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Faculty of Graduate Studies and Research كلية الدراسات العليا والأبحاث

Master Program in Clinical Laboratory Sciences

Investigation of Hematological and Biochemical Markers in COVID-19 Patients in West Bank, Palestine

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This thesis was submitted in partial fulfillment of the requirement for the Master Degree in Clinical Laboratory Sciences from the Faculty of Graduate Studies and Research at Birzeit University.

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Dedication

To Allah

To my husband

To my children

To my directors

To all my friends

For their support

Kafa Ratib Adeeb Rimawi

Declaration

I certify that this thesis submitted for the degree of Master in Clinical Laboratory Sciences, is the result of my own research, except where otherwise acknowledged, and that this study has not been submitted for higher degree to any other university or institution.

Signed

kafa rimawi

Kafa Ratib Adeeb Rimawi

Date:

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List of abbreviations

ACE2	Angiotensin converting enzyme II receptor
ABO	Blood group
ALAT	Alanine transaminase
ALP	An alkaline phosphatase
ANOVA	Analysis of variance
APTT	Activated Partial Thromboplastin Time
ARDS	Acute respiratory distress syndrome
ASAT	Aspartate aminotransfere
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CBC	Complete Blood Count
CCDC	Chinese Center for Disease Control and Prevention
CDC	Center for Disease Control and Prevention
CK	Creatine kinase
CK-MB	Creatine kinase-MB
CMs	Convoluted membranes
COV	Coronavirus
COVID-19	Coronavirus disease-2019
CRP	C-reactive protein
CT scan	A computerized tomography (CT) scan
CXCL-10	C-X-C motif chemokine ligand 10
DMSs	Double-membrane spherules
DMVs	Double-membrane vesicles
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
eGFR	Estimated glomerular filtration rate
ESR	Erythrocyte sedimentation rate
GR/Ly	Granulocytes Lymphocytes ratio
GR/Mon	Granulocytes monocyte ratio
HAV	Hepatitis A virus
HBV	Hepatitis B virus
Hb	Hemoglobin
Hct	Hematocrit
Hel	Helicase
IFN	Interferons
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
INR	International normalized ratio
LDH	Lactate dehydrogenase
LY/Mon	Lymphocytes monocyte ratio
MCH	Mean cell hemoglobin

MCHC	Mean cell hemoglobin concentration
MCV	Mean cell volume
MDW	Monocyte distribution width
MIP-1	monocyte chemoattractant protein-1
MIP-1	macrophage inflammatory protein-1
mRNA	Messenger ribonucleic acid
NCIP	Novel Coronavirus-Infected pneumonia
NP	Nucleocapsid protein
NPV	Negative predictive value
nsp	Non-structural proteins
ORFs	Open reading frames
PCR	Polymerase Chain Reaction
PCT	Procalcitonin
PLT	Platelet count
PT	Prothrombin Time
PTH	Parathyroid hormone
RBCs	Red blood cell
RBD	Receptor binding domain
RdRp	RNA-dependent RNA polymerase
RDW	Red cell distribution width
RTC	Transcription complexes
RT-PCR	Reverse transcription polymerase chain reaction
SARS	Severe acute respiratory syndrome
SSRNA	Single-Strand RNA
TG	Triglyceride
TMPRSS2	Transmembrane cellular protease
TNF-α	Tumor necrosis factor-alpha
TSH	Thyroid stimulating hormone
UK	United Kingdom
USA	United States of America
VOCs	Variants of concern
VOIs	Variants of interest
WBCs	White Blood Cells
WHO	World Health Organization

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Investigation of Hematological and Biochemical Markers in COVID-19 Patients in West Bank, Palestine

Prepared by: Kafa Ratib Adeeb Rimawi

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Abstract

Background: Recently COVID-19 disease overwhelmed even the most efficient health care systems and generated millions of deaths worldwide. The disease causes several abnormalities in serum biomarkers, thus a simple and quick stratification method for identification of disease severity are sought and should aid in prompt patient management.

Objectives: To investigate the association of anthropometric measures, comorbidities as well as hematological and biochemical markers with COVID-19 disease severity in Palestine.

Methods: This is a prospective cross-sectional study that included 400 adult COVID-19 patients grouped based on disease severity into mild/moderate (n=200), severe (n=109) and critical (n=91). Severe group required hospitalization and critical group required intensive care. A structured questionnaire was used to collect anthropometric, disease symptoms and general health data at time of enrollment and 3 weeks later. Complete blood count, coagulation and biochemical markers, Parathyroid (PTH) and Thyroid stimulating (TSH) hormones and serum IgG levels were measured.

Results: All severe patients recovered from the infection while all critical patients died except for three patients. Statistically significant association was observed between disease severity and older age, female sex, high BMI, presence of chronic diseases (diabetes, hypertension, lung and heart diseases) and Delta variant. An inverse association between disease severity and allergy/asthma was observed while no association with smoking was observed. Significant proportions of severe and critical patients were still suffering from some disease symptoms three weeks after disease inset compared to mild/moderate. A significant decrease in Hemoglobin, platelets, lymphocytes, lymphocyte/monocyte ratio, and increase in granulocytes, prothrombin time and D-dimer. No significant association between ABO or Rh (D) type and disease

severity was observed. Our findings showed a significant association between biochemical markers and disease severity including elevated levels of CRP, ferritin, IgG, PTH and decreased TSH.

Conclusion: We provide a comprehensive analysis of patient characteristics and laboratory markers of COVID-19 patients grouped based on disease severity. Older age, overweight/obesity, presence of comorbidities and abnormal hematological and biochemical markers were associated with diseases severity and they provide a simple and rapid method to triage patients with severe disease and who needs hospitalization or intensive care.

Chapter one

Introduction

1. Introduction

1.1 Background

COVID-19 disease is an emergent – infectious - respiratory disease caused by SARS-CoV2 virus and it was first recognized in November 2019 in Wuhan city, China. The disease spread rapidly worldwide and on March 11th, 2020, the WHO declared COVID-19 as a pandemic with confirmed cases in 114 countries (Wu and McGoogan, 2020). Thereafter, the disease spread rapidly and reached almost every corner of the world. According to records released by WHO, as of March 21st, 2023, the number of laboratory-confirmed cases reached more than 761 million and that of the death cases more than 6.8 million (WHO, 2023). The lockdown enforced by this pandemic in most countries as well as the restriction of movement worldwide failed to control the spread of the disease. The disease reached a peak by the end of 2021 and beginning of 2022 –in terms of the number of daily-infected cases, followed by a second peak in December 2022 and thereafter showed a sharp decline (WHO, 2023). By the end of 2020 and beginning of 2021, a number of COVID-19 vaccines became available. As of March 13th, 2023, more than 13.2 billion doses of vaccine have been administered worldwide (WHO, 2023). Population immunity resulting from natural infection and vaccination greatly reduced the risk of medically significant disease, hospitalization or death due to CIVID-19 disease for most people (CDC, 2023a).

SARS-CoV2 belongs to coronaviruses that are widely spread pathogens of humans and animals. They were firstly isolated in 1937 as the causative agents for bronchitis in birds (Ravi et al., 2022). Since, then seven coronaviruses were isolated and determined as causative agent for respiratory diseases. SARS-CoV2 (identified in December 2019). SARS-CoV2 virus was identified in Wuhan city, China in December 2019 as the causative agent for first cases of COVID-19 (Ochani et al., 2021). Other coronaviruses namely SARS-CoV (causative agent of SARS) and MERS-CoV (causative agent for MERS) were responsible for limited outbreaks in 2003 and 2014, respectively (Ravi et al., 2022). The fatality rate associated with SARS-CoV (9.6%) and MERS-CoV (34.4%) are higher than that associated with SARS-CoV2 (3.4%). But SARS-CoV2 is more contagious and thus enabled a rapid spread of the disease (Ochani et al., 2021).

RNA viruses including SARS-CoV2 usually have relatively high mutation rates compared to DNA viruses, and these mutation rates are correlated with enhanced virulence and evolution of these viruses (Duffy, 2018). Shortly after its recognition in December 2019, several SARS-CoV2 variants have been recognized and some of them showed high risk for global public health (Ravi et al., 2022). Virus variants have genetic markers that affect their transmission, virulence and their ability to evade the immune system or reduced response to therapeutics (Ravi et al., 2022).

SARS-CoV2 virus is transmitted via respiratory aerosols that binds to the nasal epithelial cells in the upper respiratory tract (Kutti-Sridharan et al., 2020). Hoffman et al. (2020) have shown that SARS-CoV2 virus uses the SARS-CoV receptor ACE2 for entry to host cells and serine protease TMPRSS2 for priming the viral S protein. ACE is highly expressed in the nasal epithelial cells and this enables the virus to establish infection of nasal epithelial cells. The host cell serine protease (Hoffman et al., 2020; Parasher, 2021, Cascella et al., 2023). Here, the virus undergoes replication and proliferation and virus progeny infects ciliated cells in conducting airways. Host immune response at this stage is limited and the viral load is low too. Despite this, the person is highly infectious and the virus can be detected via nasal swabs testing (Parasher, 2021). From nasal epithelial cells, the virus migrates to cells of upper respiratory tract and at this stage there is a greater immune response that involve releasing CXCL-10 and interferons (INF-β, INF-γ). The immune response mounted at this stage is sufficient to contain the virus in the majority of patients (Parasher, 2021).

However, in about one-fifth of all patients the virus invades type 2 alveolar epithelial cells. Infected pneumocytes initiates the so called ‘cytokine storm’ that involves the release of an array of cytokines and inflammatory markers including interleukins (IL-1, -6, -8, -120 and -12), tumor necrosis factor-alpha (TNF-α), interferons (IFN-β and -γ), CXCL-10, monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1 (MIP-1) (Parasher, 2021, Tang et al., 2005). Products of cytokine storm function as a chemoattractant for neutrophils, CD4 helper T cells, CD8 cytotoxic T cells, that accumulate in lung tissues and combat the invading virus. The accumulation of these cells contribute to inflammation and injury of the lung tissues and eventually leads to acute respiratory distress syndrome (Parasher, 2021, Xu et al., 2020). A common feature observed in patients

with severe COVID-19 diseases is eosinopenia and lymphopenia with a severe reduction in CD34+ and CD8+ T cells, B cells and NK cells (Anka et al., 2021).

In severe cases, extensive activation of coagulation and consumption of clotting factors occur. The inflamed lung tissues and pulmonary endothelial cells may contribute to microthrombi formation and account to the high incidence of thrombotic complications, like deep vein thrombosis, pulmonary embolism, and thrombotic arterial complications in critically ill patients. In fulminant disease, viral sepsis develops, defined as life threatening organ dysfunction caused by a dysregulated host response to infection, and may contribute to multiorgan failure (Wiersinga et al., 2020).

Symptoms of COVID-19 disease appear after an average incubation period of 5.2 days, ranging from 2 to 11 days. Common COVID-19 symptoms such as fever, dry cough, dyspnea, and bilateral ground-glass opacities on chest CT scans are similar to infections caused by earlier betacoronaviruses (SARS-CoV and MERS-CoV) (Adhikari et al., 2020; (Rothan and Byrareddy, 2020). Patients with mild symptoms 4and as of March 22nd, 2023, the total confirmed cases of COVID-19 were 703,228 and death cases were 5708. These data show that the case fatality rate in Palestine (0.811%) (COVID-19 in Palestine, 2023) is similar to CFR reported worldwide by the WHO (0.904%) (WHO, 2023). As of march 22nd, 2023 a total of 3.75 million doses of COVID-19 vaccine have been administered and 1.77 million individuals (51.4% of the target individuals) have received 2 doses and 9.76% of individuals received three doses of the vaccine (COVID-19 in Palestine, 2023). Despite these figures, research data and background information concerning COVID-19 disease are lacking. So studies that tackles COVID-19 disease are very much needed.

