVID-19. This information could be used to help individuals make informed decisions about their activities and lifestyle choices, and to develop new treatments for COVID-19.

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List of Abbreviations

Abbreviation	Meaning
A	Adenine
ACE2	Angiotensin-Converting Enzyme 2
Ad26.CoV.S	Adenovirus Type 26 Encoding the SARS-CoV-2 Spike Glycoprotein
ADA	Adenosine Deaminase
ADBP	Adenosine Deaminase Binding Protein
AG	Adenine Guanine
ALT	Alanine Aminotransferase
ARDS	Acute Respiratory Distress Syndrome
AS-PCR	Allele-Specific Polymerase Chain Reaction
AST	Aspartate Aminotransferase
B Cell	Bursa of Fabricius Cell
bp	Base Pair
BSL-2	Biosafety Level 2
C	Cytosine
C°	Degree Celsius
CBC	Complete Blood Count
CCL5	CC-Chemokine Ligand 5
CD	Clusters of Differentiation
CDC	Centers for Disease Control and Prevention
cDNA	Complementary Deoxyribonucleic Acid

ChAdOx1-S/nCoV-19	Chimpanzee Adenoviral Vector was Developed at Oxford University Containing the Spike Protein; Novel Coronavirus 2019
CI	Confidence Interval
CMP	Comprehensive Metabolic Panel
CMV	Cytomegalovirus
COPD	Chronic Obstructive Pulmonary Disease
CoV	Coronavirus
COVID-19	Coronavirus Disease of 2019
CRP	C-Reactive Protein
CT	Computed Tomography
CT/TT	Cytosine Thymine / Thymine Thymine
CXCL8	C-X-C Motif Chemokine Ligand 8
CXCLR	Chemokine Receptor
D614G	Glycine Residue at Position 614
dATP	Deoxyadenosine Triphosphate
dCTP	Deoxycytidine Triphosphate
ddH2O	Double-Distilled Water
DEPC	Diethyl Pyrocarbonate
dGTP	Deoxyguanosine Triphosphate
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid
DNase	Deoxyribonuclease
dNTP	Deoxyribonucleotide Triphosphate.
DPP4	Dipeptidyl Peptidase 4
DTT	Dithiothreitol

dTTP	Deoxythymidine Triphosphate
DVT	Deep Venous Thrombosis
E	Envelope
e.g.	exempli gratia “meaning “for example”
ECMO	Extracorporeal Membrane Oxygenation
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
ESR	Erythrocyte Sedimentation Rate
EUA	Emergency Use Authorization
F	Forword
FDA	Food and Drug Administration
FiO_2	Fraction of Inspired Oxygen
G	Guanine
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
GISAID	Global Initiative on Sharing All Influenza Data
GIT	Gastrointestinal Tract
GM-CSF	Granulocyte Macrophage Colony-Stimulating Factor
gp120	Envelope Glycoprotein 120
H1N1	Haemagglutinin Type 1 and Neuraminidase Type 1
Hb	Hemoglobin
HbA1c	Hemoglobin A1c
HCl	Hydrochloric Acid
HCoV	Human Coronavirus
HE	Hemagglutinin Esterase Dimer

HFNC	High Flow Nasal Cannula
HIT	Heparin-Induced Thrombocytopenia
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HRCT	High Resolution Computed Tomography
HRP	Horseradish Peroxidase
HS	Highly Significant
IBV	Infectious Bronchitis Virus
ICTV	International Committee on Taxonomy of Viruses
ICU	Intensive Care Unit
IFN-γ	Interferon Gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
IMV	Intermittent Mandatory Ventilation
IQR	Inter-Quartile Range
kb	Kilo-Base
L	Liter
LAMP	Loop-Mediated Isothermal Amplification
LDH	Lactate Dehydrogenase
M	Membrane
mA	Milliampere
MAP	Mean Arterial Pressure
MERS	Middle East Respiratory Syndrome

$MgCl_2$	Magnesium Chloride
MHC	Major Histocompatibility Complex
MHV	Murine Hepatitis Virus
min	Minute
miR or miRNA	Micro Ribonucleic Acid
ml	Milliliter
mmHg	Millimetres of Mercury
M-MLV	Murine Moloney Leukemia Virus
MOF	Multiple Organ Failure
Mpro	Main Protease
mRNA	Messenger RNA
N	Nucleocapsid
NAAT	Nucleic Acid Amplification Test
NCBI	National Center for Biotechnology Information
ng	Nanogram
NIH	National Institutes of Health
NIPPV	Nasal Intermittent Positive Pressure Ventilation
nm	Nanometer
NOD-like receptor	Nucleotide-Binding Oligomerization Domain-Like Receptors
NS	Not Significant
NSP	Nonstructural Proteins
NVX-CoV2373	Novavax Coronavirus Vaccine
OD	Optical Density
OR	Odds Ratio

ORF	Open Reading Frame
p	Probability
PAMP	Pathogen Associated Molecular Patterns
PaO_2	Partial Pressure of Oxygen
PCR	Polymerase Chain Reaction
PE	Pulmonary Embolism
pmol	Picomoles
pp1a , b	Polyprotein 1a , b
PPE	Personal Protective Equipment
PHEIC	Public Health Emergency of International Concern
qPCR	Quantitative Polymerase Chain Reaction
R	Reverse
RAAS	Renin Angiotensin Aldosterone System
RBD	Receptor Binding Domain
RBS	Random Blood Sugar
RCT	Replication Transcription Complex
RdRp	RNA dependent RNA Polymerase
RFLP-PCR	Restriction Fragment Length Polymorphism Polymerase Chain Reaction
RIG-I-Like Receptors	Retinoic Acid-Inducible Gene I Like Receptors
RNA	Ribonucleic Acid
RNase	Ribonuclease
rpm	Revolutions Per Minute
RR	Respiratory Rate
rRT-PCR	Real-Time Reverse Transcription Polymerase Chain Reaction

RTase	Reverse Transcriptase
RT-PCR	Reverse Transcription Polymerase Chain Reaction
RT-qPCR	Reverse Transcription Quantitative Polymerase Chain Reaction
S	Spike or Significant
SARS	Severe Acute Respiratory Syndrome
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SD	Standard Deviation
sgRNA	Subgenomic RNA
SNP	Single Nucleotide Polymorphism
SpO_2	Saturation of Peripheral Oxygen
SPSS	Statistical Package for The Social Sciences
SSP-PCR	Sequence Specific Primer - Polymerase Chain Reaction
ssRNA	Single Stranded Ribonucleic Acid
T Cell	Thymus Cell
T2DM	Type 2 Diabetes Mellitus
Taq	*Thermus aquaticus*
TBE	Tris/ Borate/ EDTA
TLR	Toll-Like Receptors
TMPRSS2	Transmembrane Protease, Serine 2
TNF-α	Tumor Necrosis Factor Alpha
TT	Thymine Thymine
µl	Microliter
UTR	Untranslated Region
UV	Ultraviolet

VITT	Vaccine-Induced Immune Thrombotic Thrombocytopenia
VOC	Variant of Concern
VOI	Variant of Interest
VTE	Venous Thromboembolism
WASP	Web-based Allele Specific Primer
WBC	White Blood Cell
WHO	World Health Organization
3CLpro	3 Chymotrypsin Like Protease
ΔCT	Delta Threshold Cycles

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1. Introduction and Literature Review

1.1. Introduction

Coronavirus disease 2019 (COVID-19) is a global pandemic that is caused by a novel coronavirus, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) (Cascella *et al.,* 2023).

Coronaviruses are enveloped viruses that possess a positive-sense single-stranded RNA genome 26–32 kb in length. Coronaviruses belong to the Coronaviridae subfamily Orthocoronavirinae. According to variations in the genome sequence and serological reactions, coronavirus members in the subfamily are classified into four genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus and Deltacoronavirus (Weiss and Navas-Martin, 2005 ; Masters, 2006 ; Yang and Rao, 2021)

Coronaviruses are characterized by high genetic recombination and mutation rates, which result in their ecological diversity (Cui *et al.,* 2019). They are able to infect and readily adapt to a wide range of hosts, from birds to whales and they are characterized by mild respiratory illnesses, such as the common cold (Fung and Liu, 2019). By contrast, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are able to cause severe human respiratory diseases, potentially resulting in high mortality (de Wit *et al.,* 2016 ; Fevrier, 2022).

MicroRNAs (miRNAs) are small and noncoding RNAs 18-25 nucleotides in length that target the mRNAs to degrade them or obstruct their translation thus, miRNAs act as an observer in cells. The exact mechanistic roles of cellular miRNAs in viral infections are not fully understood. However, cellular miRNA is produced at the early stage of viral infections due to the antiviral reaction. miRNAs can inhibit the viral translation after the attachment of miRNAs to

3′-UTR of the viral genome or target the receptors, structural or nonstructural proteins of SARS-CoV-2 without affecting the expression of human genes (Fani *et al.,* 2021).

The host miRNA expression has a fundamental effect on viral pathogenesis control through interfering with T cells and immune reactions to viral infections. A lot of miRNAs are correlated with increases in plasma cytokine storms such as TNF-α, IL-1β, IL-6, miR-146a, miR-146b and IL-8 in the acute respiratory distress syndrome and chronic obstructive pulmonary disease (COPD). Indeed, reducing these miRNAs expressions emphasizes on a way to improve COVID-19 severity due to the downregulation of proinflammatory cytokines that increase apoptosis protein expression. On the other hand, the expression of these miRNAs can offer promising diagnostic value to SARS-CoV-2 infection (Guterres *et al.,* 2020).

The binding of SARS-CoV-2 to the angiotensin-converting enzyme 2 (ACE2) receptors on pancreatic islets, leading to pancreatic damage with subsequent impairment of insulin secretion and development of hyperglycemia even in non-DM patients (El-Huneidi *et al.,* 2021).

However, recent evidence suggested that dipeptidyl peptidase 4 (DPP4), an enzyme that has been associated with the presence of hypertension, insulin resistance, and type 2 diabetes mellitus (T2DM), may be used as a co-receptor when SARS-CoV-2 enters the target cells (Noh *et al.,* 2021). The renin–angiotensin–aldosterone system (RAAS) component ACE2 and DPP4 (CD26) are proteins dysregulated in diabetes. It is hypothesized that diabetic patients might be more susceptible to COVID-19 due to increased presence of ACE2 and DPP4 mediating infection and viral entry and contributing to a compromised vasculature (Valencia *et al*., 2020). Besides that, previous studies found the DPP4-encoding gene is polymorphic; and an association of the enzyme levels

was found with not only some genotypes of these polymorphisms but also some diseases, such as T2DM (Posadas-Sánchez *et al.,* 2021).

The human leukocyte antigen (HLA) system orchestrates immune regulation. It is recognized that T-cell receptors recognize the conformational structure of the antigen binding-groove in the HLA molecule along with the accompanying antigen peptides. Thus, particular HLA haplotypes are associated with distinct genetic predispositions to disease. Furthermore, HLA variability in a population could be correlated with COVID-19 incidence since HLA plays such a crucial role in the immune response to pathogens and the development of infectious diseases. Likewise, the genetic variability of the MHC molecules can affect the susceptibility and severity of SARS-CoV-2 (Migliorini *et al.,* 2021).

The Aim of This Study

This study aims to provide an overview of the potential link between COVID-19 and selected biological markers (miRNAs, ACE2 and DPP4), and to evaluate the association of *DPP4* (*rs3788979* A/G) gene polymorphisms and *HLA* genotyping with the susceptibility and severity to SARS-CoV-2 infection, and to evaluate ACE2 and DPP4 levels among patients in Babylon province.
This study has been carried out to achieve the following objectives:

1. Measurement of HbA1c, random blood sugar (RBS), white blood cells count, serum ferritin, D-dimer, and hemoglobin (Hb).
2. Evaluate selected miRNAs expression profile (miR-423-5p, miR-23a-3p and miR-195-5p) by stem-loop RT-qPCR technique.
3. Analyze *DPP4* (*rs3788979* A/G) gene polymorphism among patients with COVID-19 and control groups through AS-PCR technique.
4. Genotyping of *HLA-B*07:03*, *B*46:01*, *DRB1*03:01*, *DRB1*12:02* alleles for patients and control groups by SSP-PCR technique.
5. Evaluate serological markers including ACE2 and DPP4 levels among patients with COVID-19 and control groups through ELISA technique.

1.2. Literature Review

1.2.1. Coronavirus Disease Definition

Coronavirus disease 2019 (COVID-19), the highly contagious viral illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has had a catastrophic effect on the world's demographics resulting in about 6,953,743 deaths worldwide, reported to World Health Organization (WHO) from December 2019 to July 2023, emerging as the most consequential global health crisis since the era of the influenza pandemic of 1918 (Cascella *et al.,* 2023; WHO, 2023).

After the first cases of this predominantly respiratory viral illness were first reported in Wuhan, Hubei Province, China, in late December 2019, SARS-CoV-2 rapidly disseminated across the world in a short span of time, compelling the WHO to declare it as a global pandemic on March 11, 2020. Since being declared a global pandemic, COVID-19 has ravaged many countries worldwide and has overwhelmed many healthcare systems (Cascella *et al.,* 2023 ; WHO, 2023).

The pandemic has also resulted in the loss of livelihoods due to prolonged shutdowns, which have had a rippling effect on the global economy. Even though substantial progress in clinical research has led to a better understanding of SARS-CoV-2 and the management of COVID-19, limiting the continuing spread of this virus and its variants has become an issue of increasing concern, as SARS-CoV-2 continues to wreak havoc across the world, with many countries enduring a second or third wave of outbreaks of this viral illness attributed mainly due to the emergence of mutant variants of the virus (Arya *et al.,* 2023).

1.2.2. Structure of SARS-CoV-2

Human have long been infected by coronavirus as it is one of those responsible for the common cold. It is a contagious viral infection that can be spread through inhalation or ingestion of viral droplets as a result coughing and sneezing and touching infected surface are primary sources of infection. The coronavirus genome is comprised of ~30000 nucleotides. It encodes four structural proteins, Nucleocapsid (N) protein, Membrane (M) protein, Spike (S) protein and Envelop (E) protein and several non-structural proteins (nsp) (Boopathi *et al.,* 2021).

The capsid is the protein shell, inside the capsid, there is nuclear capsid or N-protein which is bound to the virus single positive strand RNA that allows the virus to hijack human cells and turn them into virus factories (Boopathi *et al.,* 2021).

The N protein coats the viral RNA genome which plays a vital role in its replication and transcription. This is one of the important open research problems the developing of an effective drug targeting to prevent the contacts between N-terminal of N-protein and single positive RNA strand which can stop viral replication and transcription. Sarma *et al.* (2020) reported that two important class of compounds, theophylline, and pyrimidine drugs as possible inhibitors of RNA binding to the N terminal domain of N protein of coronavirus, thus opening new avenues for in vitro validations (Boopathi *et al.,* 2021).

The M-protein is most abundant in the viral surface and it is believed to be the central organizer for the coronavirus assembly.

The S-protein is integrated over the surface of the virus, it mediates attachment of the virus to the host cell surface receptors and fusion between the viral and host cell membranes to facilitate viral entry into the host cell (Kirchdoerfer *et al.,* 2016).

The E-protein is a small membrane protein composed ~76 to 109 amino-acid and minor component of the virus particle, it plays an important role in virus assembly, membrane permeability of the host cell and virus-host cell interaction (Gupta *et al*., 2020). A lipid envelop encapsulates the genetic material. Hemagglutinin-esterase dimer (HE) has been located on the surface of the viral as shown in Figure (1-1) (Lissenberg *et al.,* 2005 ; Boopathi *et al.,* 2021).

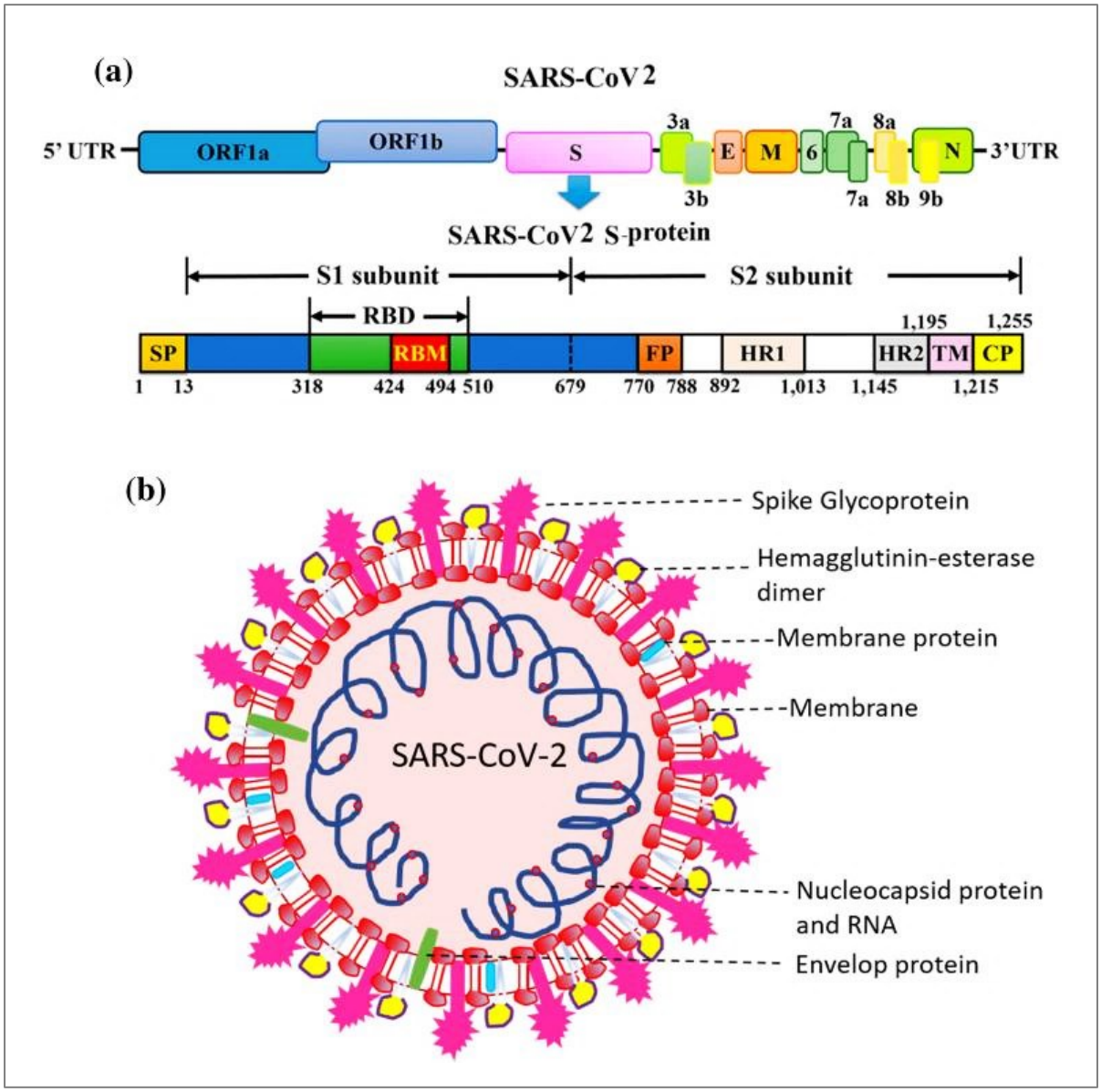


Figure (1-1): SARS-CoV-2 structure (Boopathi *et al.,* 2021).

The HE protein may be involved in virus entry, is not required for replication, but appears to be important for infection of the natural host-cell (Lissenberg *et al.,* 2005).

Such glycoprotein is made of three identical chains with 1273 amino acid each and it is composed of two well-defined protein domain regions: S1 and S2 subunits which are associated to cell recognition and the fusion of viral and cellular membranes respectively. The latter process occurs through different protein conformational changes that remain still uncharacterized (Boopathi *et al.,* 2021).

1.2.3. SARS-CoV-2 Variants

The novel coronavirus is prone to genetic evolution resulting in multiple variants that may have different characteristics compared to its ancestral strains. Periodic genomic sequencing of viral specimens is of fundamental importance, especially in a global pandemic setting, as it helps detect any new genetic variants of SARS-CoV-2 (Korber *et al.,* 2020).

Another variant was identified in humans, attributed to transmission from infected farmed mink in Denmark, which was not associated with increased transmissibility (Oreshkova *et al.,* 2020). Since then, multiple variants of SARS-CoV-2 have been described, of which a few are considered variants of concern (VOCs) due to their potential to cause enhanced transmissibility or virulence, reduction in neutralization by antibodies obtained through natural infection or vaccination, the ability to evade detection, or a decrease in therapeutics or vaccination effectiveness. With the continued emergence of multiple variants, the Centers for Disease Control and Prevention (CDC) and the WHO have independently established a classification system for distinguishing the emerging variants of SARS-CoV-2 into variants of concern (VOCs) and variants of interest (VOIs) (Cascella *et al.,* 2023).

1.2.3.1. SARS-CoV-2 Variants of Concern

1.2.3.1.1. Alpha (B.1.1.7 Lineage)

In late December 2020, a new SARS-CoV-2 variant of concern, B.1.1.7 lineage, also referred to as Alpha variant was reported in the UK based on whole-genome sequencing of specimens from patients who tested positive for SARS-CoV-2 (Galloway *et al.,* 2021 ; Volz *et al.,* 2021).

The B.1.1.7 variant includes 17 mutations in the viral spike protein which shows an increased affinity of the spike protein to ACE 2 receptors, enhancing the viral attachment and subsequent entry into host cells (Wu *et al.,* 2021 ; Davies *et al.,* 2021 ; Walensky *et al.,* 2021).

1.2.3.1.2. Beta (B.1.351 Lineage)

Another variant of SARS-CoV-2, B.1.351 also referred to as Beta variant with multiple spike mutations, resulted in the second wave of COVID-19 infections, was first detected in South Africa in October 2020 (Tegally *et al.,* 2021).

The B.1.351 variant includes nine mutations in the spike protein that are located in the RBD and increase the binding affinity for the ACE receptors (Mwenda *et al.,* 2021 ; Wibmer *et al.,* 2021 ; Wu *et al.,* 2021).

This variant is reported to have an increased risk of transmission and reduced neutralization by monoclonal antibody therapy, convalescent sera, and post-vaccination sera (Wang *et al.,* 2021).

1.2.3.1.3. Gamma (P.1 Lineage)

The third variant of concern, the P.1 variant also known as Gamma variant was identified in December 2020 in Brazil. The B.1.1.28 variant harbors ten mutations in the spike. Three mutations are located in the RBD, similar to the B.1.351 variant (Faria *et al.,* 2021).

Notably, this variant may have reduced neutralization by monoclonal antibody therapies, convalescent sera, and post-vaccination sera (Wang *et al.,* 2021).

1.2.3.1.4. Delta (B.1.617.2 Lineage)

The fourth variant of concern, B.1.617.2 also referred to as the Delta variant was initially identified in December, 2020 in India and was responsible for the deadly second wave of COVID-19 infections in April 2021 in India.

The Delta variant was initially considered a variant of interest. However, this variant rapidly spread around the world prompting the WHO to classify it as a VOC in May 2021**.** The B.1.617.2 variant harbors ten mutations in the spike protein (Cascella *et al.,* 2023).

1.2.3.1.5. Omicron (B.1.1.529 Lineage)

The fifth variant of concern B.1.1.529, also designated as the Omicron variant by the WHO was first identified in South Africa on 23 November 2021 after an uptick in the number of cases of COVID-19 (Vaughan, 2021).

Omicron was quickly recognized as a VOC due to more than 30 changes to the spike protein of the virus along with the sharp rise in the number of cases observed in South Africa (Callaway, 2021).

The reported mutations include one mutation in the envelope, six mutations in the nucleocapsid protein, three mutations in the matrix, nine mutations in the N-terminal domain of the spike, fifteen mutations in the receptor-binding domain of the spike, one mutation in the fusion peptide of the spike, three mutations in the heptad repeat 1 of the spikes as well as multiple other mutations in the non-structural proteins and spike protein (Gu *et al.,* 2022).

1.2.3.2. SARS-CoV-2 Variants of Interest

Variants of Interest (VOIs) are defined as variants with specific genetic markers that have been associated with changes that may cause enhanced transmissibility or virulence, reduction in neutralization by antibodies obtained through natural infection or vaccination, the ability to evade detection, or a decrease in the effectiveness of therapeutics or vaccination. So far since the beginning of the pandemic, WHO has described eight variants of interest (VOIs), namely Epsilon (B.1.427 and B.1.429); Zeta (P.2); Eta (B.1.525); Theta (P.3); Iota (B.1.526); Kappa (B.1.617.1); Lambda (C.37) and Mu (B.1.621) (Cascella *et al.,* 2023).

1.2.3.2.1. Epsilon (B.1.427 and B.1.429)

This variant emerged in the US around June 2020 and increased from 0% to > 50% of sequenced cases from September 1, 2020, to January 29, 2021, exhibiting an 18.6-24 % increase in transmissibility relative to wild-type circulating strains. Due to its increased transmissibility, the CDC classified this strain as a variant of concern in the US (Zhang *et al.,* 2021).

1.2.3.2.2. Zeta (P.2)

This variant has key spike mutations and was first detected in Brazil in April 2020. This variant is classified as a VOI by the WHO and the CDC due to its potential reduction in neutralization by antibody treatments and vaccine sera (Aleem *et al.,* 2023).

1.2.3.2.3. Eta (B.1.525) and Iota (B.1.526)

These variants harbor key spike mutations and were first detected in New York in November 2020 and classified as a variant of interest by CDC and the WHO due to their potential reduction in neutralization by antibody treatments and vaccine sera (Aleem *et al*., 2023).

1.2.3.2.4. Theta (P.3)

Theta variants carry key spike mutations and was first detected in the Philippines and Japan in February 2021 and is classified as a variant of interest by the WHO (Aleem *et al*., 2023).

1.2.3.2.5. Kappa (B.1.617.1)

This variant harbor key mutations and was first detected in India in December 2021 and is classified as a variant of interest by the WHO and the CDC (Aleem *et al*., 2023).

1.2.3.2.6. Lambda (C.37)

Lambda variant was first detected in Peru and has been designated as a VOI by the WHO in June 2021 due to a heightened presence of this variant in the South American region (Aleem *et al*., 2023).

1.2.3.2.7. Mu (B.1.621)

It was identified in Columbia and was designated as a VOI by the WHO in August 2021.

The CDC has designated the Epsilon (B.1.427 and B.1.429) variants as a VOC and Eta (B.1.525); Iota (B.1.526); Kappa (B.1.617.1); Zeta (P.2); Mu (B.1.621, B.1.621.1) and B.1.617.3 as variants of interest (VOIs) (Aleem *et al*., 2023).

1.2.4. Etiology

Genomic characterization has shown that bats and rodents are the probable gene sources of alphaCoVs and betaCoVs. On the contrary, avian species seem to represent the gene sources of deltaCoVs and gammaCoVs. Coronavirus has become the major pathogens of emerging respiratory disease outbreaks. Members of this large family of viruses can cause respiratory, enteric, hepatic, and neurological diseases in different animal species, including camels, cattle, cats,

and bats. For reasons yet to be explained, these viruses can cross species barriers and can cause, in humans, illness ranging from the common cold to more severe diseases such as MERS and SARS (Lei *et al.,* 2018).

Genomic characterization of the new human coronavirus (HCoV), isolated from a cluster-patient with atypical pneumonia after visiting Wuhan, had 89% nucleotide identity with bat SARS-like-CoVZXC21 and 82% with that of human SARS-CoV. Hence, it was termed SARS-CoV-2 by experts of The International Committee on Taxonomy of Viruses (ICTV). The single-stranded RNA genome of SARS-CoV-2 contains 29,891 nucleotides, encoding for 9,860 amino acids (International Committee on Taxonomy of Viruses Executive Committee, 2020).

Although the origin of SARS-CoV-2 is currently unknown, it is widely postulated to have originated from an animal implicating zoonotic transmission. Genomic analyses suggest that SARS-CoV-2 probably evolved from a strain found in bats. The genomic comparison between the human SARS-CoV-2 sequence and known animal coronaviruses indeed revealed high homology (96%) between the SARS-CoV-2 and the betaCoV of bats (*Rhinolophus affinis*) (Andersen *et al.,* 2020). Similar to SARS and MERS, it has been hypothesized that SARS-CoV-2 advanced from bats to intermediate hosts such as pangolins and minks, and then to humans (Oreshkova *et al.,* 2020 ; Zhang *et al.,* 2020a). A recently released report by the WHO describing the possible origins of SARS-CoV-2 was inconclusive as it did not clearly specify the origin of the virus; however, it did report that the circulation of SARS-CoV-2 occurred as early as December 2019. This report explored several possible hypotheses of the origin of the virus that included the origin of the virus in an animal, the transmission of the virus to an intermediate host, and subsequent passage into humans (Cascella *et al.,* 2023).

1.2.5. Transmission of SARS-CoV-2

Respiratory droplet and contact transmission are the main transmission routes for person-to-person spread of SARS-CoV-2. The fecal-oral transmissions, which has not yet been confirmed (General Office of National Health Commission of the People's Republic of China, 2020; Special Expert Group for Control of the Epidemic of Novel Coronavirus Pneumonia of the Chinese Preventive Medicine Association, 2020).

1.2.5.1. Aerosol Transmission

Biological aerosol refers to droplets containing viruses that are suspended in the air for a period of time and lose moisture; the droplets are able to transfer some distance along the air currents, resulting in potential long-distance transmission of disease. Patients with severe SARS-CoV-2 infection may shed virus to a greater degree during specific medical interventions (mask ventilation, non-invasive ventilation, and tracheal intubation); this can generate local aerosols, which put others in the environment at greater risk (Wax and Christian, 2020). Respiratory droplets are believed to be the predominant route of transmission, similar to that observed in other respiratory viral infections (Shi *et al.,* 2020)

1.2.5.2. Contact Transmission

Since SARS-CoV-2 was found to persist within the environment of infected individuals in Guangzhou, China (household surfaces, door handles, mobile phones, and such). When susceptible individuals come into contact with virus-containing body fluids (sputum, saliva, feces) from humans or animals, SARS-CoV-2 can be transmitted through the oral cavity, nasal cavity, and other mucous membranes. Likewise, when susceptible individuals come into contact with body fluid-contaminated items, indirect transmission of SARS-CoV-2 can occur (Shi *et al.,* 2020).

1.2.5.3. Fecal-Oral and Urinary Transmission

Recently, infectious SARS-CoV-2 was successfully isolated from feces and urine of patients with COVID-19. These findings indicate that the virus can survive in the digestive tract and the urethra, and suggest that SARS-CoV-2 could be transmitted via the fecal-oral or urinary route (Fang, 2020; Xinhuanet *et al.,* 2020). However, how the virus persists in feces and urine is currently under further investigation

1.2.5.4. Mother to Child Transmission

In Wuhan Tongji Hospital, reported that SARS-CoV-2-infected pregnant woman gave birth to a child who tested SARS-CoV-2-positive 36 hours after birth; these results suggested the possibility of mother-to-child transmission. Also, in Zhejiang, a pregnant woman with severe COVID-19 gave birth to a newborn who was negative on multiple subsequent nucleic acid virus tests for COVID-19. Nonetheless, a recent study revealed that fetal infection could be transmitted in late pregnancy (Chen *et al*., 2020a). These observations may relate to low expression of ACE2 among cells detected at the maternal–fetal interface (Zheng *et al*., 2020). Overall, there appears to be a minimal risk of fetal infection via known routes of vertical transmission.

1.2.5.5. Other Potential Routes of Infection

Some researchers have speculated that SARS-CoV-2 could be transmitted through conjunctiva, although a recent study refuted this possibility. The studies showed only one of the 67 patients with COVID-19 had conjunctivitis; the virus nucleic acid test of secretions from the conjunctival sac was negative (Zhou *et al*., 2020a).

Fomite transmission from contamination of inanimate surfaces with SARS-CoV-2 has been well characterized based on many studies reporting the viability of SARS-CoV-2 on various porous and nonporous surfaces (Cascella *et al.,* 2023).

Under experimental conditions, SARS-CoV-2 was noted to be stable on stainless steel and plastic surfaces compared to copper and cardboard surfaces, with the viable virus being detected up to 72 hours after inoculating the surfaces with the virus (van Doremalen *et al.,* 2020).

Viable virus was isolated for up to 28 days at 20 ºC from nonporous surfaces such as glass, stainless steel. Conversely, recovery of SARS-CoV-2 on porous materials was reduced compared with nonporous surfaces (Riddell *et al.,* 2020). A study evaluating duration of the viability of the virus on objects and surfaces showed that SARS-CoV-2 can be found on plastic and stainless steel for up to 2-3 days, cardboard for up to 1 day, copper for up to 4 hours. Moreover, it seems that contamination was higher in intensive care units (ICUs) than in general wards, and SARS-CoV-2 can be found on floors, computer mice, trash cans, and sickbed handrails as well as in the air up to 4 meters from patients implicating nosocomial transmission as well in addition to fomite transmission (Guo *et al.,* 2020).

The Centers for Disease Control and Prevention (CDC) recently released an update stating that individuals can be infected with SARS-CoV-2 via contact with surfaces contaminated by the virus, but the risk is low and is not the main route of transmission of this virus (CDC, 2021).

1.2.6. Epidemiology

According to the World Health Organization (WHO), the emergence of viral diseases represents a serious public health risk. In the past two decades, several epidemics caused by viruses such as the severe acute respiratory syndrome coronavirus (SARS-CoV) from 2002 to 2003, and H1N1 influenza in 2009, and the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 have been described which have had a significant impact on global health (Ahmad *et al.,* 2020).

Since being declared a global pandemic by the WHO to 28 June 2023, there have been 767,518,723 confirmed cases of COVID-19, including 6,947,192 deaths, reported globally to WHO. As of 26 June 2023, a total of 13,461,751,619 vaccine doses have been administered (WHO, 2023).

World Health Organization (WHO), recorded the situation of COVID-19 by regions from 31 December 2019 to 28 June 2023 (WHO, 2023).

In Americas, there have been 193,094,953 confirmed cases of COVID-19 with 2,956,943 deaths reported to WHO. In Europe, there have been 275,686,519 confirmed cases of COVID-19 with 2,243,097 deaths, reported to WHO. In Western Pacific, there have been 204,623,604 confirmed cases of COVID-19 with 413,961 deaths reported to WHO. In South-East Asia, there have been 61,189,014 confirmed cases of COVID-19 with 806,441 deaths reported to WHO. In Eastern Mediterranean, there have been 23,383,773 confirmed cases of COVID-19 with 351,341 deaths reported to WHO. In Africa, there have been 9,540,096 confirmed cases of COVID-19 with 175,396 deaths reported to WHO (WHO, 2023).

World Health Organization (WHO), recorded the situation of COVID-19 by country, territory or area from 3 January 2020 to 28 June 2023 (WHO, 2023).

In Iraq, there have been 2,465,545 confirmed cases of COVID-19 with 25,375 deaths, reported to WHO. As of 1 January 2023, a total of 19,557,364 vaccine doses have been administered. People receiving at least 1 dose 11,332,925 (28.18%) (WHO, 2023).

The daily cases recorded the highest number of cases 11,986 in July 30, 2021 and recorded daily deaths in this day was 65 deaths. The daily deaths recorded the highest number of deaths 106 in July 4, 2020 and recorded daily cases in this day was 2,227 cases according to the data repository for the 2019 Novel coronavirus visual dashboard operated by the Johns Hopkins University

Center for Systems Science and Engineering (JHU CSSE) which collecting and reporting of global COVID-19 data from WHO and CDC (JHU, 2023).

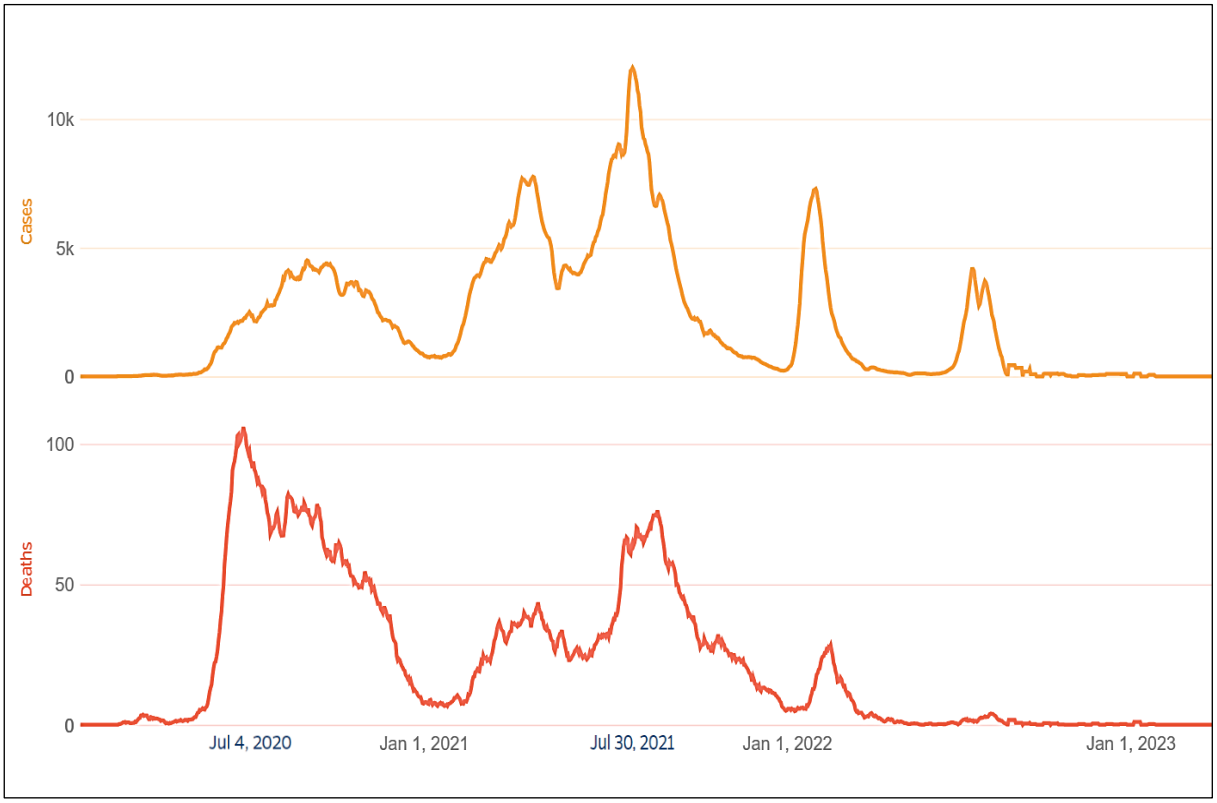


Figure (1-2): Coronaviruses daily new cases and deaths in Iraq by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU, 2023).

In Syrian Arab Republic, there have been 57,423 confirmed cases of COVID-19 with 3,163 deaths, reported to WHO. As of 17 April 2023, a total of 5,090,630 vaccine doses have been administered.

In Jordan, there have been 1,746,997 confirmed cases of COVID-19 with 14,122 deaths, reported to WHO. As of 20 August 2022, a total of 10,057,975 vaccine doses have been administered.