Worldwide, new cases of COVID-19 are still being reported daily. In addition, recent studies predict that COVID-19 to become a seasonal disease in temperate countries (D'Amico et al., 2022). Thus, understanding the clinical and laboratory predictors of disease severity are very much needed. Such predictors will allow risk stratification, provide guidance for interventional studies to target patients at enhanced risk of developing severe disease and aids in combating this ongoing pandemic. In particular, the identification of hematological and biochemical markers capable of differentiation between severe and non-severe cases, will enable the management of COVID-19 disease.

1.2 Study questions

Is there an association between COVID-19 disease severity and patient characteristics such as chronic diseases and anthropometric data?

Is there an association between COVID-19 disease severity and hematological markers?

Is there an association between COVID-19 disease severity and biochemical markers?

1.3 Study justifications

COVID-19 pandemic is ongoing since its declaration in March 2020 and recent studies predicted that COVID-19 will become a seasonal disease in temperate countries (D'Amico et al., 2022). The high morbidity and mortality and high economic burden, associated with COVID-19 disease makes this disease an important public health burden. Thus the identification of hematological and biochemical markers are important discriminating severe from non-severe cases as well as the risk of mortality will enable a better management and treatment of patients. In addition, understanding viral pathogenesis will aid in the prevention of serious complications and death of affected patients.

1.4 Study aim and objectives

The main objective of this study is to investigate the association of COVID-19 disease severity with hematological and biochemical markers.

The specific objectives are:

1. Investigate the association between comorbidities (chronic diseases) and COVID-19 disease severity.
2. Investigate the association between anthropometric data and COVID-19 disease severity.
3. Investigate the association between hematological markers and COVID-19 disease severity.
4. Investigate the association between biochemical markers and COVID-19 disease severity.
5. Investigate the association between SARS-CoV2 variant and COVID-19 disease severity.
6. Investigate the association between ABO blood group and COVID-19 disease severity.

1.5 Study hypothesis

1. There is a significant association between anthropometric data of patients and severity of COVID-19 disease.
2. There is a significant association between comorbidities (chronic diseases) and severity of COVID-19 disease.
3. There is a significant association between hematological markers (RBCs, Hb, RBC indices, WBCs, Plt, Pt, APTT and D-dimer) and severity of COVID-19 disease.
4. There is a significant association between biochemical markers (serum proteins, CRP, ferritin, LDH, FBS, calcium, AST, ALT, CK, ALP, amylase, creatinine, cholesterol, TG, TSH, PTH and anti-SARS-CoV2 antibodies) and severity of COVID-19 disease.
5. There is a significant association between ABO blood group and RhD, and severity of COVID-19 disease.
6. There is a significant association between SARS-CoV2 variant and severity of COVID-19 disease.

Chapter Two

Literature Review

2.1 Biology of SARS-CoV2 virus

Coronaviruses are widely spread pathogens of humans and animals. They were firstly isolated in 1937 as the causative agents for bronchitis in birds (Ravi et al., 2022). Since, then seven coronaviruses were isolated and determined as causative agents for respiratory diseases. From these viruses, HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1 are known to be less pathogenic than SARS-CoV (identified in 2003), MERS-CoV (identified in 2012) and SARS-CoV2 (identified in December 2019). SARS-CoV2 virus was identified in Wuhan city, China in December 2019 as the causative agent for first cases of COVID-19 (Ochani et al., 2021). The chronological discovery 760 of different coronaviruses and related pandemics are illustrated in Figure 2.1 (V'Kovski et al., 2021).

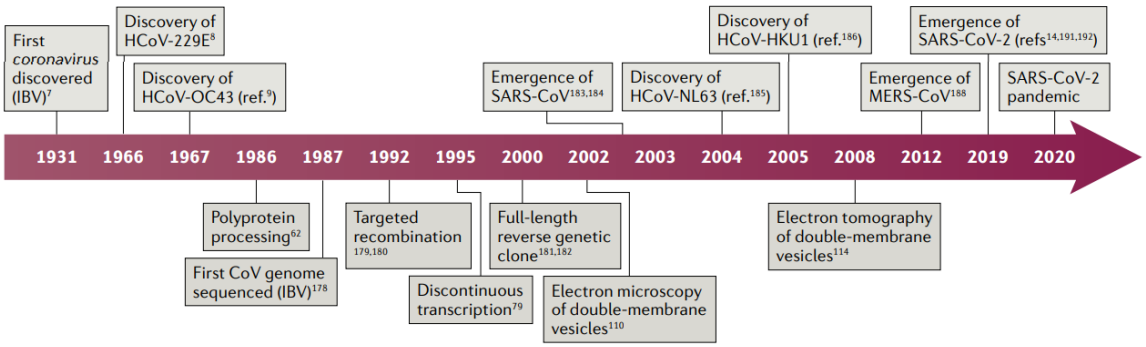

Figure 2.1: Milestones in the discovery of coronaviruses and related pandemics. Adapted from (V'Koski et al., 2021).

2.2 Emergence of coronavirus events:

Corona viruses (CoV) are zoonotic pathogens and emergent species of the genus Betacoronavirinae are responsible for three epidemics (SARS, MERS and COVID-19) in humans in the last two decades (Figure 2.1). Those viruses are thought to be originated from bats and transmitted to humans through an intermediate animal host, thus emphasizing the likelihood that the spill over was from animals to humans (Ravi et al., 2022).

2.3 Classification of SARS-CoV2 virus:

SARS-CoV2 virus is a positive sense RNA virus and it belongs to order Nidovirales, family Coronaviridae, subfamily Orthocoronavirinae, genus Betcoronavirinae and subgenus Sarbecovirus (Figure 2.2). Genetic diversity and emergence of new species is attributed to genetic recombination of same or different genera (Ravi et al., 2022). The Orthocoronavirinae subfamily is subdivided into four genera based on serology: alpha-, beta-, gamma- and delta-CoV. Alpha- and Beta-CoV infect mammals, gamma-CoV infects avian species and delta-CoV can infect mammals and avian species (Orchani et al., 2021). The three most pathogenic CoVs and that caused the recent three pandemics (SARS-CoV, MERS-CoV, SARS-CoV2) belong the same genus, Beta-CoV (Ravi et al., 2022).

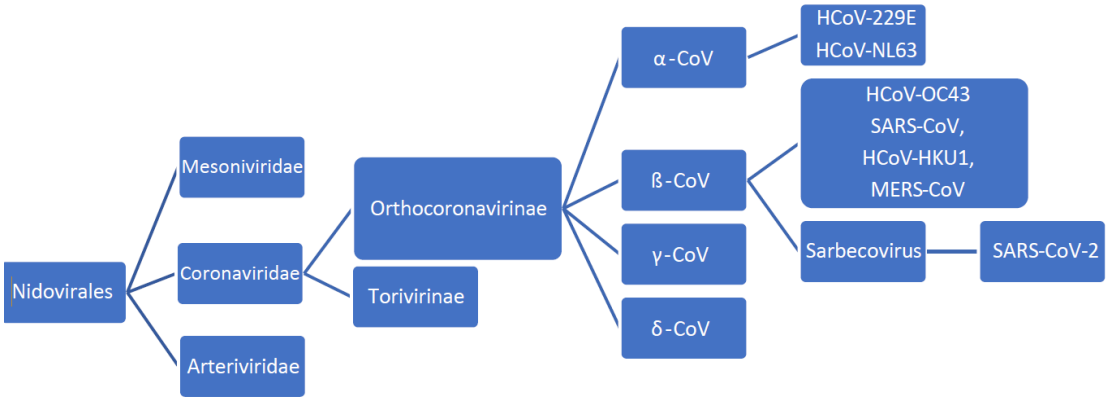

Figure 2.2: Classification of SARS-CoV2 virus and other coronaviruses. Adapted from (Ravi et al. 2022).

2.4 Genomic organization of SARS-CoV2 virus

SARS-CoV2 virus has a positive-sense single-stranded RNA genome (approximately 29.9 kbp) that contains six open reading frames (ORFs) (Figure 2.3) (Ravi et al., 2022). The first in terms of transcription (5') is ORF1a/b, which makes up approximately two- thirds of the length of the viral genome, and produces non-structural proteins (nsp) polyproteins 1a and 1b (pp1a, pp1b). The pp1a refers to NSP1 to 11, and pp1b refers to NSP12 to 16. These proteins (nsp) are cleaved by the viral 3C-like protease and papain-like protease to make viral RNA-dependent RNA polymerase (RdRp) and helicase (Hel), which guide viral genome replication, transcription, and translation (Gitman et al., 2021; Ravi et al., 2022). Fifteen of the nsps are processed individually to nsps that form the replication and

transcription complexes (RTC) of the virus that compose amongst others, RNA-processing and RNA-modifying enzymes as well as an RNA proofreading function necessary for maintaining the integrity of the large (>30Kb) viral genome. Viral ORFs that code structural proteins and interspersed ORFs that code accessory proteins are transcribed from the 3′ end of the one-third of the viral genome to form a nested set of subgenomic mRNAs (sg mRNAs) (V'Kovski et al., 2021).

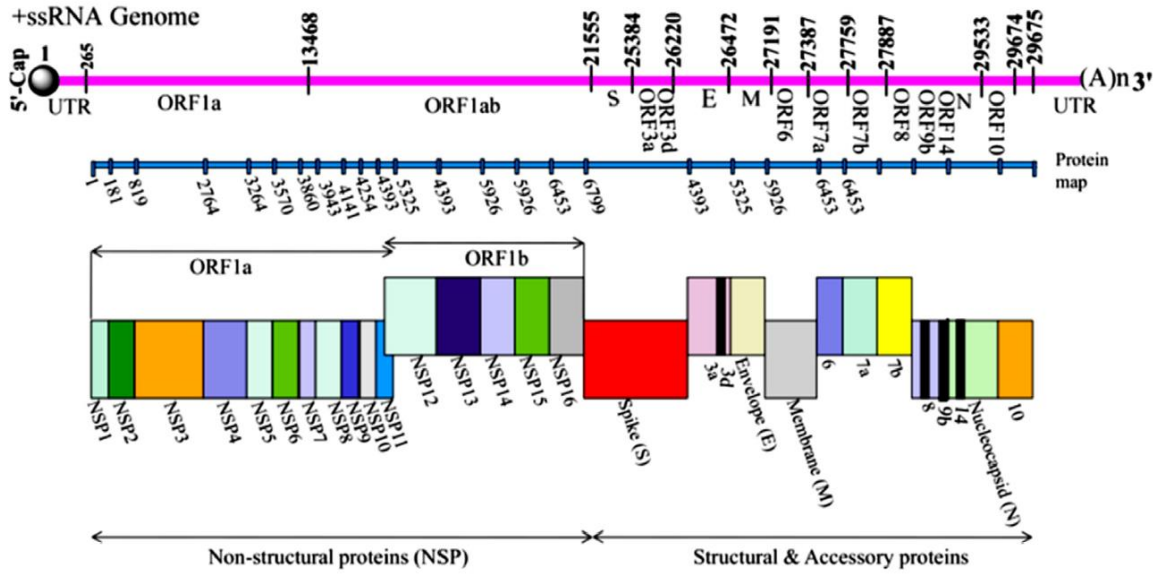

Figure 2.3: Genome structure and phylogenetics of SARS-CoV-2 virus. Genome structure of SARS-CoV-2 (upper part of the diagram); a sketch diagram of the E, S, M, and N proteins of SARS-CoV-2 (lower part of diagram). Adapted from (Ravi et al., 2022).