In Saudi Arabia, there have been 841,469 confirmed cases of COVID-19 with 9,646 deaths, reported to WHO. As of 24 April 2023, a total of 68,534,631 vaccine doses have been administered.

In Kuwait, from 3 January 2020 to 5:56pm CEST, 28 June 2023, there have been 665,974 confirmed cases of COVID-19 with 2,570 deaths, reported to WHO. As of 17 June 2023, a total of 8,260,540 vaccine doses have been administered.

In Islamic Republic of Iran, there have been 7,612,414 confirmed cases of COVID-19 with 146,292 deaths, reported to WHO. As of 19 June 2023, a total of 155,426,133 vaccine doses have been administered.

In Türkiye, there have been 17,004,677 confirmed cases of COVID-19 with 101,419 deaths, reported to WHO. As of 28 January 2023, a total of 139,694,693 vaccine doses have been administered (WHO, 2023).

In May, 5th, 2023, the WHO Director-General concurs with the advice offered by the Committee regarding the ongoing COVID-19 pandemic. WHO determines that COVID-19 is now an established and ongoing health issue which no longer constitutes a Public Health Emergency of International Concern (PHEIC) (WHO, 2023).

1.2.7. Pathophysiology

Upon entry into the host, replication of the viral RNA is initiated with the synthesis of polyprotein 1a/1ab (pp1a/pp1ab). The transcription occurs through the replication-transcription complex (RCT) organized in double-membrane vesicles and via the synthesis of subgenomic RNAs (sgRNAs) sequences. Conversely, transcription termination occurs at transcription regulatory sequences, located between the so-called open reading frames (ORFs) that work as templates for the production of subgenomic mRNAs. In an atypical CoV genome, at least six ORFs can be present. Among these, a frameshift between ORF1a and ORF1b guides the production of both pp1a and pp1ab polypeptides that are processed by virally encoded chymotrypsin-like protease (3CLpro) or main protease (Mpro), as well as one or two papain-like proteases for producing

16 with known or predicted RNA synthesis and modification functions non-structural proteins (NSPs 1-16). Besides ORF1a and ORF1b, other ORFs encode structural proteins, including spike, membrane, envelope, and nucleocapsid proteins and accessory protein chains (Biryukov *et al.,* 2021).

Different CoVs possess unique structural and accessory proteins translated by dedicated sgRNAs.The pathogenesis of coronaviruses is related to the function of the non-structural proteins and structural proteins. For example, researchers have outlined the role of non-structural proteins in blocking the host's innate immune response (Lei *et al.,* 2018).

Among functions of structural proteins, the envelope has a crucial role in virus pathogenicity as it promotes viral assembly and release. Among the structural elements of CoVs, there are the spike glycoproteins composed of two subunits (S1 and S2). Homotrimers of S proteins compose the spikes on the viral surface, guiding the link to host receptors (Song *et al.,* 2018).

1.2.8. Pathogenesis of SARS-CoV-2

The entry of SARS-CoV-2 into the hosts' cells by binding the spike or S protein (S1) to the ACE2 receptors abundantly on respiratory epithelium such as type II alveolar epithelial cells. Besides the respiratory epithelium, ACE2 receptors are also expressed by other organs such as the upper esophagus, enterocytes from the ileum, myocardial cells, proximal tubular cells of the kidney, and urothelial cells of the bladder (Xu *et al.,* 2020a). The viral attachment process is followed by priming the spike protein S2 subunit by the host transmembrane serine protease 2 (TMPRSS2) that facilitates cell entry and subsequent viral replication endocytosis with the assembly of virions (Hoffmann *et al.,* 2020a).

The spike RBD allows the binding to the ACE2 receptor in the lungs and other tissues. The spike protein of an amino acid site (polybasic site) allows the functional processing of the same by the human enzyme furin (protease). This process enables the exposure of the fusion sequences and, therefore, the fusion of

the viral and cell membranes, a necessary passage for the virus to enter the cell (Cascella *et al.,* 2023 ; Boopathi *et al.,* 2021).

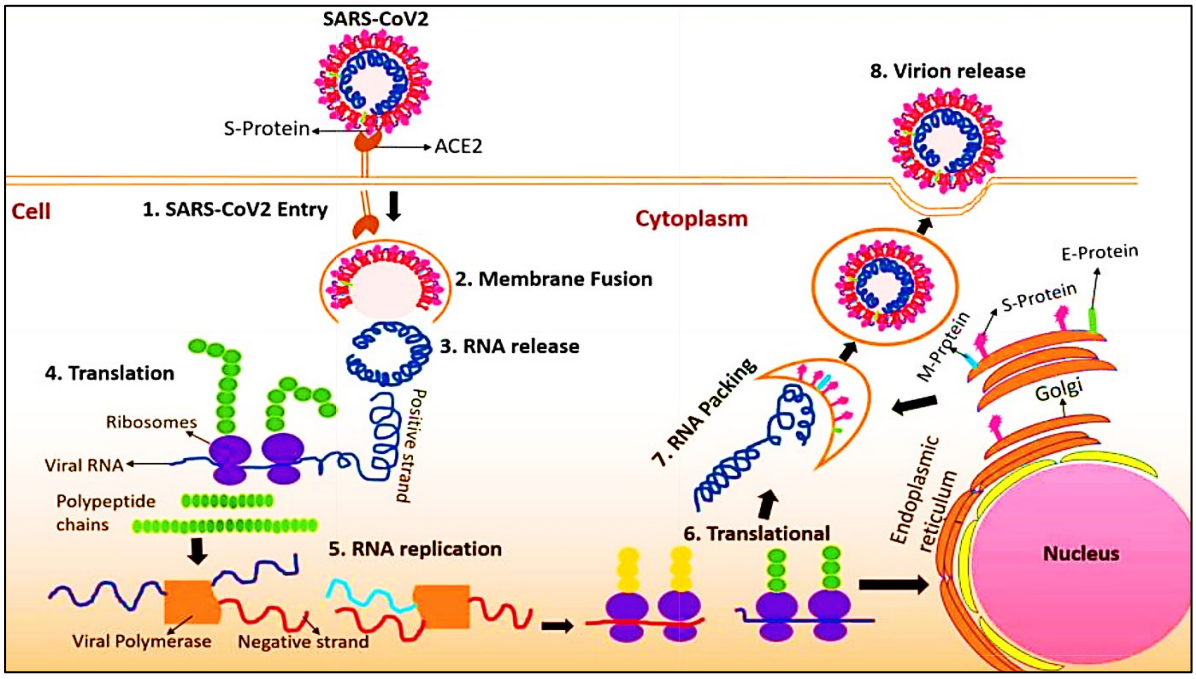


Figure (1-3): The schematic diagram of the mechanism of COVID-19 entry and viral replication and viral RNA packing in the human cell (Boopathi *et al.,* 2021).

1.2.9. Clinical Features

The clinical signs and symptoms are difference in degree of clinical severity of COVID-19. The incubation period (initial infection to symptoms) ranges from 0 to 24 days, with an average of 5 - 7 days (Guan *et al.,* 2020).

Individuals of any age are susceptible to infection, including neonates and pregnant women. Most patients present with mild to moderate symptoms. The most common symptoms are fever, dry cough, fatigue; upper respiratory tract symptoms can include pharyngalgia, headaches, and myalgia. There is also one report describing patients with gastrointestinal symptoms, including abdominal pain and diarrhea in children and adolescents (Xu *et al.,* 2020b).

In addition, asymptomatic patients have also been reported, although the frequency of this condition has not yet been determined. Approximately 20% of COVID-19 patients develop severe respiratory illness, with an overall case-fatality rate of about 2.3% (Çelik and Öztürk, 2021).

Asymptomatic infections involve individuals who test positive for SARS-CoV-2 but have no symptoms. It is estimated that 50% of persons who test positive are asymptomatic at the time of diagnosis (Oran and Topol, 2020). The overall proportion of asymptomatic infections was estimated at approximately 25% in one meta-analysis, but the proportion of asymptomatic infections is generally higher in persons with pre-existing immunity (Syangtan *et al.,* 2021).

Asymptomatic infections are more common in young and middle-aged patients (<50 years of age), women, and individuals without underlying comorbid conditions (Meng *et al.,* 2020). In an early study including 55 cases of asymptomatic infection (Wang *et al.,* 2020a), the median age at the time of diagnosis was 49 years. Despite absence of symptoms, more than half patients (37/55) in this study had evidence of pneumonia on computed tomography scans (Meng *et al.,* 2020).

Mild cases include individuals with symptoms of fever, sore throat, myalgia, and/or malaise, but without shortness of breath, dyspnea, or abnormal chest imaging indicating the presence of lower respiratory tract disease. The majority of symptomatic infections result in both mild and moderate illness with approximately (80 – 85 %) (Stokes *et al.,* 2020).

Gastrointestinal symptoms such as diarrhea, nausea, and emesis have also been reported, but with lower frequencies (<20%) than in SARS or MERS infections (Bleibtreu *et al.,* 2020). The incidence of symptoms such as loss of smell or taste varies significantly among studies, with frequencies between 10%

and 40% (Huang *et al.,* 2020). Loss of taste or smell (anosmia and ageusia, respectively), however, are often recognized by patients as a hallmark, or pathognomonic feature, of COVID-19, and frequently precede the onset of other flu-like symptoms. This peculiar manifestation has been studied, with one of the proposed theories being that the specialized cells in the olfactory bulb and olfactory epithelium have the ACE2 receptors for viral entry and subsequent infection, although there are detectable changes in brain imaging in some persons with anosmia (Brann *et al.,* 2020 ; Kandemirli *et al.,* 2021).

Most patients recover over the course of a few weeks; however, persistent anosmia and ageusia are also frequently described and remain under study (Kandakure *et al.,* 2022)

Patients with moderate disease have evidence of lower respiratory disease on physical examination or chest imaging, but maintain an oxygen saturation of equal or greater than 94% on room air at sea level. Individuals with severe disease have an oxygen saturation on room air of less than 94% at sea level, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen of less than 300 mmHg, or a respiratory rate of greater than 30 breaths/min, or lung infiltrates greater than 50% (Marczak *et al.,* 2023).

During the initial phase of the pandemic, severe disease accounted for up to 10 – 15 % of cases and critical illness was seen in about 5% of cases according to the Chinese Center for Disease Control and Prevention (Wu and McGoogan, 2020). Individuals who have SpO_2 less than 94% on room air, a ratio of partial pressure of arterial oxygen to fraction of inspired oxygen (PaO_2/FiO_2) of less than 300, marked tachypnea with a respiratory frequency of greater than 30 breaths/min, or lung infiltrates that are greater than 50% of total lung volume (Cascella *et al.,* 2023).

The rate of severe disease, however, varies depending on several factors, including history of prior infection, vaccination status, variant causing the infection and available health care resources. For example, the Omicron variant seems to be associated with milder disease compared with Delta variant (Wang *et al.,* 2022). The risk of progression to severe and critical disease is markedly decreased in persons with prior immunity, especially after vaccination. Patients with severe disease typically present with fever, dry cough, dyspnea, and bilateral pulmonary infiltrates on chest imaging. Preliminary evidence suggests that severe disease is more likely to take hold in individuals of older age, male sex, and in those with underlying co-morbidities. (Guan *et al.,* 2020).

The risk factors for disease progression have not yet been established; however, patients with severe COVID-19 illness may become critically ill with the development of acute respiratory distress syndrome (ARDS). This tends to occur approximately one week after the onset of symptoms (Clinical Spectrum COVID-19 treatment guidelines, 2021).

Critical disease is defined as respiratory failure, liver injury, acute myocardial injury, acute kidney injury, septic shock, and even multiple organ failure (MOF) (Zaim *et al.,* 2020).

Findings on chest computed tomography (CT) are frequent, reaching nearly 100% in early reports and over 80% in a recent cohort of patients outside Wuhan. Several critical points with respect to the lung parenchyma and interstitial tissue have been observed that are directly linked to the stages and severity of the disease (Fang, 2020 ; Shi *et al.,* 2020).

Lymphopenia is frequently identified among COVID-19 patients; the total white blood cell count is generally within normal limits of slightly decreased in mild cases but increased in severe or critically ill patients. Indicators of systemic inflammation, including serum levels of ferritin and C-reactive protein (CRP) as

well as erythrocyte sedimentation rate (ESR), can be elevated in association with high levels of circulating proinflammatory cytokines and chemokines. Likewise, serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), troponin, and/or creatinine may be elevated in patients with extra-pulmonary systemic complications (Shi *et al.,* 2020).

1.2.10. Acute Respiratory Distress Syndrome

Acute respiratory distress syndrome (ARDS) is a clinical entity that presents with bilateral pulmonary infiltrates and severe hypoxemia, which results from extensive damage and edema of the alveolar system owing to infiltration by inflammatory cells and mediators. Inflammatory cells, lytic enzymes, and cytokines produce thickening and fibrosis of the alveolar-blood barrier, destruction of alveoli, formation of proteinaceous hyaline membranes and severe edema of the interstitium. It is characterized by noncardiac pulmonary edema and severe hypoxemia with a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen of less than 300. The severity of disease is classified according to the ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (Thompson *et al.,* 2017).

The ARDS develops in approximately 30% to 50% of patients presenting with COVID-19 pneumonia and hypoxemia, although this number could change with the implementation of steroid therapy, vaccination, and outpatient therapeutics. In a study of 13 patients admitted to the ICU, 30% developed ARDS at a median time of 9 days and 10% required mechanical ventilation. The mortality rate of ARDS in patients with COVID-19 seems to be higher than that of other causes of ARDS (Gibson *et al.,* 2020). This could be due to multiple factors, including the added damage from the virus to the lung parenchyma and the thrombotic microangiopathy and thrombosis that develops in severe COVID-19 (Hernandez Acosta *et al.,* 2022).

1.2.11. Effect of SARS-CoV-2 on The Respiratory System

Coronavirus disease is primarily considered a viral respiratory and vascular illness as its causative agent, SARS-CoV-2, predominantly targets the respiratory and vascular systems (Cascella *et al.,* 2023).

The pathogenesis of SARS-CoV-2 induced pneumonia is best explained by two stages, an early and a late phase. The early phase is characterized by viral replication resulting in direct virus-mediated tissue damage, which is followed by a late phase when the infected host cells trigger an immune response with the recruitment of T lymphocytes, monocytes, and neutrophil recruitment which releases cytokines such as tumor necrosis factor-α (TNF α), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-1 (IL-1), interleukin-6 (IL-6), IL-1β, IL-8, IL-12 and interferon (IFN)-γ. In severe COVID-19, the immune system's overactivation results in a 'cytokine storm' characterized by the release of high levels of cytokines, especially IL-6 and TNF-α, into the circulation, causing a local and systemic inflammatory response (Azkur *et al.,* 2020 ; Wang *et al.,* 2020b).

The increased vascular permeability and subsequent development of pulmonary edema in patients with severe COVID-19 are explained by multiple mechanisms, which includes a) endotheliitis as a result of direct viral injury and perivascular inflammation leading to microvascular and microthrombi deposition b) dysregulation of the RAAS due to increased binding of virus to the ACE2 receptors and c) activation of the kallikrein- bradykinin pathway, the activation of which enhances vascular permeability, d) enhanced epithelial cell contraction causing swelling of cells and disturbance of intercellular junctions (Ackermann *et al.,* 2020 ; Teuwen *et al.,* 2020 ; van de Veerdonk *et al.,* 2020).

Besides IL-6 and TNF-α, the binding of SARS-CoV-2 to the toll-like receptor (TLR) induces release of pro-IL-1β, which is cleaved into the active mature IL-1β that mediates lung inflammation, until fibrosis (Conti *et al.,* 2020)

1.2.12. Extrapulmonary Manifestations of SARS-CoV-2

Although COVID-19 predominantly affects the respiratory system, COVID-19 can be considered a systemic viral illness given the multiple organ dysfunction associated with this illness (Cascella *et al.,* 2023).

It can affect other major organ systems such as the gastrointestinal tract (GIT), hepatobiliary, cardiovascular, renal, and central nervous system. Coronavirus induced organ dysfunction, in general, is possibly explained by either one or a combination of the proposed mechanisms such as direct viral toxicity, ischemic injury caused by vasculitis, thrombosis, or thrombo-inflammation, immune dysregulation, and renin-angiotensin-aldosterone system (RAAS) dysregulation (Coopersmith *et al.,* 2021).

1.2.13. Host Immune Responses

The innate immune response is activated when pathogen-associated molecular patterns (PAMPs) are recognized by host receptors (Carty *et al.,* 2021). The PAMPs are small molecules, such as lipopolysaccharides, peptidoglycan, lipoteichoic acid, and nucleic acids, that are present in different patterns and trigger immune cascades when recognized by the host (Rodrigues *et al.,* 2021).

The protein receptors in the host responsible for detecting PAMPs are called pattern recognition receptors. These pattern recognition receptors include Toll-like receptors, C-type lectin receptors, NOD-like receptors, and RIG-I-like receptors. Once activated, these receptors initiate downstream signaling pathways leading to secretion of type I and type III interferons, and the assembly and activation of the NOD-like receptor P3 inflammasome and other inflammasome complexes that promote secretion of proinflammatory cytokines including IL-1β and IL-18 (Rodrigues *et al.,* 2021).

The activation of antigen-presenting cells by proinflammatory cytokines also recruits the adaptive immunity to enhance viral clearance by antibody-mediated neutralization and T-cell–mediated cytotoxicity (Carty *et al.,* 2021).

The inflammasome pathway triggers the coagulation cascade, contributing to coagulopathy and the thrombotic events seen in severe COVID-19 (Wu *et al.,* 2019).

Like PAMPs, host cells are also activated by damaged or stressed cells in the setting of inflammation (Carty *et al.,* 2021), necrosis, or hypoxia even if no microbial PAMPs are present. These are called damage-associated molecular patterns. Although the activated PAMP and damage-associated molecular pattern pathways contribute to viral clearance, an overactivated response leads to a dysregulated immune system and exacerbates inflammation and damage through a cytokine storm (Wu *et al.,* 2019) as shown in figure (1-4).

One proinflammatory cytokine, IL-6, garnered attention after reports of ARDS became common in patients with severe COVID-19. IL-6 is a mediator of both innate and adaptive immune responses and acts as both a proinflammatory cytokine and an anti-inflammatory myokine. Interleukin-6 is secreted by macrophages when PAMPs bind to pattern recognition receptors. An elevated IL-6 was reported to be associated with a poor prognosis and, consequently, much emphasis was placed on the treatment of severe COVID-19 with IL-6 receptor antagonists (Vijayvargiya et al., 2020).

Clinical trials have suggested there is some benefit to limiting hyperactive immune responses through blocking IL-6 in severe cases (Gordon et al., 2021).

An early innate immune response is critical in activating the T- and B-cell immune systems and terminating the infection at asymptomatic or mild to moderate stage (Sette and Crotty, 2021). A delayed or absent innate immune response, either by immune evasion by virus or defective host immunity (or both),

fails to prime adaptive immune system and contributes to a high risk of severe or fatal COVID- 19 (Hernandez Acosta *et al.,* 2022).

Antigen-presenting cells are the initial component of antiviral response by the host. The specific mechanism of antigen presentation of SARS-CoV-2 is not well-understood; however, some of it can be extrapolated based on data from other betacoronaviruses, which mainly depends on major histocompatibility complex 1 (MHC class I) molecules. Several HLA types have been associated with increased susceptibility or protection against SARS-CoV (Chen *et al.,* 2006). It is highly likely there exist HLA alleles that predict increased susceptibility to SARS-CoV-2 and correlate with more severe outcomes, although research in diverse populations is ongoing (Hernandez Acosta *et al.,* 2022).

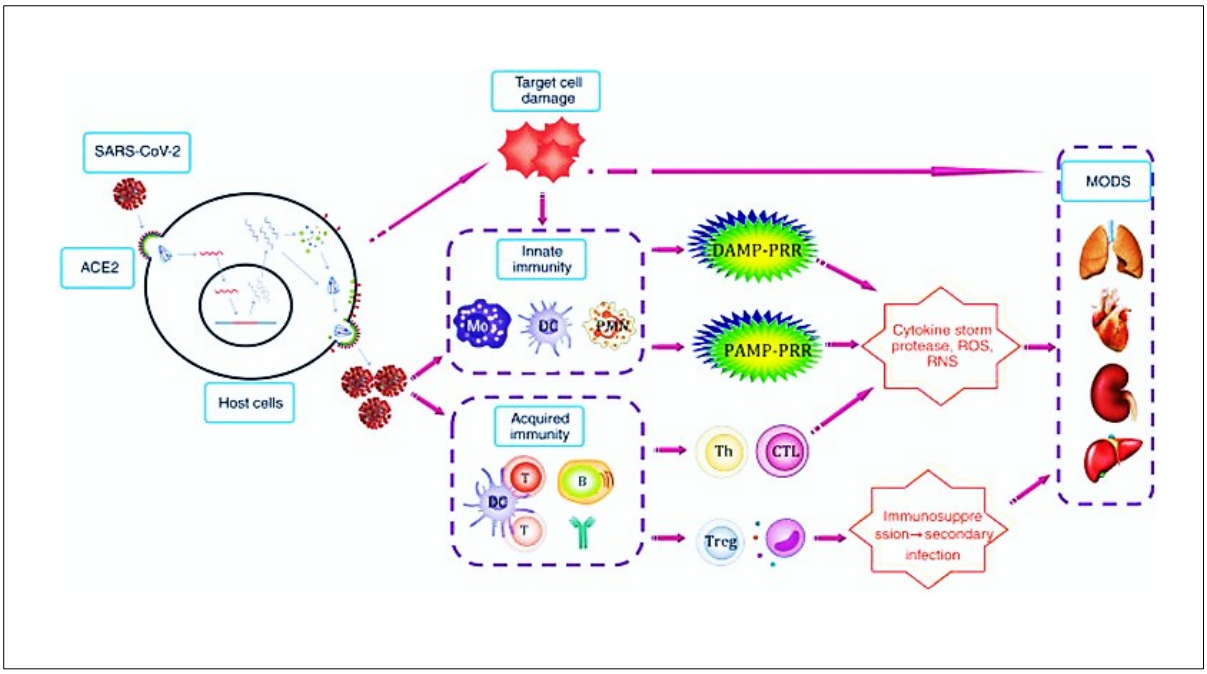


Figure (1-4): Immune response to SARS-CoV-2 infection and its role in pathogenesis. Severe acute respiratory syndrome coronavirus 2 targets cells through the S protein that binds to the ACE2 receptor, replicating and assembling in target cells before being released extracellularly. Inflammatory signaling molecules are released by infected cells and may induce organ injury through innate and acquired immunity. COVID-19, coronavirus disease 2019; S, spike; ACE2, angiotensin converting enzyme 2 (Li *et al.,* 2021).

Both humoral and cellular immune responses are activated by antigen-presenting cells as suggested by presence of virus-specific B and T cells in convalescent cases (Ni *et al.,* 2020). Coordinated humoral and cellular immune responses have been hypothesized to be protective, and an uncoordinated response has been blamed for uncontrolled disease (Rydyznski Moderbacher *et al.,* 2020).

Moreover, a delayed activation of adaptive immunity has been correlated with a higher viral burden and severe or fatal COVID-19. It has been hypothesized that the innate immune response attempts to fill the gap left by the absence of a functional adaptive immune system response, leading to an overactivated innate cytokine and chemokine responses and exacerbated neutrophil-driven lung damage, as evidenced by the presence of a substantial number of neutrophils in end-stage COVID-19 (Kuri-Cervantes *et al.,* 2020 ; Sette and Crotty, 2021).

It has been demonstrated that neutralizing antibody titers and quantity of virus-specific T cells are positively correlated (Ni *et al.,* 2020). As with other acute viral infections, IgG and IgM subtype antibodies are produced, primarily against the S and N proteins (Zhang *et al.,* 2020b).

The Immunoglobulin M (IgM) persist for 4 to 6 weeks after the onset of symptoms, whereas IgG persists for approximately 6 months after symptom onset in most cases. Persons who experienced asymptomatic infection have been shown to have lower seropositivity and delayed seroconversion when compared with persons who developed symptomatic COVID-19. Furthermore, antibodies in people who have recovered from COVID-19 may persist for well over 6 months (Ni *et al.,* 2020).

The T-cell response to SARS-CoV-2 includes both $CD4^+$ and $CD8^+$ T cells. It has been suggested that $CD4^+$ T cells are more abundant and effective against SARS-CoV-2 infection than $CD8^+$ T cells disease (Rydyznski Moderbacher *et al.,* 2020). A predominantly $CD4^+$ T-cell response is seen against S, M, and N proteins, although $CD4^+$ cells respond against almost all SARS-CoV-2 proteins. After symptom onset, a $CD4^+$ T-cell response can be detected within 2 to 4 days, whereas a $CD8^+$ T-cell response can be detected as early as 1 day after symptoms develop (Sette and Crotty, 2021**).**

Although, the overall number of CD4 and CD8 T cells in patients with COVID-19 is decreased, cells are excessively activated, with increased expression of proinflammatory HLAs and coreceptors. CD8 T cells were found to have an increased density of cytotoxic granules (Xu *et al.,* 2020b). This factor likely contributes to the cytokine storm causing ARDS and systemic inflammation, through the release of proinflammatory cytokines (including interferon, IL-6, and TNF-α) and chemokines (CCL5, CXCL8, etc.). This exaggerated immune response is what leads to the multiorgan involvement and high mortality seen in COVID-19 (Hernandez Acosta *et al.,* 2022).

Although long-term memory immunity is not yet clarified for SARS-CoV-2, it is known that CD4 and CD8 memory T cells persist for years after recovery from SARS-CoV infection and can respond to SARS-CoV antigens even after 6 years. In contrast, it seems that memory B-cell response is almost absent after 6 years after recovery from SARS. In follow-up of early SARS-CoV-2 infections and after vaccination, it also seems clear that T-cell responses are longer than antibody responses. Longer term follow-up will be required to fully elucidate the duration of post infection responses (Tang *et al.,* 2011 ; Hernandez Acosta *et al.,* 2022).

1.2.14. HLA Allele in Susceptibility to COVID-19

The human leukocyte antigen (HLA) gene complex is a locus of genes present on chromosome 6 that encodes proteins known a major histocompatibility complexes (MHC). They are a critical component of the antigen presentation pathway; they play a vital role in determining susceptibility to infectious disease. The *HLA* alleles are variable and polymorphic, and individuals with different *HLA* genotypes may trigger different immune responses against pathogens (Apanius *et al.,* 2017 ; Dendrou *et al.,* 2018).

The *HLA* alleles constitute a specific group of molecules expressed on the cell surface, crucial for the recognition of non-self-molecules by the acquired immune system (Kulski *et al.,* 2019). The essential function of MHCs is to bind and expose antigens derived from pathogens, to present them to the appropriate T lymphocytes, triggering the immune response. In particular, MHC molecules class I expose peptide antigens, present within the cytoplasm activating CD8 T-cell response (Ambagala *et al.,* 2005), while MHC molecules class II expose peptide antigens present in the extracellular space activating CD4 T-cell response. As *HLA* genes are a critical component of the antigen presentation pathway, they play a vital role in determining susceptibility to infectious disease. The *HLA* alleles are variable and polymorphic, and individuals with different *HLA* genotypes may trigger different immune responses against pathogens (Apanius *et al.,* 2017 ; Dendrou *et al.,* 2018 ; Migliorini *et al.,* 2021).

The association between the *HLA* genotypes and related polymorphisms with susceptibility, severity and progression of COVID-19 were considered. The crucial role played by HLA molecules in the immune response, especially through pathogen-derived peptide presentation, and the huge molecular variability of *HLA* alleles in the human populations could be responsible for the different rates of infection and the different patients following COVID-19 infection (Migliorini *et al.,* 2021).

1.2.15. Role of ACE2 in Patients with COVID-19

The Angiotensin-converting enzyme 2 (ACE2) has been established as the functional host receptor for SARS-CoV-2, the virus responsible for the current devastating worldwide pandemic of COVID-19. The ACE2 is abundantly expressed in a variety of cells residing in many different human organs. Many factors have been associated with both altered ACE2 expression and COVID-19 severity and progression, including age, sex, ethnicity, medication, and several co-morbidities, such as cardiovascular disease and metabolic syndrome (Bourgonje *et al.,* 2020).

Autopsies of SARS patients showed that SARS-CoV infection can cause injury to multiple organs, such as the heart, kidney, liver, skeletal muscle, central nervous system, and adrenal and thyroid glands, besides the lungs (Gu *et al.,* 2005 ; Gu, and Korteweg, 2007). Most critically ill patients with COVID-19 also had multiple organ damage, including acute lung injury, acute kidney injury, cardiac injury, liver dysfunction, and pneumothorax (Yang *et al.,* 2020). As with coronaviruses, organ injury is also frequently observed in MERS, especially the gastrointestinal tract and kidneys, while the incidence of acute cardiac injury is less common (Assiri *et al.,* 2013 ; Alsaad *et al.,* 2018 ; Hui *et al.,* 2018 ; Hwang *et al.,* 2019).

1.2.16. Role of miRNAs in Patients with COVID-19

MicroRNAs are small and noncoding RNAs 18-25 nucleotides in length that regulate gene expression at the posttranscriptional level, thus, miRNAs act as an observer in cells. The exact mechanistic roles of cellular miRNAs in viral infections are not fully understood. However, cellular miRNA is produced at the early stage of viral infections due to the antiviral reaction. miRNAs can inhibit the viral translation after the attachment of miRNAs to 3′-UTR of the viral genome or target the receptors, structural or nonstructural proteins of SARS-CoV-2 without affecting the expression of human genes (Fani *et al.,* 2021).

The host miRNA expression has a fundamental effect on viral pathogenesis control through interfering with T cells and immune reactions to viral infections. A lot of miRNAs are correlated with increases in plasma cytokine storms such as TNF-α, IL-1β, IL-6, miR-146a, miR-146b and IL-8 in the acute respiratory distress syndrome and chronic obstructive pulmonary disease (COPD). Indeed, reducing these miRNAs expressions emphasizes on a way to improve COVID-19 severity due to the downregulation of proinflammatory cytokines that increase apoptosis protein expression. On the other hand, the expression of these miRNAs can offer promising diagnostic value to ARS-CoV-2 infection (Guterres *et al.,* 2020).

The SARS-CoV-2 infection induces a miRNA response during the early stages of disease that involves three miRNAs (miR-423-5p, miR-23a-3p and miR-195-5p) that can independently identify COVID-19 cases and distinguish SARS-CoV-2 from influenza infections (Farr *et al.,* 2021). The miR-195 levels inversely correlate with the severity of the disease. This is in line with previously published data showing the down-regulation of host microRNAs, especially in severe cases of COVID-19. Of note, most of the microRNAs (including miR-195) targeting the SARS-CoV-2 genome are strongly deregulated in severe versus moderate and severe versus asymptomatic patients. A studies showed that, together with two other microRNAs (miR-423-5p and miR-23a-3p), miR-195-5p identified and distinguished COVID-19 from Influenza with an accuracy of over 95% (Farr *et al.,* 2021 ; Moatar *et al.,* 2023).

1.2.17. Role of DPP4 in Patients with COVID-19

The dipeptidyl peptidase 4 (DPP4) is also known as T-cell activation antigen CD26, or adenosine deaminase binding protein (ADBP). it is a single-pass transmembrane protein with multiple functions on glycemic control, cell migration and proliferation, and the immune system, among others. It has recently acquired an especial relevance due to the possibility to act as a receptor or co-

receptor for SARS-CoV-2, as it has been already demonstrated for other coronaviruses (Sebastián-Martín *et al.,* 2022).

The actions of DPP4 do not only rely on its catalytic activity as a peptidase, but also on its own structure, given that DPP4 can bind to several proteins, like adenosine deaminase (ADA), fibronectin, collagen, chemokine receptor CXCR4, tyrosine phosphatase CD45, and even some viral proteins such as the Human Immunodeficiency Virus (HIV) gp120 envelope protein. Thus, it regulates multiple cellular processes, playing a role in adhesion to the extracellular matrix, proliferation, and in T-cell maturation and activity (Sebastián-Martín *et al.,* 2022). The dipeptidyl peptidase 4 has acquired certain relevance in the scenario of the SARS-CoV-2 infection, due to its potential role as a cellular entry receptor or co-receptor for the virus. Although this hypothesis needs to be further elucidated, data from clinical studies indicate that DPP4 participates in virus physiopathology and therefore is a therapeutic target for this disease. The most prevalent comorbidities in SARS-CoV-2-infected patients were hypertension and diabetes, followed by cardiovascular diseases and respiratory system disease. Interestingly, DPP4 has a striking role in these disorders, especially on type 2 diabetes mellitus (T2DM) (Yang *et al.,* 2020).

1.2.18. Diagnostic Testing in COVID-19

World Health Organization declaring the laboratory testing guiding principles for patients who meet the suspect case definition. The decision to test should be based on clinical and epidemiological factors and linked to an assessment of the likelihood of infection. The PCR testing of asymptomatic or mildly symptomatic contacts can be considered in the assessment of individuals who have had contact with a COVID-19 case (WHO, 2004 ; WHO, 2020).

Rapid collection and testing of appropriate specimens from patients meeting the suspected case definition for COVID-19 is a priority for clinical

management and outbreak control and should be guided by a laboratory expert. Suspected cases should be screened for the virus with nucleic acid amplification tests (NAAT), such as RT-PCR (WHO, 2020).

All patients that meet the suspected case definition should be tested for COVID-19 virus regardless of whether another respiratory pathogen is found like community-acquired pneumonia (WHO, 2020).

Specimens should be collected from upper respiratory tract by nasopharyngeal and oropharyngeal swab or wash in ambulatory patients and/or from lower respiratory specimens like sputum (if produced) and/or endotracheal aspirate or bronchoalveolar lavage in patients with more severe respiratory disease (WHO, 2020).

For transport of specimens for viral detection, use viral transport medium (VTM) containing antifungal and antibiotic supplements. Avoid repeated freezing and thawing of specimens. If VTM is not available sterile saline may be used instead (WHO, 2020).

1.2.18.1. Nucleic Acid Amplification Tests (NAAT)

Routine confirmation of cases of COVID-19 is based on detection of unique sequences of virus RNA by NAAT such as real-time reverse transcription polymerase chain reaction (rRT-PCR) with confirmation by nucleic acid sequencing when necessary. The viral genes targeted so far include the N, E, S and *RdRp* genes. RNA extraction should be done in a biosafety cabinet in a BSL-2 or equivalent facility. Heat treatment of specimens before RNA extraction is not recommended (WHO, 2020).

One positive NAAT result for the presence of betacoronavirus, and COVID-19 virus further identified by sequencing partial or whole genome of the virus as long as the sequence target is larger or different from the amplicon probed in the NAAT assay used (WHO, 2020).

One or more negative results do not rule out the possibility of COVID-19 virus infection. A number of factors could lead to a negative result in an infected individual, including: poor quality of the specimen, the specimen was collected late or very early in the infection, the specimen was not handled and shipped appropriately or there is a technical reason inherent in the test, e.g., virus mutation or PCR inhibition (WHO, 2020). If a negative result is obtained from a patient with a high index of suspicion for COVID-19 virus infection, particularly when only upper respiratory tract specimens were collected, additional specimens, including from the lower respiratory tract, if possible, should be collected and tested (WHO, 2020).

1.2.18.2. Viral Sequencing

In addition to providing confirmation of the presence of the virus, regular sequencing of a percentage of specimens from clinical cases can be useful to monitor for viral genome mutations that might affect the performance of medical countermeasures, including diagnostic tests. Virus whole genome sequencing can also inform molecular epidemiology studies. Many public-access databases for deposition of genetic sequence data are available, including GISAID, which is the Global Initiative on Sharing All Influenza Data, (previously: Global Initiative on Sharing Avian Influenza Data), is a global science initiative established in 2008 to provide access to genomic data of influenza viruses (WHO, 2020).

1.2.18.3. Serological Testing

Serological surveys can aid investigation of an ongoing outbreak and retrospective assessment of the attack rate or extent of an outbreak. In cases where NAAT assays are negative and there is a strong epidemiological link to COVID-19 infection, paired serum specimens (in the acute and convalescent phase) could support diagnosis once validated serology tests are available. Serum specimens can be stored for these purposes (WHO, 2020).

Cross reactivity to other coronaviruses can be challenging, but commercial and non-commercial serological tests are currently under development. Serological tests play an important role in broad-based surveillance of COVID-19, and many commercial manufactured antibody testing kits are available to evaluate the presence of antibodies against SARS-CoV-2 (Bai *et al.,* 2020 ; WHO, 2020).

Despite the numerous antibody tests designed to date, serologic testing has limitations in specificity and sensitivity, and results from different tests vary. However, an antibody test with a specificity higher than 99% and a sensitivity of 96% has been developed by the CDC, which can identify past SARS-CoV-2 infection (Pandey *et al.,* 2022).

1.2.18.4. Viral Culture

Virus isolation is not recommended as a routine diagnostic procedure according to WHO guidelines (WHO, 2020).

1.2.18.5. Other Laboratory Assessment

Complete blood count (CBC), a comprehensive metabolic panel (CMP) that includes testing for renal and liver function, and a coagulation panel should be performed in all hospitalized patients (Cascella *et al.,* 2023).

Additional tests such as testing for inflammatory markers such as, C-reactive protein (CRP), ferritin, lactate dehydrogenase (LDH), random blood sugar (RBS), HbA1c, D-dimer, and procalcitonin can be considered in hospitalized patients (Cascella *et al.,* 2023).

Considering this viral illness commonly manifests itself as pneumonia, radiological imaging has a fundamental role in the diagnostic process, management, and follow-up. Imaging studies may include chest x-ray, lung ultrasound, or chest computed tomography (CT). There are no guidelines available regarding the timing and choice of pulmonary imaging studies in

patients with COVID-19, and the type of imaging should be considered based on clinical evaluation (Bao *et al.,* 2020).

1.2.19. Management

Initially, early in the pandemic, the understanding of COVID-19 and its therapeutic management was limited, creating an urgency to mitigate this new viral illness with experimental therapies and drug repurposing. Since then, due to the intense efforts of clinical researchers globally, significant progress has been made, which has led to a better understanding of not only COVID-19 and its management but also has resulted in the development of novel therapeutics and vaccine development at an unprecedented speed (Pandey *et al.,* 2022).

1.2.19.1. Pharmacologic Therapies

Currently, a variety of therapeutic options are available that include antiviral drugs (e.g., Molnupiravir, Paxlovid, Remdesivir), anti-SARS-CoV-2 monoclonal antibodies (e.g., Bamlanivimab/Etesevimab, Casirivimab/Imdevimab), anti-inflammatory drugs (e.g., Dexamethasone), immunomodulators agents (e.g., Baricitinib, Tocilizumab) are available under FDA issued Emergency Use Authorization (EUA) or being evaluated in the management of COVID-19 (Pandey *et al.,* 2022).

1.2.19.1.1. Antiviral Therapies

Molnupiravir is a directly acting broad-spectrum oral antiviral agent acting on the RdRp enzyme was initially developed as a possible antiviral treatment for influenza, alphaviruses including Eastern, Western, and Venezuelan equine encephalitic viruses (Singh *et al.,* 2021)

Paxlovid (Ritonavir in combination with Nirmatrelvir) is an oral combination pill of two antiviral agents which on an interim analysis of phase 2-3 data (reported via press release), found that the risk of COVID-19 related hospital admission or all-cause mortality was 89% lower in the Paxlovid

group when compared to placebo when started within three days of symptom onset. The FDA issued a EUA authorizing the use of Paxlovid for patients with mild to moderate COVID-19 (Mahase, 2021 ; Aleem *et al.,* 2023).

Remdesivir is a broad-spectrum antiviral agent that previously demonstrated antiviral activity against SARS-CoV-2 in vitro (Wang *et al.,* 2020c). Based on results from three randomized, controlled clinical trials that showed that remdesivir was superior to placebo in shortening the time to recovery in adults who were hospitalized with mild-to-severe COVID-19. The U.S. Food and Drug Administration (FDA) approved remdesivir for clinical use in adults and pediatric patients (over age 12 years and weighing at least 40 kilograms or more) to treat hospitalized patients with COVID-19 (Beigel *et al.,* 2020 ; Goldman *et al.,* 2020 ; Spinner *et al.,* 2020).

Hydroxychloroquine and chloroquine were proposed as antiviral treatments for COVID-19 initially during the pandemic. However, data from randomized control trials evaluating the use of Hydroxychloroquine with or without Azithromycin in hospitalized patients did not improve the clinical status or overall mortality compared to placebo. Data from randomized control trials of Hydroxychloroquine used as postexposure prophylaxis did not prevent SARS-CoV-2 infection or symptomatic COVID-19 illness (Boulware *et al.,* 2020 ; Cavalcanti *et al.,* 2020 ; Horby *et al.,* 2020 ; Mitjà *et al.,* 2021).

1.2.19.1.2. Convalescent Patient Serum

Patients who have recovered from the SARS-CoV-2 infection will develop a polyclonal immune response to viral antigens. These polyclonal antibodies may neutralize virus and prevent further infection in the recovered host (Arabi *et al*., 2015; Zhao *et al*., 2015; Al-Tawfiq *et al*., 2017). Therefore, convalescent patient serum may be a potentially effective tool for treating and preventing further disease related to this outbreak. It was reported that serum from a

convalescent SARS patient inhibited SARS-S protein-driven virus entry and reduced SARS-CoV-2 virus entry (Hoffmann *et al*., 2020b).

Passive transfer of antibodies from convalescent patient serum is currently under consideration for the treatment of COVID-19 patients with severe disease.

1.2.19.1.3. Immunomodulatory Agents

Corticosteroids, an anti-inflammatory steroid prescribed for patients with severe COVID-19, are associated with inflammation-related lung injury driven by the release of cytokines characterized by an elevation in inflammatory markers (Horby *et al.,* 2020).

The oral or intravenous dexamethasone might be considered in early stage of symptomatic patients with hypoxia ($SaO_2 \leq 94\%$ at room air), and if untreated even sudden death, especially in patients older than 60 years old (Lai *et al.,* 2020).

Dexamethasone is recommended by the World Health Organization for patients with severe and critical COVID-19 based on a prospective meta-analysis (Lamontagne *et al.,* 2020 ; Sterne *et al.,* 2020).

Interferon-β-1a (IFN- β-1a) are cytokines that are essential in mounting an immune response to a viral infection, and SARS-CoV-2 suppresses its release in vitro (Yuen *et al.,* 2020).

Interleukin (IL)-1 Antagonists, Anakinra is an interleukin-1 receptor antagonist that is FDA approved to treat rheumatoid arthritis. Its off-label use in severe COVID-19 was assessed in a small case-control study trial based on the rationale that the severe COVID-19 is driven by cytokine production, including interleukin -1β (Huet *et al.,* 2020).

Anti-IL-6 receptor monoclonal antibodies, a proinflammatory cytokine that is considered the key driver of the hyperinflammatory state associated with COVID-19. Targeting this cytokine with an IL-6 receptor inhibitor could slow

down the process of inflammation, based on case reports that showed favorable outcomes in patients with severe COVID-19 (Cellina *et al.,* 2020 ; Conti *et al.,* 2020 ; Michot *et al.,* 2020).

Tocilizumab, an anti-interleukin-6 receptor alpha receptor monoclonal antibody that has been indicated for various rheumatological diseases. The data regarding the use of this agent is mixed (Rosas *et al.,* 2021).

1.2.20. Management of COVID-19 Based on Severity

Individuals with a positive COVID-19 test without any clinical symptoms consistent with COVID-19 should be advised to isolate themselves and monitor clinical symptoms (Inui *et al.,* 2020 ; Abdul Aleem *et al.,* 2023).

Individuals with mild illness is manageable in the ambulatory setting with supportive care and isolation. Laboratory and radiographic evaluations are routinely not indicated (Abdul Aleem *et al.,* 2023).

Patients with moderate COVID-19 illness should be hospitalized for close monitoring. Clinicians and healthcare staff should do appropriate personal protective equipment (PPE) while interacting or taking care of the patient (Abdul Aleem *et al.,* 2023). All hospitalized patients should receive supportive care with isotonic fluid resuscitation if volume-depleted, and supplemental oxygen therapy must be initiated if SpO_2 and be maintained no higher than 96% (Alhazzani *et al.,* 2020).

Patients with severe or critical COVID-19 illness require hospitalization. Considering that patients with severe COVID-19 are at increased risk of prolonged critical illness and death, discussions regarding care goals, reviewing advanced directives, and identifying surrogate medical decision-makers must be made. All patients should be maintained on prophylactic anticoagulation, considering COVID-19 is associated with a prothrombotic state (Alhazzani *et al.,* 2020 ; Cook *et al.,* 2020 ; Coopersmith *et al.,* 2021)

1.2.21. Prevention of COVID-19

Besides the importance of imposing public health and infection control measures to prevent or decrease the transmission of SARS-CoV-2, the most crucial step to contain this global pandemic is by vaccination to prevent SARS-CoV-2 infection in communities across the world. Extraordinary efforts by clinical researchers worldwide during this pandemic have resulted in the development of novel vaccines against SARS-CoV-2 at an unprecedented speed to contain this viral illness that has devastated communities worldwide (Abdul Aleem *et al.,* 2023).

Vaccination triggers the immune system leading to the production of neutralizing antibodies against SARS-CoV-2 (Polack *et al.,* 2020). Globally, vaccination statistics was reported by WHO since being declared a global pandemic to 28 June 2023, there have been 13,461,751,619 vaccine doses administered of COVID-19, including 5,579,548,776 persons vaccinated with at least one dose, and 5,137,861,772 persons vaccinated with a complete primary series (WHO, 2023).

The Pfizer BioNTech (BNT162b2) COVID-19 vaccine is results of an ongoing multinational, placebo-controlled, observer-blinded, pivotal efficacy trial reported that individuals 16 years of age or older receiving two-dose regimen the trial vaccine BNT162b2 (mRNA-based, BioNTech/Pfizer) when given 21 days apart conferred 95% protection against COVID-19 with a safety profile similar to other viral vaccines (Polack *et al.,* 2020). Based on the results of this vaccine efficacy trial, the FDA issued a EUA on December 11, 2020, granting the use of the BNT162b2 vaccine to prevent COVID-19 (FDA, 2021a).

The Moderna COVID-19 (mRNA-1273) vaccine is results from another multicenter, phase 3, randomized, observer-blinded, placebo-controlled trial demonstrated that individuals who were randomized to receive two doses of mRNA-1273 (mRNA based, Moderna) vaccine given 28 days apart showed

94.1% efficacy at preventing COVID-19 illness and no safety concerns were noted besides transient local and systemic reactions. Based on the results of this vaccine efficacy trial, the FDA issued a EUA on December 18, 2020, granting the use of the mRNA-1273 vaccine to prevent COVID-19 (Wu *et al.,* 2021).

The Janssen Ad26.COV2.S vaccine is a third vaccine for the prevention of COVID-19 received EUA by the FDA on February 27, 2021, based on the results of an international multicenter, randomized, placebo-controlled multicenter, phase 3 trial showed that a single dose of Ad26.COV2.S vaccine conferred 73.1% efficacy in preventing COVID-19 in adult participants who were randomized to receive the vaccine (Sadoff *et al.,* 2021).

AstraZeneca ChAdOx1-S/nCoV-19 recombinant vaccine is an interim analysis of an ongoing multicenter randomized control trial demonstrated an acceptable safety profile and clinical efficacy of 70.4% against symptomatic COVID-19 after two doses and 64 % protection against COVID-19 after at least one standard dose (Voysey *et al.,* 2021).The ChAdOx1 nCoV-19 vaccine has been approved or granted emergency use authorization to prevent COVID-19 in many countries across the world but has not yet received a EUA or approval from the FDA for use in the U.S (FDA, 2021b).

Novavax NVX-CoV2373 vaccine is preliminary results from a randomized, observer-blinded, placebo-controlled, phase 2 trial in South Africa evaluating the efficacy and safety of NVX-CoV2373 (Novavax), a recombinant SARS-CoV-2 nanoparticle genetically engineered vaccine, reported that NVX-CoV2373 vaccine was efficacious in preventing COVID-19. This trial was conducted when the country was experiencing a second wave of infection due to the Beta (B.1.351) variant implying efficacy against this virus. A single dose of NVX-CoV2373, which is an adjuvanted, recombinant spike protein nanoparticle vaccine, demonstrated 92.6% (95% CI, 83.6 to 96.7) vaccine efficacy against any variant of concern based on results from a randomized observer-blinded placebo-

controlled trial in the United States and Mexico involving more than 29,000 participants (Marchese *et al.,* 2023).

In addition to the vaccines mentioned above, as many as seven other vaccines, including protein-based and inactivated vaccines, have been developed indigenously in India (Covaxin), Russia (Sputnik V), and China (CoronaVac) and have been approved or granted emergency use authorization to prevent COVID-19 in many countries around the world (Pandey *et al.,* 2022).

A third dose (booster dose) has been included in the vaccination schedule of various nations, with studies showing some amount of waning of immunity after 2 doses and a third dose offering higher protection levels (Goldberg *et al.,* 2021 ; Saiag *et al.,* 2021). A phase 2 randomized controlled trial from the United Kingdom which compared various combinations of boosting regimens concluded that mixing vaccine types boosted antibody as well as neutralizing responses for all seven vaccines studied, which included most major commercially available vaccines (Munro *et al.,* 2021).

CHAPTER TWO

MATERIALS AND METHODS

2. Materials and Methods

2.1. Materials

2.1.1. Instruments and Equipment

The instruments and equipment that were used in this study is shown in table (2-1).

Table (2-1): Instruments and equipment that were used in this study.

No.	Instruments and Equipment	Company	Country
1	Centrifuge	PLC Series	Taiwan
2	Deep Freezer -20° C	Haier Company	China
3	Deep Freezer -80° C	Haier Company	China
4	Digital Camera	Nikon	Japan
5	Disposable Syringe 5ml	LUER LOCK	China
6	EDTA Tube 2ml	Ab Medical	Korea
7	ELISA Microplate Reader	PARAMEDICAL	Italy
8	Eppendorf Tubes	Sigma	England
9	Exispin Vortex Centrifuge	Bioneer	Korea
10	Gel Electrophoresis	Bioneer	Korea
11	Gel/ Clot Activator Vacuum Tube 6ml	Ab Medical	Korea
12	High Speed Cold Centrifuge	Eppendorf	Germany
13	Hot Plat Stirrer	Labtech	Korea
14	Incubator	Memmert	Germany
15	Magnesia® 16 Automated Nucleic Acid Extraction Instrument	Anatolia Geneworks	Turkey
16	Micropipettes (different volumes)	Eppendorf	Germany
17	Miniopticon Real Time PCR	Bio-Rad	USA
18	Nanodrop	Thermo Scientific	UK
19	Refrigerator	Arçelik	Turkey
20	Reusable Ice Packs	J.L. CHILDRESS	USA
21	Sensitive Balance	Sartorius	Germany
22	Sterile Latex Gloves	Broche	Armenia
23	T100 Thermal Cycler PCR	BioRad	USA
24	Tips for Micropipette	ALS	China
25	UV Transilluminator	ATTA	Korea
26	Vortex	CYAN	Belgium
27	Water Bath	Kottermann	Germany

2.1.2. Rapid Test Kit

The COVID-19 Ag rapid test device was used in this study with companies and countries of origin is shown in table (2-2).

Table (2-2): Panbio™ COVID-19 Ag rapid test device was used in this study for healthy control group.

No.	PANBIO™ COVID-19 Ag Rapid Test Device	Company	Country
1	25 Test devices with desiccant in individual foil pouch	Abbott	USA
2	Buffer (1 x 9 ml/bottle)		
3	25 Extraction tubes		
4	25 Extraction tube caps		
5	1 Positive control swab		
6	1 Negative control swab		
7	25 Sterilized nasal swabs for sample collection		
8	1 Tube rack		

2.1.3. Gene Expression Kits

The kits that were used in this study with companies and countries of origin is shown in table (2-3).

Table (2-3): The kits were used in this study with companies and origins.

No.	Kit	Company	Country
1	**AccuZol™ Total RNA Extraction Kit**	Bioneer	Korea
	Trizol Reagent 100 ml		
2	DNase I Enzyme Kit	Promega	USA
	DNase I Enzyme		
	10x Buffer		
	Free Nuclease Water		
	Stop Reaction		
3	**M-MLV Reverse Transcriptase Kit**	Bioneer	Korea
	M-MLV Reverse Transcriptase (10,000 U)		
	5X M-MLV RTase Reaction Buffer		
	dNTP		
	100 mM DTT		
	RNase Inhibitor		
4	**GoTaq ® qPCR Master Mix**	Promega	USA
	qPCR Master Mix for SYBR Green Dye, Taq DNA Polymerase dNTPs (dATP, dCTP, dGTP, dTTP) and 10X Buffer		

2.1.4. Gene Polymorphism Detection Kit

The Magnesia 16 Genomic DNA Whole Blood Kit is used for DNA extraction (Anatolia Geneworks, Turkey). Also, GoTaq® G2 Green Master Mix kit is used for the gene polymorphism (Promega, Korea) in this study are shown in table (2-4).

Table (2-4): Gene polymorphism detection kit was used in this study.

No.	GoTaq® G2 Green Master Mix kit	Company	Country
1	Taq DNA Polymerase	Promega	Korea
	dNTPs (dATP, dCTP, dGTP, dTTP)		
	Tris. HCl pH 9.0		
	KCl		
	$MgCl_2$		
	Loading Dye		

2.1.5. Primers

2.1.5.1. miRNA Gene Expression Primers

The qPCR Primers for miRNA (miR-423-5p, miR-23a-3p and miR-195-5p) were design in this study by using (The Sanger Center miRNA Database Registry) to selected miRNA sequence and using miRNA Primer Design Tool (http://www.srnaprimerdb.com). Whereas, qPCR housekeeping gene (GAPDH) were design in this study by using NCBI-Database and Primer3 plus design online. These primers were provided by (Macrogen Company, Korea) as following table:

Table (2-5): The qPCR primers for miRNA were designed in this study.

Primer	Sequence (5'-3')		Genbank Code
miR-423-5p qPCR primer	F	AACAAGTGAGGGGCAGAGAG	MIMAT0004748
	R	GTCGTATCCAGTGCAGGGT	
RT primer (specific) miR-423-5p	GTCGTATCCAGTGCAGGGTCCGAGGT ATTCGCACTGGATACGACAAAGTC		
miR-23a-3p qPCR primer	F	AACACGCATCACATTGCCAG	MIMAT0000078
	R	GTCGTATCCAGTGCAGGGT	
RT primer (specific) miR-23a-3p	GTCGTATCCAGTGCAGGGTCCGAGGT ATTCGCACTGGATACGACGGAAAT		
miR-195-5p qPCR primer	F	AACCGGTAGCAGCACAGAA	MIMAT0000461
	R	GTCGTATCCAGTGCAGGGT	
RT primer (specific) miR-195-5p	GTCGTATCCAGTGCAGGGTCCGAGGTA TTCGCACTGGATACGACGCCAAT		
GAPDH qPCR primer	F	TCTGACTTCAACAGCGACAC	NM_001256799.3
	R	TGACAAAGTGGTCGTTGAGG	

2.1.5.2. Sequence-Specific Primers PCR Primers

Sequence-specific primers PCR (SSP-PCR) primers for *HLA* typing were designed in this study using NCBI-Database and Primer3 plus design online. These primers were provided from (Macrogen Company, Korea) as following table:

Table (2-6): The SSP-PCR primers with their sequence and amplicon size.

Primer	Sequence (5'-3')		Product Size	Genbank Code
HLA-B*07:03	F	CCAAAGACACACGTGACCCA	208 bp	LT616965.1
	R	CTTCTCCAGAAGGCACCACC		
HLA-B*46:01	F	CCAGTTCGTGAGGTTCGACA	391 bp	MH124609.1
	R	CTCCAGGTAGGCTCTCCACT		
HLA-DRB1*03:0	F	ACTGCAGACACAACTACGGG	463 bp	MH645234.1
	R	GGCTGAAGTCCAGAGTGTCC		
DRB1*12:02	F	GGACACCAGACCACGTTTCT	570 bp	LC257798.1
	R	CGTGCTCTCCATTCCACTGT		

2.1.5.3. Allele Specific PCR Primers

Allele specific PCR (AS-PCR) primers for *DPP4* (*rs3788979* A/G) gene polymorphism were designed in this study using NCBI-SNP data base and WASP Web-based Allele Specific Primer design tool. These primers were provided from (Macrogen Company, Korea) as following tables:

Table (2-7): The AS-PCR primers with their sequence and amplicon size.

Primer	Sequence (5'-3')	Product Size
Wild Type Forward Primer	CCAACACTGCTGTACTCACA	180 bp
Mutant Type Forward Primer	CCAACACTGCTGTACTCACG	
Common Reverse Primer	AAGTTGGTACAAGATTCCGA	

2.1.6. Chemicals

The chemical and biological materials are listed in following table.

Table (2-8): The chemical and biological materials were used in this work.

No.	Chemicals	Company	Country
1	Absolute Ethanol	Scharlau	Spain
2	Agarose	BioBasic	Canada
3	Chloroform	Labort	India
4	DEPC Water	Bioneer	Korea
5	DNA Marker Ladder 100bp	INtRON	Korea
6	Ethidium Bromide10mg/ml	BioBasic	Canada
7	Isopropanol	Labort	India
8	RNase Free Water	Bioneer	Korea
9	TBE Buffer 10X	Bioneer	Korea
10	RNAlater Stabilization Solution	Thermo Fisher Scientific	USA

2.2. Methods

2.2.1. Subjects of The Study

Whole blood specimens were collected from 100 patients with COVID-19 who were diagnosed by RT-qPCR previously and classified according to severity of infection through symptoms, saturation of peripheral oxygen (SpO_2) and CT imaging into two groups; mild cases (fever <38 ℃, decreased without treatment, with or without cough, no dyspnea, no gasping, no chronic disease, no imaging findings of pneumonia) and severe cases (respiratory distress, respiratory rate (RR) ≥ 30 times / min, SpO_2 < 93% at rest, patients showing a rapid progression > 50 % on CT imaging within 24 - 48 hours) (Fang, 2020), who were attended to Merjan Medical City and Al-Imam Al-Sadiq Hospital in Babylon province from February to April of 2022. Patients' specimens were collected in two groups based on severity of infection: 50 patients with mild symptoms and 50 patients with severe symptoms. Control specimens were collected from 50 people who were healthy.

2.2.2. Study Design

In this a case-control comparative study, patients with COVID-19 were diagnosed by previously RT-qPCR were included. Patients were grouped into two groups regarding the severity of COVID-19 according to clinical features and laboratory tests including; CRP, CBC, HbA1c, RBS, D-dimer, and ferritin levels.

Apparently healthy subjects enrolled as control group selected depending on the results of COVID-19 rapid test (COVID-19 antigen test).

This study included 100 specimens collected from patients (50 specimens collected from patients were suffering from mild symptoms, 50 patients with severe symptoms who were clinically classified according to SpO_2 < 93% and CT imaging), and 50 healthy individuals as control group.

2.2.3. Inclusion Criteria

Individuals with confirmed positive COVID-19 by RT-PCR of any age, sex, and even individuals with diabetes mellitus type 2 (T2DM) and hypertension were included in this study.

2.2.4. Exclusion Criteria

Individuals suffering from autoimmune diseases (Graves' disease, Hashimoto's autoimmune thyroiditis, Addison disease, type I diabetes, etc.) and connective tissue diseases (rheumatoid arthritis, scleroderma, systemic lupus erythematosus, ankylosing spondylitis, etc.) were excluded from this study by past medical history.

2.2.5. Ethical Approval

All of the patients and the healthy control subjects are counseled and vocally agreed on the inclusion in the study before participation in the study. This study is performed and being facilitated with permission from Babylon university, College of Medicine, and the General Health Directorate of Babylon province.

2.2.6. Patient's Information Sheet

Information Sheet included general data and laboratory tests as listed in Appendix (1) and (2).

2.2.7. Specimens Collection

2.2.7.1. Blood Specimens Collection

The whole blood specimens were collected in two types of tubes, EDTA tube as anticoagulation tube for molecular analysis. A 500 µl of RNAlater was added to 500 µl of anticoagulated blood from an EDTA tube into an Eppendorf tube to stabilize RNA specimens and stored at -20 °C. The gel and clot activator vacuum tubes with collected blood were centrifuged to separate sera at supernatant, which were evacuated into Eppendorf tubes and stored in a deep freezer at -20 °C to be used in ELISA technique.

2.2.7.2. Swab Specimens Collection

Swab specimens were collected from healthy individuals as a control group to confirm negative result for COVID-19, and was done according to test procedure described by Abbott company, USA instructions as follow:

1. The patient's head was Tilted back 70 degrees. While gently rotating the swab, swab was inserted less than one inch (about 2 cm) into nostril (until resistance is met at the turbinates).
2. The swab was rotated five times against the nasal wall then slowly remove from the nostril.
3. Using the same swab repeat the collection procedure with the second nostril. specimen collection with a new swab.
4. The swab tip was swirled in the buffer fluid inside the extraction tube, pushing into the wall of the extraction tube at least five times and then the swab was squeeze out by the extraction tube with fingers.
5. The swab was broken at the breakpoint and close the cap of extraction tube.

2.2.8. Schematic Diagram of The Study

The schematic diagram of the study is shown in figure (2-1).

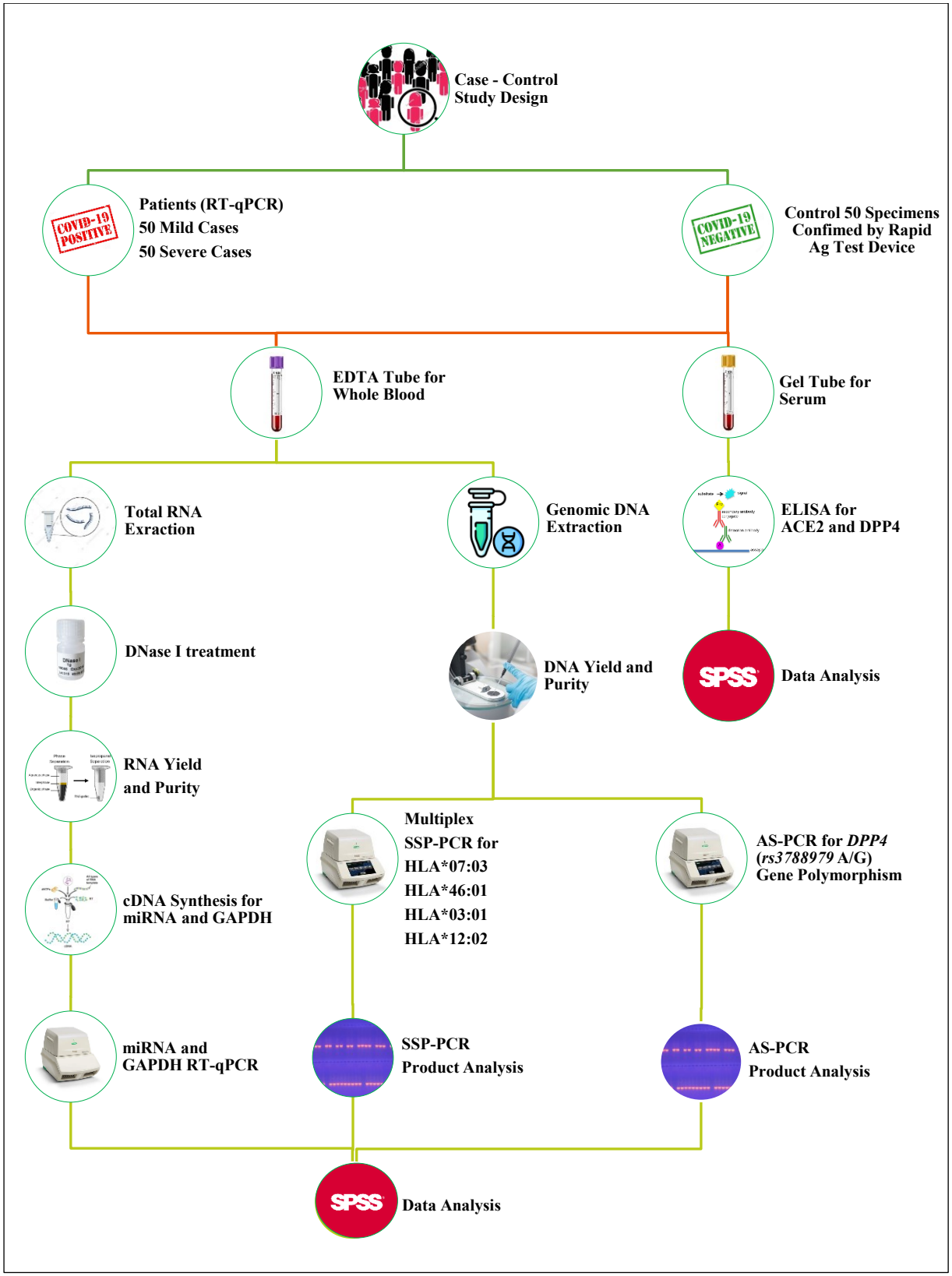


Figure (2-1): Schematic diagram of the study.

2.2.9. Rapid Test Procedure

Swab specimens were checked by Panbio™ COVID-19 Ag rapid test device to confirm negative result for COVID-19 from healthy individuals as a control group and done according to test procedure described by Abbott company, USA instructions as following steps:

1. All kit components allowed to reach a temperature between 15-30 °C prior to testing for 30 minutes.
2. The test device was removed from the foil pouch prior to use. In a flat, horizontal and clean surface should be placed.
3. The buffer bottle was hold vertically and the extraction tube was filled with buffer fluid until it flows up to the Fill-line of the extraction tube (300 µl).
4. The extraction tube was placed in the tube rack.
5. The dropping nozzle cap was opened at the bottom of the extraction tube.
6. Five drops of extracted specimens were dispensed vertically into the specimen well (S) on the device. The test device should not handle or move until the test is complete and ready for reading.
7. The nozzle was closed and disposed of the extraction tube containing the used swab according to the local regulations and biohazard waste disposal protocol.
8. The result was read at 15 minutes. The result shouldn't read results after 20 minutes.
9. The used device was disposed according to biohazard waste disposal protocol.

2.2.10. Stem Loop RT-qPCR

The stem loop RT-qPCR was used in expression analysis quantification of miR-423-5p, miR-23a-3p and miR-195-5p, that normalized with housekeeping gene (GAPDH) in blood specimens of patients with COVID-19 and heathy control by using Real-Time PCR technique and this method was carried out according to described by (Farr *et al.,* 2021) by the following steps:

2.2.10.1. Total RNA Extraction

Total RNA was extracted from blood specimens by using AccuZol™ reagent kit and done according to company instructions as following steps:

1. A 250 µl blood specimens were placed in 1.5 microcentrifuge tube then 750 µl AccuZol™ reagent kit was added to each tube.
2. Then, 200 µl chloroform was added to each tube and mixed vigorously for 60 seconds.
3. The mixture was incubated on ice for 5 minutes. Then it was centrifuged at 12000 rpm, 4C°, for 15 minutes.
4. Supernatant was transferred into a new Eppendorf tube, and 500 µl isopropanol was added. Then, mixture was mixed by inverting the tube 4-5 times and incubated at 4 °C for 10 minutes. Then, it was centrifuged at 12,000 rpm, 4 °C for 10 minutes.
5. Supernatant was discarded, and 1ml 80% Ethanol with DEPC was added and mixed by vortex again. Then, it was centrifuge at 12000 rpm, 4 °C for 5 minutes.
6. The supernatant was discarded and the RNA pellet was left to air to dry.
7. A 100 µl free nuclease water was added to each specimen to dissolve the RNA pellet, Then, the extracted RNA specimen was kept at -80 °C in deep freezer.

2.2.10.2. Estimation of Total RNA Yield and Quality

The extracted total RNA was checked by using Nanodrop spectrophotometer (Thermo Scientific. USA) that check RNA concentration and estimation of RNA purity through reading the absorbance in at (260 / 280 nm) as following steps:

1. After opening up the Nanodrop software, chosen the appropriate application (Nucleic acid, RNA).
2. A dry wipe was taken and cleaned the measurement pedestals several times. Then carefully pipet 2µl of ddH_2O onto the surface of the lower measurement pedestal.
3. The sampling arm was lowered and clicking OK to blank the Nanodrop, then cleaning off the pedestals.
4. After that, the pedestals are cleaned and pipet 1 µl of RNA specimen for measurement.

2.2.10.3. DNase I Treatment

The extracted total RNA was treated with DNase I enzyme to remove the trace amounts of genomic DNA from the eluted total RNA by using specimens (DNase I enzyme kit) and done according to method described by Promega Company, USA instructions as following table (2-9):

Table (2-9): Eluted total RNA by using DNase I enzyme kit.

No	Mix	Volume
1	Total RNA 100 ng/µl	10 µl
2	DNase I Enzyme	1 µl
3	10X Buffer	4 µl
4	DEPC Water	5 µl
Total		20 µl

After that, the mixture was incubated at 37 °C for 30 minutes. Then, 1µl stop solution was added and incubated at 65 °C for 10 minutes for inactivation of DNase enzyme action.

2.2.10.4. cDNA Synthesis

2.2.10.4.1. cDNA Synthesis for miRNA

The DNase I treated RNA specimens were used in miRNA cDNA synthesis step by using M-MLV Reverse Transcriptase kit and done according to company instructions as following tables:

Table (2-10): Step 1 in cDNA synthesis for miRNA.

No.	**RT Master Mix**	**Volume**
1	Total RNA 100 ng/µl	8 µl
2	hsa-miR-21RT Primer	1 µl
3	DEPC Water	1 µl
Total		10 µl

Then RNA and primer were denatured for 10 min at 75 ˚C, after that immediately cool on ice.

Table (2-11): Step 2 in cDNA synthesis for miRNA.

No	RT Master Mix	Volume
1	Step 1 RT Master Mix	10 µl
2	M-MLV RTase (200u)	1 µl
3	5X M-MLV RTase Reaction Buffer	4 µl
4	100 mM DTT	2 µl
5	dNTP	2 µl
6	RNase Inhibitor	1 µl
Total		20 µl

Then the tubes were placed in vortex and briefly spinning down. The RNA converted into cDNA in thermocycler under the following thermocycler conditions, as shown in table (2-12).

Table (2-12): The thermocycler condition in step 2 of cDNA synthesis for miRNA.

Step	Temperature	Time
cDNA Synthesis (RT Step)	42 °C	1 hour
Heat Inactivation	95 °C	5 minutes

2.2.10.4.2. cDNA Synthesis for *GAPDH* Gene

The DNase I treated RNA specimens were also used cDNA synthesis step for *GAPDH* gene by using M-MLV Reverse Transcriptase kit and done according to company instructions as following tables:

Table (2-13): Step 1 in cDNA synthesis for *GAPDH* Gene.

No.	RT Master Mix	Volume
1	Total RNA 100 ng/µl	8 µl
2	Random Hexamer Primer	1 µl
3	DEPC Water	1 µl
Total		10 µl

Then RNA and primer was denatured for 10 min at 65 °C, after that immediately cool on ice.

Table (2-14): Step 2 in cDNA synthesis for *GAPDH* Gene.

No.	RT Master Mix	Volume
1	Step 1 RT Master Mix	10 µl
2	M-MLV RTase (200u)	1 µl
3	5X M-MLV RTase reaction buffer	4 µl
4	100 mM DTT	2 µl
5	dNTP	2 µl
6	RNase inhibitor	1 µl
Total		20 µl

Then the tubes were placed in vortex and briefly spinning down. The RNA converted into cDNA in thermocycler under the following thermocycler conditions as shown in table (2-15).

Table (2-15): Thermocycler conditions in cDNA synthesis for *GAPDH* Gene.

Step	**Temperature**	**Time**
cDNA synthesis (RT step)	42 °C	1 hour
Heat inactivation	95 °C	5 minutes

2.2.10.5. qPCR Master Mix Preparation

2.2.10.5.1. miRNA qPCR Master Mix Preparation

MicroRNA qPCR master mix was prepared by using GoTaq® qPCR master mix kit that dependent on SYBR Green dye detection of gene amplification in Real-Time PCR system as shown in table (2-16).

Table (2-16): Standard protocol in cDNA synthesis for *GAPDH* Gene.

No.	**qPCR Master Mix**	**Volume**
1	miRNA cDNA Template (Final Cocentration100 ng)	5 µL
2	Forward Primer (10 pmol)	1 µL
3	Reverse Primer (10 pmol)	1 µL
4	qPCR Master Mix	10 µL
5	DEPC Water	3 µL
Total		20 µL

After that, these qPCR master mix components that mentioned above were placed in qPCR strip standard plate tubes and mixed by Exispin vortex centrifuge for 3 minutes, then they were placed in Miniopticon Real-Time PCR system.

2.2.10.5.2. miRNA qPCR Thermocycler Conditions

After that, the qPCR plate was loaded and the following thermocycler protocol in the following table:

Table (2-17): Thermocycler conditions in cDNA synthesis for *GAPDH* Gene.

qPCR Step	Temperature	Time	Repeat Cycle
Initial Denaturation	95 °C	10 min	1
Denaturation	95 °C	20 sec	45
Annealing / Extension Detection (Scan)	60 °C	30 sec	

2.2.10.5.3. *GAPDH* qPCR Master Mix Preparation

The *GAPDH* qPCR master mix was prepared by using GoTaq® qPCR master mix kit that dependent on SYBR Green dye detection of gene amplification in Real-Time PCR system as shown in table (2-18).

Table (2-18): Standard protocol in cDNA synthesis for *GAPDH* Gene.

No.	qPCR Master Mix	Volume
1	cDNA Template (100 ng)	5 µl
2	*GAPDH* Forward Primer (10 pmol)	1 µl
3	*GAPDH* Reverse Primer (10 pmol)	1 µl
4	qPCR Master Mix	10 µl
5	DEPC Water	3 µl
Total		20 µl

After that, these qPCR master mix components that mentioned above placed in qPCR strip plate tubes and mixed by Exispin vortex centrifuge for 3 minutes, then they were placed in Miniopticon Real-Time PCR system.

2.2.10.5.4. *GAPDH* qPCR Thermocycler Conditions

After that, the qPCR plate was loaded and the following thermocycler protocol in the following table:

Table (2-19): Thermocycler conditions in cDNA synthesis for *GAPDH* Gene.

qPCR Step	Temperature	Time	Repeat Cycle
Initial Denaturation	95 °C	10 min	1
Denaturation	95 °C	20 sec	45
Annealing / Extention Detection (Scan)	58 °C	30 sec	

2.2.10.5.5. Data Analysis of qPCR

The data results of qRT-PCR for target and housekeeping gene were analyzed by the relative quantification gene expression levels (fold change) (The ΔCT method using a reference gene) that described by (Livak and Schmittgen, 2001) as following equation:

Gene expression ratio (reference / target) = $2^{CT\ (reference) - CT\ (target)}$

2.2.11. Sequence-Specific Primers PCR

The Sequence-Specific Primers PCR (SSP-PCR) technique was performed for *HLA* typing of *HLA-B*07:03*, *B*46:01*, *DRB1*03:01*, and *DRB1 *12:02* alleles in blood specimens of patients with COVID-19 and heathy control. This method was carried out according to a company instruction as following steps:

2.2.11.1. Automated Genomic DNA Extraction

Genomic DNA from blood specimens were extracted by using Magnesia 16 Genomic DNA whole blood kit contains pre-filled cartridge has been designed for purification of total genomic DNA from whole blood by using automated instrument Magnesia 16 (Anatolia Geneworks, Turkey) and done according to company instructions as following steps:

1. The method was performed by using pre-filled cartridge contains proteinase K and a chaotropic salt, guanidine hydrochloride to lysis cells and degrade protein as showed in figure below:

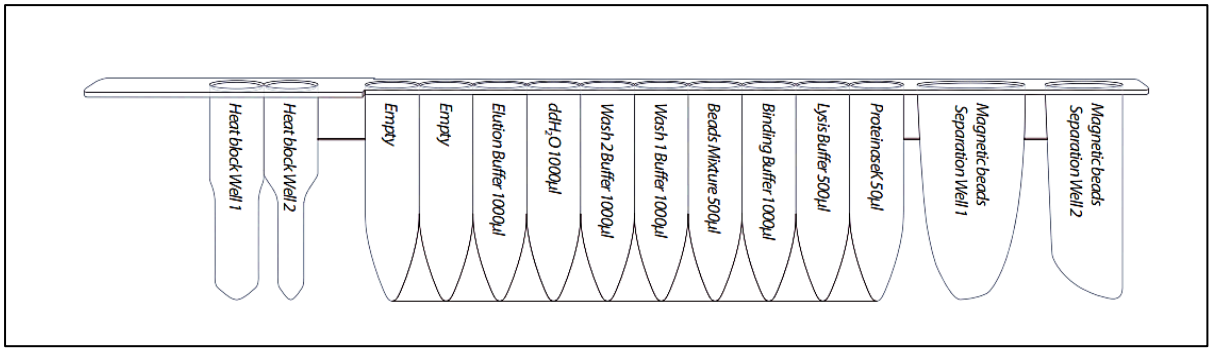


Figure (2-2): Pre-filled cartridge of Magnesia 16 Genomic DNA whole blood kit.

2. A 200µl whole blood sample was transferred in to a microcentrifuge tube, then the Sample Tube was put into hole 4 of T-Rack of automated DNA extraction machine.
3. The blood DNA in chaotropic salt will binds to cellulose coated magnetic beads. After washing off the contaminants, the purified DNA is eluted by 100µl low salt elution buffer or water. Purified DNA of approximately 20-30 kb in length is suitable for PCR or other enzymatic reactions.
4. Running Time: 44 min (sample volume :200 µl) for 16 blood sample.