The other ORFs, designated S, E, M and N (Figure 3) are located at the 3'end and encode the following structural proteins: Envelop glycoprotein spike S (recognizes host cell receptors), Membrane (M) proteins (shapes the virions and envelop), E proteins (virion assembly and release) and Nucleocapsid (N) proteins for packaging of the RNA genome and pathogenicity of the virus as Interferon (IFN) inhibitor (Ravi et al., 2022; Tafanidou and Gkintzi, 2022). Additionally, there are nine species-specific ORFs (ORF3a, ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF14 and ORF10) located near the 3'end and encode accessory proteins (Figures 2.3 and 2.4) (Gitman et al., 2021; Ravi et al., 2022). Coronavirus accessory proteins are highly variable sets of virus-specific proteins that display limited conservation even within individual species but they are principally thought to contribute to modulating host responses to infection and are determinants of viral pathogenicity. Nevertheless, the molecular functions of many accessory proteins remain

largely unknown owing to the lack of homologies to accessory proteins of other coronaviruses or to other known proteins (V'Kovski et al., 2021).

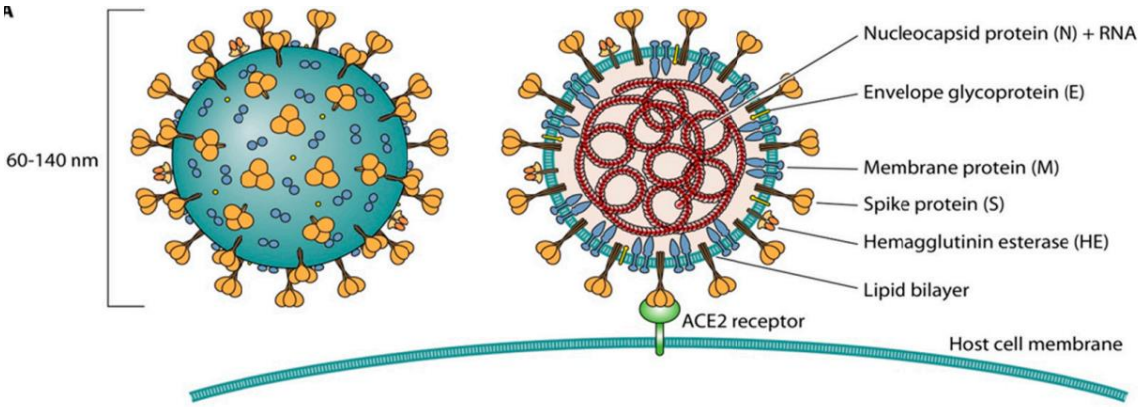

Figure 2.4: Structure of SARS-CoV2 virus. Adapted from (Gitman et al., 2021).

2.5 Life cycle of SARS-CoV2 virus

SAS-CoV2 uses Angiotensin converting enzyme II receptor (ACE2) as a cellular entry receptor. ACE2 is expressed on the epithelial cells of the alveoli, bronchi, trachea, serous bronchial glands, alveolar monocytes and macrophages in the respiratory tract. ACE2 is also generally expressed on the mucosa such as the nasal, eyelid, lips, and oral cavity (Hoffman et al., 2020; Ochani et al., 2021). The pathogenic mechanism of SARS-CoV2 is illustrated in Figure 2.5. Virions enter target cells via binding of viral Spike (S) protein to cellular ACE2 together with the cell surface serine protease TMPRSS2, and then release their ssRNA, which combines with the ribosome within the target cell and is translated to yield RNA replicase (Hoffman et al., 2020; V'Kovski et al., 2021). The RNA replicase copies the ssRNA to produce negative-strand RNA, positive-strand RNA and RNA fragments, which combine with the ribosome to produce a protein shell. Alongside the expression of viral nsps, the viral replication organelles made of perinuclear double-membrane vesicles (DMVs), convoluted membranes (CMs) or small open double-membrane spherules (DMSs) provide a protective environment for viral genomic RNA replication and transcription of subgenomic mRNA (sg mRNA). Translated structural proteins enter the endoplasmic reticulum, transit to Golgi complex, where they interact with N-encapsidated and newly synthesized viral genomic RNA, and eventually virions bud into the lumen of vesicular compartments. Then viral particles, protein shell and the newly

synthesized positive-strand RNA, are packed into secretory vesicles, arrive at the cytoplasmic site of plasma membrane and are released via exocytosis to infect more target cells (V'Kovski et al., 2021).

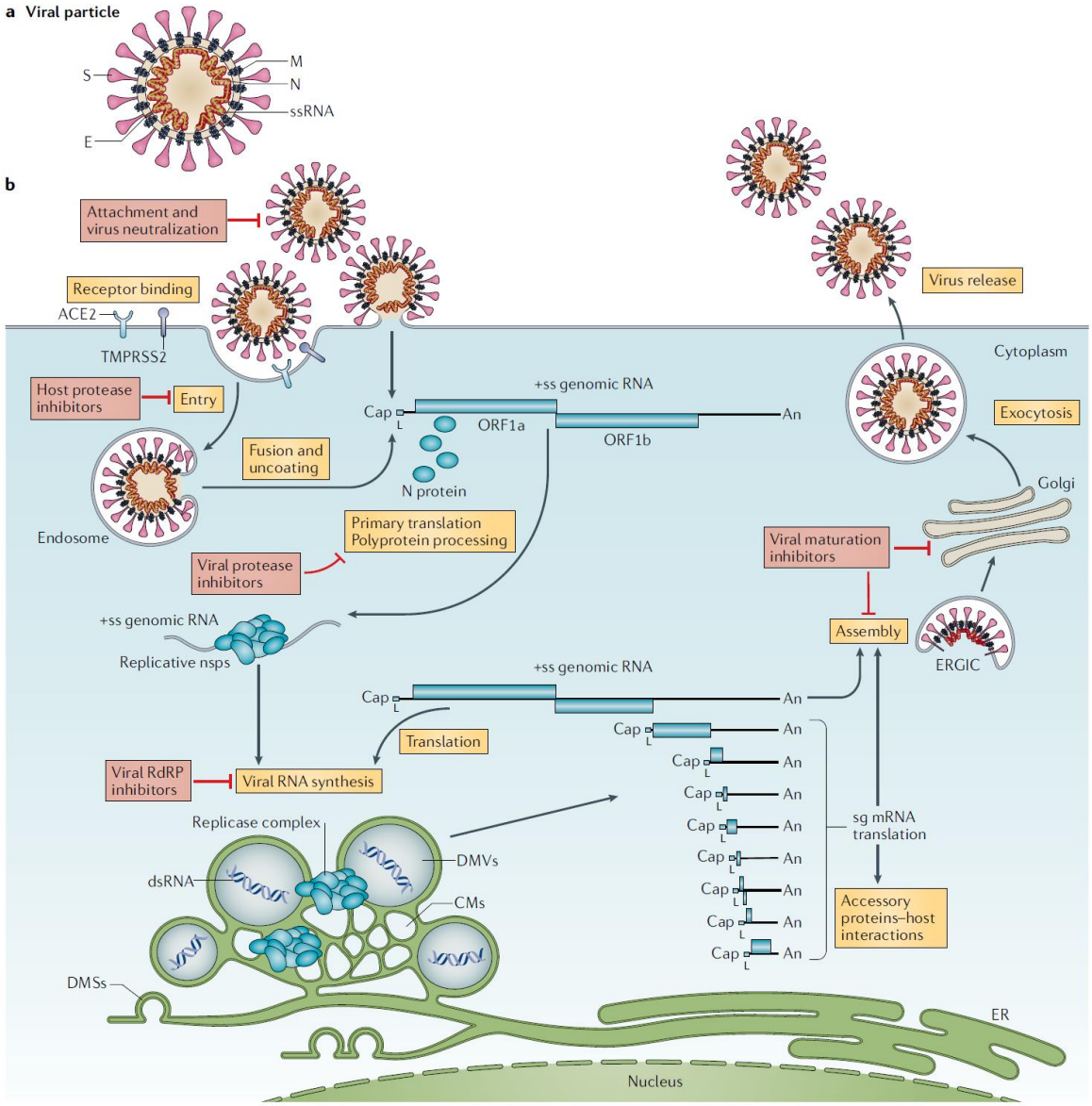

Figure 2.5: SARS-CoV2 virion and life cycle. Adapted from (V'Kovski et al., 2021)

2.6 SARS-CoV2 virus genetic variants

RNA viruses have relatively high mutation rates compared to DNA viruses, and these mutation rates are correlated with enhanced virulence and evolution of these viruses (Duffy, 2018). Lack of reduced efficiency of proofreading RNA polymerases contributes to the high mutation rates in RNA viruses (Kaur et al., 2021). Coronaviruses have genetic

proofreading mechanism and thus SARS-CoV2 genetic diversity should be low (Sallard et al., 2021; Akkiz, 2021). Despite this low mutation rate in SARS-CoV2, researchers have reported more than 12,706 mutations in its genome, the majority of which are single nucleotide polymorphisms (Akkiz, 2021). Indeed, shortly after its recognition in December 2019, several SARS-CoV2 variants have been recognized and some of them showed high risk for global public health (Ravi et al., 2022).

SARS-CoV2 variants originate during virus replication and their emergence is correlated with the number of infected individuals. Variants that usually predominate are those that can evade the immune system, have higher replication rate or have higher transmission rate (Canton et al., 2021). Variants that have clinical significance are those affecting the Spike region because the virus binds via its Spike to host cell receptor (ACE2) and thus this process can enhance the virus ability to colonize the respiratory tract and increase its transmission rate (Canton et al., 2021).

Virus variants are described as variants of interest (VOIs) or variants of concern (VOCs). VOIs have genetic markers that affect their transmission, virulence and their ability to evade the immune system or reduced response to therapeutics (Ravi et al., 2022). Examples of VOIs include 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) (Ravi et al., 2022).

While VOCs are similar to VOIs but with enhanced transmission (Ravi et al., 2022). VOCs harbor mutations in Spike protein responsible for increased receptor binding (N501Y, P681R) and immune escape to host response (L452R, E484K/Q, T478K/R) (Nayak et al., 2021).

Several VOCs were recognized and below a summary of some of these variants (Ravi et al., 2022). Although these variants were classified as VOCs at the beginning, they are classified as VBM (variants being monitored) at present (CDC, 2023b).

- Alpha (B.1.1.7 lineage; UK variant): It was first reported in UK and has 17 mutations and 8 of them are in the Spike protein. The Spike protein mutation N501Y increases affinity of virus S protein to ACE2 receptor.
- Beta (B.1.351 lineage or GH501Y.V2; South African variant): It was first reported in South Africa in October 2020 and was responsible for the second wave of

COVID-19. It contains 9 mutations in S protein and 3 mutations in receptor binding domain (RBD) that increase affinity to ACE2 receptor.

- Gamma (P.1 lineage or GR/501Y.V3): First detected in Brazil in December 2020. It contains 10 mutations in S protein and 4 mutations in RBD.
- Delta (B.1.617.2 lineage): first reported in India in December 2020 and was responsible for the deadly second wave of COVID-19. It harbors 10 S proteins and 3 RBD mutations.
- Omicron (B.1.1.529 lineage): First reported in South Africa in November 2021 and then spread worldwide and became the most dominant variant even in India. It harbors at least 50 mutations and 30 of them are in the S gene.