2.2.11.2 Genomic DNA Estimation

The extracted blood genomic DNA was checked by using Nanodrop spectrophotometer (THERMO, USA), which measured DNA concentration (ng/µl) and check the DNA purity by reading the absorbance at (260 / 280 nm) as following steps:

1. After opening up the Nanodrop software, the appropriate application (Nucleic acid, DNA) was chosen.
2. A dry wipe was taken and cleaned the measurement pedestals several times. Then 2 µl was carefully pipetted of free nuclease water onto the surface of the lower measurement pedestals for blank the system.
3. The sampling arm was lowered and clicking OK to initialized the Nanodrop, then cleaning off the pedestals and 1 µl of blood genomic DNA was added to measurement.

2.2.11.3. Sequence-Specific Primers PCR Master Mix Preparation

The multiplex Sequence-Specific Primers PCR (SSP-PCR) master mix was prepared by using GoTaq® G2 Green Master Mix kit and this master mix done two reactions for each specimen according to company instructions as following tables:

Table (2-20): Multiplex Sequence-Specific Primers PCR master mix.

No.	Multiplex SSP-PCR Master Mix	Volume
1	DNA template 5-50 ng	5 µl
2	HLA-B*07:03 Forward primers (20 pmol)	0.5 µl
3	HLA-B*07:03 Reverse primers (20 pmol)	0.5 µl
4	HLA- B*46:01 Forward primers (20 pmol)	0.5 µl
5	HLA- B*46:01 Reverse primers (20 pmol)	0.5 µl
6	HLA- DRB1*03:01Forward primers (20 pmol)	0.5 µl
7	HLA- DRB1*03:01Reverse primers (20 pmol)	0.5 µl
8	HLA- DRB1 *12:02 Forward primers (20 pmol)	0.5 µl
9	HLA- DRB1 *12:02 Reverse primers (20 pmol)	0.5 µl
10	GoTaq® G2 Green Master Mix	12.5 µl
11	Free Nuclease Water	3.5 µl
Total Volume		25 µl

After that, these SSP-PCR master mix components that mentioned in table (2-20) were transferred into Exispin vortex centrifuge at 3000 rpm for 3 minutes. Then they were placed in PCR thermocycler (BioRad, USA).

2.2.11.4. Sequence-Specific Primers PCR Thermocycler Conditions

Sequence-Specific Primers PCR (SSP-PCR) Primers thermocycler conditions were done as following table:

Table (2-21): SSP-PCR thermocycler conditions.

No.	SSP-PCR Step	Temperature	Time	Repeat
1	Initial Denaturation	95 °C	5 min.	1
2	Denaturation	95 °C	30 Sec.	35 Cycle
3	Annealing	55 °C	30 Sec.	
4	Extension	72 °C	30 Sec.	
5	Final Extension	72 °C	5 min	1
6	Hold	4 °C	Forever	-

2.2.11.5. Sequence-Specific Primers PCR Product Analysis

Sequence-Specific Primers PCR (SSP-PCR) products were analyzed by agarose gel electrophoresis following steps:

1. A 2% Agarose gel was prepared in using 1X TBE and dissolving in water bath at 100 °C for 15 minutes, after that, it was left to cool at 50 °C.
2. Then 3 µl of ethidium bromide stain (10 mg/µl) was added into agarose gel solution.
3. Agarose gel solution was poured in tray after fixed the comb in proper position after that, it was left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray.
4. The gel tray was fixed in electrophoresis chamber and fill by 1X TBE buffer.

5. A 10 µl SSP-PCR product was added in to each comb well and 3 µl of (100 bp Ladder) in first well.
6. The electric current was performed at 85 volt and 80 mA for 1.5 hour.
7. The SSP-PCR products were visualized by using UV Transilluminator.

2.2.12. Allele Specific PCR

The Allele Specific PCR **(**AS-PCR) technique was performed for detection and genotyping of *DPP4* (*rs3788979* A/G) gene polymorphism in blood specimens of patients with COVID-19 and heathy control. This method was carried out according to company instructions as following steps:

2.2.12.1. Allele Specific PCR Master Mix Preparation

Allele specific PCR (AS-PCR) master mix was prepared by using GoTaq® G2 Green Master Mix kit and this master mix was done including two reactions (wild type allele and mutant type allele) for each specimen according to company instructions as following table:

Table (2-22): Wild type allele AS-PCR reaction mix.

No.	AS PCR Master Mix	Volume
1	DNA template	5 µl
2	Wild type Forward primers (10pmol)	2 µl
3	Common Reverse Primer (10pmol)	2 µl
4	G2 Green Master Mix	12.5 µl
5	PCR Water	3.5 µl
Total Volume		25 µl

Table (2-23): Mutant type allele AS-PCR reaction mix.

No.	AS PCR Master Mix	Volume
1	DNA template	5 µl
2	Mutant Type Forward Primers (10 pmol)	2 µl
3	Common Reverse Primer (10 pmol)	2 µl
4	G2 Green Master Mix	12.5 µl
5	PCR water	3.5 µl
Total volume		25 µl

After that, these AS-PCR master mix component that mentioned in table (2-22) and (2-23) were transferred into Exispin vortex centrifuge at 3000 rpm for 3 minutes. Then it was placed in PCR Thermocycler (BioRad, USA).

2.2.12.2. Allele Specific PCR Thermocycler Conditions

The PCR thermocycler conditions were done for each gene independent as following table:

Table (2-24): The PCR thermocycler conditions for AS-PCR.

PCR Step	Temperature	Time	Repeat
Initial Denaturation	95 °C	5 min.	1
Denaturation	95 °C	30 sec.	35 Cycle
Annealing	58 °C	30 sec.	
Extension	72°C	30 sec.	
Final extension	72°C	5 min	1
Hold	4°C	Forever	-

2.2.12.3. Allele Specific PCR Product Analysis

The Allele Specific PCR (AS-PCR) products were analyzed by agarose gel electrophoresis following steps:

1. A 2% Agarose gel was prepared in using 1X TBE and dissolving in water bath at 100 °C for 15 minutes, after that, it was left to cool at 50 °C.
2. Then 3 µl of ethidium bromide stain (10 mg/µl) was added into agarose gel solution.
3. Agarose gel solution was poured in tray after fixed the comb in proper position after that, it was left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray.
4. The gel tray was fixed in electrophoresis chamber and filled by 1X TBE buffer.
5. A 10 µl AS-PCR product was added in to each comb well and 3 µl of (100 bp Ladder) in first well.
6. The electric current was performed at 85 volt and 80 mA for 1.5 hour.
7. The AS-PCR products were visualized by using UV transilluminator.

2.2.13. ELISA Method

The ELISA method was used in this study for quantitative determination of ACE2 and DPP4 levels from in patient and healthy control serum specimens and done according to company instruction (BT-LAB, China) as the following table:

Table (2-25): ELISA components.

No	Reagent	Quantity
1	Standard Solution	0.5 ml x1
2	Pre-coated ELISA Plate	12 * 8 well strips x1
3	Standard Diluent	3 ml x1
4	Streptavidin-HRP	6 ml x1
5	Stop Solution	6 ml x1
6	Substrate Solution A	6 ml x1
7	Substrate Solution B	6 ml x1
8	Wash Buffer Concentrate (25x)	20 ml x1
9	Biotinylated Human Antibody	1 ml x1

2.2.13.1. ELISA Procedure

1. All reagents, standard solutions and specimens were prepared according to kit instruction and bring at room temperature before use.
2. A 50 µl standard serial dilution was added into standard well. Without added antibody to standard well because the standard solution contains biotinylated antibody.
3. A 40 µl specimen was added to sample wells and then 10 µl antibody was added to sample wells, then 50 µl streptavidin-HRP was added in to sample wells and standard wells (not blank control well).
4. The ELISA was mixed well and covered the plate with a sealer. Then it was incubated at 60 minutes at 37 °C.
5. Sealer was removed and wash plate 5 times with wash buffer. Wells were socked with at least 350 µl wash buffer for 30 sec to 1 min for each wash.
6. A 50 µl substrate solution A was added to each well and then 50 µl substrate solution B was added to each well.
7. The plate was covered with a new sealer and incubated for 10 minutes at 37 °C in the dark.
8. A 50 µl Stop Solution was added to each well, then the blue color will change into yellow immediately.
9. The optical density (OD value) was determined using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

2.2.13.2. Calculation of ELISA Results:

The ELISA results were calculated depend on the optical density reading for each standard and samples optical density. Then the standard curve was plotted by the mean OD value for each standard on the X-axis against the concentration on the Y-axis and draw a best fit curve through the points on the graph as shown in appendix (7) and (8).

2.2.14. Statistical Analysis

Data were collected, summarized, analyzed and presented using statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. Qualitative (categorical) variables were expressed as number and percentage, whereas, quantitative (numeric) variables were first evaluated for normality distribution using Kolmogorov-Smirnov test, and then accordingly normally distributed numeric variables were expressed as mean (an index of central tendency) and standard deviation (an index of dispersion), while those numeric variables that are not normally distributed were expressed as median (an index of central tendency) and inter-quartile range (an index of dispersion).

The following statistical tests were used:

1. Chi-square Test was use to evaluate association between any two categorical variables provided that less than 20 % of cells have expected count of less than 5. However, Yates correction test was used instead if less than 20 % of cells had expected count of less than 5 and Fischer exact test was chosen in cases of zero observed count.
2. Independent samples *t*-test was used to evaluate difference in mean of numeric variables between two groups provided that these numeric variables were normally distributed; but Mann Whitney U test was chosen in case of non-normally distributed variables.
3. Hardy Weinberg Equilibrium Test was used to evaluate frequency distribution of various genotypes.
4. The risk analysis was based on calculation of Odds ratio and the corresponding 95 % confidence interval.

The level of significance was considered at *p*-value of equal or less than 0.05.

CHAPTER THREE

RESULTS AND DISCUSSION

3. Results and Discussion

3.1. Comparison of Characteristics Between Patients with COVID-19 and Control Subjects

3.1.1. The Demographic Characteristics of Patients with COVID-19 and Control Group

The demographic characteristics of patients with COVID-19 and control group are shown in table (3-1). The mean age of patients was 43.44 ± 19.35 years and the range was between 10 - 80 years. The mean age of control group was 46.24 ± 19.26 years and the range was between 15 -80 years. There was no significant difference in mean age between patients and control group ($p = 0.404$).

The frequency distribution of patients with COVID-19 according to age group is shown in figure (3-1), which revealed that the disease is most frequently seen in the age group from 40 to 49 years (24 %) and is less frequently encountered in the age group between 30 to 39 years (4 %).

With respect to sex, there was no significant difference in the frequency distribution of subjects between patients group and control group ($p = 0.639$). The patients group included 60 (60.0 %) males and 40 (40.0 %) females, as shown in figure (3-1) making the male to female ratio at 1.5:1.

With respect to residency, patients with COVID-19 were categorized into 72 (72.0 %) and 28 (28.0 %) as belonging to urban areas and rural areas, respectively. Control group included 36 (72.0 %) subjects from urban areas and 14 (28.0 %) subjects from rural areas. There was no significant difference between patients group and control group in the frequency distribution of participants based on residency ($p = 1.000$).

Table (3-1): The demographic characteristics of patients with COVID-19 and control group.

Characteristic	Patients Group *n* = 100	Control Group *n* = 50	*p*	Interpretation
Age (years)				
Mean ± SD	43.44 ± 19.35	46.24 ± 19.26	0.404 I	Not Significant
Range	10 -80	15 -80		
Sex				
Male, *n* (%)	60 (60.0 %)	28 (56.0 %)	0.639 C	Not Significant
Female, *n* (%)	40 (40.0 %)	22 (44.0 %)		
Residency Site				
Urban, *n* (%)	72 (72.0 %)	36 (72.0 %)	1.000 C	Not Significant
Rural, *n* (%)	28 (28.0 %)	14 (28.0 %)		

n: number of cases; **SD**: standard deviation; **I:** independent samples *t*-test; **C**: chi-square test

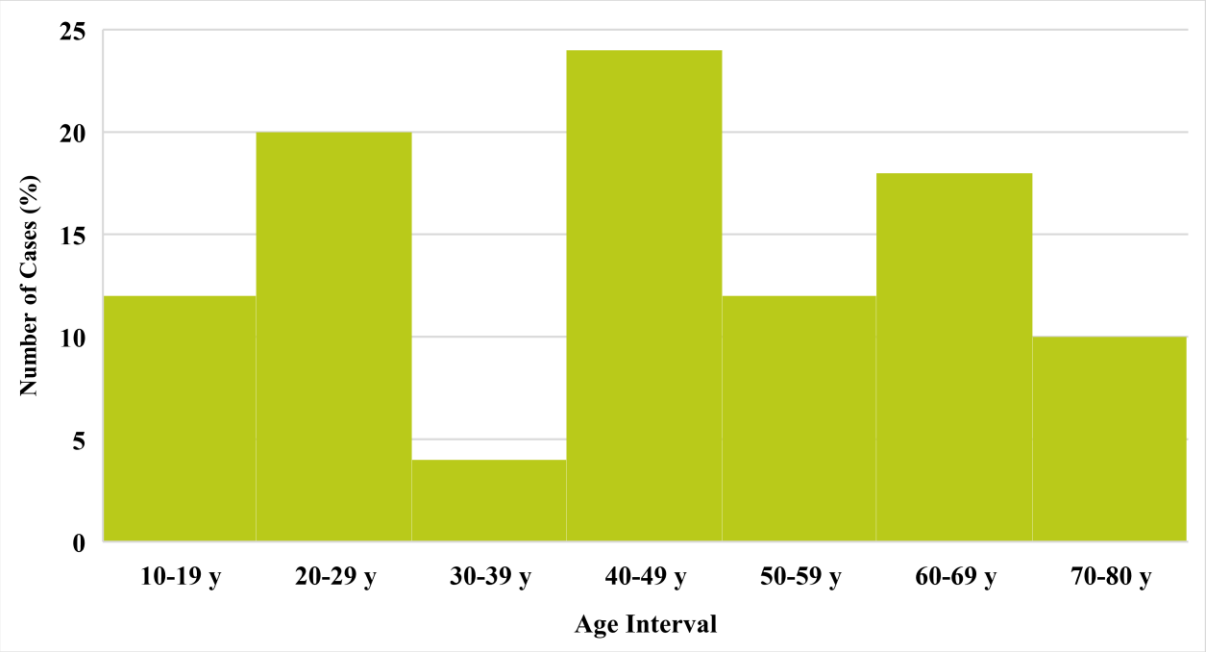


Figure (3-1): Histogram showing the frequency distribution of patients with COVID-19 according to 10 years age interval.

In this study, the mean age of patients with COVID-19 was 43.44 ± 19.35 years and the range was between 10 - 80 years and the most frequent age interval enrolled was from 40 to 49 years. The age of COVID-19 patients in a prior study conducted in Iraq ranged from one year to 103 years, with a mean of 36 years (Merza *et al.,* 2021), which is younger than the age of patients in this study. The average age in another Iraqi study conducted in Baghdad was 44.3 ± 17.0 years (Abbas *et al.,* 2021), which is comparable to the age found in this study. In current study, there was also no significant difference in mean age between patients and control group; as a result, any bias in the results can be avoided because results influenced by age will be excluded, which is necessary in this type of comparison study.

The patients group included 60 (60.0 %) males and 40 (40.0 %) females, making the male to female ratio at 1.5:1 and there was no significant difference in sex proportions between patients and control groups. Numerous studies support this observation by pointing to higher hospitalization rates for males with COVID-19, even after adjusting for age and other comorbidities (Fortunato *et al.,* 2021 ; Gomez *et al.,* 2021).

In the Rush University Health System, from 1 March 2020 to 21 June 2020, Gomez *et al.* (2021) conducted a multicenter, retrospective, cohort study to examine disparities in hospitalization and ICU admission rates between males and females. Males were hospitalized at higher rates than females, according to the authors' multivariable logistic model, which controlled for age and a number of comorbidities. In the Apulian District of Foggia, Italy, between February and June of 2020, Fortunato *et al*. (2021) conducted a retrospective epidemiological investigation of hospitalization rates, variations in viral clearance, and case fatality rates in a group of patients diagnosed with COVID-19. Compared to women, men had higher hospitalization rates. In Brescia, Italy, Quaresima *et al.* (2021) conducted retrospective study on COVID-19 patients, and the results

corroborated the current study observation that the rate was higher in men than in women. Initial reports from China and data from a number of European nations showed comparable numbers of confirmed cases involving men and women (Chen *et al.,* 2020b; Gebhard *et al*., 2020).

Additionally, there hasn't been a noticeable difference in the sex distribution compared to COVID-19. The proportions of males and females with COVID-19 did not significantly differ in Jin *et al's* 2020 study, therefore the present study findings are somewhat inconsistent with their findings (Jin *et al.,* 2020).

With respect to residency, most patients were from urban areas. Indeed, this finding is self-explanatory because of the higher population density in urban areas in comparison with rural areas in Babylon province.

3.1.2. Comparison of The Frequency Rate of Chronic Medical Illnesses Between Patients and Control Groups

Comparison of the frequency rate of chronic medical illnesses between patients and control groups is shown in table (3-2). Essential hypertension was seen only in patients group accosting for 34 % and therefore statistically the difference was significant ($p < 0.001$).

In addition, diabetes mellitus (DM) was only seen in patients group when contrasted to control group, 8 % versus 0 %, respectively; however, the difference was statistically not significant ($p = 0.052$)**.**

Table (3-2): Comparison of the frequency rate of chronic medical illnesses between patients and control groups.

Characteristic	Patients Group *n* = 100	Control Group *n* = 50	*p*	Interpretation
Hypertension				
Positive, *n* (%)	34 (34.0 %)	0 (0.0 %)	<0.001 C	Significant
Negative, *n* (%)	66 (66.0 %)	50 (100.0 %)		
Diabetes Mellitus				
Positive, *n* (%)	8 (8.0 %)	0 (0.0 %)	0.052 F	Not Significant
Negative, *n* (%)	92 (92.0 %)	50 (100.0 %)		

n: number of cases; **C**: chi-square test; **F**: Fischer exact test

In the present study, systemic hypertension and diabetes mellitus were seen in patients with COVID-19 and not in control group. While essential hypertension showed significant association, diabetes mellitus did not. According to the observations, Guan *et al*. (2020) published data from 1099 patients with confirmed COVID-19, of whom hypertension was the primary infection risk factor with 15% of patients reporting it. Additionally, 30% of the 140 individuals investigated by Zhang *et al*. (2020a) who have COVID-19 did so. Additionally, a meta-analysis of six trials with 1527 patients revealed that 17.1% of infection-related patients had hypertension (Li *et al*., 2020). Actually, the reason for this apparent association remains unclear. Many causes have been put forth, including pre-existing hypertensive cardiac end-organ damage, interactions between

COVID-19 and frequently prescribed antihypertensive drugs, and simply because older people tend to have high blood pressure more often (Kulkarni *et al*., 2020).

With respect to association between diabetes mellitus (DM) and COVID-19, in COVID-19 patients in China, a meta-analysis found 9.7% prevalence of DM (Li *et al*., 2020), which it is comparable to the DM prevalence in China.

Patients with COVID-19, both those with and without diabetes, have a rise in hyperglycemic symptoms and consequences. In particular, new-onset diabetes has been seen after COVID-19 infection (Rubino *et al*., 2020), including acute hyperglycemia in COVID-19 patients who did not already have diabetes, diabetic ketoacidosis in COVID-19 patients who did (Chee *et al*., 2020; Fadini *et al*., 2020), and new-onset diabetes in COVID-19 patients. It is currently unknown if newly developed SARS-CoV-2-induced diabetes results from recognized pathways in T1DM or T2DM or instead reflects an unusual kind of diabetes due to the COVID-19 pandemic's changing nature. Furthermore, it is yet unknown whether COVID-19 individuals are still more likely to experience newly appearing diabetes or other related issues after the virus has been cleared and recovered (Metwally *et al*., 2021).

3.1.3. Comparison of The Frequency Rate of Vaccination Between Patients and Control Groups

Comparison of the frequency rate of vaccination between patients and control groups is shown in table (3-3). The rate of vaccination in the patients group was 4 %, whereas, the control group included 52 % rate of vaccination thus the difference was statistically significant ($p < 0.001$). Single dose of vaccine was reported in 2 % versus 4 %, in patients group and control group, respectively. Two doses of vaccine were reported in 2 % versus 44 %, in patients group and control group, respectively. Three doses of vaccine were reported in 4 % of control group only.

Table (3-3): Comparison of the frequency rate of vaccination between patients and control groups.

Characteristic	Patients Group *n* = 100	Control Group *n* = 50	*p*	Interpretation
Vaccination Status				
Negative, *n* (%)	96 (96.0 %)	24 (48.0 %)	< 0.001 C	Significant
Positive, *n* (%)	4 (4.0 %)	26 (52.0 %)		
Single dose, *n* (%)	2 (2.0 %)	2 (4.0 %)	-	
Two doses, *n* (%)	2 (2.0 %)	22 (44.0 %)		
Three doses, *n* (%)	0 (0.0 %)	2 (4.0 %)		

n: number of cases; **C**: chi-square test.

In this study, the rate of vaccination in the patients group was 4 %, whereas, the control group included 52 % rate of vaccination thus the difference was statistically significant. This is a clue to the benefit of vaccination in reducing the rate of infection with coronavirus.

Recent systematic reviews and meta-analyses of vaccine effectiveness have been published (Harder *et al*., 2021; Kow and Hasan, 2021); these studies show that full immunization against SARS-CoV-2 infection has an average effectiveness of 85 % - 95 % within a few months of vaccination (Kow and Hasan, 2021). But many of the investigations in these reviews were done before the problematic variations appeared. While the Alpha form was predominant, studies

in Israel, Europe, and the United Kingdom showed that two doses of the Pfizer-BioNTech COVID-19 vaccine had a high real-world efficiency (> 85 %). (Hall *et al*., 2021; Lopez Bernal *et al*., 2021; Regev-Yochay *et al*., 2021; Sheikh *et al*., 2021).

Studies from Qatar have shown that Pfizer-BioNTech and Moderna vaccines were 96 % - 100 % effective against severe, critical, or fatal disease, regardless of strain, against documented infection with Alpha and Beta 14 days after receiving the Pfizer-BioNTech vaccine (90% and 75%, respectively), and the Moderna vaccine (100% and 96%, respectively (Chemaitelly *et al*., 2021; Abu-Raddad *et al*., 2021).

Three trials from Canada showed in the first of them that mRNA vaccines were 79% effective against confirmed infection when Alpha and Gamma represented the majority of infections, while the other two showed that they were 84% and 88% effective against symptomatic infection brought on by Gamma / Beta (Chung *et al*., 2021; Nasreen *et al*., 2021; Yassi *et al*., 2021).

3.1.4. Comparison of miRNA Expression Level Between Patients and Control Groups

Comparison of miRNA expression level between patients and control groups is shown in table (3-4). The level of miR-423 was significantly higher in patients with COVID-19 in comparison with control group, 1.81 (6.12) versus 0.45 (1.89), respectively ($p < 0.001$), as shown in figure (3-2). In addition, the level of miR-195 was significantly higher in patients with COVID-19 in comparison with control group, 10.74 (17.68) versus 6.95 (10.02), respectively ($p = 0.005$), as shown in figure (3-3). However, the level of miR-23a was significantly lower in patients with COVID-19 in comparison with control group, 8.33 (14.71) versus 19.13 (23.69), respectively ($p < 0.001$), as shown in figure (3-4).

Table (3-4): Comparison of miRNA expression level between patients and control groups**.**

Characteristic	Patients Group *n* = 100	Control Group *n* = 50	*p*	Interpretation
miR-423				
Median (IQR)	1.81 (6.12)	0.45 (1.89)	<0.001 M	Significant
Range	0.02 -28.32	0.03 -13.73		
miR-195				
Median (IQR)	10.74 (17.68)	6.95 (10.02)	0.005 M	Significant
Range	1.18 -177	1.05 -29.86		
miR-23a				
Median (IQR)	8.33 (14.71)	19.13 (23.69)	< 0.001 M	Significant
Range	0.39 -142.67	3.48 -90.56		

n: number of cases; **IQR**: inter-quartile range; **M:** Mann Whitney U test.

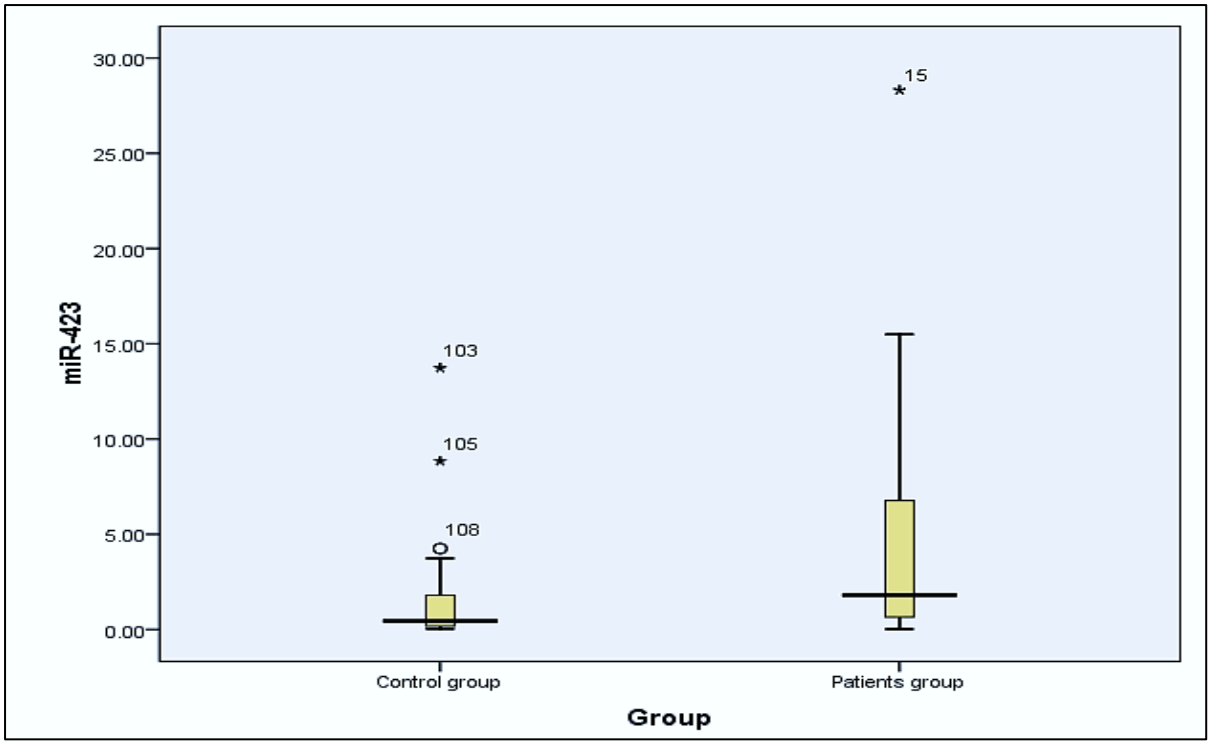


Figure (3-2): Box plot showing comparison of miR-423 expression level between patients group and control group.

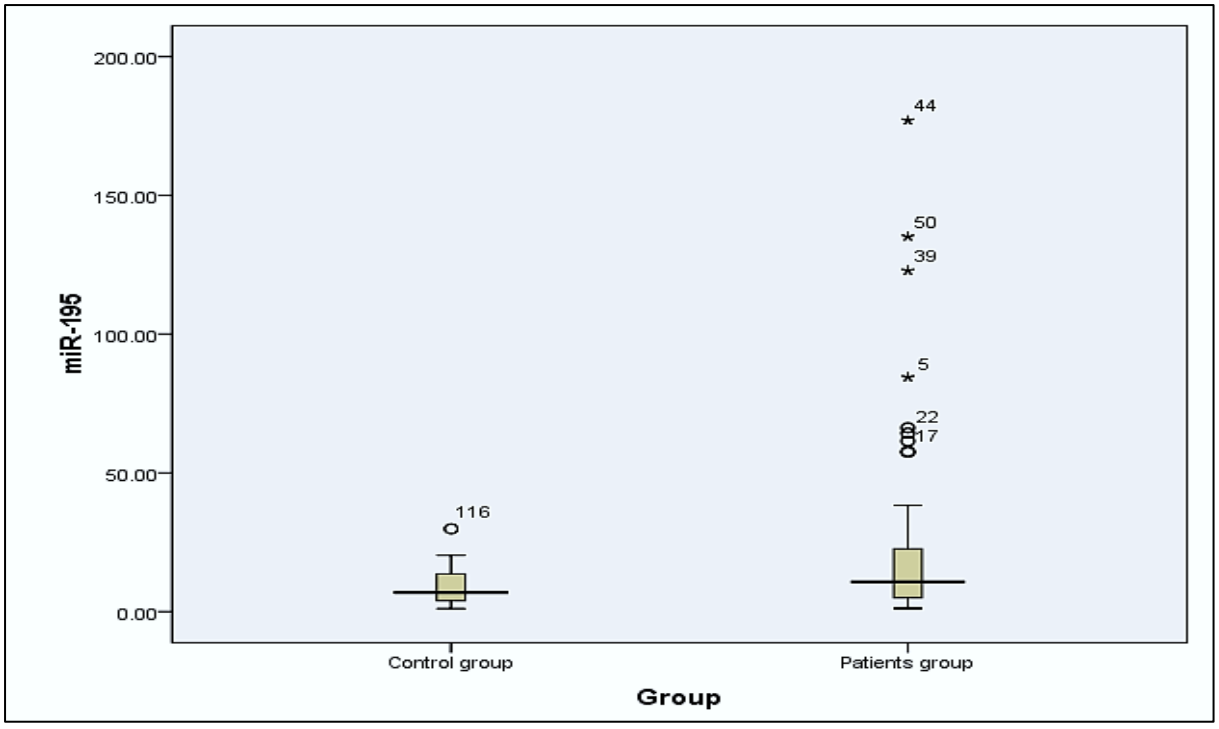


Figure (3-3): Box plot showing comparison of miR-195 expression level between patients group and control group.

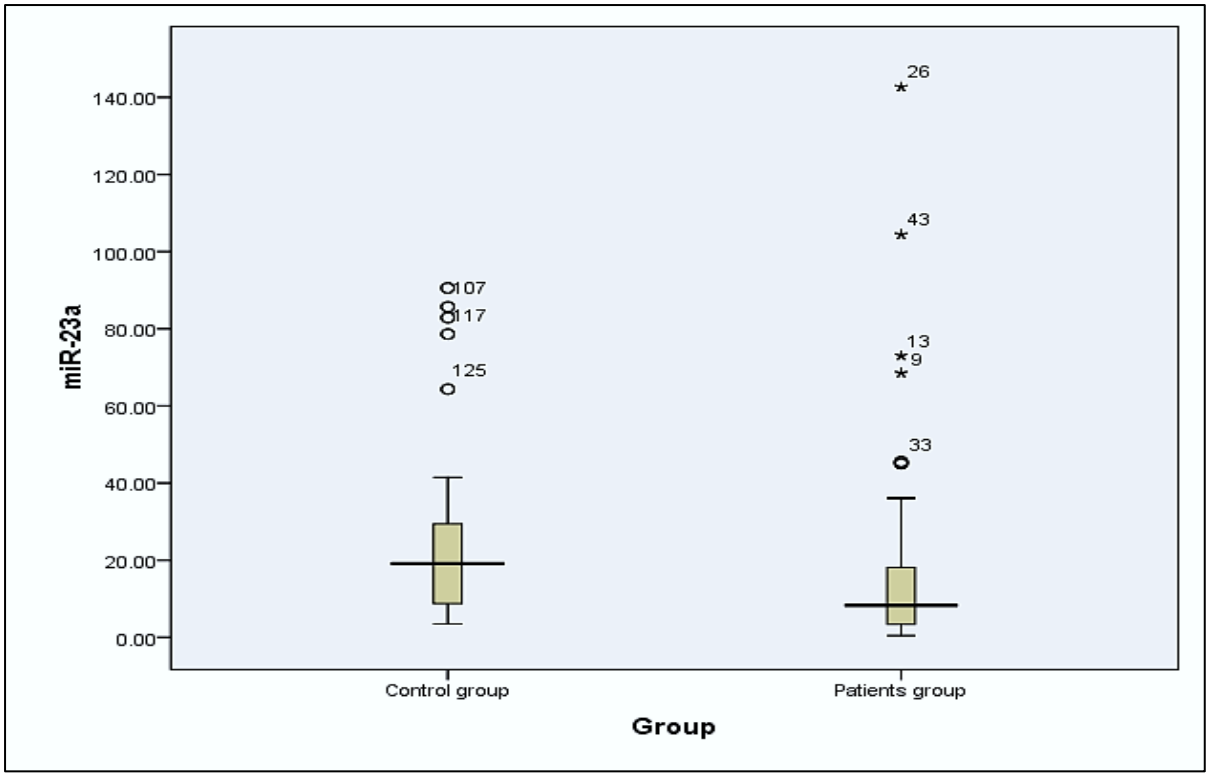


Figure (3-4): Box plot showing comparison of miR-23a expression level between patients group and control group.

Understanding the host response to SARS-CoV-2 infection can help with patient care and understanding viral pathogenesis. But it's still unclear how the SARS-CoV-2 infection affects the host-encoded microRNA (miRNA) response (Farr *et al*., 2021). In the current study, the levels of miR-423 and miR-195 were significantly higher in patients with COVID-19 in comparison with control group; however, the level of miR-23a was significantly lower in patients with COVID-19 in comparison with control group. According to the study of Farr *et al*., (2021), the levels of 55 miRs were studied in 10 patients with COVID-19 and compared to 10 age and sex matched control group; they observed that miR-423 and miR-195 were significantly higher and that miR-23a was significantly lower in patients in comparison with control.

It should be emphasized that significant changes in the levels of individual miRs is not specific because it has been reported that increased expression of circulating miR-423-5p is observed during heart failure (Tijsen *et al*., 2010) and pulmonary tuberculosis (Tu *et al*., 2019). Increases in the amount of miR-195-5p in the blood have been linked to osteosarcoma, autism, and gestational diabetes mellitus (Mundalil *et al*., 2014 ; Lian *et al*., 2015 ; Wang *et al*., 2020d). A four-miRNA signature that may accurately detect HIV-1 infection includes miR-195-5p, which is interestingly found to have enhanced plasma expression during HIV-1 infection (Biswas *et al*., 2019).

However, in present study, the measurement of the levels of three miRs simultaneously may provide strong prognostic evidence to the state of SARS-CoV-2 infection and become an auxiliary prognosis tool with relatively high accuracy rate in patients with clinically suspected manifestations.

Viral RNA is the target of the current COVID-19 molecular assays for detection. Unfortunately, a somewhat high viral load for SARS-CoV-2 is necessary for even the most sophisticated contemporary molecular diagnostic methods (such as PCR or LAMP amplifying viral RNA) to reliably detect

infection (Kucirka *et al*., 2020). Since the viral load is still low during the early presymptomatic phase of the disease (incubation period), their sensitivity is weak. It is challenging to diagnose infections in many cases that are presymptomatic and in some cases that are asymptomatic because the overall sensitivity of current PCR testing has been reported to be as low as 30-70% (Ai *et al*., 2020 ; Kanne *et al*., 2020).

The significance of miRNAs in COVID-19 pathogenesis is little understood, despite the fact that host responses to infection are known to be crucial in the diverse outcomes of SARS-CoV-2 infection. Therefore, future histopathological studies in conjunction with serum evaluation of miRs levels may help revealing such pathogenic role.

3.1.5. Comparison of Serum DPP4 and ACE2 Levels Between Patients and Control Groups

Comparison of serum DPP4 and ACE2 levels between patients and control groups is shown in table (3-5). The level of DPP4 was significantly lower in patients with COVID-19 in comparison with control group, 161.09 (96.35) versus 235.88 (107.15), respectively ($p < 0.001$), as shown in figure (3-5).

Moreover, the level of ACE2 was significantly higher in patients with COVID-19 in comparison with control group, 11.97 (2.59) versus 6.28 (1.47), respectively ($p < 0.001$), as shown in figure (3-6).

Table (3-5): Comparison of serum DPP4 and ACE2 levels between patients and control groups**.**

Characteristic	Patients group *n* = 100	Control group *n* = 50	*p*	Interpretation
DPP4				
Median (IQR)	161.09 (96.35)	235.88 (107.15)	<0.001 M	Significant
Range	82.08 -643.32	99.74 -455.9		
ACE2				
Median (IQR)	11.97 (2.59)	6.28 (1.47)	<0.001 M	Significant
Range	5.92 -23.96	4.89 -20.79		

n: number of cases; **IQR**: inter-quartile range; **M:** Mann Whitney U test.

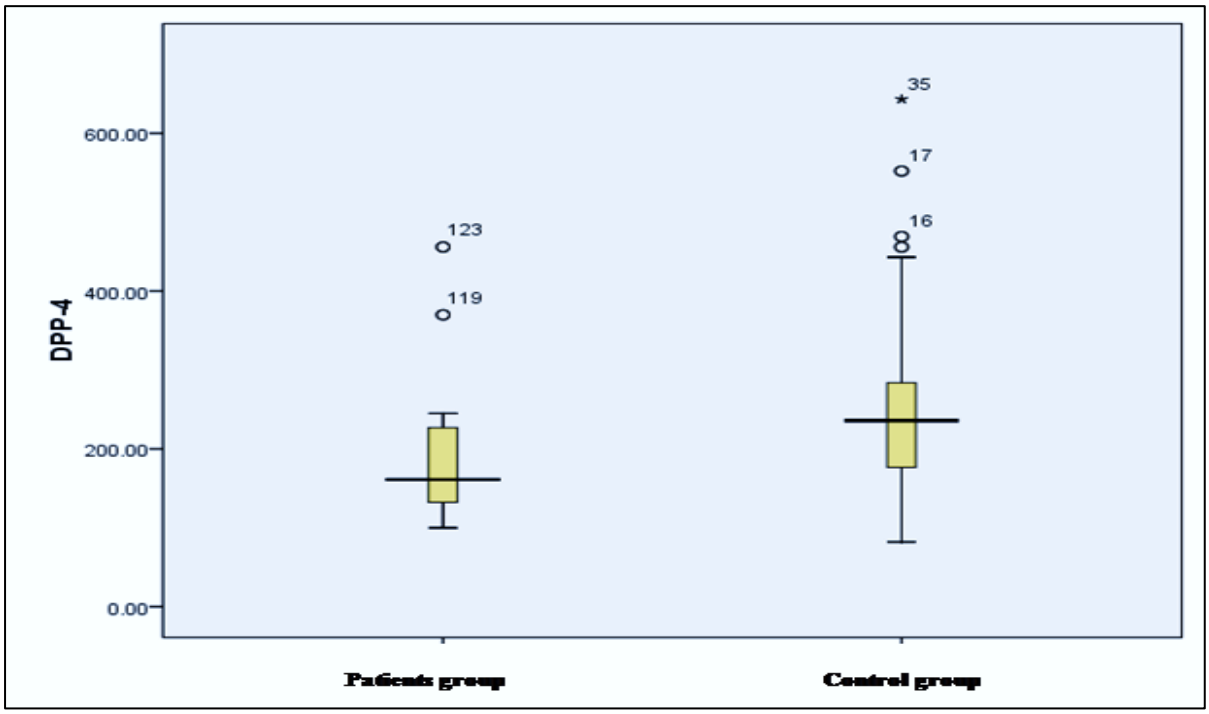


Figure (3-5): Box plot showing comparison of DPP4 level between patients group and control group**.**

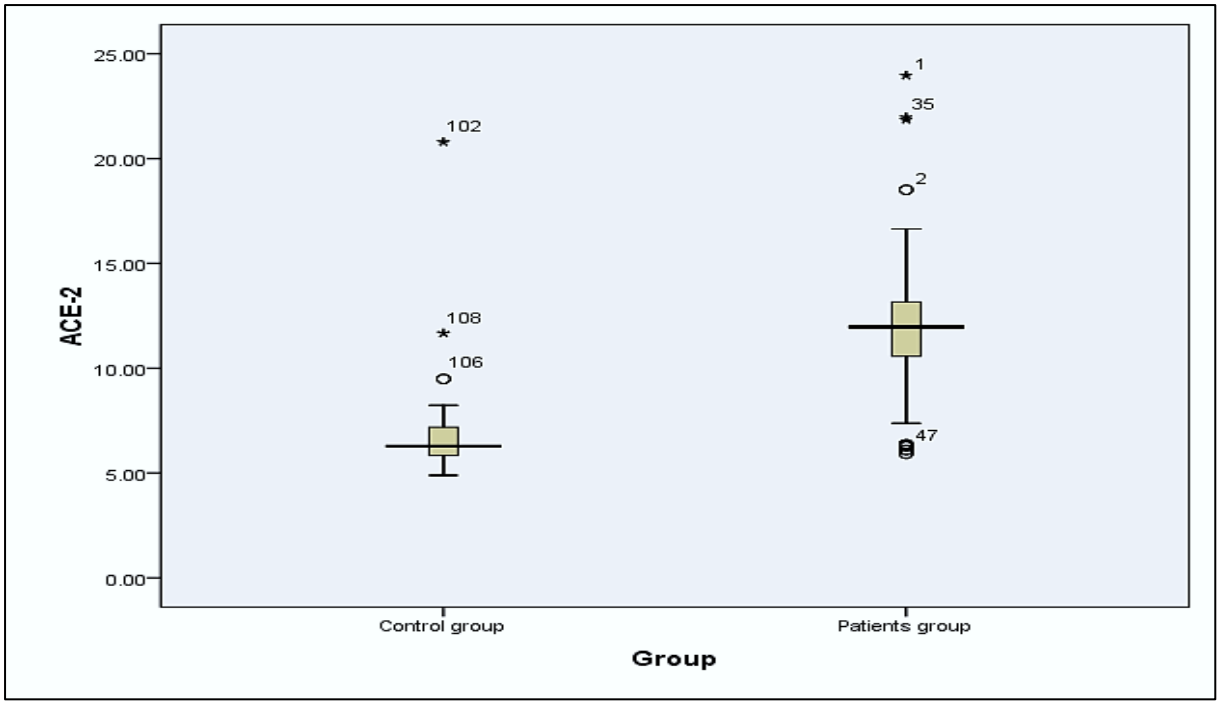


Figure (3-6): Box plot showing comparison of ACE2 level between patients group and control group.

In current study, the level of DPP4 was significantly lower and ACE2 was significantly higher in patients with COVID-19 in comparison with control group. In the study of Schlicht *et al*., (2020), serum level of DPP4 was significantly lower in cases with COVID-19 in comparison with healthy controls. Similarly, Posadas-Sánchez *et al.,* (2021) reported that the level of serum DDP4 was significantly lower in patients with COVID-19 in comparison with control group.

According to reports, the corona-like receptor for viruses like MERS is the DPP4 protein (Wang *et al.,* 2013). Recently, it was shown through bioinformatics research that the SARS-CoV-2 spike protein interacts with DPP4 (Vankadari and Wilce, 2020), indicating a potential involvement of this protein in the infection and severity of the illness. Schlicht *et al*. (2020) revealed low levels of DPP4 in a small number of COVID-19 patients relative to healthy controls; this result has previously been reported in MERS-CoV infected subjects (Inn *et al.,* 2018). According to Widagdo *et al.,* (2016), DPP4 is a widely distributed serine

peptidase that is expressed in endothelium, bronchiolar, alveolar, and blood cells (especially lymphocytes) (Gorrell *et al*., 1991). The two forms of this protein are one attached to the cell membrane and the other dispersed in the plasma. DPP4 may have a number of roles in the SARS infection, one of which may be facilitating the virus' entrance into the host cell.

Given that it is produced by adipose tissue as a proinflammatory adipokine with a significant impact on T cell activation, cell adhesion, and death, another role could be the systemic impact that it may have (Boonacker and Van Noorden, 2003). Given that T2DM and obesity are two key risk factors for a severe course of a COVID-19 condition, its connection to the inflammatory process and insulin resistance is pertinent (Mesas *et al*., 2020). Recently, Solerte *et al*. (2020) demonstrated the benefit of the DPP4 inhibitor Sitagliptin in COVID-19 due to the up-regulation of DPP4 expression and release that occurs as a result of this type of DPP4 inhibitor (Solerte *et al*., 2020).

3.1.6. Genetic Results Contrasted Between Patients and Control Groups

3.1.6.1. *DPP4* Gene Polymorphism in Patients Group and Control Group

Hardy Weinberg equilibrium in all enrolled subjects, patients group and control group is shown in table (3-6). The frequency distribution of all subjects according to *DPP4* (*rs3788979* A/G) genotypes was as following: 74, 66 and 10 as AA, AG and GG genotypes. The frequency distribution of patients according to *DPP4* (*rs3788979* A/G) genotypes was as following: 48, 52 and 0 as AA, AG and GG genotypes. The frequency distribution of control subjects according to *DPP4* (*rs3788979* A/G) genotypes was as following: 26, 14 and 10 as AA, AG and GG genotypes.

Comparison with expected counts as calculated by Hardy Weinberg equilibrium revealed no significant difference in cases of all enrolled subjects ($p = 0.353$), but both control group and patients group showed significant variation ($p = 0.008$ and < 0.001, respectively).

Table (3-6): Hardy Weinberg equilibrium in all enrolled subjects.

DPP4 (***rs3788979*** A/G) **Gene Polymorphism**	**All Cases**		**Control Group**		**Patients Group**	
	Observed Count	**Expected Count**	**Observed Count**	**Expected Count**	**Observed Count**	**Expected Count**
AA	74	76.3	26	21.8	48	54.8
AG	66	61.4	14	22.4	52	38.4
GG	10	12.3	10	5.8	0	6.8
χ^2	0.863		7.073		12.345	
p	0.353		0.008		< 0.001	
Interpretation	Not Significant		Significant		Significant	

χ^2: chi square value.

Comparison of *DPP4* (*rs3788979* A/G) genotypes between patients group and control group is shown in table (3-7). The rate of genotype AA was 48 % in patients group and 52 % in control group. The rate of genotype AG was 52 % in patients group and 28 % in control group. The rate of genotype GG was 0 % in patients group and 20 % in control group. Thus, Genotype AA was approximately the same in both groups, whereas, genotype AG was more frequent and genotype GG was less frequent in patients group in comparison with control group and overall, the difference was statistically significant ($p < 0.001$).

Comparison of risk association of *DPP4* (*rs3788979* A/G) genotypes with COVID-19 is shown in table (3-7). Genotype AA was considered as reference because it was the most frequently encountered genotype in all cases as well as in control group. Genotype AG was most frequently seen in patients group in comparison with control group, 52 % versus 28 %, respectively and the difference was significant; the odds ratio was 2.79, thus this genotype can be considered as

a risk factor. Genotype GG was less frequently seen in patients group in comparison with control group, 0 % versus 20 %, respectively and the difference was significant; the odds ratio was 0.03, thus this genotype can be considered as a protective factor.

Allelic analysis is also shown in table (3-7). Allele A was more frequent in patients group in comparison with control group, 74 % versus 66 %, respectively and allele G was less frequent in patients group in comparison with control group, 26 % versus 34 %, respectively, but the difference was statistically not significant (p = 0.149) and therefore, none of the alleles can be considered as a risk factor for the disease and this was confirmed further when odds ratio was calculated which in both alleles ranged between less than 1 and more than 1 a condition in which a factor statistically cannot be considered as a risk factor.

These results pointed to the fact that in most enrolled subjects genotype AA was the most frequent, whereas, genotype GG was the least frequent with respect to *DPP4* (*rs3788979* A/G).

The significant deviation from Hardy Weinberg equilibrium by patients may indicate a possible role for *DPP4* (*rs3788979* A/G) in susceptibility to COVID-19. Genotype AG was considered as a risk factor with an odds ratio of 2.79. Genotype GG was a protective factor with an odds ratio of 0.03. Alleles A and G showed no significant association with the disease susceptibility.

Table (3-7): Comparison of risk association of *DPP4* (*rs3788979* A/G) genotypes with COVID-19.

Genotypes	**Patients Group** ***n* = 100**	**Control Group** ***n* = 50**	***p***	**OR**	**95 % CI**
***DPP4* (*rs3788979* A/G) Gene Polymorphism**					
AA, *n* (%)	48 (48.0 %)	26 (52.0 %)	< 0.001 C ***	Reference	
AG, *n* (%)	52 (52.0 %)	14 (28.0 %)		2.79	1.34 -5.79
GG, *n* (%)	0 (0.0 %)	10 (20.0 %)		0.03 [A]	-
Alleles	**Patients Group** ***n* = 200**	**Control Group** ***n* = 100**	***p***	**OR**	**95 % CI**
A, *n* (%)	148 (74 %)	66 (66 %)	0.149 C NS	1.47	0.87 -2.47
G, *n* (%)	52 (26 %)	34 (34 %)		0.68	0.41 -1.15

n: number of cases; **C**: chi-square; **OR**: odds ratio; **CI**: confidence interval; ***: significant at $p \leq 0.001$; **NS**: not significant; **A**: approximate odds ratio.

Posadas-Sánchez *et al.,* in 2021 performed a study on the association between COVID-19 and *DPP4* gene polymorphism and their study enrolled 107 patients with COVID-19 and 263 matched-healthy controls and they found that The *DPP4 rs3788979* polymorphism associated with a high risk of COVID-19 disease and, the TT genotype carriers had the lowest DPP4 levels. Therefore, the current study agrees with the finding of Posadas-Sánchez *et al.,* (2021) with respect to susceptibility of disease with gene polymorphism of DPP4.

3.1.6.2. *HLA* Genotypes in Patients Group and Control Group

Comparison of *HLA* genotypes between patients and control groups is shown in table (3-8). The rate of expression of *HLA-B*07:03* was significantly more frequent in patients group in comparison with control group, 36 % versus 16 %, respectively (p = 0.011). There was no significant difference in rate of expression of *HLA-B*46:01* between patients and control groups (p = 0.086). There was also no significant difference in the rate of expression of *HLA-DRB1*03:01* between patients group and control group (p = 0.602). The rate of expression of *DRB1*12:02* was significantly more frequent in patients group in comparison with control group, 88 % versus 64 %, respectively (p = 0.001).

Table (3-8): Comparison of *HLA* genotypes between patients and control groups.

Characteristic	Patients Group *n* = 100	Control Group *n* = 50	*p*	Interpretation
HLA-B*07:03				
Negative	64 (64.0 %)	42 (84.0 %)	0.011 C	Significant
Positive	36 (36.0 %)	8 (16.0 %)		
HLA-B*46:01				
Negative	62 (62.0 %)	38 (76.0 %)	0.086 C	Not Significant
Positive	38 (38.0 %)	12 (24.0 %)		
HLA-DRB1*03:01				
Negative	72 (72.0 %)	38 (76.0 %)	0.602 C	Not Significant
Positive	28 (28.0 %)	12 (24.0 %)		
HLA-DRB1*12:02				
Negative	12 (12.0 %)	18 (36.0 %)	0.001 C	Significant
Positive	88 (88.0 %)	32 (64.0 %)		

n: number of cases; **C**: chi-square test.

In this study, human leukocyte antigen distribution study revealed that *HLA-B*07:03* and *DRB1*12:02* were significantly associated with COVID-19 and can be considered as risk factors. *HLA-B*46:01* and *HLA-DRB1*03*:01 were not significantly associated with the disease.

Previous studies have linked the susceptibility of SARS-CoV to *HLA-B*07:03* but not to the severity of disease (Lin *et al*., 2003; Ng *et al*., 2004). Recently, studies have found that *HLA-B*07:03* is associated with susceptibility and severity of disease (Iturrieta-Zuazo *et al*., 2020 ; Pisanti *et al*., 2020 ; Tavasolian *et al*., 2021), thus this study agrees with them. In line with the current observation, several recent reports, concluded that *HLA-B*46:01* is associated with susceptibility of COVID-19 (Too *et al*., 2019 ; Iturrieta-Zuazo *et al*., 2020 ; Pisanti *et al.,* 2020;; Tavasolian *et al*., 2021). Previous reports have linked the severity and susceptibility of the disease to *HLA-DRB1*03:01* and *DRB1*12:02* (Pisanti *et al*., 2020; Littera *et al*., 2021; Tavasolian *et al*., 2021). So, there is much controversy about the association between severity and susceptibility of COVID-19 and the *HLA*- genes and much research work may be needed to validate the claims of contradictory reports.

Human leukocyte antigen (HLA) plays an important role in immune responses to infections, especially in the development of acquired immunity. Given the high degree of polymorphisms that HLA molecules present, some will be more or less effective in controlling SARS-CoV-2 infection (Gutiérrez-Bautista *et al.,* 2022).

Of special interest are the human leukocyte antigens (HLA), which are highly polymorphic proteins that play a crucial role in the function of adaptive immunity. HLA presents pathogen-derived peptides on the surface of the infected cell, which are then recognized by specific T lymphocytes, inducing an immune response against the pathogen (Shiina *et al.,* 2009; Dendrou *et al.,* 2018; Olwenyi *et al.,* 2020).

The high level of polymorphism found in both HLA class I (HLA-A, -B and -C) and class II (HLA-DP, -DQ, -DR) molecules increases the variety of peptides that can be presented and recognized by the immune system. This is due to variations in their physical/chemical properties that increases or decreases their ability to bind and present certain peptides. Because of this, an allele can be a good or poor presenter of peptides derived from a pathogen. There is evidence of HLA alleles that can play a protective or susceptible role in infections (Blackwell *et al.,* 2009; Dutta *et al.,* 2018).

It has been suggested that the differences observed in the number of cases and severity of COVID-19 between different regions of the world, may in part be due to a skewed distribution of HLA alleles involved in protective immunity against SARS-CoV-2 (Debnath *et al.,* 2020 ; Ishii, 2020).

Gutiérrez-Bautista *et al.,* 2022 performed a study on the association between *HLA* gene polymorphism and SARS-CoV-2 and found that *HLA-A∗25:01* and *DRB1∗11:01* alleles were more common in control group; whereas, *HLA-A66:01*, *HLA-B55:01*, *HLA-DRB114:04* and *HLA-DQB105:03* were more common in COVID-19 group, but the corrected *p*-value was not significant. Indeed, these HLA-alleles were not considered in current study.

However, numerous studies have reported alleles of protection or susceptibility to COVID-19. A study conducted with 82 Chinese patients found that the *HLA-C∗07:29* and *HLA-B∗15:27* alleles were more frequently detected in the COVID-19 group than in the control population (Wang *et al.,* 2020e). Novelli *et al.,* (2020), found that the *HLA-B∗27:07*, *DQB1∗06:02* and *DRB1∗15:01* alleles were significantly increased in a group of 99 COVID-19 Italian patients compared to the control group. In addition, the alleles HLA-B∗44 and C∗01 were positively and individually associated with COVID-19 in the Italian population (Correale *et al.,* 2020).

The *HLA-A∗02:01* allele has a possible positive association with the risk of COVID-19 (Tomita *et al.,* 2020). Another study, with 190 Chinese patients, found a positive correlation between the HLA-B22 serotype and COVID-19 susceptibility (Yung *et al.,* 2021).

3.2. Comparison of Patients' Characteristics According to Severity of Disease

3.2.1. Comparison of Demographic Characteristics of Patients with COVID-19 According to Severity of Disease

Comparison of demographic characteristics of patients with COVID-19 according to severity of disease is shown in table (3-9). Severe disease characterized by significantly higher mean age in comparison with mild cases, 55.84 ± 12.42 years versus 31.04 ± 16.98 years, respectively ($p < 0.001$). Males were more frequently seen in, 72 % versus 48 %, respectively ($p = 0.014$). There was no significant difference in the frequency distribution of participants according residence between severe cases and mild cases ($p = 1.000$).

In current study, severe disease characterized by significantly higher mean age and male sex in comparison with mild cases, but not related to residence. Previous studies have shown an association between aging and severity of disease since ageing is linked with increasing decline and dysregulation in immune functions (Hamza *et al*., 2022). Age stands as one of the risk factors of diseases, plays an essential role in severity and mortality of infectious diseases (Du *et al.,* 2020 ; Zhou *et al.,* 2020b). Previous studies had identified that age ≥ 65 years is one of the risk factors that predicting mortality of COVID-19 patients (Du *et al*., 2020). Recent studies emphasized that women are more likely to be infected by COVID-19; however, severe and fatal outcomes are more commonly seen among male patients (Qian *et al*., 2020). It is reported that the male predominance phenomena of COVID-19 might be due to the androgen-driven pathogen of SARS-CoV-2 (Brenner *et al*., 2020).

Table (3-9): Demographic characteristics of patients with COVID-19 according to severity of disease.

Characteristic	Mild *n* = 50	Severe *n* = 50	*p*	Interpretation
Age (years)				
Mean ± SD	31.04 ± 16.98	55.84 ± 12.42	< 0.001	Significant
Range	10 -70	34 -80		
Sex				
Male	24 (48.0 %)	36 (72.0 %)	0.014 C	Significant
Female	26 (52.0 %)	14 (28.0 %)		
Residency				
Urban	36 (72.0 %)	36 (72.0 %)	1.000 C	Not Significant
Rural	14 (28.0 %)	14 (28.0 %)		

n: number of cases; **SD**: standard deviation; **I**: independent samples *t*-test; **C**: chi-square test.

3.2.2. Comparison of Rate of Hypertension and Rate of Diabetes Mellitus According to Severity of Disease

Comparison of rate of hypertension and rate of diabetes mellitus according to severity of disease is shown in table (3-10). Rate of hypertension was significantly higher in severe group in comparison with mild group, 52 % versus 16 %, respectively ($p < 0.001$); however, there was no significant difference in rate of diabetes mellitus between both groups ($p = 1.000$).

Table (3-10): Comparison of rate of hypertension and rate of diabetes mellitus according to severity of disease.

Characteristic	Mild *n* = 50	Severe *n* = 50	*p*	Interpretation
Hypertension				
Negative	42 (84.0 %)	24 (48.0 %)	< 0.001 C	Significant
Positive	8 (16.0 %)	26 (52.0 %)		
Diabetes Mellitus				
Negative	46 (92.0 %)	46 (92.0 %)	1.000 Y	Not Significant
Positive	4 (8.0 %)	4 (8.0 %)		

***n**:* number of cases; **C**: chi-square test; **Y**: Yates correction test.

In this study, essential hypertension, but not diabetes mellitus, was significantly associated with COVID-19; however, high RBS and HbA1c% were significantly associated with severe cases. Hypertension has been identified as the most prevalent cardiovascular comorbidity in patients infected with COVID-19 that demonstrably increases the risk of hospitalization and death. Initial studies implied that renin–angiotensin–aldosterone system inhibitors might increase the risk of viral infection and aggravate disease severity, thereby causing panic given the high global prevalence of hypertension (Peng *et al*., 2021).

Diabetes mellitus is associated with an increased risk and severity of COVID-19. While interacting with various other risk factors, high blood sugar was found to reduce immunity and increase the replication of SARS-CoV-2. Oxidative stress and the release of proinflammatory cytokines are greater in diabetic individuals than in healthy people, worsening the outcome of SARS-CoV-2 infection in diabetics (Sen *et al*., 2021).

3.2.3. Comparison of Disease Characteristics According to Severity of COVID-19 Infection

Comparison of disease characteristics according to severity of COVID-19 infection is shown in table (3-11). There was no significant difference in all other characteristics ($p > 0.05$).

Table (3-11): Comparison of disease characteristics according to the severity of infection.

Characteristic	Mild *n* = 50	Severe *n* = 50	*p*	Interpretation
Recurrent Infection				
0	24 (48.0 %)	28 (56.0 %)	0.687 C	Not significant
1	6 (12.0 %)	6 (12.0 %)		
2	20 (40.0 %)	16 (32.0 %)		
Vaccination Status				
0	48 (96.0 %)	48 (96.0 %)	1.000 Y	Not significant
1	2 (4.0 %)	0 (0.0 %)		
2	0 (0.0 %)	2 (4.0 %)		
Antibiotic Treatment				
No	24 (48.0 %)	24 (48.0 %)	1.000 C	Not significant
Yes	26 (52.0 %)	26 (52.0 %)		
Anti-viral Treatment				
No	44 (88.0 %)	48 (96.0 %)	0.269 Y	Not significant
Yes	6 (12.0 %)	2 (4.0 %)		

***n**:* number of cases; **C**: chi-square test; **Y**: Yates correction test; **I**: independent samples t-test.

3.2.4. Comparison of Biochemical, Serological and Hematological Characteristics of Patients with COVID-19 According to Severity of Disease

Comparison of biochemical, serological, and hematological characteristics of patients with COVID-19 according to severity of disease is shown in table (3-12). Mean random blood sugar was significantly higher in severe group compared to mild group ($p = 0.014$). Mean HbA1c % was significantly higher in severe group compared to mild group ($p = 0.020$). There was no significant difference in the level of serum ferritin between severe group and mild group ($p = 0.793$). The D-dimer level was significantly higher in severe group compared to mild group ($p < 0.001$). There was no significant difference in the level of Hb between severe group and mild group ($p = 0.594$). The WBC count was significantly higher in severe group compared to mild group ($p = 0.008$). The lymphocyte % was significantly lower in severe group compared to mild group ($p = 0.001$). The C-reactive protein (CRP) was significantly higher in severe group compared to mild group ($p < 0.001$).

This study has an observation that serum ferritin was not associated but high d-dimer was predictive of severe disease. Therefore, and based on this study observation it can be suggested that serum ferritin can aid in the diagnosis, whereas, d-dimer level can be used as a marker of prognosis to predict severity of disease and tailor treatment measures accordingly. Yu *et al*. (2020), performed a meta-analysis study on 1561 patients with COVID-19 (severe cases = 365 and mild cases = 1196) and found that d-dimer level was higher in patients with severe disease in comparison with patients with mild disease and this finding is supportive to the finding.

Table (3-12): Comparison of biochemical, serological and hematological characteristics of patients with COVID-19 according to severity of disease.

Characteristic	Mild *n* = 50	Severe *n* = 50	*p*	Interpretation
RBS (mg/dL)				
Mean ± SD	130.12 ± 54.67	166.08 ± 85.99	0.014 I	Significant
Range	95 -360	95 - 410		
HbA1c (%)				
Mean ± SD	5.95 ± 0.86	6.35 ± 0.82	0.020 I	Significant
Range	5.3 -8.7	4.5 - 9.6		
Serum Ferritin (ng/ml)				
Median (IQR)	420.00 (464.50)	540.00 (696.00)	0.793 M	Not Significant
Range	40.08 -1000	95 -1000		
D-Dimer (ng/ml)				
Median (IQR)	840 (1280)	3000 (8360)	< 0.001 M	Significant
Range	170 -16000	260 -16000		
Hb (g/dL)				
Mean ± SD	11.96 ± 2.39	11.70 ± 2.46	0.594 I	Not Significant
Range	7.4 -17.8	5.9 -16.7		
WBCs (/L)				
Median (IQR)	8800.00 (4025.00)	11500.00 (8350.00)	0.008 M	Significant
Range	4000.00 -26500.00	4200.00 -23600.00		
Lymphocytes (/L)				
Median (IQR)	8.60 (8.55)	4.60 (6.92)	0.001 M	Significant
Range	1.6 -23	1.1 -17.3		
CRP				
Positive	10 (20.0 %)	50 (100.0 %)	< 0.001 Y	Significant
Negative	40 (80.0 %)	0 (52.0 %)		

***n**:* number of cases; **M**: Mann Whitney U test; **I**: independent samples t-test.

Elevated D-dimer may be related to COVID-19 illness development, according to recent study reporting the laboratory abnormalities in individuals with confirmed COVID-19. Patients with COVID-19 admitted to the ICU were found to have considerably higher levels of D-dimer (Huang *et al*., 2020). Patients with severe COVID-19, who were frequently bedridden and displayed impaired coagulation function, should receive special clinical attention due to their risk of venous thromboembolism (Chen *et al*., 2020c). In situations where the D-dimer level considerably increased as the disease progressed, rapid decline was shown. When patients exhibit clinical symptoms such a sharp drop in blood pressure, a quick deterioration in oxygenation, or respiratory distress, pulmonary embolism following deep vein thrombosis separation should be taken into consideration and detected right away (Yu *et al*., 2020).

In the present study, high WBC and low lymphocyte count were significantly associated with severe disease. According to a previous report by Palladino (2021), it was reported that high WBC count and low lymphocyte count is associated with severe disease and this study is in agreement with Palladino (2021) with this regard.

The most prevalent hematological abnormality in COVID-19 infected patients is lymphopenia, which can occur in up to 85% of severe cases and is correlated with prognosis (Qin *et al*., 2020). Most published series report the occurrence of lymphopenia, which is characterized by an absolute lymphocyte count of less than 1.0 x 109 /L and is typically thought to be an inadequate immune response to viral infection (van Wolfswinkel *et al*., 2013).

It is well known that the number of total lymphocytes and the subsets change depending on the virus type, suggesting a possible connection between the changing of the lymphocyte subset and viral pathogenic mechanisms (Li *et*

al., 2004). Lymphopenia may be brought on directly by immunological damage from inflammatory mediators or indirectly by virus attachment (Palladino, 2021).

In the present study, anemia was not significant predictor of the severity of disease. Actually, conflicting results about the association between low hemoglobin and outcome and severity of COVID-19, but in general low hemoglobin may be associated with severity of disease and further large specimen studies may require to reach a final consensus about this point (Palladino, 2021).

In this study, recurrent infection, vaccination status, and type of treatment were not associated with severity of disease, but CRP was associated with severe cases. Some COVID-19 patients have contracted the virus again as a result of the ongoing pandemic. According to viral gene sequencing, some of these individuals relapsed with distinct strains while others relapsed with the same variants. This has sparked doubts regarding the efficacy of post-infection immunity and the validity of vaccines (Ren *et al*., 2022).

In line with observation, a meta-analysis was carried out by Ikeagwulonu *et al.,* (2021) and the results pointed out to the fact that high CRP is associated with severe disease. One retrospective single-center investigation in China showed the clinical significance of CRP for COVID-19. According to the study, most individuals with severe stages had significantly higher CRP levels than the non-severe cohort (Luo *et al.,* 2020). The CRP is a sign of widespread inflammation. Therefore, the level of CRP may be used to predict the recovery or negative prognosis of severe patients as well as the progression of weakly infected individuals (Stringer *et al.,* 2021).

3.2.5. Comparison of miRNA Expression of Patients with COVID-19 According to Severity of Disease

Comparison of miRNA expression of patients with COVID-19 according to severity of disease is shown in table (3-13). There was no significant difference in the levels of miR-423, miR-195 and miR-23a between mild group and severe group ($p > 0.05$).

Table (3-13): Comparison of miRNA expression of patients with COVID-19 according to severity of disease.

Characteristic	Mild *n* = 50	Severe *n* = 50	*p*	Interpretation
miR-423				
Median (IQR)	1.99 (5.98)	1.31 (6.25)	0.689 M	Not Significant
Range	0.02 -13.62	0.11 -28.32		
miR-195				
Median (IQR)	10.30 (13.77)	11.17 (25.59)	0.923 M	Not Significant
Range	2.01 -135.16	1.18 -177		
miR-23a				
Median (IQR)	8.50 (11.14)	7.95 (16.60)	0.348 M	Not Significant
Range	0.39 -142.67	0.91 -104.4		

***n**:* number of cases; **M**: Mann Whitney U test.

In present study, there was no significant difference in the levels of miR-423, miR-195 and miR-23a between mild group and severe group. Far *et al* in 2021 evaluated serum levels of 55 miRs in 10 patients with COVID-19 and 10 healthy controls and found some of them to be associated with severity, but, levels of miR-423, miR-195 and miR-23a were not significantly associated with severity of disease and this study agrees with current study with this regard.

The significant difference in serum levels of these miRs when contrasted between controls and patients and lack of correlation of them with clinical severity suggested their important diagnostic role and limited prognostic value, thus this study recommends these markers to be used for early detection of disease but do not recommend them to assess disease severity and clinically established diagnosis.

3.2.6. Comparison of Serum DPP4 and ACE2 Levels of Patients with COVID-19 According to Severity of Disease

Comparison of serum DPP4 and ACE2 levels of patients with COVID-19 according to severity of disease is shown in table (3-14). The level of DPP4 was significantly higher in mild group in comparison with severe group, 264.00 (133.70) versus 203.39 (111.01), respectively ($p = 0.011$). The level of ACE2 was significantly higher in severe group in comparison with mild group, 12.27 (2.67) versus 11.67 (4.94), respectively ($p = 0.004$).

Table (3-14): Comparison of serum DPP4 and ACE2 levels of patients with COVID-19 according to severity of disease.

Characteristic	Mild *n* = 50	Severe *n* = 50	*p*	Interpretation
DPP4 (ng/ml)				
Median (IQR)	264.00 (133.70)	203.39 (111.01)	0.011 M	Significant
Range	82.08 -643.32	112.27 -552.18		
ACE2 (ng/ml)				
Median (IQR)	11.67 (4.94)	12.27 (2.67)	0.004 M	Significant
Range	5.92 -13.85	6.28 -23.96		

***n**:* number of cases; **M**: Mann Whitney U test.

The level of DPP4 was significantly lower in severe group in comparison with mild group. This indicates that lower serum level of DPP4 is associated with severe disease and this finding is in line with the observation of Posadas-Sánchez *et al*., (2021). Posadas-Sánchez *et al*., (2021) evaluated the serum level of DPP4 in 107 COVID-19 patients and 263 matched-healthy controls and they found that patients with severe disease showed lower DPP4 levels than those with mild disease significantly.

Although it is difficult to determine whether reduced DPP4 levels in patients are a result of or a cause of SARS-CoV-2 infection, the fact that uninfected control people had higher levels of this enzyme raises the possibility that this variation may be due to the illness. Low serum levels of DPP4 in COVID-19 patients may result from a number of factors, one of which being the enzyme acting as a viral receptor, which may change the protein's structure at the cell membrane and stop its proteolytic cleavage. Therefore, as shown by this study, those with SARS will therefore have decreased serum DPP4 levels. Alterations at the intracellular level brought on by the virus may also have an impact on the soluble enzyme concentration by preventing DPP4 from assembling properly. The amount of biomolecule on the cell membrane and, consequently, in the patients' serum could both decrease as a result of this effect (Posadas-Sánchez *et al*., 2021).

Dipeptidyl Peptidase 4 is expressed in a variety of blood cells; however, it is mostly found in T lymphocytes, and more specifically in CD4 T cells (Gorrell *et al*., 1991; Boonacker *et al*., 2002). Given these data, it is possible that the decreased lymphocyte count (the main cell source of soluble DPP4) accounts for the low blood DPP4 levels observed in COVID-19 patients.

With respect to serum level of ACE2 in this study, it was, as mentioned previously, higher in patients with severe disease in comparison with patients with mild disease. In line with this findings, Bani Hani *et al*., (2022), found

similar results when compared serum level of ACE2 in patients with severe disease in comparison with mild disease when they evaluated 144 COVID-19 patients and 123 healthy controls.

Analysis of the ACE2 levels in 306 COVID-19 positive patients and 78 COVID-19 negative patients revealed that high admission plasma ACE2 in COVID-19 patients was associated with an increase in maximal illness severity within 28 days, suggesting that measuring plasma ACE2 may be useful in predicting COVID-19 outcomes (Kragstrup *et al*., 2021). Similar findings were observed on the degree of ACE2 level and COVID-19 severity (Kaur *et al*., 2021 ; Patel *et al*., 2021 ; Reindl-Schwaighofer *et al*., 2021 ; van Lier *et al*. 2021). Due to the lysis of ACE2-expressing cells brought on by severe lung infection, increasing levels of ACE2 may therefore be a result of increased ACE2 shedding, and as a result, the amount rises with the degree of lung involvement (Filbin *et al*., 2021; Henry *et al*., 2021; Kragstrup *et al*., 2021).

3.2.7. Comparison of Gene Expression Rate Between Severe and Mild Groups

Comparison of rate of gene expression between severe and mild groups is shown in table (3-15), figure (3-7) and (3-8).

There was no significant difference in the frequency of *DPP4* (*rs3788979* A/G) gene polymorphism between severe group and mild group ($p = 0.109$).

The expression of *HLA-B*07:03* was significantly less in severe group in comparison with mild group ($p = 0.012$). The expression of *HLA-B*46:01* was significantly more in severe group in comparison with mild group ($p = 0.039$). There was no significant difference in rates of *HLA-DRB1*03:01* and *DRB1*12:02* genes expression between severe group and mild group ($p > 0.05$).

Table (3-15): Comparison of rate of genes expression between severe and mild groups.

Characteristic	Mild *n* = 50	Severe *n* = 50	*p*	Interpretation
***DPP4 (rs3788979* A/G)**				
AA	28 (56.0 %)	20 (40.0 %)	0.109 C	Not Significant
AG	22 (44.0 %)	30 (60.0 %)		
HLA-B*07:03				
Negative	26 (52.0 %)	38 (76.0 %)	0.012 C	Significant
Positive	24 (48.0 %)	12 (24.0 %)		
HLA-B*46:01				
Negative	36 (72.0 %)	26 (52.0 %)	0.039 C	Significant
Positive	14 (28.0 %)	24 (48.0 %)		
HLA-DRB1*03:01				
Negative	32 (64.0 %)	40 (80.0 %)	0.075 C	Not Significant
Positive	18 (36.0 %)	10 (20.0 %)		
DRB1*12:02				
Negative	6 (12.0 %)	6 (12.0 %)	1.000 C	Not Significant
Positive	44 (88.0 %)	44 (88.0 %)		

C: Chi-square test.

There was no significant difference in the frequency of *DPP4* (*rs3788979* A/G) genotypes between severe group and mild group ($p = 0.109$). In contrary to the current results, Posadas-Sánchez *et al.* (2021), performed a study on the association between severity of COVID-19 and *DPP4* (*rs3788979* A/G) genotypes and found that significant association and they stated that "The *DPP4 rs3788979* polymorphism was associated with a high risk of COVID-19 disease and, the TT genotype carriers had the lowest DPP4 levels". In addition, and in disagreement with the current results, Aghili *et al*. in 2012 reported that individuals with the *rs3788979* CT/TT genotypes presented with low levels of DPP4 in his study on association between gene polymorphism and myocardial infarction; whereas in this study, there was no significant difference in serum level of DPP4 with respect to *DPP4* gene polymorphism

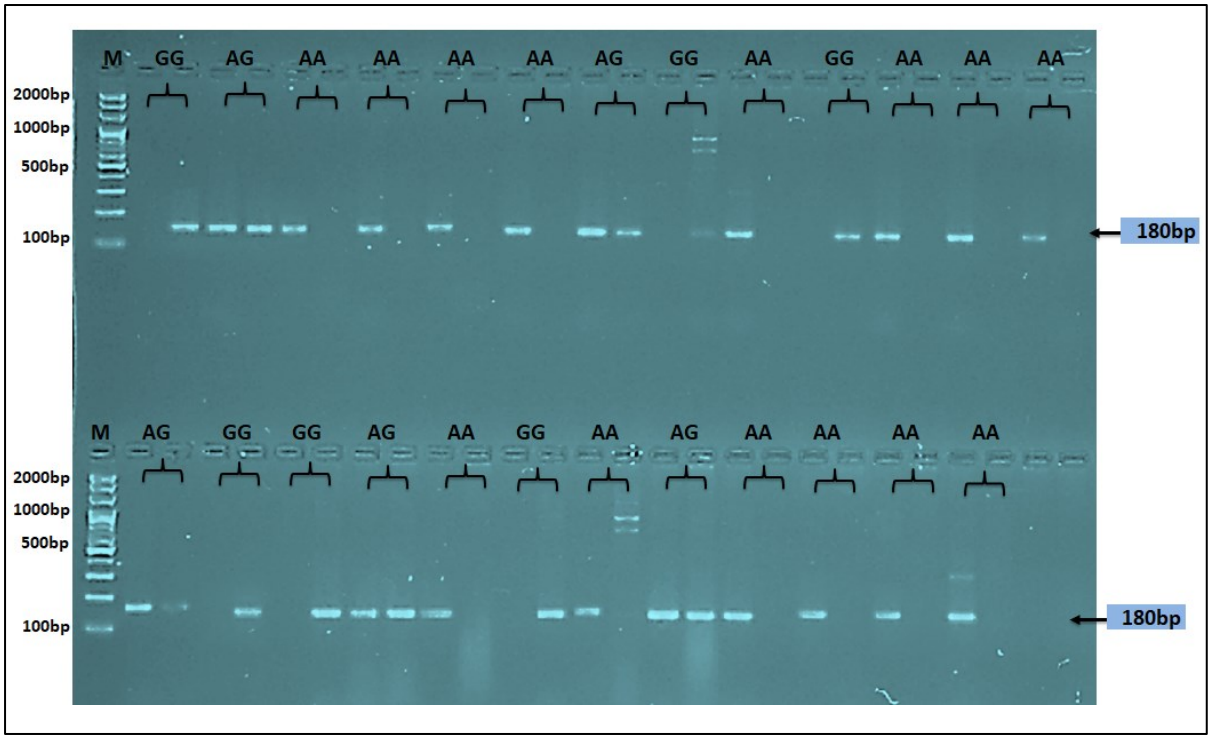


Figure (3-7): Agarose gel electrophoresis image that showed the AS-PCR product analysis of *DPP4* (*rs3788979* A/G) gene polymorphism. Where M: marker (2000 - 100bp). The (AA) wild type homozygote was showed in A allele only, the (GG) mutant type homozygote were showed in G allele only, whereas the (AG) heterozygote were showed in both A and G allele. The presence of A or G allele were observed at 180bp product size.