2.7 Mode of transmission

The SARS-CoV-2 is essentially transmitted through respiratory droplets or aerosols and fomites (Kutti-Sridharan et al., 2020). Indeed, nasal and oropharyngeal swabs have high viral loads (Kutti-Sridharan et al., 2020) and are usually preferred for viral detection. Droplets are small particles that carry viable and/or bacteria and fall to ground after they are released due to their relatively large size (>5µm). Aerosols are similar to droplets but usually 5 µm in size. Droplets and aerosols are usually generated through coughing, sneezing, talking and even normal breathing. Analysis of SARS-CoV2 aerosols' distribution showed that its transmission distance may reach up to 4 meters (Kutti-Sridharan et al., 2020) and they remain airborne for about 3 hours (Ochani et a., 2021). Close contact is an additional source of transmission of SARS-CoV-2. For example, SARS-CoV-2 can be transmitted through direct or indirect contact with mucous membranes in the eyes, mouth or nose or body fluids like saliva and tears. Transmission via healthcare settings is possible especially through procedures that generate aerosols like positive pressure ventilation. In addition, feco-oral route is an accepted route of transmission for coronaviruses, and transmission via seminal fluid is not uncommon (Kutti-Sridharan et al., 2020). There is also a possibility of aerosol transmission in a relatively closed environment with continuous exposure to high concentrations of aerosol (Zhang et al., 2020).

2.8 COVID-19 disease

COVID-19 disease is an emergent – infectious - respiratory disease caused by SARS-CoV2 virus and it was first recognized in November 2019 in Wuhan city, China. The disease spread rapidly worldwide and on March 11th, 2020, the WHO declared COVID-19 as a pandemic with confirmed cases in 114 countries (Wu and McGoogan, 2020). As of March 16th, more than 760 million confirmed cases and more than 6.8 million confirmed deaths have been reported on the WHO dashboard (WHO, 2023/ https://covid19.who.int/). Symptoms of COVID-19 disease appear after an average incubation period of 5.2 days, ranging from 2 to 11 days. Common COVID-19 symptoms such as fever, dry cough, dyspnea, and bilateral ground-glass opacities on chest CT scans are similar to infections caused by earlier betacoronaviruses (SARS-CoV and MERS-CoV) (Adhikari et al., 2020; (Rothan and Byrareddy, 2020). Other disease symptoms that have been observed among COVID-19 patients include: fatigue, headache, diarrhea, myalgia, pneumonia, acute respiratory disease, anosmia (loss of smell), taste dysfunction (dysgeusia), ageusia, ear pain, chest pain, dysphagia, face pain (heaviness), rhinorrhea, nasal obstruction and myocardial infarction (Lechien et al., 2020; Ochani et al., 2021). Patients with mild symptoms were reported to recover after one week while severe cases were reported to experience progressive respiratory failure due to alveolar damage from virus, which may lead to death (Adhikri et al., 2020).

The average case fatality rate for SARS-CoV2 is 3.4% compared to 9.6% and 34.4% for SARS-CoV and MERS-CoV viruses, respectively (Ochani et al., 2021). A split of the case fatality shows that it ranges from 2.3% of all confirmed cases, 14% in patients' >80 years old, 8% in patients aged 70 to 79 and 49% in critical cases (Wu and Mcgoogan, 2020).

The Chinese CDC has categorized the clinical manifestations of COVID-19 disease according to the severity of symptoms. This grouping was derived by reporting the analysis of 72,314 cases during the first wave of COVID-19 (Wu and McGoogan, 2020). In this cohort, most patients were 30 to 79 years of age (87%), 1% were aged 9 years or younger, 1% were aged 10 to 19 years and 3% were 80 years old or older. According to the later report, three groups were recognized:

- Mild: represent 81% of cases and patients showed either no symptoms or mild symptoms like mild pneumonia.

- Severe: represent 14% of all cases and patients showed dyspnea, respiratory frequency ≥ 30/min, blood oxygen saturation ≤ 93%, partial pressure of arterial oxygen to fraction of inspired oxygen ratio < 300, and/ or lung infiltrates, and > 50% of cases showed deterioration of symptoms within 24-48 hours.
- Critical: represent 5% of all cases, and patients showed respiratory failure, septic shock, and/ or multiple organ dysfunction or failure.

2.9 The host defense against SARS-CoV-2 virus

ACE2 is the functional receptor for SARS-CoV2 entry into host cells (Li et al., 2003; Hoffmann et al., 2020) and the serine protease TMPRSS2 is required for priming of the viral S protein (Hoffmann et al., 2020). According to this model, the S1 surface subunit of S protein binds to cellular ACE2 receptor and the transmembrane cellular protease (TMPRSS2) cleaves the S protein at the S1/S2 and S2' sites and allows fusion of viral and cellular membranes, a process mediated by the viral S2 subunit (Amirfakhryan and Safari, 2021). ACE2 is expressed in multiple tissues such as the lungs, heart, gut, kidneys and has many physiological functions (Magadum and Kishore, 2020).

ACE2 and TMPRSS2 are expressed in host target cells and in particular, the alveolar epithelial type 2 cells (pnuemocytes). SARS-CoV2 virus like other respiratory viral diseases, such as influenza viruses, causes profound lymphopenia and it infects and kills T lymphocytes. Host cells responds to the invading virus by mounting an inflammatory response, consisting of the innate and the adaptive immune response (comprising humoral and cell-mediated immunity), that impairs lymphopoiesis and increases lymphocyte apoptosis. When the viral replication accelerates in later stages of infection, the integrity of epithelial-endothelial barrier is compromised. In addition to the epithelial cells, SARS-CoV-2 infects pulmonary capillary endothelial cells; accentuating the inflammatory response and triggering an influx of neutrophils and monocytes. This leads to diffuse thickening of the alveolar wall with mononuclear cells and macrophages infiltrating airspaces in addition to endothelialitis. An interstitial mononuclear inflammatory infiltrates and edema develop and appear as ground-glass opacities on CT imaging. After that, pulmonary edema comprising hyaline membrane formation fills alveolar spaces, a state that is compatible with early-phase acute respiratory distress syndrome (ARDS) (Wiersinga et al., 2020). A Bradykinin-dependent lung angioedema may also contribute to the disease.

Overall, endothelial barrier disruption, dysfunctional alveolar-capillary oxygen transmission, and impaired oxygen diffusion capacity are characteristic features of COVID-19 (Wiersinga et al., 2020). In addition to the aforementioned immune response, infected pneumocytes initiates the so called 'cytokine storm' that involves the release of an array of cytokines and inflammatory markers including interleukins (IL-1, -6, -8, -120 and -12), tumor necrosis factor-alpha (TNF-α), interferons (IFN-β and -γ), CXCL-10, monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1 (MIP-1) (Parasher, 2021, Tang et al., 2005). Products of cytokine storm function as a chemoattractant for neutrophils, CD4 helper T cells, CD8 cytotoxic T cells, that accumulate in lung tissues and combat the invading virus. The accumulation of these cells contribute to inflammation and injury of the lung tissues and eventually leads to acute respiratory distress syndrome (Parasher, 2021, Xu et al., 2020).

In severe cases, extensive activation of coagulation and consumption of clotting factors occur. The inflamed lung tissues and pulmonary endothelial cells may contribute to microthrombi formation and account to the high incidence of thrombotic complications, like deep vein thrombosis, pulmonary embolism, and thrombotic arterial complications in critically ill patients. In fulminant disease, viral sepsis develops, defined as life threatening organ dysfunction caused by a dysregulated host response to infection, and may contribute to multiorgan failure (Wiersinga et al., 2020).

There is no protective immunity against SARS-CoV2 virus and that the virus can escape innate immune responses. However, the virus can activate both innate and adaptive immunity. A common feature observed in patients with severe COVID-19 diseases is eosinopenia and lymphopenia with a severe reduction in CD34+ and CD8+ T cells, B cells and NK cells (Anka et al., 2021). SARS-CoV2 induces a robust humoral immune response (B cell response) as evidenced by the near-universal detection of virus- IgA and neutralizing IgG antibodies in the days following infection (Hou et al., 2020; Anka et al., 2021).

2.10 Prevention and control

The emergence and high transmission rate of COVID-19 has challenged the health care systems all over the world. General hygiene precautions, social distancing, lockdown,

laboratory testing and tracking of infections were enforced in almost all countries in an attempt to control the spread of COVID-19 disease. These measures may have slowed down the spread of the disease but they did not manage to stop it as evidenced by the continued rise in the number of cases reported daily worldwide. According to WHO dashboard of COVID-19, and as of March 21st, 2023 there have been 761,071,826 confirmed cases of COVID-19 and 6,879,677 deaths reported to WHO (WHO, 2023). The WHO dashboard shows that the number of confirmed COVID-19 cases increased continuously starting from March 2020 and reached a peak in December 2020 and January 2021 (reached approximately 16-20 million confirmed cases weekly in January 2021) and showed a gradual decline after that. However, in December 2022 there was a second peak and thereafter there was a sharp decline the reported cases (WHO, 2023).

For combating the COVID-19 disease, there has been a race for the development of anti-SARS-CoV2 vaccines. Because of these efforts, several anti-SARS-CoV2 became available by the end of 2020 and beginning of 2021. At present, there are six vaccines that are authorized and commercially available for immunization against COVID-19, namely: Pfizer-BioNTech, Moderna, AstraZeneca-Oxford, Johnson & Johnson, Sputnik V, Sinopharm and Sinovac (Al-Hatamleh et al., 2023). As of March 18th, 2023, more than 13 billion disease of vaccine have been administered (WHO, 2023). The high levels of vaccination and population immunity gained through vaccination and natural infection greatly reduced the risk of medically significant diseases, hospitalization and death due to COVID-19 for most people (CDC, 2023a).

2.11 Diagnosis of COVID-19 disease

Laboratory diagnosis of COVID-19 disease is essential for establishing the disease state, monitoring and prognosis at every stage of disease. Lab tests used for detection of SARS-CoV2 infection falls into three areas: detection of viral RNA by RT-PCR, detection of viral antigens and anti-SARS-CoV2 antibodies.

The application of reverse transcriptase real time PCR for the detection of viral RNA in clinical samples such as nasopharyngeal sample is the reference standard for diagnosis of SARS-CoV2 infection (WHO, 2021). This method allows detection of acute infection and can provide an estimate of the viral load that can aid in the determination of diseases stage.

RT-PCR provided a vital role in screening, monitoring and contact tracing for COVI-19 cases (Gdoura et al., 2022). A diverse array of RT-PCR protocols are available and they target a range of SARS-CoV2 genomic regions. The genome targets affect the performance and sensitivity of the method. RT-PCR usually involves detection of one to three viral RNA targets, which increases the sensitivity and specificity of the method. The most commonly used targets are S, N, and E genes (Niu et al., 2020; Safiabadi Tali et al., 2021).

Serological testing of SARS-CoV2 antigens in clinical samples provides a rapid and easily performed test and does not require expensive machines. The two main viral proteins targeted by antigen testing are S and N proteins (Safiabadi Tali et al., 2021). However, its sensitivity is lower than RT-PCR and thus negative results should be repeated by RT-PCR based on WHO recommendations. However, Ag testing provides availability and speed of testing in appropriate scenarios or where screening of highly infected population is required (WHO, 2021).

The detection of ani-SARS-CoV2 antibodies indicates acute infection if IgM is present or past infection if IgG only detected. However, testing for antibodies is less sensitive than either antigen or RT-PCR. In addition, the WHO does not recommended the use of antibody testing for COVID-19 diagnosis as it takes around 1-2 weeks to detect immune responses to SARS-CoV2 infection (Safiabadi Tali et al., 2021). The lifetime and effectiveness of immune response against SARS-CoV2 is still vague which restricts its application as a diagnostic tool or for surveillance studies (Yuce et al., 2021). Nevertheless, it is useful for identification of past infection or research surveillance (WHO, 2021).

In addition to the above laboratory diagnostic methods, radiological, ultrasound and CT imaging proved to be useful in the diagnosis and/ or staging of COVID-19 states. Chest X-ray was the most commonly used imaging method for COVID-19 patients (Buda et al., 2020; Salehi et al., 2020; Cozzi et al., 2020). Radiological examination of patients with clinical suspicion of COVID-19 is mandatory, especially in the emergency department while waiting for RT-PCR results. Chest X-ray provides a quick evaluation of the thoracic involvement because COVID-19 can progress to severe pneumonia and aggressive acute respiratory distress syndrome (ARDS) (Cozzi et al., 2020).

2.12 Risk Factors

A systematic review by (Wolff et al. (2021) analyzed 28 records published between the end of 2019 and first months of 2020. Most studies were conducted in China (n=24) and the remaining were conducted in Italy, franc end the USA. The sample size in these studies ranged between 25 and 62800 patients. The authors concluded that risk factors such as high age, obesity, diabetes and hypertension were associated with severe and fatal COVID-19 disease course.

Williamson et al. (2020) analyzed a British cohort of over 17 million adult records that were pseudonymously linked to 10,926 COVID-19 related deaths. COVID-19 related deaths in this cohort were associated with being male, older age, deprivation diabetes, severe asthma and various other comorbidities such as respiratory disease, chronic heart disease, liver disease, stroke, neurological diseases, reduced kidney function and autoimmune diseases (rheumatoid arthritis, lupus and psoriasis). Black and South Asian people were at higher risk compared to white ethnicity.

Huang et al. (2021) investigated the long-term health consequences of 1733 (48% women, 52% men) COVID-19 patients at 6-months after discharge from hospital in Wuhan city (China) between January 7th and May 29th, 2020. The follow up period was between June 16th to September 3rd, 2020 with a median follow up time after disease onset of 186 days (Range 175-199 days). The most common symptoms reported were fatigue or muscle weakness (63%), sleep difficulties (26%) and anxiety or depression (23%). Patients who suffered from severe illness during their hospital stay had more severe impaired pulmonary diffusion capacity and abnormal chest imaging manifestations and are the main target group for intervention of long-term recovery. A subgroup of this cohort (n=94) were tested for anti-SARS-CoV2 antibodies and showed a high seropositivity (96.2%) and their median neutralizing antibodies were significantly lower compared to the baseline titer at the acute phase. In addition, patients without acute kidney injury and had an eGFR of 90 ml/min per 1.73 m^2 or more at acute phase had eGFR less than 90 ml/min per 1.73 m2 at follow up.

2.13 Immune response to SARS-CoV2

A study was conducted by(Zhou et al. (2020) and showed that COVID-19 patients exhibited nucleocapsid Protein (NP) – Specific antibody response, and in one patient, immunoglobulins (IgM), peaked at day 9 after disease onset and then switched to IgG by the second week. Another study by Hou et al. (2020) assessed the immune response to SARS-CoV2 infection in 338 COVID-19 patients. The study reported that the IgM levels increased during the first week after onset of infection peaked after weeks and returned to near-background levels in most patients. Schultheib et al, (2020) showed that almost all individuals with COVID-19 or a history of COVID-19 developed antibodies to SARS-CoV-2, including patients with major disturbances in B cell/Ig levels at active disease. The immune response included high IgA and normal or low IgG and IgM amounts, as well as B lymphocytosis. The authors also noted a pronounced deregulation in cytokines and soluble factors exclusively increased at recovery (IL-12, APRIL, and sCD40L), while others were elevated independently of the disease state (TNF-β, IFN-γ1, IFN-α2, and IFN-γ2/3) (Shultheib et al., 2020).

A meta-analysis of potential biomarkers associated with severity of COVID-19 by Danwang et al. (2020) showed that inflammatory (procalcitonin, CRP), haematologic (lymphocyte, Thrombocytes), and biochemical (CK-MB, Troponin I, D-dimer, aspartate aminotransfere (ASAT), alanine transaminase (ALAT), lactate dehydrogenase (LDH) LDH, γ-GT) biomarkers are significantly associated with severe COVID-19 disease.

An observational study conducted in Italy by Ognibene et al. (2020) evaluated the contribution of monocyte distribution width (MDW) for diagnosis of adult patients entering in the emergency department setting and tested for SARS-COV-2 in 147 patients. The authors concluded that 106 patients were negative and 41 were positive for SARS-CoV-2. The performance of MDW in differentiating SARS-COV-2 positive patients from negative ones showed an evident efficacy in terms of sensitivity and negative predictive value (NPV). Interestingly, 23 hospitalized patients in the intensive care unit showed significantly higher MDW.

A retrospective observational study Poggiali et al. (2020) investigated tissue damage and inflammatory status of 123 Italian hospitalized COVID-19 patients. The most common

laboratory abnormalities among this Italian cohort were lymphocytopenia and elevated levels of CRP and LDH. Liver (AST and ALT) and cardiac biomarkers (AST, CK) were also increased. Respiratory performance (PaO2/FiO2) revealed a strong inverse correlation with LDH, and a significant inverse correlation with age, WBCs, AST and neutrophil count. The authors concluded that elevated CRP and LDH are related respiratory function and may be used as a predictor of respiratory failure in COVID-19 patients.

A Chinese meta-analysis study by Bao et al. (2020) conducted a comparative analysis of laboratory findings for severe and non-severe COVID-19 patients. The authors analyzed 35 articles comprising 5912 patients. The later study reported higher levels of WBCs (1.2 folds), neutrophils (1.33 folds), CRP 3.04 folds), PCT (2 folds), ESR (1.44 folds), AST (1.4 folds), ALT (1.34 folds), CK (1.44 folds), CK-MB (1.39 folds), total bilirubin (1.14 folds), urea (1.28 folds), creatinine (1.09 folds), PT (1.03 folds) and D-dimer (2.74 folds) as well as lower levels of lymphocytes (1.44 folds), eosinophils (2 folds), monocytes (1.08 folds), Hb (1.53 folds), platelets (1.15 folds), albumin (1.15 folds), APTT (1.02 folds), CD4 T cells (2.1 folds and CD8 T cells (2 folds). Higher levels of inflammatory cytokines in severe patients compared to non-severe patients were also reported such as IL-1B (1.02 folds), IL-6 (1.93 folds) and IL-10 (1.55 folds).

Chapter Three
Materials and Methods

3.1 Materials

Reagents and equipment used in this study are listed in Table 1.

Table 3.1: Reagents and equipment used in this study.

#	Item name	Manufacturer, country
1	EDTA tubes	Vacutest kima, Italy
2	Plain tubes	Vacutest kima, Italy
3	Sodium Citrate tubes	Vacutest kima, Italy
4	Nihon Kohden Automated hematology (Cell Counter) analyzer	Nihon Kohden, Japan
5	Architest-i1000 Automated Immunoassay Analyzer	Abbott, USA
6	Cobas c311 Automated Chemistry Analyzer	Roche, Germany
7	Stago Compact Max2 Automated Coagulometer Analyzer	Diagnostica Stago, France
8	Cobas e411 Automated Immunoassay Analyzer	Roche, Switzerland
9	Quant Studio 5 Real-Time PCR System	Sansure Biotech, China

3.2 Study design

A prospective cross-sectional study of COVID-19 patients.

3.3 Study setting

Patients seeking testing for SARS-CoV2 infection at one of the SARS-CoV2 testing centers or admitted to one of the MoH hospitals that were included in this study. Three SARS-CoV2 virus-testing centers and four hospitals representing the northern, middle and southern regions of West Bank were used for recruitment of patients. The SARS-CoV2 testing centers/ hospitals included in this study were: Ramallah SARS-CoV2 testing center, Palestine Medical Complex and Hugo Schafez Hospital (middle region), Hebron city SARS-CoV2 testing center, Alia Hospital- Hebron (south) and Nablus SARS-CoV2 testing center and Nablus Military Hospital – Nablus (north).

3.4 Study population

Inclusion criteria: All laboratory-confirmed SARS-CoV-2 infection cases presented at the MoH COVID-19 testing centers or admitted to the MoH hospitals between February and October 2021 were enrolled in the study. The study protocol was approved by the Research Ethics Committee at Birzeit University (Reference number: BZUPNH2103) and Palestinian MOH. Laboratory confirmation for SARS-Cov-2 is defined as a positive result of real-time reverse transcriptase–polymerase chain reaction (RT-PCR) assay of upper or lower respiratory tract samples.

Exclusion criteria: Patients negative for SARS-CoV2 infection by real-time RT-PCR, as well as pediatric patients lower than 14 years of age. Also patients who died before completing the second questionnaire were excluded.

Patients were grouped into three groups according to COVID-19 disease severity based on the criteria of the Palestinian National COVID-19 Management Protocol (MOH, 2020) as follows:

- Mild/ moderate: Patients with positive Real-Time PCR test for SARS-CoV2 virus. Patients present with flu-like symptoms with varying severity (mostly mild to moderate), without organ involvement and they did not require hospitalization.
- Severe: Patients were hospitalized due to fever, suspected respiratory infection, plus one of the following: respiratory rate ≥30 times/ minute, SpO2 ≤93% at rest.
- Critical: Hospitalized patients showed one of the following: worsening of respiratory symptoms or respiratory failure, requirement for mechanical ventilation. Intensive care unit is needed.

3.5 Data and sample collection

All COVID-19 patients who fulfilled the study criteria were asked to take part in this study. All patients or their legal guardians in case of minors were asked to provide a written informed consent. Then patients were asked to provide anthropometric data and data about their health status to complete the study questionnaire at first time of enrollment. The response rate from patients was more than 95%. Three weeks later, general health data for

all patients were collected again to complete the second questionnaire; where non-hospitalized patients were contacted via telephone while data concerning hospitalized patients were collected from medical records at the respective hospital. Additionally, patients who agreed to participate in this study were asked to donate blood samples at time of interview (for non-hospitalized patients) and to give a permission to collect their data from medical records (for hospitalized patients). All patients were briefed about the study objective and asked to give a written informed consent for enrolment in the study. In case of minors or unconscious patients in the ICU ward an adult family member/ guardian was asked to give the written informed consent on behalf of the patient. The family guardian accompanied the patient or minor during the interview and sample collection. Patients were informed that they have the right to withdraw from the study at any point.

Laboratory findings for hematological and biochemical tests for hospitalized patients were collected from medical records. Additionally, 2-3 mL of venous blood were collected in plain tubes, and the harvested serum was used for measurement of anti-SARS-CoV2 antibodies.

For non-hospitalized patients, medical records at SARS-CoV2 testing centers were reviewed and those who have had positive results were contacted via telephone within 1-2 days within their positive SARS-CoV2 test or their visit to the testing center, and asked to participate in this study. Patients were interviewed at the SARS-CoV2 testing center, provided information for completing the study questionnaire and donated 8-10 mL of blood. Blood samples was collected in three tubes: calcium citrate, EDTA tube and plain tube. Citrated plasma was separated within one hour, stored at -30°C and used later for measurement of coagulation parameters. EDTA blood was used for Complete Blood Count (CBC) analysis. Plain tube was allowed to clot at room temperature; serum was collected by centrifugation, stored at -20°C and used later for measurement of biochemical tests.