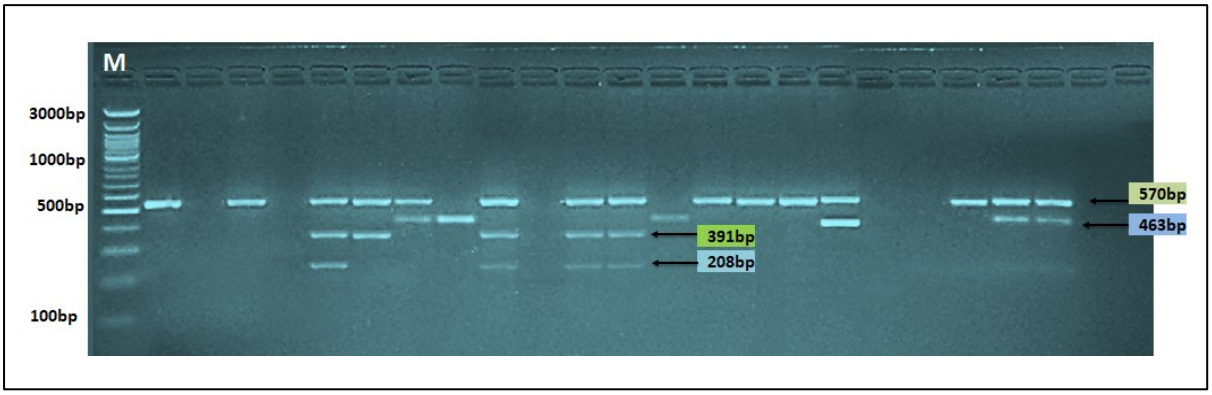


Figure (3-8): Agarose gel electrophoresis image that showed the SSP-PCR product analysis of *HLA* mutations. Where M: marker (3000 - 100 bp). The positive lanes for *HLA-DRB1*12:02* mutants were showed at 570bp product size, the *HLA-DRB1*03:01* positive lanes were showed at 463bp SSP-PCR product size, the *HLA-B*46:01* positive lanes were showed at 391bp SSP-PCR product size, and the *HLA-B*07:03* positive lanes were showed at 208bp SSP-PCR product size.

Actually, the study of Posadas-Sánchez *et al*. (2021) is the only study that investigated the association between susceptibility to and severity of COVID-19 and gene polymorphism related to DPP4; and the lack of consensus between the present study and their study suggested the need for further research work to clearly identify such association.

In the present study, the expression of *HLA-B*07:03* was significantly less in severe group in comparison with mild group ($p = 0.012$). Previous studies have linked the susceptibility of SARS-CoV to *HLA-B*07:03* but not to the severity of disease (Lin *et al*., 2003; Ng *et al*., 2004). Recently, studies have found that *HLA-B*07:03* is associated with susceptibility and severity of disease (Iturrieta-Zuazo *et al*., 2020 ; Pisanti *et al.,* 2020 ; Tavasolian *et al*., 2021), thus the current study disagreed with them.

In addition, the expression of *HLA-B*46:01* was significantly more in severe group in comparison with mild group ($p = 0.039$). In line with the current observation, several recent reports, concluded that *HLA-B*46:01* is associated with severity of COVID-19 (Too *et al*., 2019 ; Iturrieta-Zuazo *et al.,* 2020 ; Pisanti *et al.,* 2020 ; Tavasolian *et al.,* 2021). Furthermore, the present study found no significant difference in rates of *HLA-DRB1*03:01* and *DRB1*12:02* genes between severe group and mild group ($p > 0.05$). Previous reports have linked the severity and susceptibility of the disease to *HLA-DRB1*03:01* and *DRB1*12:02* (Littera *et al.,* 2020 ; Pisanti *et al.,* 2020 ; Tavasolian *et al.,* 2021) in clear disagreement to current study. So, there is much controversy about the association between severity and susceptibility of COVDI-19 and the *HLA* genes and much research work may be needed to validate the claims of contradictory reports.

The effectiveness of a person's immune system, particularly the T-cell immunity, has a significant impact on how the SARS-CoV-2 virus behaves and the results of the infection. Given that the *HLA* haplotype occurs variably in different cultures, it is hypothesized that the effectiveness of SARS-CoV-2 viral clearance and illness progression will vary. The role of cytotoxic CD8+ T and helper CD4+ T cells in SARS-CoV-2 and HLA studies has been emphasized since these responses are crucial for the first viral clearance, the development of immunologic memory, and ultimately for coordinating the adaptive immune responses (Khanolkar *et al*., 2007 ; Lani *et al*., 2022;).

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. The levels of miR-423 and miR-195 are significantly higher in patients with COVID-19 in comparison with control group. Conversely, the level of miR-23a is significantly lower in patients with COVID-19 in comparison with control group. There is no significant difference in the levels of miR-423, miR-195 and miR-23a between mild group and severe group.
2. The rate of expression of *HLA-B*07:03* and *DRB1*12:02* are significantly more frequent in patients group in comparison with control group quite the opposite to *HLA-B*46:01* and *HLA-DRB1*03:01.* The expression of *HLA-B*07:03* is significantly less in severe group in comparison with mild group in contrast to that of *HLA-B*46:01*. There was no significant difference in rates of *HLA-DRB1*03:01* and *DRB1*12:02* genes expression between severe group and mild group.
3. The level of serum DPP4 is significantly higher in mild group in comparison with severe group.
4. The level of serum ACE2 is significantly higher in severe group in comparison with mild group.
5. Essential hypertension is significantly associated with COVID-19.
6. Elevation in random blood sugar, hemoglobin A1c, D-dimer, white blood cell counts and C-reactive protein levels are significantly associated with severe cases. The lymphocyte percent was significantly lower in severe group compared to mild group.

Recommendations

1. Vaccination is highly recommended in reducing the rate of infection with SARS-CoV-2.
2. Performing Real-Time quantitative reverse transcription PCR or rapid antigen tests assays were recommended for testing of suspected cases, including those asymptomatic individuals in close contact with confirmed COVID-19 patients and also, for the symptomatic individuals.
3. Conductance of multicentric-studies dealing with larger sample size in order to study the association of COVID-19 with cytokines expression, genetic polymorphism and demographic characteristics impact among different populations.
4. A comparative study between various societies based on differences in ethnicity and COVID-19 severity of infection.
5. Conductance of follow-up study for patients with severe COVID-19 after one year in the event of the appearance of autoimmune diseases.
6. In particular, efforts should be made to provide an appropriate environment and educate on adequate preventive measures by organizing campaigns for individuals to follow the rules to prevent COVID-19 regardless of their social status.
7. However, in present study, the measurement of the levels of three miRNAs simultaneously may provide strong prognostic evidence to the state of SARS-CoV-2 infection and become an auxiliary prognosis tool with relatively high accuracy rate in patients with clinically suspected manifestations.
8. D-dimer serum level can be used as a marker of prognosis to predict severity of disease and tailor treatment measures accordingly.

REFERENCES

References

Abbas, H. M., Al-Jumaili, A. A., Nassir, K. F., Al-Obaidy, M. W., Al Jubouri, A. M., Dakhil, B. D., Abdulelah, M. M., & Al Khames, Q. A. (2021). Assessment of COVID-19 Treatment containing both Hydroxychloroquine and Azithromycin: A natural clinical trial. *International Journal of Clinical Practice*, 75(4), e13856.

Abu-Raddad, L. J., Chemaitelly, H., Butt, A. A., & National Study Group for COVID-19 Vaccination (2021). Effectiveness of the BNT162b2 Covid-19 Vaccine against the B.1.1.7 and B.1.351 Variants. *The New England Journal of Medicine*, 385(2), 187–189.

Ackermann, M., Verleden, S. E., Kuehnel, M., Haverich, A., Welte, T., Laenger, F., Vanstapel, A., Werlein, C., Stark, H., Tzankov, A., Li, W. W., Li, V. W., Mentzer, S. J., & Jonigk, D. (2020). Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in COVID-19. *The New England Journal of Medicine*, 383(2), 120–128.

Aghili, N., Devaney, J. M., Alderman, L. O., Zukowska, Z., Epstein, S. E., & Burnett, M. S. (2012). Polymorphisms in dipeptidyl peptidase IV gene are associated with the risk of myocardial infarction in patients with atherosclerosis. *Neuropeptides*, 46(6), 367–371.

Ahmad, F. B., Cisewski, J. A., Miniño, A., & Anderson, R. N. (2021). Provisional Mortality Data - United States, 2020. MMWR. *Morbidity and Mortality Weekly Report*, 70(14), 519–522.

Ai, T., Yang, Z., Hou, H., Zhan, C., Chen, C., Lv, W., Tao, Q., Sun, Z., & Xia, L. (2020). Correlation of Chest CT and RT-PCR Testing for Coronavirus Disease 2019 (COVID-19) in China: A Report of 1014 Cases. *Radiology*, 296(2), E32–E40.

Aleem, A., Akbar Samad, A. B., & Vaqar, S. (2023). Emerging Variants of SARS-CoV-2 and Novel Therapeutics Against Coronavirus (COVID-19). In StatPearls. *StatPearls Publishing*.

Alhazzani, W., Møller, M. H., Arabi, Y. M., Loeb, M., Gong, M. N., Fan, E., Oczkowski, S., Levy, M. M., Derde, L., Dzierba, A., Du, B., Aboodi, M., Wunsch, H., Cecconi, M., Koh, Y., Chertow, D. S., Maitland, K., Alshamsi, F., Belley-Cote, E., Greco, M., *et al.* (2020). Surviving Sepsis Campaign: Guidelines on the Management of Critically Ill Adults with Coronavirus Disease 2019 (COVID-19). *Critical Care Medicine*, 48(6), e440–e469.

Alsaad, K. O., Hajeer, A. H., Al Balwi, M., Al Moaiqel, M., Al Oudah, N., Al Ajlan, A., AlJohani, S., Alsolamy, S., Gmati, G. E., Balkhy, H., Al-Jahdali, H. H., Baharoon, S. A., & Arabi, Y. M. (2018). Histopathology of Middle East respiratory syndrome coronovirus (MERS-CoV) infection - clinicopathological and ultrastructural study. *Histopathology*, 72(3), 516–524.

Al-Tawfiq, J. A., Alfaraj, S. H., Altuwaijri, T. A., & Memish, Z. A. (2017). A cohort-study of patients suspected for MERS-CoV in a referral hospital in Saudi Arabia. *The Journal of Infection*, 75(4), 378–379.

Ambagala, A. P., Solheim, J. C., & Srikumaran, S. (2005). Viral interference with MHC class I antigen presentation pathway: the battle continues. *Veterinary Immunology and Immunopathology*, 107(1-2), 1–15.

Andersen, K. G., Rambaut, A., Lipkin, W. I., Holmes, E. C., & Garry, R. F. (2020). The proximal origin of SARS-CoV-2. *Nature Medicine*, 26(4), 450–452.

Apanius, V., Penn, D., Slev, P. R., Ruff, L. R., & Potts, W. K. (2017). The Nature of Selection on the Major Histocompatibility Complex. *Critical Reviews in Immunology*, 37(2-6), 75–120.

Arabi, Y., Balkhy, H., Hajeer, A. H., Bouchama, A., Hayden, F. G., Al-Omari, A., Al-Hameed, F. M., Taha, Y., Shindo, N., Whitehead, J., Merson, L., AlJohani, S., Al-Khairy, K., Carson, G., Luke, T. C., Hensley, L., Al-Dawood, A., Al-Qahtani, S., Modjarrad, K., Sadat, M., *et al.*,. (2015). Feasibility, safety, clinical, and laboratory effects of convalescent plasma therapy for patients with Middle East respiratory syndrome coronavirus infection: a study protocol. *SpringerPlus*, 4, 709.

Arya, S., Maqsood, T., Ushmani, R., Prakash, V., Biswas, S., & Dar, M. A. (2023). Assessment on serum CRP and serum ferritin level in COVID-19 patients of Dehradun Uttarakhand, India. *International Journal of Reproduction, Contraception, Obstetrics and Gynecology*, 12(3), 623-628.

Assiri, A., McGeer, A., Perl, T. M., Price, C. S., Al Rabeeah, A. A., Cummings, D. A., Alabdullatif, Z. N., Assad, M., Almulhim, A., Makhdoom, H., Madani, H., Alhakeem, R., Al-Tawfiq, J. A., Cotten, M., Watson, S. J., Kellam, P., Zumla, A. I., Memish, Z. A., & KSA MERS-CoV Investigation Team (2013). Hospital outbreak of Middle East respiratory syndrome coronavirus. *The New England Journal of Medicine*, 369(5), 407–416.

Azkur, A. K., Akdis, M., Azkur, D., Sokolowska, M., van de Veen, W., Brüggen, M. C., O'Mahony, L., Gao, Y., Nadeau, K., & Akdis, C. A. (2020). Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. *Allergy*, 75(7), 1564-1581.

Bai, S. L., Wang, J. Y., Zhou, Y. Q., Yu, D. S., Gao, X. M., Li, L. L., & Yang, F. (2020). *Chinese Journal of Preventive Medicine*, 54(0), E005. Advance online publication.

Bani Hani, A., Abu Tarboush, N., Bani Ali, M., Alabhoul, F., Alansari, F., Abuhani, A., Al-Kawak, M., Shamoun, B., Albdour, S., Abu Abeeleh, M., & Ahram, M. (2022). Serum ACE2 Level is Associated With Severe SARS-CoV-2 Infection: A Cross-Sectional Observational Study. *Biomarker Insights*, 17, 11772719221125123.

Bao, C., Liu, X., Zhang, H., Li, Y., & Liu, J. (2020). Coronavirus Disease 2019 (COVID-19) CT Findings: A Systematic Review and Meta-analysis. *Journal of the American College of Radiology*, 17(6), 701–709.

Beigel, J. H., Tomashek, K. M., Dodd, L. E., Mehta, A. K., Zingman, B. S., Kalil, A. C., Hohmann, E., Chu, H. Y., Luetkemeyer, A., Kline, S., Lopez de Castilla, D., Finberg, R. W., Dierberg, K., Tapson, V., Hsieh, L., Patterson, T. F., Paredes, R., Sweeney, D. A., Short, W. R., Touloumi, G., ACTT-1 Study Group Members (2020). Remdesivir for the Treatment of COVID-19 - Final Report. *The New England Journal of Medicine*, 383(19), 1813–1826.

Biryukov, J., Boydston, J. A., Dunning, R. A., Yeager, J. J., Wood, S., Ferris, A., Miller, D., Weaver, W., Zeitouni, N. E., Freeburger, D., Dabisch, P., Wahl, V., Hevey, M. C., & Altamura, L. A. (2021). SARS-CoV-2 is rapidly inactivated at high temperature. *Environmental Chemistry Letters*, 19(2), 1773–1777.

Biswas, S., Haleyurgirisetty, M., Lee, S., Hewlett, I., & Devadas, K. (2019). Development and validation of plasma miRNA biomarker signature panel for the detection of early HIV-1 infection. *EBioMedicine*, 43, 307–316.

Blackwell, J. M., Jamieson, S. E., & Burgner, D. (2009). HLA and infectious diseases. *Clinical Microbiology Reviews*, 22(2), 370–385.

Bleibtreu, A., Bertine, M., Bertin, C., Houhou-Fidouh, N., & Visseaux, B. (2020). Focus on Middle East respiratory syndrome coronavirus (MERS-CoV). *Medecine Et Maladies Infectieuses*, 50(3), 243–251.

Boonacker, E. P., Wierenga, E. A., Smits, H. H., & Van Noorden, C. J. (2002). CD26/DPPIV signal transduction function, but not proteolytic activity, is directly related to its expression level on human Th1 and Th2 cell lines as detected with living cell cytochemistry. *The Journal of Histochemistry and Cytochemistry : Official Journal of The Histochemistry Society*, 50(9), 1169–1177.

Boonacker, E., & Van Noorden, C. J. (2003). The multifunctional or moonlighting protein CD26/DPPIV. *European Journal of Cell Biology*, 82(2), 53–73.

Boopathi, S., Poma, A. B., & Kolandaivel, P. (2021). Novel 2019 coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment. *Journal of Biomolecular Structure & Dynamics*, 39(9), 3409–3418.

Boulware, D. R., Pullen, M. F., Bangdiwala, A. S., Pastick, K. A., Lofgren, S. M., Okafor, E. C., Skipper, C. P., Nascene, A. A., Nicol, M. R., Abassi, M., Engen, N. W., Cheng, M. P., LaBar, D., Lother, S. A., MacKenzie, L. J., Drobot, G., Marten, N., Zarychanski, R., *et al.,* (2020). A Randomized Trial of Hydroxychloroquine as Postexposure Prophylaxis for COVID-19. *The New England Journal of Medicine*, 383(6), 517–525.

Bourgonje, A.R., Abdulle, A.E., Timens, W., Hillebrands, J.-L., Navis, G.J., Gordijn, S.J., Bolling, M.C., Dijkstra, G., Voors, A.A., Osterhaus, A.D., van der Voort, P.H., Mulder, D.J. and van Goor, H. (2020), Angiotensin-converting enzyme 2 (ACE2), SARS-CoV-2 and the pathophysiology of coronavirus disease 2019 (COVID-19). *The Journal of Pathology*., 251: 228-248.

Brann, D. H., Tsukahara, T., Weinreb, C., Lipovsek, M., Van den Berge, K., Gong, B., *et al.* (2020). Non-neuronal expression of SARS-CoV-2 entry genes in the olfactory system suggests mechanisms underlying COVID-19-associated anosmia. *Science Advances*, 6(31), eabc5801.

Brenner S. R. (2020). COVID-19, TMPRSS2, and whether android regulation affects pandemic virus gender incidence and age distribution of disease. Medical hypotheses, 140, 109773. *Advance Online Publication*.

Callaway E. (2021). Heavily mutated Omicron variant puts scientists on alert. *Nature*, 600(7887), 21.

Carty, M., Guy, C., & Bowie, A. G. (2021). Detection of Viral Infections by Innate Immunity. *Biochemical Pharmacology*, 183, 114316.

Cascella, M., Rajnik, M., Aleem, A., Dulebohn, S. C., & Di Napoli, R. (2023). Features, Evaluation, and Treatment of Coronavirus (COVID-19). In StatPearls. *StatPearls Publishing*.

Cavalcanti, A. B., Zampieri, F. G., Rosa, R. G., Azevedo, L. C. P., Veiga, V. C., Avezum, A., Damiani, L. P., Marcadenti, A., Kawano-Dourado, L., Lisboa, T., Junqueira, D. L. M., de Barros E Silva, P. G. M., Tramujas, L., Abreu-Silva, E. O., Laranjeira, L. N., Soares, A. T., Echenique, L. S., Pereira, A. J., Freitas, F. G. R., Gebara, O. C. E., Coalition COVID-19 Brazil I Investigators (2020). Hydroxychloroquine with or without Azithromycin in Mild-to-Moderate COVID-19. *The New England Journal of Medicine*, 383(21), 2041–2052.

CDC. (2021). Science Brief: SARS-CoV-2 and Surface (Fomite) Transmission for Indoor Community Environments.https://www.cdc.gov/coronavirus/2019-ncov/more/science-and-research/surface-transmission.html#ref1

Cellina, M., Orsi, M., Bombaci, F., Sala, M., Marino, P., & Oliva, G. (2020). Favorable changes of CT findings in a patient with COVID-19 pneumonia after treatment with tocilizumab. *Diagnostic and Interventional Imaging*, 101(5), 323–324.

Chee, Y. J., Ng, S. J. H., & Yeoh, E. (2020). Diabetic ketoacidosis precipitated by COVID-19 in a patient with newly diagnosed diabetes mellitus. *Diabetes Research and Clinical Practice*, 164, 108166.

Chemaitelly, H., Yassine, H. M., Benslimane, F. M., Al Khatib, H. A., Tang, P., Hasan, M. R., Malek, J. A., Coyle, P., Ayoub, H. H., Al Kanaani, Z., Al Kuwari, E., Jeremijenko, A., *et al.*. (2021). mRNA-1273 COVID-19 vaccine effectiveness against the B.1.1.7 and B.1.351 variants and severe COVID-19 disease in Qatar. *Nature Medicine*, 27(9), 1614–1621.

Chen, H., Guo, J., Wang, C., Luo, F., Yu, X., Zhang, W., Li, J., Zhao, D., Xu, D., Gong, Q., Liao, J., Yang, H., Hou, W., & Zhang, Y. (2020a). Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. *Lancet* (London, England), 395(10226), 809–815.

Chen, J., Bai, H., Liu, J., Chen, G., Liao, Q., Yang, J., Wu, P., Wei, J., Ma, D., Chen, G., Ai, J., & Li, K. (2020b). Distinct Clinical Characteristics and Risk Factors for Mortality in Female Inpatients With Coronavirus Disease 2019 (COVID-19): A Sex-stratified, Large-scale Cohort Study in Wuhan, China. *Clinical Infectious Diseases : an official publication of the Infectious Diseases Society of America*, 71(12), 3188–3195.

Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., Qiu, Y., Wang, J., Liu, Y., Wei, Y., Xia, J., Yu, T., Zhang, X., & Zhang, L. (2020c). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* (London, England), 395(10223), 507–513.

Chen, Y. M., Liang, S. Y., Shih, Y. P., Chen, C. Y., Lee, Y. M., Chang, L., Jung, S. Y., Ho, M. S., Liang, K. Y., Chen, H. Y., Chan, Y. J., & Chu, D. C. (2006). Epidemiological and genetic correlates of severe acute respiratory syndrome coronavirus infection in the hospital with the highest nosocomial infection rate in Taiwan in 2003. *Journal of Clinical Microbiology*, 44(2), 359–365.

Chung, H., He, S., Nasreen, S., Sundaram, M. E., Buchan, S. A., Wilson, S. E., Chen, B., Calzavara, A., Fell, D. B., Austin, P. C., Wilson, K., Schwartz, K. L., Brown, K. A., Gubbay, J. B., Basta, N. E., Mahmud, S. M., Righolt, C. H., *et al.* (2021). Effectiveness of BNT162b2 and mRNA-1273 COVID-19 vaccines against symptomatic SARS-CoV-2 infection and severe COVID-19 outcomes in Ontario, Canada: test negative design study. *British Medical Journal* (Clinical research ed.), 374, n1943.

Clinical Spectrum COVID-19 treatment guidelines. https://www.covid19treatmentguidelines.nih.gov/overview/clinical-spectrum/ Available at: Accessed June 6, 2023.

Conti, P., Ronconi, G., Caraffa, A., Gallenga, C. E., Ross, R., Frydas, I., & Kritas, S. K. (2020). Induction of pro-inflammatory cytokines (IL-1 and IL-6) and lung inflammation by Coronavirus-19 (COVI-19 or SARS-CoV-2): anti-inflammatory strategies. *Journal of Biological Regulators and Homeostatic Agents*, 34(2), 327–331.

Cook, T. M., El-Boghdadly, K., McGuire, B., McNarry, A. F., Patel, A., & Higgs, A. (2020). Consensus guidelines for managing the airway in patients with COVID-19: Guidelines from the Difficult Airway Society, the Association of Anaesthetists the Intensive Care Society, the Faculty of Intensive Care Medicine and the Royal College of Anaesthetists. *Anaesthesia*, 75(6), 785-799.

Coopersmith, C. M., Antonelli, M., Bauer, S. R., Deutschman, C. S., Evans, L. E., Ferrer, R., Hellman, J., Jog, S., Kesecioglu, J., Kissoon, N., Martin-Loeches, I., Nunnally, M. E., Prescott, H. C., Rhodes, A., Talmor, D., Tissieres, P., & De Backer, D. (2021). The Surviving Sepsis Campaign: Research Priorities for Coronavirus Disease 2019 in Critical Illness. *Critical Care Medicine*, 49(4), 598-622.

Correale, P., Mutti, L., Pentimalli, F., Baglio, G., Saladino, R. E., Sileri, P., & Giordano, A. (2020). HLA-B*44 and C*01 Prevalence Correlates with COVID19 Spreading across Italy. *International Journal of Molecular Sciences*, 21(15), 5205.

Cui, J., Li, F., & Shi, Z. L. (2019). Origin and evolution of pathogenic coronaviruses. *Nature Reviews Microbiology*, 17(3), 181–192.

Çelik, I., & Öztürk, R. (2021). From asymptomatic to critical illness: decoding various clinical stages of COVID-19. *Turkish Journal of Medical Sciences*, 51(SI-1), 3284–3300.

Davies, N. G., Abbott, S., Barnard, R. C., Jarvis, C. I., Kucharski, A. J., Munday, J. D., Pearson, C. A. B., Russell, T. W., Tully, D. C., Washburne, A. D., Wenseleers, T., Gimma, A., Waites, W., Wong, K. L. M., van Zandvoort, K., Silverman, J. D., CMMID COVID-19 Working Group, COVID-19 Genomics UK (COG-UK) Consortium, Diaz-Ordaz, K., Keogh, R., Edmunds, W. J. (2021). Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. *Science* (New York, N.Y.), 372(6538), eabg3055.

de Wit, E., van Doremalen, N., Falzarano, D., & Munster, V. J. (2016). SARS and MERS: recent insights into emerging coronaviruses. *Nature Reviews Microbiology*, 14(8), 523–534.

Debnath, M., Banerjee, M., & Berk, M. (2020). Genetic gateways to COVID-19 infection: Implications for risk, severity, and outcomes. *FASEB Journal : official publication of the Federation of American Societies for Experimental Biology*, 34(7), 8787–8795.

Dendrou, C. A., Petersen, J., Rossjohn, J., & Fugger, L. (2018). HLA variation and disease. *Nature Reviews Immunology*, 18(5), 325–339.

Du, R. H., Liang, L. R., Yang, C. Q., Wang, W., Cao, T. Z., Li, M., Guo, G. Y., Du, J., Zheng, C. L., Zhu, Q., Hu, M., Li, X. Y., Peng, P., & Shi, H. Z. (2020). Predictors of mortality for patients with COVID-19 pneumonia caused by SARS-CoV-2: a prospective cohort study. *The European Respiratory Journal*, 55(5), 2000524.

Dutta, M., Dutta, P., Medhi, S., Borkakoty, B., & Biswas, D. (2018). Polymorphism of HLA class I and class II alleles in influenza A (H1N1) pdm09 virus infected population of Assam, Northeast India. *Journal of Medical Virology*, 90(5), 854–860.

El-Huneidi, W., Hamad, M., & Taneera, J. (2021). Expression of SARS-CoV-2 receptor "ACE2" in human pancreatic β cells: to be or not to be!. *Islets*, 13(5-6), 106–114.

Fadini, G. P., Morieri, M. L., Boscari, F., Fioretto, P., Maran, A., Busetto, L., Bonora, B. M., Selmin, E., Arcidiacono, G., Pinelli, S., Farnia, F., Falaguasta, D., Russo, L., Voltan, G., Mazzocut, S., Costantini, G., Ghirardini, F., Tresso, S., Cattelan, A. M., Vianello, A., … Vettor, R. (2020). Newly-diagnosed diabetes and admission hyperglycemia predict COVID-19 severity by aggravating respiratory deterioration. *Diabetes Research and Clinical Practice*, 168, 108374.

Fang, Q. (2020). Nan-shan ZHONG's team isolated neocoronavirus from a patient'surfline specimen. *Guangzhou Daily*.

Fani, M., Zandi, M., Ebrahimi, S., Soltani, S., & Abbasi, S. (2021). The role of miRNAs in COVID-19 disease. *Future Virology*, 10.2217/fvl-2020-0389.

Faria, N. R., Mellan, T. A., Whittaker, C., Claro, I. M., Candido, D. D. S., Mishra, S., Crispim, M. A. E., Sales, F. C., Hawryluk, I., McCrone, J. T., Hulswit, R. J. G., Franco, L. A. M., Ramundo, M. S., *et al.* (2021). Genomics and epidemiology of a novel SARS-CoV-2 lineage in Manaus, Brazil. *medRxiv* : the preprint server for health sciences, 2021.02.26.21252554.

Farr, R. J., Rootes, C. L., Rowntree, L. C., Nguyen, T. H. O., Hensen, L., Kedzierski, L., Cheng, A. C., Kedzierska, K., Au, G. G., Marsh, G. A., Vasan, S. S., Foo, C. H., Cowled, C., & Stewart, C. R. (2021). Altered microRNA expression in COVID-19 patients enables identification of SARS-CoV-2 infection. *PLoS Pathogens*, 17(7), e1009759.

FDA. (2021a). FDA Approves First COVID-19 Vaccine. https://www.fda.gov/news-events/press-announcements/fda-approves-first-covid-19-vaccine

FDA. (2021b). Coronavirus (COVID-19) Update: August 6, 2021. https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-august-6-2021

Fevrier, B. (2022). Severe Acute Respiratory Syndromes and Coronaviruses (SARS-CoV, MERS-CoV, and SARS-CoV-2). *IntechOpen*.

Filbin, M. R., Mehta, A., Schneider, A. M., Kays, K. R., Guess, J. R., Gentili, M., Fenyves, B. G., Charland, N. C., Gonye, A. L. K., Gushterova, I., *et al.* (2021). Longitudinal proteomic analysis of severe COVID-19 reveals survival-associated signatures, tissue-specific cell death, and cell-cell interactions. *Cell Reports Medicine*, 2(5), 100287.

Fortunato, F., Martinelli, D., Lo Caputo, S., Santantonio, T., Dattoli, V., Lopalco, P. L., & Prato, R. (2021). Sex and gender differences in COVID-19: an Italian local register-based study. *British Medical Journal*, 11(10), e051506.

Fung, T. S., & Liu, D. X. (2019). Human Coronavirus: Host-Pathogen Interaction. *Annual Review of Microbiology*, 73, 529–557.

Galloway, S. E., Paul, P., MacCannell, D. R., Johansson, M. A., Brooks, J. T., MacNeil, A., Slayton, R. B., Tong, S., Silk, B. J., Armstrong, G. L., Biggerstaff, M., & Dugan, V. G. (2021). Emergence of SARS-CoV-2 B.1.1.7 Lineage - United States, December 29, 2020-January 12, 2021. MMWR. *Morbidity and Mortality Weekly Report*, 70(3), 95–99.

Gebhard, C., Regitz-Zagrosek, V., Neuhauser, H. K., Morgan, R., & Klein, S. L. (2020). Impact of sex and gender on COVID-19 outcomes in Europe. Biology of sex differences, 11(1), 29.

General Office of National Health Commission of the People's Republic of China. 2020. Diagnosis and treatment for novel coronavirus (Version 5). http://www.nhc.gov.cn/xcs/zhengcwj/202002/3b09b894ac9b4204a79db5b8912d4440.shtml [Accessed on June. 6, 2023]

Gibson, P. G., Qin, L., & Puah, S. H. (2020). COVID-19 acute respiratory distress syndrome (ARDS): clinical features and differences from typical pre-COVID-19 ARDS. The Medical journal of Australia, 213(2), 54–56.e1.

Goldberg, Y., Mandel, M., Bar-On, Y. M., Bodenheimer, O., Freedman, L., Haas, E. J., Milo, R., Alroy-Preis, S., Ash, N., & Huppert, A. (2021). Waning Immunity after the BNT162b2 Vaccine in Israel. The New England journal of medicine, 385(24), e85.

Goldman, J. D., Lye, D. C. B., Hui, D. S., Marks, K. M., Bruno, R., Montejano, R., Spinner, C. D., Galli, M., Ahn, M. Y., Nahass, R. G., Chen, Y. S., *et al.* (2020). Remdesivir for 5 or 10 Days in Patients with Severe COVID-19. The New England journal of medicine, 383(19), 1827–1837.

Gomez, J. M. D., Du-Fay-de-Lavallaz, J. M., Fugar, S., Sarau, A., Simmons, J. A., Clark, B., Sanghani, R. M., Aggarwal, N. T., Williams, K. A., Doukky, R., & Volgman, A. S. (2021). Sex Differences in COVID-19 Hospitalization and Mortality. Journal of women's health (2002), 30(5), 646–653.

Gordon, A. C., Mouncey, P. R., Al-Beidh, F., Rowan, K. M., Nichol, A. D., Arabi, Y. M., Annane, D., Beane, A., van Bentum-Puijk, W., Berry, L. R., Bhimani, Z., Bonten, M. J. M., Bradbury, C. A., *et al.* (2021). Interleukin-6 Receptor Antagonists in Critically Ill Patients with COVID-19. The New England journal of medicine, 384(16), 1491–1502.

Gorrell, M. D., Wickson, J., & McCaughan, G. W. (1991). Expression of the rat CD26 antigen (dipeptidyl peptidase IV) on subpopulations of rat lymphocytes. Cellular immunology, 134(1), 205–215.

Gu, H., Krishnan, P., Ng, D. Y. M., Chang, L. D. J., Liu, G. Y. Z., Cheng, S. S. M., Hui, M. M. Y., Fan, M. C. Y., Wan, J. H. L., Lau, L. H. K., Cowling, B. J., Peiris, M., & Poon, L. L. M. (2022). Probable Transmission of SARS-CoV-2 Omicron Variant in Quarantine Hotel, Hong Kong, China, November 2021. Emerging infectious diseases, 28(2), 460–462.

Gu, J., & Korteweg, C. (2007). Pathology and pathogenesis of severe acute respiratory syndrome. The American journal of pathology, 170(4), 1136–1147.

Gu, J., Gong, E., Zhang, B., Zheng, J., Gao, Z., Zhong, Y., Zou, W., Zhan, J., Wang, S., Xie, Z., Zhuang, H., Wu, B., Zhong, H., Shao, H., Fang, W., Gao, D., Pei, F., Li, X., He, Z., Xu, D., … Leong, A. S. (2005). Multiple organ infection and the pathogenesis of SARS. The Journal of experimental medicine, 202(3), 415–424.

Guan, W. J., Ni, Z. Y., Hu, Y., Liang, W. H., Ou, C. Q., He, J. X., Liu, L., Shan, H., Lei, C. L., Hui, D. S. C., Du, B., Li, L. J., Zeng, G., Yuen, K. Y., Chen, R. C., Tang, C. L., Wang, T., Chen, P. Y., Xiang, J., Li, S. Y., *et al.* (2020). Clinical Characteristics of Coronavirus Disease 2019 in China. The New England journal of medicine, 382(18), 1708–1720.

Guo, Z. D., Wang, Z. Y., Zhang, S. F., Li, X., Li, L., Li, C., Cui, Y., Fu, R. B., Dong, Y. Z., Chi, X. Y., Zhang, M. Y., Liu, K., Cao, C., Liu, B., Zhang, K., Gao, Y. W., Lu, B., & Chen, W. (2020). Aerosol and Surface Distribution of Severe Acute Respiratory Syndrome Coronavirus 2 in Hospital Wards, Wuhan, China, 2020. Emerging infectious diseases, 26(7), 1583–1591.

Gupta, M. K., Vemula, S., Donde, R., Gouda, G., Behera, L., & Vadde, R. (2021). In-silico approaches to detect inhibitors of the human severe acute respiratory syndrome coronavirus envelope protein ion channel. Journal of biomolecular structure & dynamics, 39(7), 2617–2627.

Guterres, A., de Azeredo Lima, C. H., Miranda, R. L., & Gadelha, M. R. (2020). What is the potential function of microRNAs as biomarkers and therapeutic targets in COVID-19?. Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases, 85, 104417.

Gutiérrez-Bautista, J. F., Rodriguez-Nicolas, A., Rosales-Castillo, A., López-Ruz, M. Á., Martín-Casares, A. M., Fernández-Rubiales, A., Anderson, P., Garrido, F., Ruiz-Cabello, F., & López-Nevot, M. Á. (2022). Study of HLA-A, -B, -C, -DRB1 and -DQB1 polymorphisms in COVID-19 patients. Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi, 55(3), 421–427.

Hall, V. J., Foulkes, S., Saei, A., Andrews, N., Oguti, B., Charlett, A., Wellington, E., Stowe, J., Gillson, N., Atti, A., Islam, J., Karagiannis, I., Munro, K., Khawam, J., Chand, M. A., Brown, C. S., Ramsay, M., Lopez-Bernal, J., Hopkins, S., & SIREN Study Group (2021). COVID-19 vaccine coverage in health-care workers in England and effectiveness of BNT162b2 mRNA vaccine against infection (SIREN): a prospective, multicentre, cohort study. Lancet (London, England), 397(10286), 1725–1735.

Hamza, A., Shah, N. N., Azad, A. M., Ghanshyam, O. S., & Khan, Z. (2022). Impact of age, gender and comorbidities affecting the severity of COVID-19 infection in Kashmir. Journal of family medicine and primary care, 11(4), 1519–1524.

Harder, T., Koch, J., Vygen-Bonnet, S., Külper-Schiek, W., Pilic, A., Reda, S., Scholz, S., & Wichmann, O. (2021). Efficacy and effectiveness of COVID-19 vaccines against SARS-CoV-2 infection: interim results of a living systematic review, 1 January to 14 May 2021. Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin, 26(28), 2100563.

Henry, B. M., Cheruiyot, I., Benoit, J. L., Lippi, G., Prohászka, Z., Favaloro, E. J., & Benoit, S. W. (2021). Circulating Levels of Tissue Plasminogen Activator and Plasminogen Activator Inhibitor-1 Are Independent Predictors of Coronavirus Disease 2019 Severity: A Prospective, Observational Study. Seminars in thrombosis and hemostasis, 47(4), 451–455.

Hernandez Acosta, R. A., Esquer Garrigos, Z., Marcelin, J. R., & Vijayvargiya, P. (2022). COVID-19 Pathogenesis and Clinical Manifestations. Infectious disease clinics of North America, 36(2), 231–249.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T. S., Herrler, G., Wu, N. H., Nitsche, A., Müller, M. A., Drosten, C., & Pöhlmann, S. (2020a). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell, 181(2), 271–280.e8.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T. S., Herrler, G., Wu, N. H., Nitsche, A., Müller, M. A., Drosten, C., & Pöhlmann, S. (2020b). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell, 181(2), 271–280.e8.

Horby, P., Mafham, M., Linsell, L., Bell, J. L., Staplin, N., Emberson, J. R., Wiselka, M., Ustianowski, A., Elmahi, E., Prudon, B., Whitehouse, T., *et al.* (2020). Effect of Hydroxychloroquine in Hospitalized Patients with COVID-19. The New England journal of medicine, 383(21), 2030–2040.

Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., Cheng, Z., Yu, T., Xia, J., Wei, Y., Wu, W., Xie, X., Yin, W., Li, H., Liu, M., Xiao, Y., *et al.* (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet (London, England), 395(10223), 497–506.

Huet, T., Beaussier, H., Voisin, O., Jouveshomme, S., Dauriat, G., Lazareth, I., Sacco, E., Naccache, J. M., Bézie, Y., Laplanche, S., Le Berre, A., Le Pavec, J., Salmeron, S., Emmerich, J., Mourad, J. J., Chatellier, G., & Hayem, G. (2020). Anakinra for severe forms of COVID-19: a cohort study. The Lancet. Rheumatology, 2(7), e393–e400.

Hui, D. S., Azhar, E. I., Kim, Y. J., Memish, Z. A., Oh, M. D., & Zumla, A. (2018). Middle East respiratory syndrome coronavirus: risk factors and determinants of primary, household, and nosocomial transmission. The Lancet. Infectious diseases, 18(8), e217–e227.

Hwang, S. M., Na, B. J., Jung, Y., Lim, H. S., Seo, J. E., Park, S. A., Cho, Y. S., Song, E. H., Seo, J. Y., Kim, S. R., Lee, G. Y., Kim, S. J., Park, Y. S., & Seo, H. (2019). Clinical and Laboratory Findings of Middle East Respiratory Syndrome Coronavirus Infection. Japanese journal of infectious diseases, 72(3), 160–167.

Ikeagwulonu, R. C., Ugwu, N. I., Ezeonu, C. T., Ikeagwulonu, Z. C., Uro-Chukwu, H. C., Asiegbu, U. V., Obu, D. C., & Briggs, D. C. (2021). C-Reactive Protein and COVID-19 Severity: A Systematic Review. West African journal of medicine, Vol. 38(10), 1011–1023.

Inn, K. S., Kim, Y., Aigerim, A., Park, U., Hwang, E. S., Choi, M. S., Kim, Y. S., & Cho, N. H. (2018). Reduction of soluble dipeptidyl peptidase 4 levels in plasma of patients infected with Middle East respiratory syndrome coronavirus. Virology, 518, 324–327.

International Committee on Taxonomy of Viruses Executive Committee (2020). The new scope of virus taxonomy: partitioning the virosphere into 15 hierarchical ranks. Nature microbiology, 5(5), 668–674.

Inui, S., Fujikawa, A., Jitsu, M., Kunishima, N., Watanabe, S., Suzuki, Y., Umeda, S., & Uwabe, Y. (2020). Chest CT Findings in Cases from the Cruise Ship Diamond Princess with Coronavirus Disease (COVID-19). Radiology. Cardiothoracic imaging, 2(2), e200110.

Ishii, T. (2020). Human Leukocyte Antigen (HLA) Class I Susceptible Alleles Against COVID-19 Increase Both Infection and Severity Rate. Cureus, 12(12), e12239.

Iturrieta-Zuazo, I., Rita, C. G., García-Soidán, A., de Malet Pintos-Fonseca, A., Alonso-Alarcón, N., Pariente-Rodríguez, R., Tejeda-Velarde, A., Serrano-Villar, S., Castañer-Alabau, J. L., & Nieto-Gañán, I. (2020). Possible role of HLA class-I genotype in SARS-CoV-2 infection and progression: A pilot study in a cohort of COVID-19 Spanish patients. Clinical immunology (Orlando, Fla.), 219, 108572.

JHU. 2023. Cases and deaths data from JHU CSSE. https://coronavirus.jhu.edu/region/iraq

Jin, J. M., Bai, P., He, W., Wu, F., Liu, X. F., Han, D. M., Liu, S., & Yang, J. K. (2020). Gender Differences in Patients With COVID-19: Focus on Severity and Mortality. Frontiers in public health, 8, 152.

Kandakure, V. T., Valvi, H. R., Khokle, P., More, M. S., & Chouhan, R. (2022). Prevalence and Recovery from Newly Onset Anosmia and Ageusia in COVID 19 Patients at our Teritary Care Centre. Indian journal of otolaryngology and head and neck surgery : official publication of the Association of Otolaryngologists of India, 74(Suppl 2), 3399–3406.

Kandemirli, S. G., Altundag, A., Yildirim, D., Tekcan Sanli, D. E., & Saatci, O. (2021). Olfactory Bulb MRI and Paranasal Sinus CT Findings in Persistent COVID-19 Anosmia. Academic radiology, 28(1), 28–35.

Kanne, J. P., Little, B. P., Chung, J. H., Elicker, B. M., & Ketai, L. H. (2020). Essentials for Radiologists on COVID-19: An Update-Radiology Scientific Expert Panel. Radiology, 296(2), E113–E114.

Kaur, G., Yogeswaran, S., Muthumalage, T., & Rahman, I. (2021). Persistently Increased Systemic ACE2 Activity Is Associated With an Increased Inflammatory Response in Smokers With COVID-19. Frontiers in physiology, 12, 653045.

Khanolkar, A., Badovinac, V. P., & Harty, J. T. (2007). CD8 T cell memory development: CD4 T cell help is appreciated. Immunologic research, 39(1-3), 94–104.

Kirchdoerfer, R. N., Cottrell, C. A., Wang, N., Pallesen, J., Yassine, H. M., Turner, H. L., Corbett, K. S., Graham, B. S., McLellan, J. S., & Ward, A. B. (2016). Pre-fusion structure of a human coronavirus spike protein. Nature, 531(7592), 118–121.

Korber, B., Fischer, W. M., Gnanakaran, S., Yoon, H., Theiler, J., Abfalterer, W., Hengartner, N., Giorgi, E. E., Bhattacharya, T., Foley, B., Hastie, K. M., Parker, M. D., Partridge, D. G., Evans, C. M., Freeman, T. M., de Silva, T. I., Sheffield COVID-19 Genomics Group, McDanal, C., Perez, L. G., Tang, H., *et al.* (2020). Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. Cell, 182(4), 812–827.e19.

Kow, C. S., & Hasan, S. S. (2021). Real-world effectiveness of BNT162b2 mRNA vaccine: a meta-analysis of large observational studies. Inflammopharmacology, 29(4), 1075–1090.

Kragstrup, T. W., Singh, H. S., Grundberg, I., Nielsen, A. L., Rivellese, F., Mehta, A., Goldberg, M. B., Filbin, M. R., Qvist, P., & Bibby, B. M. (2021). Plasma ACE2 predicts outcome of COVID-19 in hospitalized patients. PloS one, 16(6), e0252799.

Kucirka, L. M., Lauer, S. A., Laeyendecker, O., Boon, D., & Lessler, J. (2020). Variation in False-Negative Rate of Reverse Transcriptase Polymerase Chain Reaction-Based SARS-CoV-2 Tests by Time Since Exposure. Annals of internal medicine, 173(4), 262–267.

Kulkarni, S., Jenner, B. L., & Wilkinson, I. (2020). COVID-19 and hypertension. Journal of the renin-angiotensin-aldosterone system : JRAAS, 21(2), 1470320320927851.

Kulski, J. K., Shiina, T., & Dijkstra, J. M. (2019). Genomic Diversity of the Major Histocompatibility Complex in Health and Disease. Cells, 8(10), 1270.

Kuri-Cervantes, L., Pampena, M. B., Meng, W., Rosenfeld, A. M., Ittner, C. A. G., Weisman, A. R., Agyekum, R. S., Mathew, D., Baxter, A. E., Vella, L. A., Kuthuru, O., Apostolidis, S. A., Bershaw, L., Dougherty, J., Greenplate, A. R., Pattekar, A., Kim, J., Han, N., Gouma, S., Weirick, M. E., … Betts, M. R. (2020). Comprehensive mapping of immune perturbations associated with severe COVID-19. Science immunology, 5(49), eabd7114.

Lamontagne, F., Agarwal, A., Rochwerg, B., Siemieniuk, R. A., Agoritsas, T., Askie, L., Lytvyn, L., Leo, Y. S., Macdonald, H., Zeng, L., Amin, W., da Silva, A. R. A., Aryal, D., Barragan, F. A. J., Bausch, F. J., Burhan, E., Calfee, C. S., Cecconi, M., Chacko, B., Chanda, D., … Vandvik, P. O. (2020). A living WHO guideline on drugs for COVID-19. BMJ (Clinical research ed.), 370, m3379.

Lani, R., Senin, N. A., AbuBakar, S., & Hassandarvish, P. (2022). Knowledge of SARS-CoV-2 Epitopes and Population HLA Types Is Important in the Design of COVID-19 Vaccines. Vaccines, 10(10), 1606.

Lei, J., Kusov, Y., & Hilgenfeld, R. (2018). Nsp3 of coronaviruses: Structures and functions of a large multi-domain protein. Antiviral research, 149, 58–74.

Li, B., Yang, J., Zhao, F., Zhi, L., Wang, X., Liu, L., Bi, Z., & Zhao, Y. (2020). Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. Clinical research in cardiology : official journal of the German Cardiac Society, 109(5), 531–538.

Li, C., He, Q., Qian, H., & Liu, J. (2021). Overview of the pathogenesis of COVID-19 (Review). Experimental and therapeutic medicine, 22(3), 1011.

Li, H., Tian, S., Chen, T., Cui, Z., Shi, N., Zhong, X., Qiu, K., Zhang, J., Zeng, T., Chen, L., & Zheng, J. (2020). Newly diagnosed diabetes is associated with a higher risk of mortality than known diabetes in hospitalized patients with COVID-19. Diabetes, obesity & metabolism, 22(10), 1897–1906.

Li, T., Qiu, Z., Zhang, L., Han, Y., He, W., Liu, Z., Ma, X., Fan, H., Lu, W., Xie, J., Wang, H., Deng, G., & Wang, A. (2004). Significant changes of peripheral T lymphocyte subsets in patients with severe acute respiratory syndrome. The Journal of infectious diseases, 189(4), 648–651.

Lian, F., Cui, Y., Zhou, C., Gao, K., & Wu, L. (2015). Identification of a plasma four-microRNA panel as potential noninvasive biomarker for osteosarcoma. PloS one, 10(3), e0121499.

Lin, M., Tseng, H. K., Trejaut, J. A., Lee, H. L., Loo, J. H., Chu, C. C., Chen, P. J., Su, Y. W., Lim, K. H., Tsai, Z. U., Lin, R. Y., Lin, R. S., & Huang, C. H. (2003). Association of HLA class I with severe acute respiratory syndrome coronavirus infection. BMC medical genetics, 4, 9.

Lissenberg, A., Vrolijk, M. M., van Vliet, A. L., Langereis, M. A., de Groot-Mijnes, J. D., Rottier, P. J., & de Groot, R. J. (2005). Luxury at a cost? Recombinant mouse hepatitis viruses expressing the accessory hemagglutinin esterase protein display reduced fitness in vitro. Journal of virology, 79(24), 15054–15063.

Littera, R., Campagna, M., Deidda, S., Angioni, G., Cipri, S., Melis, M., Firinu, D., Santus, S., Lai, A., Porcella, R., Lai, S., Rassu, S., Scioscia, R., Meloni, F., Schirru, D., Cordeddu, W., Kowalik, M. A., Serra, M., Ragatzu, P., *et al.* (2020). Human Leukocyte Antigen Complex and Other Immunogenetic and Clinical Factors Influence Susceptibility or Protection to SARS-CoV-2 Infection and Severity of the Disease Course. The Sardinian Experience. Frontiers in immunology, 11, 605688.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods (San Diego, Calif.), 25(4), 402–408.

Lopez Bernal, J., Andrews, N., Gower, C., Gallagher, E., Simmons, R., Thelwall, S., Stowe, J., Tessier, E., Groves, N., Dabrera, G., Myers, R., Campbell, C. N. J., Amirthalingam, G., Edmunds, M., *et al*. (2021). Effectiveness of COVID-19 Vaccines against the B.1.617.2 (Delta) Variant. The New England journal of medicine, 385(7), 585–594.

Luo, X., Zhou, W., Yan, X., Guo, T., Wang, B., Xia, H., Ye, L., Xiong, J., Jiang, Z., Liu, Y., Zhang, B., & Yang, W. (2020). Prognostic Value of C-Reactive Protein in Patients With Coronavirus 2019. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 71(16), 2174–2179.

Mahase E. (2021). COVID-19: Pfizer's paxlovid is 89% effective in patients at risk of serious illness, company reports. BMJ (Clinical research ed.), 375, n2713.

Marchese, A. M., Zhou, X., Kinol, J., Underwood, E., Woo, W., McGarry, A., Beyhaghi, H., Áñez, G., Toback, S., & Dunkle, L. M. (2023). NVX-CoV2373 vaccine efficacy against hospitalization: A post hoc analysis of the PREVENT-19 phase 3, randomized, placebo-controlled trial. Vaccine, 41(22), 3461–3466.

Marczak, H., Chruściel, J., Kulus, M., & Krenke, K. (2023). COVID-19 in patients with persistent tachypnea of infancy. Pediatric pulmonology, 58(3), 934–940.

Masters P. S. (2006). The molecular biology of coronaviruses. Advances in virus research, 66, 193–292.

Meng, H., Xiong, R., He, R., Lin, W., Hao, B., Zhang, L., Lu, Z., Shen, X., Fan, T., Jiang, W., Yang, W., Li, T., Chen, J., & Geng, Q. (2020). CT imaging and clinical course of asymptomatic cases with COVID-19 pneumonia at admission in Wuhan, China. The Journal of infection, 81(1), e33–e39.

Merza, M. A., Aswad, S. M., Sulaiman, H. M., Abdulah, D. M., Rasheed, W. S., & Taib, N. I. (2021). Clinical and epidemiological characteristics and outcomes of Coronavirus disease-19 patients in a large longitudinal study. International journal of health sciences, 15(4), 29–41.

Mesas, A. E., Cavero-Redondo, I., Álvarez-Bueno, C., Sarriá Cabrera, M. A., Maffei de Andrade, S., Sequí-Dominguez, I., & Martínez-Vizcaíno, V. (2020). Predictors of in-hospital COVID-19 mortality: A comprehensive systematic review and meta-analysis exploring differences by age, sex and health conditions. PloS one, 15(11), e0241742.

Metwally, A. A., Mehta, P., Johnson, B. S., Nagarjuna, A., & Snyder, M. P. (2021). COVID-19-Induced New-Onset Diabetes: Trends and Technologies. Diabetes, 70(12), 2733–2744.

Michot, J. M., Albiges, L., Chaput, N., Saada, V., Pommeret, F., Griscelli, F., Balleyguier, C., Besse, B., Marabelle, A., Netzer, F., Merad, M., Robert, C., Barlesi, F., Gachot, B., & Stoclin, A. (2020). Tocilizumab, an anti-IL-6 receptor antibody, to treat COVID-19-related respiratory failure: a case report. Annals of oncology : official journal of the European Society for Medical Oncology, 31(7), 961–964.

Migliorini, F., Torsiello, E., Spiezia, F., Oliva, F., Tingart, M., & Maffulli, N. (2021). Association between HLA genotypes and COVID-19 susceptibility, severity and progression: a comprehensive review of the literature. European journal of medical research, 26(1), 84.

Mitjà, O., Corbacho-Monné, M., Ubals, M., Alemany, A., Suñer, C., Tebé, C., Tobias, A., Peñafiel, J., Ballana, E., Pérez, C. A., Admella, P., Riera-Martí, N., Laporte, P., Mitjà, J., Clua, M., Bertran, L., *et al.* (2021). A Cluster-Randomized Trial of Hydroxychloroquine for Prevention of COVID-19. The New England journal of medicine, 384(5), 417–427.

Moatar, A. I., Chis, A. R., Romanescu, M., Ciordas, P., Nitusca, D., Marian, C., Oancea, C., & Sirbu, I. (2023). Plasma Mir-195-5p predicts the severity of COVID-19 in hospitalized patients. Scientific Reports, 13(1).

Mundalil Vasu, M., Anitha, A., Thanseem, I., Suzuki, K., Yamada, K., Takahashi, T., Wakuda, T., Iwata, K., Tsujii, M., Sugiyama, T., & Mori, N. (2014). Serum microRNA profiles in children with autism. Molecular autism, 5, 40.

Munro, A. P. S., Janani, L., Cornelius, V., Aley, P. K., Babbage, G., Baxter, D., Bula, M., Cathie, K., Chatterjee, K., Dodd, K., Enever, Y., Gokani, *et al.* (2021). Safety and immunogenicity of seven COVID-19 vaccines as a third dose (booster) following two doses of ChAdOx1 nCov-19 or BNT162b2 in the UK (COV-BOOST): a blinded, multicentre, randomised, controlled, phase 2 trial. Lancet (London, England), 398(10318), 2258–2276.

Mwenda, M., Saasa, N., Sinyange, N., Busby, G., Chipimo, P. J., Hendry, J., Kapona, O., Yingst, S., Hines, J. Z., Minchella, P., Simulundu, E., Changula, K., Nalubamba, K. S., Sawa, H., Kajihara, M., Yamagishi, J., Kapin'a, M., Kapata, N., Fwoloshi, S., Zulu, P., *et al.* (2021). Detection of B.1.351 SARS-CoV-2 Variant Strain - Zambia, December 2020. MMWR. Morbidity and mortality weekly report, 70(8), 280–282.

Nasreen, S., Chung, H., He, S., Brown, K. A., Gubbay, J. B., Buchan, S. A., Fell, D. B., Austin, P. C., Schwartz, K. L., Sundaram, M. E., Calzavara, A., Chen, B., Tadrous, M., Wilson, K., Wilson, S. E., Kwong, J. C., & Canadian Immunization Research Network (CIRN) Provincial Collaborative Network (PCN) Investigators (2022). Effectiveness of COVID-19 vaccines against symptomatic SARS-CoV-2 infection and severe outcomes with variants of concern in Ontario. Nature microbiology, 7(3), 379–385.

Ng, M. H., Lau, K. M., Li, L., Cheng, S. H., Chan, W. Y., Hui, P. K., Zee, B., Leung, C. B., & Sung, J. J. (2004). Association of human-leukocyte-antigen class I (B*0703) and class II (DRB1*0301) genotypes with susceptibility and resistance to the development of severe acute respiratory syndrome. The Journal of infectious diseases, 190(3), 515–518.

Ni, L., Ye, F., Cheng, M. L., Feng, Y., Deng, Y. Q., Zhao, H., Wei, P., Ge, J., Gou, M., Li, X., Sun, L., Cao, T., Wang, P., Zhou, C., Zhang, R., Liang, P., Guo, H., Wang, X., Qin, C. F., Chen, F., *et al.* (2020). Detection of SARS-CoV-2-Specific Humoral and Cellular Immunity in COVID-19 Convalescent Individuals. Immunity, 52(6), 971–977.e3.

Noh, Y., Oh, I. S., Jeong, H. E., Filion, K. B., Yu, O. H. Y., & Shin, J. Y. (2021). Association Between DPP-4 Inhibitors and COVID-19-Related Outcomes Among Patients With Type 2 Diabetes. Diabetes care, 44(4), e64–e66.

Novelli, A., Andreani, M., Biancolella, M., Liberatoscioli, L., Passarelli, C., Colona, V. L., Rogliani, P., Leonardis, F., Campana, A., Carsetti, R., Andreoni, M., *et al.* (2020). HLA allele frequencies and susceptibility to COVID-19 in a group of 99 Italian patients. HLA, 96(5), 610–614.

Olwenyi, O. A., Dyavar, S. R., Acharya, A., Podany, A. T., Fletcher, C. V., Ng, C. L., Reid, S. P., & Byrareddy, S. N. (2020). Immuno-epidemiology and pathophysiology of coronavirus disease 2019 (COVID-19). Journal of molecular medicine (Berlin, Germany), 98(10), 1369–1383.

Oran, D. P., & Topol, E. J. (2020). Prevalence of Asymptomatic SARS-CoV-2 Infection : A Narrative Review. Annals of internal medicine, 173(5), 362–367.

Oreshkova, N., Molenaar, R. J., Vreman, S., Harders, F., Oude Munnink, B. B., Hakze-van der Honing, R. W., Gerhards, N., *et al.*. (2020). SARS-CoV-2 infection in farmed minks, the Netherlands, April and May 2020. Euro surveillance: bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin, 25(23), 2001005.

Palladino, M. (2021). Complete blood count alterations in COVID-19 patients: A narrative review. Biochemia medica, 31(3), 030501.

Pandey, L., Bhatt, A., Singh, J., & Rajkumar, B. (2022). A pandemic COVID-19: In Dentistry (1st ed., Vols. 1–183). Book Rivers.

Patel, S. K., Juno, J. A., Lee, W. S., Wragg, K. M., Hogarth, P. M., Kent, S. J., & Burrell, L. M. (2021). Plasma ACE2 activity is persistently elevated following SARS-CoV-2 infection: implications for COVID-19 pathogenesis and consequences. The European respiratory journal, 57(5), 2003730.

Peng, M., He, J., Xue, Y., Yang, X., Liu, S., & Gong, Z. (2021). Role of Hypertension on the Severity of COVID-19: A Review. Journal of cardiovascular pharmacology, 78(5), e648–e655.

Pisanti, S., Deelen, J., Gallina, A. M., Caputo, M., Citro, M., Abate, M., Sacchi, N., Vecchione, C., & Martinelli, R. (2020). Correlation of the two most frequent HLA haplotypes in the Italian population to the differential regional incidence of COVID-19. Journal of translational medicine, 18(1), 352.

Polack, F. P., Thomas, S. J., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Perez, J. L., Pérez Marc, G., Moreira, E. D., Zerbini, C., Bailey, R., Swanson, K. A., Roychoudhury, S., Koury, K., Li, P., Kalina, W. V., *et al.* (2020). Safety and Efficacy of the BNT162b2 mRNA COVID-19 Vaccine. The New England journal of medicine, 383(27), 2603–2615.

Posadas-Sánchez, R., Sánchez-Muñoz, F., Guzmán-Martín, C. A., Hernández-Díaz Couder, A., Rojas-Velasco, G., Fragoso, J. M., & Vargas-Alarcón, G. (2021). Dipeptidylpeptidase-4 levels and DPP4 gene polymorphisms in patients with COVID-19. Association with disease and with severity. Life sciences, 276, 119410.

Qian, J., Zhao, L., Ye, R. Z., Li, X. J., & Liu, Y. L. (2020). Age-dependent Gender Differences in COVID-19 in Mainland China: Comparative Study. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 71(9), 2488–2494.

Qin, C., Zhou, L., Hu, Z., Zhang, S., Yang, S., Tao, Y., Xie, C., Ma, K., Shang, K., Wang, W., & Tian, D. S. (2020). Dysregulation of Immune Response in Patients With Coronavirus 2019 (COVID-19) in Wuhan, China. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 71(15), 762–768.

Quaresima, V., Scarpazza, C., Sottini, A., Fiorini, C., Signorini, S., Delmonte, O. M., Signorini, L., Quiros-Roldan, E., & Imberti, L. (2021). Correction to: Sex differences in a cohort of COVID-19 Italian patients hospitalized during the first and second pandemic waves. Biology of sex differences, 12(1), 48.

Regev-Yochay, G., Amit, S., Bergwerk, M., Lipsitch, M., Leshem, E., Kahn, R., Lustig, Y., Cohen, C., Doolman, R., Ziv, A., Novikov, I., Rubin, C., Gimpelevich, I., Huppert, A., Rahav, G., Afek, A., & Kreiss, Y. (2021). Decreased infectivity following BNT162b2 vaccination: A prospective cohort study in Israel. The Lancet regional health. Europe, 7, 100150.

Reindl-Schwaighofer, R., Hödlmoser, S., Eskandary, F., Poglitsch, M., Bonderman, D., Strassl, R., Aberle, J. H., Oberbauer, R., Zoufaly, A., & Hecking, M. (2021). ACE2 Elevation in Severe COVID-19. American journal of respiratory and critical care medicine, 203(9), 1191–1196.

Ren, H., Yang, Y., Wang, F., Yan, Y., Shi, X., Dong, K., Yu, X., & Zhang, S. (2020). Association of the insulin resistance marker TyG index with the severity and mortality of COVID-19. Cardiovascular diabetology, 19(1), 58.

Ren, X., Zhou, J., Guo, J., Hao, C., Zheng, M., Zhang, R., Huang, Q., Yao, X., Li, R., & Jin, Y. (2022). Reinfection in patients with COVID-19: a systematic review. Global health research and policy, 7(1), 12.

Riddell, S., Goldie, S., Hill, A., Eagles, D., & Drew, T. W. (2020). The effect of temperature on persistence of SARS-CoV-2 on common surfaces. Virology journal, 17(1), 145.

Rodrigues, T. S., de Sá, K. S. G., Ishimoto, A. Y., Becerra, A., Oliveira, S., Almeida, L., Gonçalves, A. V., Perucello, D. B., Andrade, W. A., Castro, R., Veras, F. P. *et al.* (2021). Inflammasomes are activated in response to SARS-CoV-2 infection and are associated with COVID-19 severity in patients. The Journal of experimental medicine, 218(3), e20201707.

Rosas, I. O., Bräu, N., Waters, M., Go, R. C., Hunter, B. D., Bhagani, S., Skiest, D., Aziz, M. S., Cooper, N., Douglas, I. S., Savic, S., Youngstein, T., Del Sorbo, L., Cubillo Gracian, A., De La Zerda, D. J., Ustianowski, A., *et al.* (2021). Tocilizumab in Hospitalized Patients with Severe COVID-19 Pneumonia. The New England journal of medicine, 384(16), 1503–1516.

Rubino, F., Amiel, S. A., Zimmet, P., Alberti, G., Bornstein, S., Eckel, R. H., Mingrone, G., Boehm, B., Cooper, M. E., Chai, Z., Del Prato, S., Ji, L., Hopkins, D., Herman, W. H., Khunti, K., Mbanya, J. C., & Renard, E. (2020). New-Onset Diabetes in COVID-19. The New England journal of medicine, 383(8), 789–790.

Rydyznski Moderbacher, C., Ramirez, S. I., Dan, J. M., Grifoni, A., Hastie, K. M., Weiskopf, D., Belanger, S., Abbott, R. K., Kim, C., Choi, J., Kato, Y., Crotty, E. G., Kim, C., Rawlings, S. A., Mateus, J., Tse, L. P. V., Frazier, A., Baric, R., Peters, B., Greenbaum, J., *et al.* (2020). Antigen-Specific Adaptive Immunity to SARS-CoV-2 in Acute COVID-19 and Associations with Age and Disease Severity. Cell, 183(4), 996–1012.e19.

Sadoff, J., Gray, G., Vandebosch, A., Cárdenas, V., Shukarev, G., Grinsztejn, B., Goepfert, P. A., Truyers, C., Fennema, H., Spiessens, B., Offergeld, K., Scheper, G., Taylor, K. L., Robb, M. L., Treanor, J., Barouch, D. H., Stoddard, J., Ryser, M. F., Marovich, M. A., Neuzil, K. M., *et al.* (2021). Safety and Efficacy of Single-Dose Ad26.COV2.S Vaccine against COVID-19. The New England journal of medicine, 384(23), 2187–2201.

Saiag, E., Goldshmidt, H., Sprecher, E., Ben-Ami, R., & Bomze, D. (2021). Immunogenicity of a BNT162b2 vaccine booster in health-care workers. The Lancet. Microbe, 2(12), e650.

Sarma, P., Shekhar, N., Prajapat, M., Avti, P., Kaur, H., Kumar, S., Singh, S., Kumar, H., Prakash, A., Dhibar, D. P., & Medhi, B. (2021). In-silico homology assisted identification of inhibitor of RNA binding against 2019-nCoV N-protein (N terminal domain). Journal of biomolecular structure & dynamics, 39(8), 2724–2732.

Schlicht, K., Rohmann, N., Geisler, C., Hollstein, T., Knappe, C., Hartmann, K., Schwarz, J., Tran, F., Schunk, D., Junker, R., Bahmer, T., Rosenstiel, P., Schulte, D., Türk, K., Franke, A., Schreiber, S., & Laudes, M. (2020). Circulating levels of soluble Dipeptidylpeptidase-4 are reduced in human subjects hospitalized for severe COVID-19 infections. International journal of obesity (2005), 44(11), 2335–2338.

Sebastián-Martín, A., Sánchez, B. G., Mora-Rodríguez, J. M., Bort, A., & Díaz-Laviada, I. (2022). Role of Dipeptidyl Peptidase-4 (DPP4) on COVID-19 Physiopathology. Biomedicines, 10(8), 2026.

Sen, S., Chakraborty, R., Kalita, P., & Pathak, M. P. (2021). Diabetes mellitus and COVID-19: Understanding the association in light of current evidence. World journal of clinical cases, 9(28), 8327–8339.

Sette, A., & Crotty, S. (2021). Adaptive immunity to SARS-CoV-2 and COVID-19. Cell, 184(4), 861–880.

Sheikh, A., McMenamin, J., Taylor, B., Robertson, C., & Public Health Scotland and the EAVE II Collaborators (2021). SARS-CoV-2 Delta VOC in Scotland: demographics, risk of hospital admission, and vaccine effectiveness. Lancet (London, England), 397(10293), 2461–2462.

Shi, Y., Wang, G., Cai, X. P., Deng, J. W., Zheng, L., Zhu, H. H., Zheng, M., Yang, B., & Chen, Z. (2020). An overview of COVID-19. Journal of Zhejiang University. Science. B, 21(5), 343–360.

Shiina, T., Hosomichi, K., Inoko, H., & Kulski, J. K. (2009). The HLA genomic loci map: expression, interaction, diversity and disease. Journal of human genetics, 54(1), 15–39.

Singh, A. K., Singh, A., Singh, R., & Misra, A. (2021). Molnupiravir in COVID-19: A systematic review of literature. Diabetes & metabolic syndrome, 15(6), 102329.

Solerte, S. B., D'Addio, F., Trevisan, R., Lovati, E., Rossi, A., Pastore, I., Dell'Acqua, M., Ippolito, E., Scaranna, C., Bellante, R., Galliani, S., Dodesini, A. R., Lepore, G., *et al.* (2020). Sitagliptin Treatment at the Time of Hospitalization Was Associated With Reduced Mortality in Patients With Type 2 Diabetes and COVID-19: A Multicenter, Case-Control, Retrospective, Observational Study. Diabetes care, 43(12), 2999–3006.

Song, W., Gui, M., Wang, X., & Xiang, Y. (2018). Cryo-EM structure of the SARS coronavirus spike glycoprotein in complex with its host cell receptor ACE2. PLoS pathogens, 14(8), e1007236.

Special Expert Group for Control of the Epidemic of Novel Coronavirus Pneumonia of the Chinese Preventive Medicine Association (2020). An update on the epidemiological characteristics of novel coronavirus pneumonia (COVID-19) Chinese Journal of Epidemiology, 41(2):139–144.

Spinner, C. D., Gottlieb, R. L., Criner, G. J., Arribas López, J. R., Cattelan, A. M., Soriano Viladomiu, A., Ogbuagu, O., Malhotra, P., Mullane, K. M., Castagna, A., Chai, L. Y. A., Roestenberg, M., *et al.* (2020). Effect of Remdesivir vs Standard Care on Clinical Status at 11 Days in Patients With Moderate COVID-19: A Randomized Clinical Trial. The Journal of the American Medical Association, 324(11), 1048–1057.

Stokes, E. K., Zambrano, L. D., Anderson, K. N., Marder, E. P., Raz, K. M., El Burai Felix, S., Tie, Y., & Fullerton, K. E. (2020). Coronavirus Disease 2019 Case Surveillance - United States, January 22-May 30, 2020. MMWR. Morbidity and mortality weekly report, 69(24), 759–765.

Stringer, D., Braude, P., Myint, P. K., Evans, L., Collins, J. T., Verduri, A., Quinn, T. J., Vilches-Moraga, A., Stechman, M. J., Pearce, L., Moug, S., McCarthy, K., Hewitt, J., Carter, B., & COPE Study Collaborators (2021). The role of C-reactive protein as a prognostic marker in COVID-19. International journal of epidemiology, 50(2), 420–429.

Syangtan, G., Bista, S., Dawadi, P., Rayamajhee, B., Shrestha, L. B., Tuladhar, R., & Joshi, D. R. (2021). Asymptomatic SARS-CoV-2 Carriers: A Systematic Review and Meta-Analysis. Frontiers in public health, 8, 587374.

Tang, F., Quan, Y., Xin, Z. T., Wrammert, J., Ma, M. J., Lv, H., Wang, T. B., Yang, H., Richardus, J. H., Liu, W., & Cao, W. C. (2011). Lack of peripheral memory B cell responses in recovered patients with severe acute respiratory syndrome: a six-year follow-up study. Journal of immunology (Baltimore, Md. : 1950), 186(12), 7264–7268.

Tavasolian, F., Rashidi, M., Hatam, G. R., Jeddi, M., Hosseini, A. Z., Mosawi, S. H., Abdollahi, E., & Inman, R. D. (2021). HLA, Immune Response, and Susceptibility to COVID-19. Frontiers in immunology, 11, 601886.

Tegally, H., Wilkinson, E., Giovanetti, M., Iranzadeh, A., Fonseca, V., Giandhari, J., Doolabh, D., Pillay, S., San, E. J., Msomi, N., Mlisana, K., von Gottberg, A., Walaza, S., Allam, M., Ismail, A., Mohale, T., Glass, A. J., Engelbrecht, S., Van Zyl, G., *et al.* (2021). Detection of a SARS-CoV-2 variant of concern in South Africa. Nature, 592(7854), 438–443.

Teuwen, L. A., Geldhof, V., Pasut, A., & Carmeliet, P. (2020). COVID-19: the vasculature unleashed. Nature reviews. Immunology, 20(7), 389–391.

Thompson, B. T., Chambers, R. C., & Liu, K. D. (2017). Acute Respiratory Distress Syndrome. The New England journal of medicine, 377(6), 562–572.

Tijsen, A. J., Creemers, E. E., Moerland, P. D., de Windt, L. J., van der Wal, A. C., Kok, W. E., & Pinto, Y. M. (2010). MiR423-5p as a circulating biomarker for heart failure. Circulation research, 106(6), 1035–1039.

Tomita, Y., Ikeda, T., Sato, R., & Sakagami, T. (2020). Association between HLA gene polymorphisms and mortality of COVID-19: An in silico analysis. Immunity, inflammation and disease, 8(4), 684–694.

Too, C., Tan, L., Heselynn, H., Nor-Shuhaila, S., Eashwary, M., Wahinuddin, S., Lau, I. S., Gun, S. C., Mohd-Shahrir, M., Ainon, M., Azmillah, R., Muhaini, O., & Shahnaz, M. (2019). HLA-A,-B,-C,-DRB1 and-DQB1 alleles and haplotypes in 194 Southeast Asia Chinese from Peninsular Malaysia. Human Immunology, 80(11), 906-907.

Tu, H., Yang, S., Jiang, T., Wei, L., Shi, L., Liu, C., Wang, C., Huang, H., Hu, Y., Chen, Z., Chen, J., Li, Z., & Li, J. (2019). Elevated pulmonary tuberculosis biomarker miR-423-5p plays critical role in the occurrence of active TB by inhibiting autophagosome-lysosome fusion. Emerging microbes & infections, 8(1), 448–460.

Valencia, I., Peiró, C., Lorenzo, Ó., Sánchez-Ferrer, C. F., Eckel, J., & Romacho, T. (2020). DPP4 and ACE2 in Diabetes and COVID-19: Therapeutic Targets for Cardiovascular Complications?. Frontiers in pharmacology, 11, 1161.

van de Veerdonk, F. L., Netea, M. G., van Deuren, M., van der Meer, J. W., de Mast, Q., Brüggemann, R. J., & van der Hoeven, H. (2020). Kallikrein-kinin blockade in patients with COVID-19 to prevent acute respiratory distress syndrome. eLife, 9, e57555.

van Doremalen, N., Bushmaker, T., Morris, D. H., Holbrook, M. G., Gamble, A., Williamson, B. N., Tamin, A., Harcourt, J. L., Thornburg, N. J., Gerber, S. I., Lloyd-Smith, J. O., de Wit, E., & Munster, V. J. (2020). Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. The New England journal of medicine, 382(16), 1564–1567.

van Lier, D., Kox, M., Santos, K., van der Hoeven, H., Pillay, J., & Pickkers, P. (2021). Increased blood angiotensin converting enzyme 2 activity in critically ill COVID-19 patients. ERJ open research, 7(1), 00848-2020.

van Wolfswinkel, M. E., Vliegenthart-Jongbloed, K., de Mendonça Melo, M., Wever, P. C., McCall, M. B., Koelewijn, R., van Hellemond, J. J., & van Genderen, P. J. (2013). Predictive value of lymphocytopenia and the neutrophil-lymphocyte count ratio for severe imported malaria. Malaria journal, 12, 101.

Vankadari, N., & Wilce, J. A. (2020). Emerging WuHan (COVID-19) coronavirus: glycan shield and structure prediction of spike glycoprotein and its interaction with human CD26. Emerging microbes & infections, 9(1), 601–604.

Vaughan A. (2021). Omicron emerges. New scientist (1971), 252(3363), 7.

Vijayvargiya, P., Esquer Garrigos, Z., Castillo Almeida, N. E., Gurram, P. R., Stevens, R. W., & Razonable, R. R. (2020). Treatment Considerations for COVID-19: A Critical Review of the Evidence (or Lack Thereof). Mayo Clinic proceedings, 95(7), 1454–1466.

Volz, E., Mishra, S., Chand, M., Barrett, J. C., Johnson, R., Geidelberg, L., Hinsley, W. R., Laydon, D. J., Dabrera, G., O'Toole, Á., Amato, R., Ragonnet-Cronin, M., Harrison, I., Jackson, B., Ariani, C. V., Boyd, O., Loman, N. J., McCrone, J. T., Gonçalves, S., Jorgensen, D., *et al.* (2021). Assessing transmissibility of SARS-CoV-2 lineage B.1.1.7 in England. Nature, 593(7858), 266–269.

Voysey, M., Clemens, S. A. C., Madhi, S. A., Weckx, L. Y., Folegatti, P. M., Aley, P. K., Angus, B., Baillie, V. L., Barnabas, S. L., Bhorat, Q. E., Bibi, S., Briner, C., Cicconi, P., Collins, A. M., Colin-Jones, R., Cutland, C. L., Darton, T. C., Dheda, K., Duncan, C. J., *et al.* (2021). Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet (London, England), 397(10269), 99–111.

Walensky, R. P., Walke, H. T., & Fauci, A. S. (2021). SARS-CoV-2 Variants of Concern in the United States-Challenges and Opportunities. The Journal of the American Medical Association, 325(11), 1037–1038.

Wang, J., Jiang, M., Chen, X., & Montaner, L. J. (2020b). Cytokine storm and leukocyte changes in mild versus severe SARS-CoV-2 infection: Review of 3939 COVID-19 patients in China and emerging pathogenesis and therapy concepts. Journal of leukocyte biology, 108(1), 17–41.

Wang, J., Pan, Y., Dai, F., Wang, F., Qiu, H., & Huang, X. (2020d). Serum miR-195-5p is upregulated in gestational diabetes mellitus. Journal of clinical laboratory analysis, 34(8), e23325.

Wang, L., Berger, N. A., Kaelber, D. C., Davis, P. B., Volkow, N. D., & Xu, R. (2022). Comparison of outcomes from COVID infection in pediatric and adult patients before and after the emergence of Omicron. medRxiv : the preprint server for health sciences, 2021.12.30.21268495.

Wang, M., Cao, R., Zhang, L., Yang, X., Liu, J., Xu, M., Shi, Z., Hu, Z., Zhong, W., & Xiao, G. (2020c). Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell research, 30(3), 269–271.

Wang, N., Shi, X., Jiang, L., Zhang, S., Wang, D., Tong, P., Guo, D., Fu, L., Cui, Y., Liu, X., Arledge, K. C., Chen, Y. H., Zhang, L., & Wang, X. (2013). Structure of MERS-CoV spike receptor-binding domain complexed with human receptor DPP4. Cell research, 23(8), 986–993.

Wang, P., Casner, R. G., Nair, M. S., Wang, M., Yu, J., Cerutti, G., Liu, L., Kwong, P. D., Huang, Y., Shapiro, L., & Ho, D. D. (2021). Increased Resistance of SARS-CoV-2 Variant P.1 to Antibody Neutralization. bioRxiv : the preprint server for biology, 2021.03.01.433466.

Wang, W., Zhang, W., Zhang, J., He, J., & Zhu, F. (2020e). Distribution of HLA allele frequencies in 82 Chinese individuals with coronavirus disease-2019 (COVID-19). HLA, 96(2), 194–196.