3.6 Study variables:

- Anthropometric and general health data collected from patients to complete the study questionnaire were: age, sex, residence place, level of education, professions or occupation, marital status, general health status (chronic diseases) and current disease symptoms relevant to COVID-19 disease. Three weeks later, non-

hospitalized patients were contacted via telephone and asked to state their current disease symptoms related to COVID-19 disease to assess disease duration.

- Hematological parameters: Complete Blood Count (CBC).
- Biochemical parameters: Cholesterol, Triglycerides, Calcium, Fasting blood sugar, Creatine kinase (CK), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Lactate dehydrogenase (LDH), Amylase, Alkaline phosphatase (ALP), Creatinine, Bilirubin (total and direct), BUN, Albumin, Total protein and serum Ferritin .
- Hormonal parameters: TSH, and PTH.
- Coagulation parameters: PT, INR, APTT, and D-dimer.
- Serological parameters: C - reactive protein, ABO and Rh (D) blood group phenotyping.
- Immunological parameters: anti-SARS-CoV2 antibodies.
- SARS-CoV2 virus and variants detection: detection of SARS-CoV2 virus as well as its variants were performed using real-time RT-PCR test for all patients at the SARS-CoV2 virus testing centers. These results were collected from medical records of the respective center.

3.7 Laboratory analysis

- CBC: It was measured by Nihon Kohden automated hematology analyzers. All four governmental hospitals participated in this study used the same instrument.
- Biochemical parameters: all biochemical parameters were measured using the fully Cobas c311 automated chemistry analyzer, using standard laboratory procedures.
- Anit-SARS-CoV2 antibodies: total antibodies (IgG + IgM) were measured using anti-SARS CoV-2 S reagents by the fully Cobas e411 automated immunoassay analyzer instrument.
- SARS-CoV2 virus detection: virus detection was determined using real-time RT-PCR and virus variants using commercially available kits (The Novel Corona (2019-nCov) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing), Sansure Biotech – China).
- SARS-CoV2 virus mutation genotyping by RT-PCR: genotyping was performed using home-made protocol at the Central Public Health Laboratory, MOH.

- Quality control: For all tests, appropriate quality control procedures were implemented and quality control materials were performed according to the respective SOPs approved by the MOH.

3.8 Statistical analysis

Descriptive statistics including (mean and standard deviation) were calculated for all variables using SPSS software (Version 23). Outliers were eliminated without exceeding 5% of the data in order to ensure normal distribution. Inferential statistical methods were used to reach conclusions and generalizations about the characteristics of study population based on data collected in this study. Independent sample *t*-test and one-way ANOVA were used to compare means of continuous variables, and Chi-Square test was for non-continuous variables. A *P*-value < 0.05 was considered statistically significant.

3.9 Ethical consideration

All patients participating in this study, were briefed about the study objectives, asked to provide information necessary to complete the study questionnaire and then asked to give a written informed consent. Privacy was secured during sample and data collection and after that, all data were made anonymous. The study protocol was approved by the Committee on Research Ethics (file # BZUPNH2103) at Birzeit University. In case of minors or unconscious patients in the ICU ward an adult family member/ guardian was asked to give the written informed consent on behalf of the patient. The family guardian accompanied the patient or minor during the interview and sample collection. Patients were informed that they have the right to withdraw from the study at any point. In addition, the guidelines of Helsinki declaration were implemented throughout this study.

Chapter four

Results of the study

A total of 400 COVID-19 patients were enrolled in this study. Patients were recruited for participation in this study from five COVID-19 testing and treatment centers representing the northern, middle and southern regions of West Bank, Palestine. Patients were grouped into three categories based on disease severity: mild/moderate (n=200), severe (n=109) and critical (n=91) based on recommendations of the Palestinian MOH (MOH, 2020) and Chinese CDC (Wu and McGoogan, 2020). All patients tested positive for SARS-CoV2 virus by RT-PCR. Additional criteria used for this classification are listed in section 3.4 of this study. Briefly, patients in the mild/ moderate group showed flu-like symptoms with varying severity (mostly mild to moderate), without organ involvement and they did not require hospitalization. Patients in the severe groups were hospitalized due to fever, suspected respiratory infection, plus one of the following: respiratory rate ≥30 times/ minute, SpO2 ≤93% at rest. While patients in the critically ill group were hospitalized and showed one of the following: worsening of respiratory symptoms or respiratory failure, requirement for mechanical ventilation and patients required hospitalization in the intensive care unit.

4.1 Anthropometric data and general characteristics of patients

Anthropometric and general health data were collected from patients using the study questionnaire and are summarized in Table 4.1. There was a significant difference among the average ages of the three groups, mild/moderate, severe and critical. The average age of the mild/moderate group was the youngest one (39.2±16.1 years) while that for severe and critical groups were 55.6±15.2 and 63.3±14.2 years, respectively. These data indicate that older age is associated with disease severity. This finding was also confirmed by analysis of age groups (Table 4.1) where younger patients (age group 14-39 years) represented 54% of the mild/ moderate group while it represented 15.6% and 9.9% of the severe and critical group, respectively. In contrast, older patients represented 11.5%, 41.3% and 71.4% of the mild/moderate, severe and critical groups, respectively.

Table 4.1: Epidemiological characteristics of study patients. Data are shown as mean ± SD for age and number (n) and percentages (between brackets) for all other parameters.

Disease severity	Age (mean±SD)	Age group (years), n (%)			Sex, n (%)		BMI kg/m2 (mean±SD)	BMI group, n (%)		
		14-39 (n=134)	40-59 (n=133)	≥60 (n=133)	Females (n=196)	Males (n=204)		≤24,9 (n=101)	25-29.9 (n=152)	≥30 (n=147)
Mild/ moderate (n=200)	39.2±16.1 [a,b]	108 (80.6)	69 (51.9)	23 (17.3)	106 (54.1)	94 (46.1)	27.3 ± 4.8 [a,b]	61 (60.4)	78 (51.3)	61 (41.5)
Severe (n=109)	55.6±15.2[c]	17 (12.7)	47 (35.3)	45 (33.8)	41 (20.9)	68 (33.3)	29.72± 5.2	20 (19.8)	41 (27.0)	48 (32.7)
Critical (n=91)	63.3±14.2	9 (6.7)	17 (12.8)	65 (48.9)	49 (25.0)	42 (20.6)	28.72± 5.2	20 (19.8)	33 (21.7)	38 (25.8)
P-value	0.000			0.000		0.020	0.000			0.059

Disease severity	Residence place, n (%)			Smoking, n (%)		Family members with COVID-19, n (%)		Vaccination	
	North	Middle	South	No	Yes	Yes	No	Yes	No
Mild/ moderate (n=200)	35 (52.2)	139 (51.9)	26 (40.0)	42 (53.1)	158 (49.2)	22 (50.0)	178 (50.0)	15 (68.2))	185 (48.9)
Severe (n=109)	23 (34.3)	59 (22.0)	27 (41.5)	21 (26.6)	88 (27.4)	10 (22.7)	99 (27.8)	2 (9.1)	107 (28.3)
Critical (n=91)	9 (13.4)	70 (26.1)	12 (18.5)	16 (20.3)	75 (23.4)	12 (27.3)	79 (22.2))	5 (22.7)	86 (22.8)
P-value	0.004			0.785		0.734		0.147	

Disease severity	Occupation				SARS-CoV2 variant	
	Health	Education	Others	Jobless	Alpha	Delta
Mild/ moderate (n=200)	18 (52.9)	20 (36.4)	47 (37.6)	115 (61.8)	186 (54.2)	14 (24.6)
Severe (n=109)	6 (17.7)	27 (4.1))	37 (29.6)	39 (21.0)	96 (28.0)	13 (22.8)
Critical (n=91)	10 (29.4)	8 (14.5)	41 (32.8)	32 (17.2)	61 (17.8)	30 (52.6)
P-value	0.000				0.000	

For continuous variables, one way ANOVA was used for mean comparisons among groups, and differences within each group was analyzed by LSD Post Hoc test. The Post hoc results are shown as superscript letters as follows: (a) mild/moderate vs. sever; (b) mild vs. critical; (c) sever vs. critical. For non-continuous variables, Chi-square was used for analysis. *Refers to family members sharing same home and at time of infection of proband.

Almost half of the patients were females (n=196/400 or 49%) and they represented slightly more than half of patients in the mild/moderate (53%) and critical groups (53.8%) but they were less represented in the severe group (37.6%). These data indicate that COVID-19 disease is more common among females in the mild/moderate and critical groups and that progression of the disease toward the critical stage was more common among females. Although there were no statistical differences within groups based on sex. Analysis of BMI, showed that higher BMI values were associated with disease severity. Also the highest percentage (39%) of patients in the mild moderate group was in the range 25-29.9 Kg/m^2, while the highest percentage of patients in the severe (42.2%) and critical (40.7%) groups have a BMI ≥ 30 kg/m^2 (Table 4.1).

Collection of patients from the three residence places was intended to be representative but not necessarily equal because, many patients who were also residents of the middle region were either - for example- living in the south or north and continuously move between two different regions. Nevertheless, including different regions aimed to avoid possible bias in sample collection. Our results showed that there was a significant differences among groups based on residence place (Table 4.1).

Our data showed that there were no association between disease severity and smoking (p-value = 0.785) (Table 4.1). In addition, when patients were asked to declare the source of infection and whether they have a family member affected with COVID-19 or not. The data indicated that there was no significant difference between presence of infected family members and disease severity (p-value= 0.734). There was no significant difference between those who were vaccinated and those who were not, but there was a small percentage of patients vaccinated among the study sample and only 2 to 15% of each group received the vaccine before participation in this study. Indeed, at the time of sample collection, the vaccination was not widely available.

Analysis of the association between occupation and disease severity showed a significant difference. Patients working in health professions or education represented 5.5% to 24.8% in the three groups (Table 4.1), but sample collection was not representative of all professions and thus we cannot deduce further conclusions about the association between occupation and disease severity.

Most patients (n=343/400; 85.8%) were infected with Alpha variant (British variant) of SARS-CoV2 virus as this variant was the most prevalent at time of sample collection, and by the end of sample collection, the Delta variant (Indian variant) emerged and was detected in (n=57/400; 14.3%) of patients. Remarkably, 30 patients out of 57 who were infected with the Delta variant were in the critical group (Table 4.1). Also the number of Delta infections in the critical group (n=30) is 4.7-folds and 2.8-folds of that observed in the mild/moderate (n=14) or severe group (n=13), respectively. These data indicate that the Delta variant was more virulent than the Alpha variant.

4.2 Chronic diseases affecting patients before onset of COVID-19

Investigation of the association of chronic diseases and COVID-19 disease severity, revealed that there was an association between chronic diseases such as diabetes mellitus (p-value= 0.000), lung diseases (p-value= 0.004), hypertension (p-value= 0.000), heart diseases (p-value= 0.000) and COVID-19 disease severity (Table 4.2). In contrast, there was an inverse association between allergy/asthma (p-value= 0.039) and COVID-19 disease severity. There was no statistically significant differences between the rest of chronic diseases analyzed in this study and COVID-19 disease severity (Table 4.2).

Table 4.2: Medical history of patients. Chronic diseases presented in this table refer to diseases that were affecting study subjects before onset of current COVID-19 infection. Data are shown in numbers (n) and percentages (between brackets).