Wang, Y., Liu, Y., Liu, L., Wang, X., Luo, N., & Li, L. (2020a). Clinical outcomes in 55 patients with severe acute respiratory syndrome coronavirus 2 who were asymptomatic at hospital admission in shenzhen, china. The Journal of infectious diseases, 221(11), 1770–1774.

Wax, R. S., & Christian, M. D. (2020). Practical recommendations for critical care and anesthesiology teams caring for novel coronavirus (2019-nCoV) patients. Directives concrètes à l'intention des équipes de soins intensifs et d'anesthésiologie prenant soin de patients atteints du coronavirus 2019-nCoV. Canadian journal of anaesthesia = Journal canadien d'anesthesie, 67(5), 568–576.

Weiss, S. R., & Navas-Martin, S. (2005). Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. Microbiology and molecular biology reviews : MMBR, 69(4), 635–664.

WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group, Sterne, J. A. C., Murthy, S., Diaz, J. V., *et al.* (2020). Association Between Administration of Systemic Corticosteroids and Mortality Among Critically Ill Patients With COVID-19: A Meta-analysis. JAMA, 324(13), 1330–1341.

WHO. (2004). WHO laboratory biosafety manual, third edition. Geneva: World Health Organization; 2004. http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/.

WHO. (2020). Laboratory testing for coronavirus disease (COVID-19) in suspected human cases: interim guidance, 19 March 2020. World Health Organization. https://apps.who.int/iris/handle/10665/331501.

WHO. (2023). WHO Coronavirus (COVID-19) Dashboard. www.covid19.who.int

Wibmer, C. K., Ayres, F., Hermanus, T., Madzivhandila, M., Kgagudi, P., Oosthuysen, B., Lambson, B. E., de Oliveira, T., Vermeulen, M., van der Berg, K., Rossouw, T., Boswell, M., Ueckermann, V., Meiring, S., von Gottberg, A., Cohen, C., Morris, L., Bhiman, J. N., & Moore, P. L. (2021). SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. Nature medicine, 27(4), 622–625.

Widagdo, W., Raj, V. S., Schipper, D., Kolijn, K., van Leenders, G. J. L. H., Bosch, B. J., Bensaid, A., Segalés, J., Baumgärtner, W., Osterhaus, A. D. M. E., Koopmans, M. P., van den Brand, J. M. A., & Haagmans, B. L. (2016). Differential Expression of the Middle East Respiratory Syndrome Coronavirus Receptor in the Upper Respiratory Tracts of Humans and Dromedary Camels. Journal of virology, 90(9), 4838–4842.

Wu, C., Lu, W., Zhang, Y., Zhang, G., Shi, X., Hisada, Y., Grover, S. P., Zhang, X., Li, L., Xiang, B., Shi, J., Li, X. A., Daugherty, A., Smyth, S. S., Kirchhofer, D., Shiroishi, T., Shao, F., Mackman, N., Wei, Y., & Li, Z. (2019). Inflammasome Activation Triggers Blood Clotting and Host Death through Pyroptosis. Immunity, 50(6), 1401–1411.e4.

Wu, K., Werner, A. P., Moliva, J. I., Koch, M., Choi, A., Stewart-Jones, G. B. E., Bennett, H., *et al.* (2021). mRNA-1273 vaccine induces neutralizing antibodies against spike mutants from global SARS-CoV-2 variants. bioRxiv : the preprint server for biology, 2021.01.25.427948.

Xinhuanet Nan-shan ZHONG's and Lan-juan LI's teams. (2020). Isolated the virus from the stools of patients with new coronary pneumonia. http://m.xinhuanet.com/hb/2020-02/13/c_1125570909.htm [Accessed on June. 6, 2023].

Xu, H., Zhong, L., Deng, J., Peng, J., Dan, H., Zeng, X., Li, T., & Chen, Q. (2020a). High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. International journal of oral science, 12(1), 8.

Xu, Z., Shi, L., Wang, Y., Zhang, J., Huang, L., Zhang, C., Liu, S., Zhao, P., Liu, H., Zhu, L., Tai, Y., Bai, C., Gao, T., Song, J., Xia, P., Dong, J., Zhao, J., & Wang, F. S. (2020b). Pathological findings of COVID-19 associated with acute respiratory distress syndrome. The Lancet. Respiratory medicine, 8(4), 420–422.

Yang, H., & Rao, Z. (2021). Structural biology of SARS-CoV-2 and implications for therapeutic development. Nature reviews. Microbiology, 19(11), 685–700.

Yang, J., Zheng, Y., Gou, X., Pu, K., Chen, Z., Guo, Q., Ji, R., Wang, H., Wang, Y., & Zhou, Y. (2020). Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis. International journal of infectious diseases. Official Publication of the International Society for Infectious Diseases, 94, 91–95.

Yang, X., Yu, Y., Xu, J., Shu, H., Xia, J., Liu, H., Wu, Y., Zhang, L., *et al*. (2020). Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. The Lancet. Respiratory medicine, 8(5), 475–481.

Yassi, A., Grant, J. M., Lockhart, K., Barker, S., Sprague, S., Okpani, A. I., Wong, T., Daly, P., Henderson, W., Lubin, S., & Kim Sing, C. (2021). Infection control, occupational and public health measures including mRNA-based vaccination against SARS-CoV-2 infections to protect healthcare workers from variants of concern: A 14-month observational study using surveillance data. PloS one, 16(7), e0254920.

Yu, H. H., Qin, C., Chen, M., Wang, W., & Tian, D. S. (2020). D-dimer level is associated with the severity of COVID-19. Thrombosis research, 195, 219–225.

Yuen, C. K., Lam, J. Y., Wong, W. M., Mak, L. F., Wang, X., Chu, H., Cai, J. P., Jin, D. Y., To, K. K., Chan, J. F., Yuen, K. Y., & Kok, K. H. (2020). SARS-CoV-2 nsp13, nsp14, nsp15 and orf6 function as potent interferon antagonists. Emerging microbes & infections, 9(1), 1418–1428.

Yung, Y. L., Cheng, C. K., Chan, H. Y., Xia, J. T., Lau, K. M., Wong, R. S. M., Wu, A. K. L., Chu, R. W., Wong, A. C. C., *et al*. (2021). Association of HLA-B22 serotype with SARS-CoV-2 susceptibility in Hong Kong Chinese patients. HLA, 97(2), 127–132.

Zaim, S., Chong, J. H., Sankaranarayanan, V., & Harky, A. (2020). COVID-19 and Multiorgan Response. Current problems in cardiology, 45(8), 100618.

Zhang, J. J., Dong, X., Cao, Y. Y., Yuan, Y. D., Yang, Y. B., Yan, Y. Q., Akdis, C. A., & Gao, Y. D. (2020a). Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. Allergy, 75(7), 1730–1741.

Zhang, W., Davis, B. D., Chen, S. S., Sincuir Martinez, J. M., Plummer, J. T., & Vail, E. (2021). Emergence of a Novel SARS-CoV-2 Variant in Southern California. The Journal of the American Medical Association, 325(13), 1324–1326.

Zhang, X., Lu, S., Li, H., Wang, Y., Lu, Z., Liu, Z., Lai, Q., Ji, Y., Huang, X., Li, Y., Sun, J., Wu, Y., Xu, X., & Hou, J. (2020b). Viral and Antibody Kinetics of COVID-19 Patients with Different Disease Severities in Acute and Convalescent Phases: A 6-Month Follow-Up Study. Virologica Sinica, 35(6), 820–829.

Zhao, J., Perera, R. A., Kayali, G., Meyerholz, D., Perlman, S., & Peiris, M. (2015). Passive immunotherapy with dromedary immune serum in an experimental animal model for Middle East respiratory syndrome coronavirus infection. Journal of virology, 89(11), 6117–6120.

Zheng, Q., Duan, T., & Jin, L. (2020). Single-cell RNA expression profiling of ACE2 and AXL in the human maternal-fetal interface. Reproductive and Developmental Medicine, 4(1), 7-10.

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., Xiang, J., Wang, Y., Song, B., Gu, X., Guan, L., Wei, Y., Li, H., Wu, X., Xu, J., Tu, S., Zhang, Y., Chen, H., & Cao, B. (2020b). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet (London, England), 395(10229), 1054–1062.

Zhou, Y., Zeng, Y., Tong, Y., & Chen, C. (2020a). Ophthalmologic evidence against the interpersonal transmission of 2019 novel coronavirus through conjunctiva. medRxiv.

APPENDICES

Appendix (1): Questionnaire sheet sample for patients in this study.

PATIENT QUESTIONNAIRE

General Data

Sample ID: _____ Patient Name: _____

Age: _____ yrs Gender: Male ☐ Female ☐

Residency Site: Urban ☐ Rural ☐

COVID-19 Severity: Mild ☐ Sever ☐ COVID-19 Variant: _____

Recurrent Infection: No ☐ Yes ☐ _____

Vaccination Status: Unvaccinated ☐ 1st Dose ☐ 2nd Dose ☐

3rd Booster Dose ☐

Vaccine Manufacturer: Pfizer ☐ Moderna ☐ AstraZeneca ☐ Sinopharm ☐

Medications: Antibiotic Treatments: No ☐ Yes ☐ _____

Anti-viral Treatments: No ☐ Yes ☐ _____

Immune Modulators (Biologics Drugs): No ☐ Yes ☐ _____

Chronic Diseases: No ☐ Yes ☐ _____

Date of Infection: _____ Date of Sampling: _____ Period: _____ days

Laboratory Tests

RBS: _____ mg/dL HbA1c: _____ % CRP: _____ mg/L

S. Ferritin: _____ ng/L D-dimer: _____ ng/ml IL-6: _____ pg/ml

CBC: Hb: _____ g/dL WBCs: _____ $*10^6$ /L Neutrophils: _____%

Lymphocytes: _____ % Monocytes: _____%

X _____

Signature

Appendix (2): Questionnaire sheet sample for healthy control individuals in this study.

CONTROL QUESTIONNAIRE

General Data

Sample ID: _____ Patient Name: _____

Age: _____ yrs Gender: Male ☐ Female ☐

Residency Site: Urban ☐ Rural ☐

Vaccination Status: Unvaccinated ☐ 1st Dose ☐ 2nd Dose ☐

3rd Booster Dose ☐

Vaccine Manufacturer: Pfizer ☐ Moderna ☐ AstraZeneca ☐ Sinopharm ☐

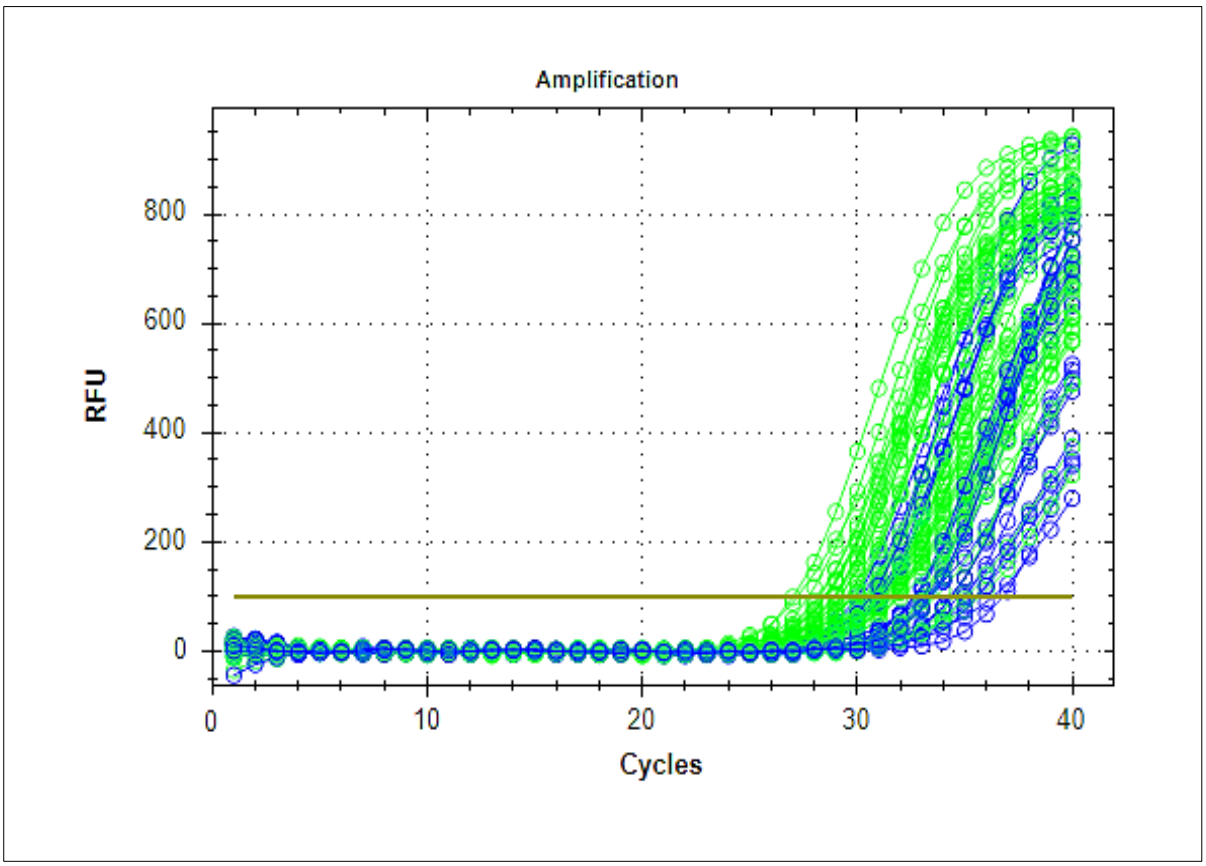
Chronic Diseases: No ☐ Yes ☐ _____

Date of Sampling: _____

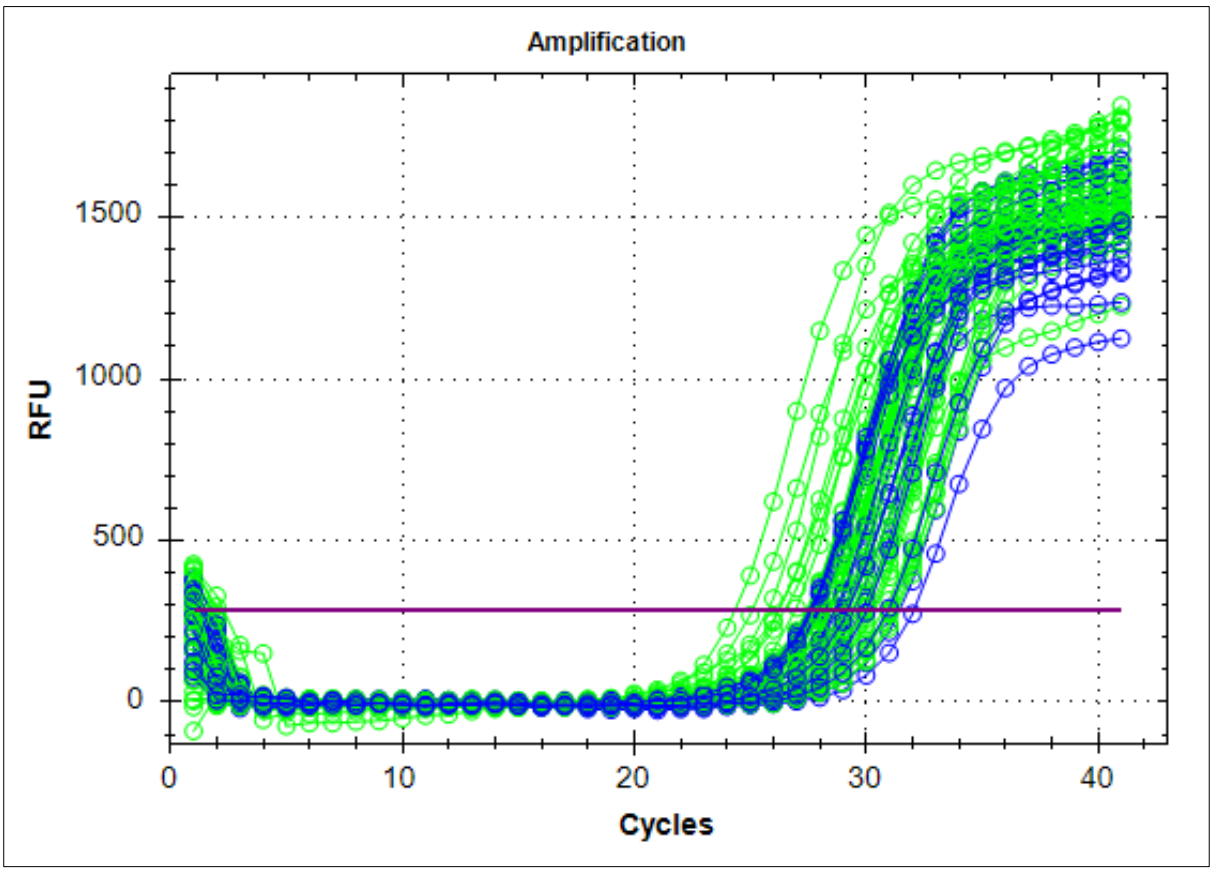
X _____

Signature

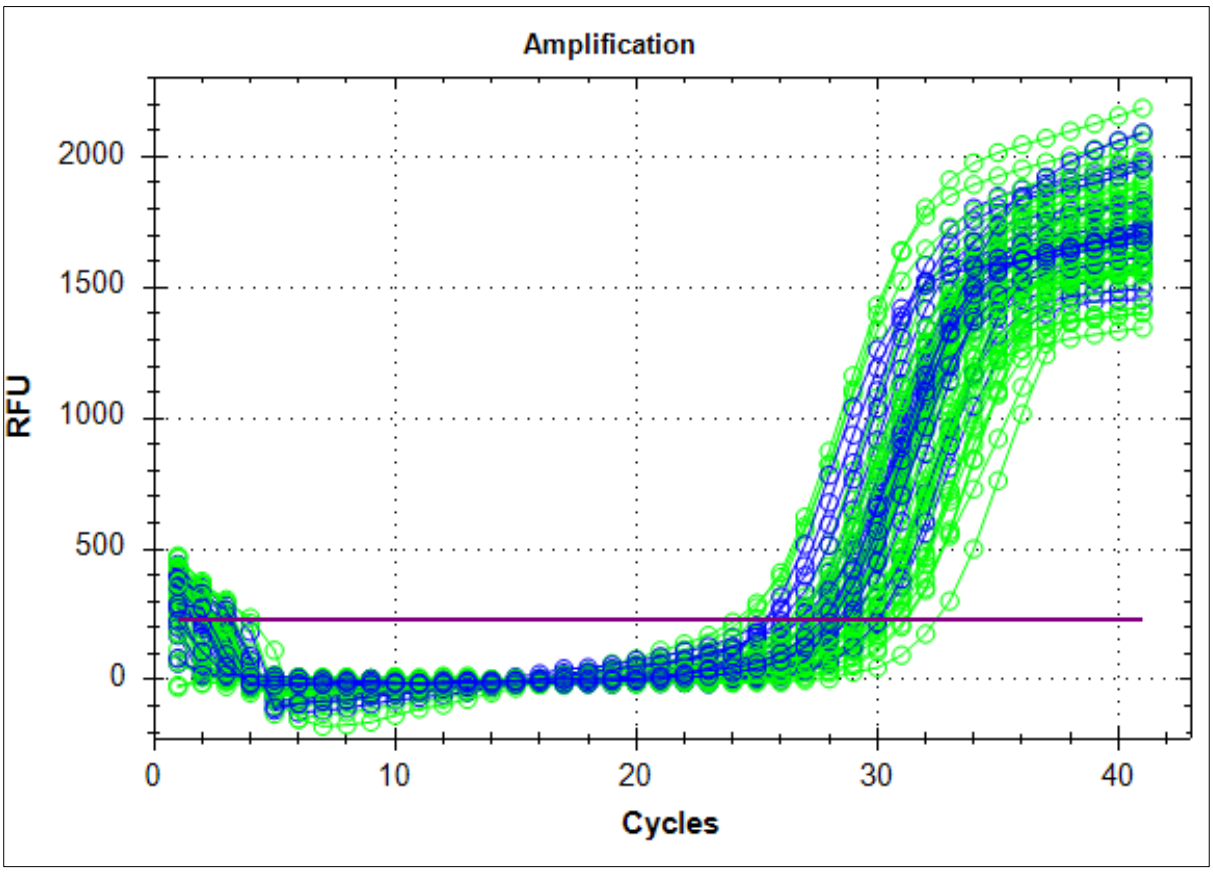
Appendix (3): Showed the Real Time amplification plots of miR-423 gene expression in COVID-19 patients (Green plots) and healthy control (Blue plots).



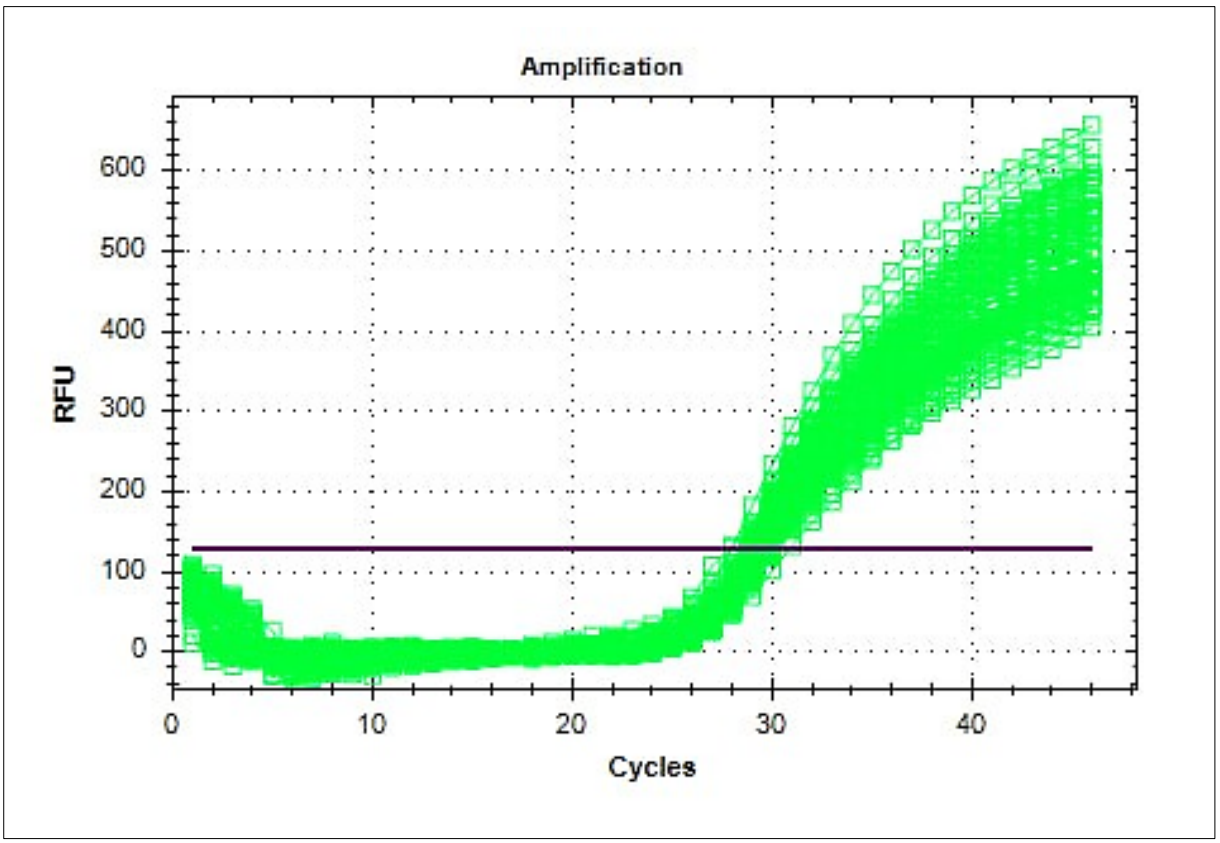
Appendix (4): Showed the Real Time amplification plots of miR-195 gene expression in COVID-19 patients (Green plots) and healthy control (Blue plots).



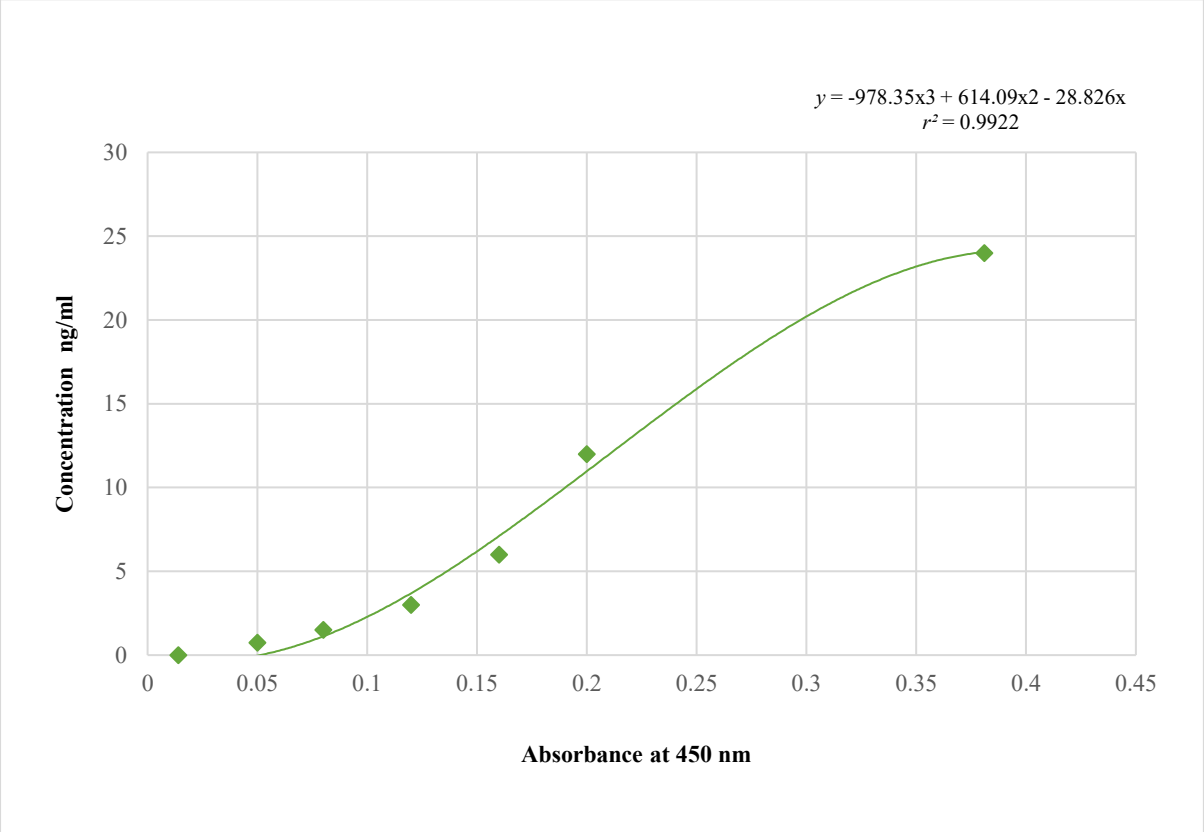
Appendix (5): Showed the Real Time amplification plots of miR-23a gene expression in COVID-19 patients (Green plots) and healthy control (Blue plots).



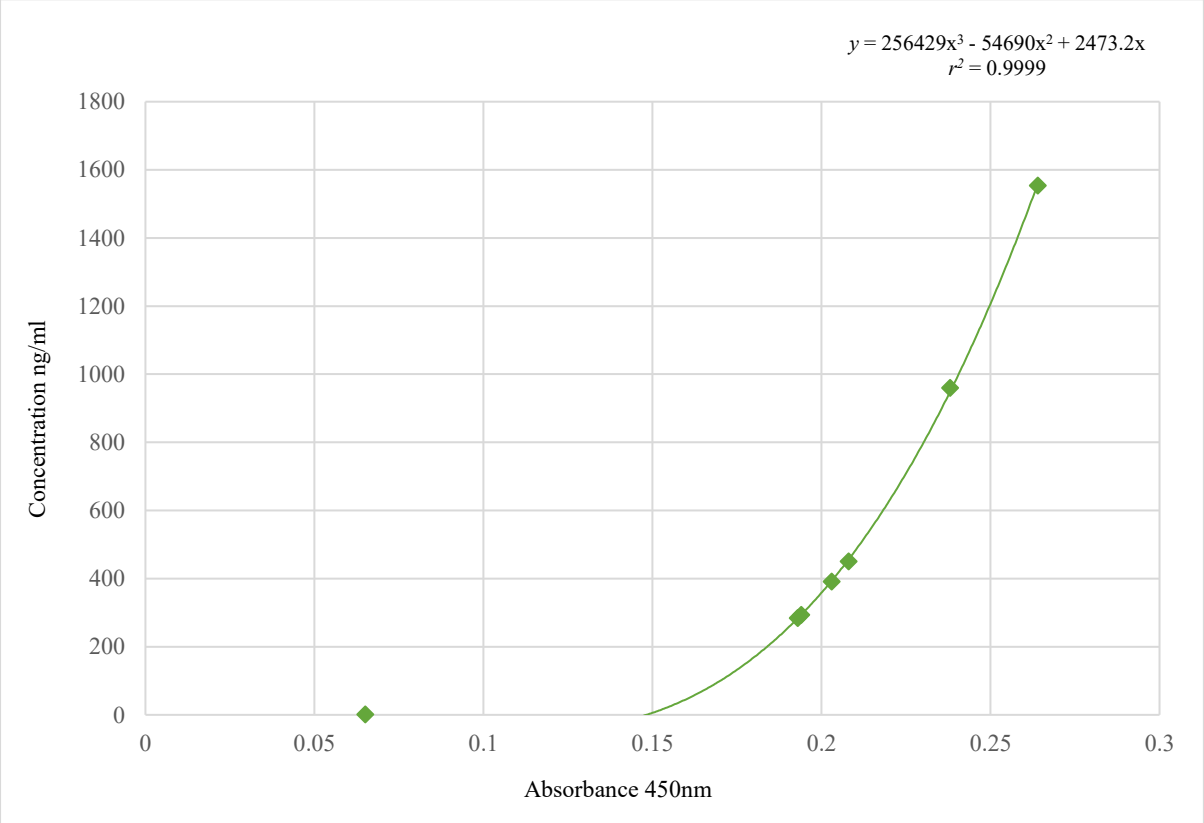
Appendix (6): Real Time PCR amplification plots for housekeeping (*GAPDH*) gene.



Appendix (7): ACE2 Standard Curve that showed the Concentration (ng/ml) and optical density OD: 450 nm.



Appendix (8): DPP4 Standard Curve that showed the Concentration (ng/ml) and optical density OD: 450 nm.



الخلاصة

يعرف مرض فيروس كورونا ٢٠١٩ (كوفيد-١٩) بأنه مرض فيروسي شديد العدوى يسببه فيروس المتلازمة التنفسية الحادة الوخيمة فيروس كورونا ٢، لا يزال الباحثون يتعلمون عن دور العلامات الجينية في التنبؤ بهذا المرض، لكن العديد من الدراسات حددت الارتباطات بين متغيرات جينية محددة وزيادة خطر الإصابة بمرض كورونا ٢٠١٩ الشديد. وتهدف هذه الدراسة إلى تقديم لمحة عامة عن العلاقة المحتملة بين كوفيد-١٩ ومجموهة من العوامل البيولوجية بما في ذلك الحمض النووي الريبوزي الميكروي، والإنزيم المحول للأنجيوتنسين 2، ثنائي ببتيديل ببتيداز ٤، ومعرفة علاقة تعدد الاشكال لمثبط ثنائي ببتيديل ببتيداز ٤ والتنميط الجيني للأليلات مستضد الكريات البيض البشرية مع قابلية الإصابة وشدة الإصابة بعدوى فيروس كورونا ومستويات مثبط ثنائي ببتيديل ببتيداز ٤ بين المرضى في محافظة بابل.

كشف التوزيع التكراري للمرضى المصابين بمرض كوفيد-١٩ بحسب الفئة العمرية أن المرض يظهر بشكل متكرر في الفئة العمرية من ٤٠ إلى ٤٩ عامًا (٢٤ ٪) ويكون أقل شيوعاً في الفئة العمرية بين ٣٠ إلى ٣٩عاماً (٤ ٪). ولوحظ ان المرضى الذكور هم اكثر تكراراً في المجموعة الشديدة المرض مقارنة بالمجموعة الخفيفة المرض ٧٢٪ مقابل ٤٨٪ على التوالي. لم يكن هناك اختلاف كبير في توزيع تكرار المشاركين وفقاً لحالات الاصابة الشديدة والحالات الخفيفة. فيما يتعلق بالجنس، لم يكن هناك اختلاف كبير في التوزيع التكراري بين مجموعة المرضى ومجموعة الأصحاء. حيث كانت مجموعة المرضى ٦٠ (٦٠٪) من الذكور و٤٠ (٤٠٪) من الإناث، مما يجعل نسبة الذكور إلى الإناث ١:١،٥.

فيما يتعلق بمكان السكن، تم تصنيف المرضى المصابين بمرض كوفيد-١٩ إلى ٧٢ (٧٢ ٪) من سكان المدينة و ٢٨ (٢٨٪) من المناطق الريفية. وتضمنت مجموعة الأصحاء ٣٦ (٧٢ ٪) من المناطق سكان المدينة و ١٤ (٢٨ ٪) من المناطق الريفية. لذلك لم يكن هناك فرق كبير بين مجموعة المرضى ومجموعة الأصحاء في توزيع تكرار الافراد المتضمنين في الدراسة على أساس مكان السكن.

تم ملاحظة ارتفاع ضغط الدم فقط في مجموعة المرضى ٣٤ ٪ وبالتالي، كان الفرق كبيراً إضافة إلى ذلك، شوهد الإصابة بداء السكري فقط في مجموعة المرضى بالمقارنة مع مجموعة الأصحاء ٨ ٪ مقابل ٠ ٪ على التوالي ومع ذلك، كان الفرق غير معنوي إحصائياً.

كانت نسبة الملقحين في مجموعة المرضى ٤ ٪، في حين كانت نسبة الملقحين في مجموعة الأصحاء ٥٢ ٪ وبالتالي كان الفرق معنوي إحصائياً.

أظهرت مقارنة خصائص الكيميائية والمصلية والدموية للمرضى المصابين بكوفيد-١٩ حسب شدة المرض أن متوسط نسبة السكر التراكم ومستوى السكر العشوائي في الدم ومستوى D-dimer في المصل والبروتين التفاعلي C في المصل وعدد كرات الدم البيضاء كان أعلى بشكل ملحوظ في المجموعة الشديدة الإصابة مقارنة بالمجموعة الخفيفة الإصابة. بينما كانت نسبة الخلايا الليمفاوية أقل بشكل ملحوظ في المجموعة الشديدة الإصابة مقارنة بالمجموعة الخفيفة الاصابة.

كانت مقارنة مستوى تعبير الحمض الريبوزي النووي الميكروبي miR-423 و miR-195 بين المرضى والأصحاء أعلى بكثير في المرضى الذين يعانون من كوفيد-١٩ مقارنة بمجموعة الاصحاء، في حين كان مستوى الحمض الريبوزي النووي الميكروبي miR-23a أقل بشكل ملحوظ في المرضى الذين يعانون من كوفيد-١٩ مقارنة بمجموعة الأصحاء. ولم يكن هناك فرق كبير في مستويات miR-423 وmiR-195 وmiR-23a بين المجموعة الخفيفة الإصابة والمجموعة الشديدة الإصابة.

أظهرت مقارنة الأنماط الجينية لمثبط ثنائي ببتيديل ببتيداز ٤ (*rs3788979* A/G) بين مجموعة المرضى ومجموعة الأصحاء أن معدل النمط الجيني AA تم اعتباره مرجعاً لأنه كان النمط الجيني الأكثر شيوعاً في جميع الحالات وكذلك في مجموعة الأصحاء. لوحظ ان النمط الجيني AG في أغلب الأحيان في مجموعة المرضى مقارنة بمجموعة الأصحاء، ٥٢٪ مقابل ٢٨٪ على التوالي، وكان الفرق كبيراً؛ وكانت نسبة الأرجحية ٢،٧٩ وبالتالي يمكن اعتبار هذا النمط الجيني كعامل خطر. بينما كان النمط الجيني GG أقل ظهوراً في مجموعة المرضى مقارنةً بمجموعة الأصحاء ٠٪ مقابل ٢٠٪ على التوالي، وكان الفرق كبيراً؛ ونسبة الأرجحية 0.03، وبالتالي يمكن اعتبار هذا النمط الجيني كعامل وقائي. وكان مستوى مثبط ثنائي ببتيديل ببتيداز-٤ أقل بشكل ملحوظ في المرضى الذين يعانون من كوفيد-١٩ مقارنة بمجموعة الاصحاء.

كشفت هذه الدراسة ان توزيع مستضد الكريات البيض البشرية *HLA-B*07:03* و*DRB1*12:02* له ارتبطاً بشكل كبير بكوفيد-١٩ ويمكن اعتبارهما من عوامل الخطر. ولم يرتبط *HLA-B*46:01* و*HLA-DRB1*03:01* بشكل كبير بالمرض. كان معدل تكرار *HLA-B*07:03* أقل بشكل ملحوظ في المجموعة الشديدة الإصابة مقارنة الإصابة بالمجموعة الخفيفة الإصابة. كان التعبير عن *HLA-B*46:01* أكثر بشكل ملحوظ في المجموعة الشديدة الإصابة مقارنة بالمجموعة الخفيفة الإصابة. لم يكن هناك اختلاف كبير في معدلات التعبير الجيني *HLA-DRB1*03:01* و

*DRB1*12:02* بين المجموعة الشديدة الإصابة والمجموعة الخفيفة الإصابة. وأخيراً كان مستوى ACE2 في المصل أعلى بشكل ملحوظ في المرضى المصابين بمرض كورونا ٢٠١٩ مقارنة بالمجموعة الضابطة.

في الختام، يمكن استخدام هذه العوامل الجينية لتطوير الاختبارات الجينية لتحديد الأشخاص المعرضين لخطر كبير للإصابة بمرض كورونا ٢٠١٩ ويمكن استخدام هذه المعلومات لمساعدة الأفراد على اتخاذ قرارات مستنيرة بشأن نشاطهم وخيارات نمط حياتهم، ولتطوير علاجات جديدة لمرض كورونا ٢٠١٩.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل
كلية الطب
فرع الأحياء المجهرية

دور بعض العوامل الوراثية في توقع الإصابة بمرض كورونا ٢٠١٩

أطروحة
مقدمة الى مجلس كلية الطب / جامعة بابل
وهي جزء من متطلبات نيل درجة دكتوراه فلسفة في العلوم/ الاحياء المجهرية الطبية

من قبل

علي طالب عبد الحمزة محمد

بكالوريوس علوم الحياة (٢٠١٥) / كلية العلوم / جامعة بابل

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الأستاذ المساعد
د. محمد حسن الحمداني

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