Chronic disease	**Mild /moderate (n=200)**		**Severe (n=109)**		**Critical (n=91)**		**P-value**
	n	%	n	%	N	%	
Vascular disease	8	4.0%	11	10.1%	8	8.8%	0.085
Diabetes mellitus	14	7.0%	32	29.4%	41	45.1%	0.000
Allergy/ asthma	22	11.0%	15	13.8%	3	3.3%	0.039
Lung diseases	3	1.5%	5	4.6%	9	9.9%	0.004
Cancer (current state)	4	2.0%	4	3.7%	5	5.5%	0.285
Hypertension	28	14.0%	47	43.1%	49	53.8%	0.000
HBV/HAV	5	2.5%	1	0.9%	1	1.1%	0.517
Chronic kidney failure	1	0.5%	1	0.9%	3	3.3%	0.129
Immune suppressive disorders	27	13.5%	20	18.3%	21	23.1%	0.215
Heart disease	15	7.5%	22	20.2%	25	27.5%	0.000
Hypothyroidism	11	5.5%	7	6.4%	6	6.6%	0.914
Neurological diseases	5	2.5%	1	0.9%	5	5.5%	0.137
Depression	27	13.5%	20	18.3%	21	23.1%	0.119

Analysis of chronic diseases based on sex and their association with COVID-19 severity showed no statistical differences between females and males for all chronic diseases studied (Table 4.3) except for vascular diseases among severe patients' group. However, in the severe group, there were one female and 10 males and thus the statistical analysis for this point is not reliable due to the small sample size.

Table 4.3: Medical history of patients distributed based on sex. Data are shown as numbers (n) and percentages (between brackets).

Chronic disease	Mild/ moderate, N (%)			Severe, N (%)			Critical, N (%)		
	Female (n=106)	Male (n=94)	P-value	Female (n=41)	Male (n=68)	P-value	Female (n=49)	Male (n=42)	P-value
Vascular disease	5 (4.7%)	3 (3.2%)	0.583	1 (2.4%)	10 (14.7%)	0.039	4 (8.2%)	4 (9.5%)	0.819
Diabetes mellitus	8 (7.5%)	6 (6.4%)	0.747	13 (31.7%)	19 (27.9%)	0.676	26 (53.1%)	15 (35.7%)	0.097
Allergy/ asthma	13 (12.3%)	9 (9.6%)	0.544	6 (14.6%)	9 (12.3%)	0.837	1 (2.0%)	2 (4.8%)	0.469
Lung diseases	1 (0.5%)	2 (2.1%)	0.492	3 (7.3%)	2 (2.9%)	0.290	6 (12.2%)	3 (7.1%)	0.416
Cancer (current state)	2 (1.9%)	2 (2.1%)	0.903	1 (2.4%)	3 (4.4%)	0.596	4 (8.2%)	1 (2.4%)	0.228
Hypertension	18 (17%)	10 (10.6%)	0.197	20 (48.8%)	27 (39.7%)	0.354	27 (55.1%)	22 (52.4%)	0.795
HBV/HAV	1 (0.9%)	4 (4.3%)	0.134	1 (2.4%)	0 (0.0%)	0.196	1 (2.0%)	0 (0.0%)	0.651
Chronic kidney failure	1 (0.9%)	0 (0.0%)	0.345	1 (2.4%)	0 (0.0%)	0.196	0 (0.0%)	0 (0.0%)	0.651
Immune suppressive disorders	4 (3.8%)	1 (1.1%)	0.606	2 (4.9%)	5 (7.4%)	0.148	1 (2.0%)	2 (4.8%)	0.232
Heart disease	8 (7.5%)	7 (7.4%)	0.979	10 (24.4%)	12 (17.6%)	0.396	13 (26.5%)	12 (28.6%)	0.828
Hypothyroidism	5 (4.7%)	6 (6.4%)	0.606	3 (7.3%)	4 (5.9%)	0.767	4 (8.2%)	2 (4.8%)	0.515
Neurological diseases	2 (1.9%)	3 (3.2%)	0.555	0 (0.0%)	1 (1.5%)	0.435	1 (2.0%)	4 (9.5%)	0.118
Depression	17 (16.0%)	10 (10.6%)	0.265	11 (26.8%)	9 (12.3%)	0.076	9 (18.4%)	12 (28.6%)	0.249

4.3 Clinical symptoms of patients at baseline and after three weeks

Patients presented with a range of clinical symptoms and some of these symptoms namely fever (>38°C), myalgia, sore throat, cough, dyspnea, ear pain, chest pain, headache, nausea/vomiting, abdominal pain, loss of appetite, face pain and retro-ocular pain showed a statistically significant association with disease severity (Table 4.4).

Three weeks after onset of COVID-19 disease, patients were asked to state the clinical symptoms that they still suffer from and these data are summarized in Table 4.8. In general, a higher proportion of patients in severe and critical groups experienced disease symptoms after weeks of disease onset and a statistically significant association of diseases severity with the following symptoms was observed: chills, fatigue, myalgia, cough, dyspnea, ear pain, chest pain, arthralgia, nausea/ vomiting, loss of appetite, face pain, mental disorders and retro-orbital headache (Table 4.4). Interestingly, fatigue, myalgia, dyspnea and retro-orbital headache were found in more than 80% of patients in severe and critical patients. Dyspnea in particular is found in 85.3% and 92.3% of severe and critical patients, respectively.

More than 50% of patients in the mild/ moderate group still experience fatigue, myalgia, arthralgia and retroocular headache, while 25-50% of patients were still experiencing loss of smell, taste dysfunction, cough, dyspnea, chest pain, headache and mental disorder (Table 4.8). These data suggest that some disease symptoms or disease sequelae may last for at least three weeks after onset of the disease.

Table 4.4: Clinical symptoms of patients due to current COVID-19 infection at time of diagnosis (at registration or baseline) and after three weeks.
Data are shown number (n) and percentages (between brackets).

Symptoms	Mild / moderate (n=200)			Severe (n=109)			Critical (n=91)			P-value[1] at Baseline	P-value[2] After 3 weeks
	Baseline, n (%)	After 3 weeks, n (%)	P-value	Baseline, n (%)	After 3 weeks, n (%)	P-value	Baseline, n (%)	After 3 weeks, n (%)	P-value		
Fever (>38°C)	103 (51.5)	21 (10.5)	0.000	78 (71.6)	16 (14.7)	0.000	71 (78.0)	16 (17.6)	0.000	0.000	0.224
Loss of smell	108 (54.0)	77 (38.5)	0.001	53 (48.6)	46 (42.2)	0.227	47 (51.6)	43 (43.7)	0.281	0.663	0.367
Taste dysfunction	107 (53.5)	80 (40.0)	0.002	51 (46.8)	46 (42.2)	0.346	45 (49.5)	43 (43.7)	0.431	0.509	0.510
Chills /shivering	135 (67.5)	43 (21.5)	0.000	81 (74.3)	25 (22.9)	0.000	73 (49.5)	44 (48.4)	0.000	0.068	0.000
Fatigue	**158 (79.0)**	**116 (58.0)**	**0.000**	**92 (84.4)**	**91 (83.5)**	**0.736**	**77 (84.6)**	**74 (81.3)**	0.432	0.363	0.000
Myalgia	**150 (75.0)**	**105 (52.0)**	**0.000**	**91 (83.5)**	**91 (83.5)**	**0.877**	**83 (91.2)**	**78 (85.7)**	0.184	0.004	0.000
Sore throat	112 (56.6)	37 (18.5)	0.000	74 (67.9)	20 (18.3)	0.000	62 (68.1)	23 (25.3)	0.000	0.047	0.359
Cough	131 (65.5)	53 (26.5)	0.000	**85 (78.0)**	**60 (55.0)**	**0.000**	**80 (87.9)**	**62 (68.1)**	0.000	0.000	0.000
Nasal obstruction	107 (53.5)	26 (13.0)	0.000	54 (49.5)	15 (13.8)	0.000	52 (57.1)	14 (15.4)	0.000	0.560	0.861
Rhinorrhea	73 (36.5)	26 (13.0)	0.000	42 (38.5)	15 (13.8)	0.000	44 (48.4)	13 (14.3)	0.000	0.153	0.953
Dyspnea	**92 (46.0)**	**62 (32.5)**	**0.008**	**96 (88.1)**	**93 (85.3)**	**0.462**	**85 (93.4)**	**84 (92.3)**	0.677	0.000	0.000
Ear pain	49 (24.5)	36 (10.0)	0.193	33 (30.3)	35 (32.1)	0.819	39 (42.9)	38 (41.8)	0.587	0.007	0.000
Chest pain	93 (46.5)	65 (32.5)	0.015	**75 (68.8)**	**78 (71.6)**	**0.826**	**66 (72.5)**	**72 (79.1)**	0.433	0.000	0.000
Arthralgia	**141 (70.5)**	**103 (51.0)**	**0.001**	**87 (79.8)**	**88 (80.7)**	**0.999**	**70 (76.9)**	**69 (75.8)**	0.665	0.166	0.000
Headache	155 (77.5)	76 (38.0)	0.000	68 (62.4)	47 (43.1)	0.003	62 (68.1)	45 (49.5)	0.018	0.015	0.179
Nausea /vomiting	54 (27.0)	35 (17.5)	0.047	47 (43.1)	35 (17.5)	0.030	52 (57.1)	36 (39.6)	0.006	0.000	0.000
Abdominal pain	80 (40.0)	13 (6.5)	0.000	50 (45.9)	8 (7.3)	0.000	51 (56.0)	10 (11.0)	0.000	0.038	0.407
Diarrhea	98 (49.0)	28 (14.0)	0.000	49 (45.0)	9 (8.3)	0.000	49 (53.8)	10 (11.0)	0.000	0.456	0.315
Loss of appetite	111 (55.5)	30 (15.0)	0.000	73 (67.0)	28 (25.7)	0.000	68 (74.7)	28 (30.8)	0.000	0.004	0.005
Face pain/ heaviness	56 (28.0)	24 (12.0)	0.000	41 (37.6)	26 (23.9)	0.009	46 (50.0)	28 (30.8)	0.001	0.001	0.000
Mental disorders	**5 (2.5)**	**93 (46.5)**	**0.000**	**1 (0.9)**	**75 (68.8)**	**0.000**	**5 (5.5)**	**66 (72.5)**	0.000	0.137	0.000
Retro-ocular headache	**161 (80.5)**	**166 (83.0)**	**0.048**	**108 (99.1)**	**96 (88.1)**	**0.014**	**90 (98.9)**	**78 (85.7)**	0.023	0.000	0.000

P-value for differences between groups at [1]Baseline or [2]After 3 weeks.

In addition to the above results, clinical symptoms were sub-grouped based on sex (females vs. males, Table 4.5), age groups (14-39, 40-59, ≥60 years, Table 4.6) and virus variants (Alpha vs. Delta variants, Table 4.7) and statistically analyzed to find any association with COVID-19 severity. The data shown in Table 4.5 shows that in general, females in the mild/ moderate and critical groups manifested more symptoms compared to men, while in the severe groups, the contrary was observed. To some extent, the data in Table 4.5 confirm the findings presented in Table 4.4 where the proportion of patients suffering from disease symptoms increased parallel to the increase in disease severity.

Further analysis of COVID-19 disease symptoms and their association with age groups (Table 4.6) showed that there were in general no statistical differences within age groups, however, within the severe group there was a significant difference among age groups concerning dyspnea and ear pain. However, these data shows that older patients are more associated with disease severity and thus confirms our data presented in Table 4.1. Investigation of the SARS-CoV2 variants and their association with disease severity and symptoms revealed that the Delta-variant was more associated with the critical disease stage (Table 4.7) and thus confirms the data presented in Table 4.1. Again, a limitation of these data is that the number of patients with Delta-variants is very small (n=57; 14.3%) and thus statistical analysis in this case is limited.

Further analysis of disease symptoms at three weeks after disease onset and their association with disease severity based on sex, age group and SARS-CoV2 variants did not yield significant differences from the data recorded at onset of disease (data not shown).

Table 4.5: Clinical symptoms of COVID-19 patients at time of diagnosis (at registration or baseline) based on disease severity and sex. Data are shown as numbers (n) and percentages (between brackets).

Symptoms	Mild/ moderate, n (%)			Severe, n (%)			Critical, n (%)		
	Female (n=106)	Male (n=94)	P-value	Female (n=41)	Male (n=68)	P-value	Female (n=49)	Male (n=42)	P-value
Fever (>38°C)	67 (63.2%)	36 (38.3%)	0.000	29 (70.7%)	49 (72.1%)	0.177	43 (87.8%)	28 (66.7%)	0.015
Loss of smell	60 (56.6%)	48 (61.7%)	0.433	12 (29.3%)	19 (27.9%)	0.674	6 (12.2%)	16 (38.1%)	0.017
Taste dysfunction	58 (54.7%)	49 (52.1%)	0.714	22 (53.7%)	29 (42.6%)	0.264	31 (63.3%	14 (33.3%)	0.004
Chills or shivering	76 (71.7%)	59 (62.8%)	0.178	33 (80.5%)	48 (70.6%)	0.252	44 (89.9%)	29 (69.0%)	0.013
Fatigue	91 (85.8%)	67 (71.3%)	0.012	36 (87.8%)	56 (82.4%)	0.447	45 (91.8%)	32 (76.2%)	0.039
Myalgia (Muscle ache)	84 (79.2%)	66 (70.2%)	0.141	36 (87.8%)	55 (80.9%)	0.345	47 (95.9%)	36 (85.7%)	0.087
Sore throat	62 (58.5%)	50 (53.2%)	0.451	31 (75.6%)	43 (63.2%)	0.180	38 (77.6%)	24 (57.1%)	0.037
Cough	78 (73.6%)	53 (56.4%)	0.011	36 (87.8%)	49 (72.1%)	0.055	43 (87.8%)	37 (88.1%)	0.960
Nasal obstruction	57 (53.8%)	50 (53.2%)	0.934	24 (58.5%)	30 (44.1%)	0.145	32 (65.3%)	22 (52.4%)	0.089
Rhinorrhea (runny nose	41 (38.7%)	32 (34.0%)	0.497	22 (53.7%)	20 (29.4%)	0.012	28(57.1%)	16 (38.1%)	0.07
Dyspnea (shortness of breath)	49 (46.2%)	43 (45.7%)	0.946	36 (87.8%)	60 (88.2%)	0.946	48 (98.0%)	37 (88.1%)	0.059
Ear pain	32 (30.2%)	17 (18.1%)	0.047	15 (36.6%)	18 (26.5%)	0.266	28 (57.1%)	11 (26.2%)	0.003
Chest pain	56 (52.8%)	37 (39.4%)	0.057	31 (75.6%)	44 (64.7%)	0.266	39 (79.6%)	27 (64.3%)	0.003
Arthralgia (Joints pain)	80 (75.5%)	61 (64.9%)	0.102	36 (87.8%)	51 (75.0%)	0.107	43 (87.8%)	27 (64.3%)	0.008
Headache	90 (84.9%)	65 (69.1%)	0.008	30 (73.2%)	38 (55.9%)	0.071	41 (83.7%)	21 (50.0%)	0.001
Nausea /vomiting	32 (30.2%)	22 (23.4%)	0.281	24 (58.5%)	23 (33.8%)	0.012	35 (71.4%)	17 (40.5%)	0.003
Abdominal pain	46 (43.4%)	34 (36.2%)	0.288	23 (56.1%)	27 (39.7%)	0.096	35 (71.4%)	16 (38.1%)	0.001
Diarrhea	51 (48.1%)	47 (50.0%)	0.790	24 (58.5%)	25 (36.8%)	0.027	32 (65.3%)	17 (40.5%)	0.018
Loss of appetite	68 (64.2%)	43 (45.7%)	0.009	30 (73.2%)	43 (63.2%)	0.285	42 (85.7%)	26 (61.9%	0.009
Face pain/ heaviness	34 (32.1%)	22 (23.4%)	0.173	20 (48.8%)	21 (30.9%)	0.062	31 (63.3%)	15 (35.7%)	0.009
Mental disorders	2 (1.9%)	3 (3.2%)	0.555	0 (0.0%)	1 (1.5%)	0.435	1 (2.0%)	4 (9.5%)	0.118
Retro-ocular headache	92 (86.8%)	69 (73.4%)	0.017	41 (100.0%)	67 (98.5%)	0.435	49 (100.0%)	41 (97.6%)	0.277

Table 4.6: Clinical symptoms of COVID-19 patients at time of diagnosis (at registration or baseline) based on disease severity and age groups (years).

Data are shown in numbers (n) and percentages (between brackets).

Symptoms	Mild / moderate, n (%)				Severe, n (%)				Critical, n (%)			
	14-39 (n=108)	40-59 (n=69)	≥60 (n=23)	P-value	14-39 (n=17)	40-59 (n=47)	≥60 (n=45)	P-value	14-39 (n=9)	40-59 (n=17)	≥60 (n=65)	P-value
Fever (>38°C)	50 (46.3)	40 (58.0)	13 (56.5)	0.278	14 (82.4)	35 (74.5)	29 (64.4)	0.319	8 (88.9)	13 (76.5)	50 (76.9)	0.708
Loss of smell	57 (52.8)	40 (58.0)	11(47.8)	0.652	8 (47.1)	21 (44.7)	24 53.5()	0.702	7 (77.8)	11 (64.7)	29 (44.6)	0.086
Taste dysfunction	54 (50.0)	42 (60.9)	11 (47.8)	0.311	8 (47.1)	19 (40.4)	24 (53.3)	0.463	6 (66.7)	10 (58.8)	29 (44.6)	0.321
Chills or shivering	71 (65.7)	50 (72.5)	14 (60.9)	0.500	14 (82.4)	37 (78.7)	30 (66.7)	0.296	8 (88.9)	15 (88.2)	50 (76.9)	0.458
Fatigue	83 (76.9)	56 (81.2)	19 (82.6)	0.714	14 (82.4)	41 (87.2)	37 (82.2)	0.778	8 (88.9)	14 (82.4)	55 (84.6)	0.908
Myalgia (Muscle ache)	80 (74.1)	53 (76.87)	17 (73.9)	0.912	16 (94.1)	40 (85.1)	35 (77.1)	0.280	8 (88.9)	16 (94.1)	59 (90.8)	0.880
Sore throat	62 (57.4)	37 (53.6)	13 (56.5)	0.884	11 (64.7)	33 (70.2)	30 (66.7)	0.983	6 (66.7)	10 (58.8)	46 (70.8)	0.639
Cough	68 (63.0)	47 (68.1)	16 (69.6)	0.710	13 (76.5)	36 (76.6)	36 (80.0)	0.913	9 (100)	17 (100.)	54 (83.1)	0.082
Nasal obstruction	59 (54.6)	35 (50.7)	13 (56.5)	0.838	10 (58.8)	21 (44.7)	23 (51.1)	0.584	4 (44.4)	9 (52.9)	39 (60.0)	0.628
Rhinorrhea (runny nose	41 (38.0)	24 (34.8)	8 (34.8)	0.897	8 (47.1)	17 (36.2)	17 (37.8)	0.725	5(55.6)	6 (35.3)	33 (50.8)	0.472
Dyspnea (shortness of breath)	46 (42.6)	33 (47.8)	13 (56.5)	0.444	11 (64.7)	41 (87.2)	44 (97.8)	0.002	9 (100)	16 (94.1)	60 (92.3)	0.678
Ear pain	30 (27.8)	13 (18.)	6 (26.1)	0.396	8 (47.1)	8 (17.0)	17 (37.8)	0.025	4 (44.4)	7 (41.2)	28 (43.1)	0.985
Chest pain	49 (45.4)	34 (31.5)	10 (43.5)	0.838	12 (70.6)	34 (72.3)	29(64.4)	0.706	7 (77.8)	11 (64.7)	48 (73.8)	0.704
Arthralgia (Joints pain)	74 (68.5)	51 (73.9)	16 (69.6)	0.741	12 (70.6)	41 87.2)	34 (75.6)	0.222	8 (88.9)	12 (70.6)	50 (76.9)	0.574
Headache	87 (80.6)	52 (75.4)	16 (69.6)	0.452	13 (76.5)	29 (61.7)	26 (57.8)	0.396	7 (77.8)	11 (64.7)	44 (67.7)	0.785
Nausea /vomiting	27 (25.0)	17 (24.6)	7 (30.4)	0.167	4 (23.5)	18 (38.3)	19 (42.2)	0.640	2 (22.2)	6 (35.3)	21 (32.3)	0.392
Abdominal pain	42 (38.9)	26 (37.7)	12 (52.2)	0.443	10 (58.8)	19 (40.4)	21 (46.7)	0.423	6 (66.7)	10 (58.8)	35 (53.8)	0.743
Diarrhea	52 (48.1)	33 (47.8)	13 (56.5)	0.744	7 (41.2)	23 (48.9)	19 (42.2)	0.765	6 (66.7)	9 (52.9)	34 (52.3)	0.718
Loss of appetite	61 (56.5)	34 (49.3)	16 (69.6)	0.277	12 (70.6)	30 (63.8)	31 (68.9)	0.825	7 (77.8)	13 (76.5)	48 (73.8)	0.952
Face pain/ heaviness	29 (26.9)	19 (27.5)	8 (34.8)	0.740	7 (41.2)	18 (38.3)	16 (35.6)	0.913	4 (44.4)	9 (52.9)	33 (50.8)	0.917
Mental disorders	2 (1.9)	1 (1.4)	2 (8.7)	0.127	1 (5.9)	0 (0.0)	0 (0.0)	0.065	0 (0.0)	0 (0.0)	0 (0.0)	0.747
Retro-ocular headache	85 (78.7)	57 (82.6)	19 (82.6)	0.786	16 (94.1)	47 (100.0)	45 (100.0)	0.065	9 (100)	17 (100)	64 (98.1)	0.817

Table 4.7: Clinical symptoms of COVID-19 patients at time of diagnosis (at registration or baseline) based on disease severity and SARS-CoV2 variants, Alpha versus Delta. Data are shown in numbers (n) and percentages (between brackets).

Symptoms	Mild / moderate, n (%)			Severe, n (%)			Critical, n (%)		
	Alpha (n=186)	Delta (n=14)	P- value	Alpha (n=96)	Delta (n=13)	P-value	Alpha (n=61)	Delta (n=30)	P-value
Fever (>38°C)	91 (48.9)	12 (85.7)	0.008	65 (67.7)	13 (100.0)	0.015	41 (67.2)	30 (100.0)	0.000
Loss of smell	97 (51.1)	11 (78.6)	0.056	42 (43.8)	11 (84.6)	0.006	22 (36.0)	25 (83.0)	0.000
Taste dysfunction	96 (51.6)	11 (78.6)	0.051	41 (42.7)	10 (76.9)	0.020	20 (33.0)	25 (83.0)	0.000
Chills or shivering	128 (68.0)	7 (50.0)	0.147	68 (70.8)	13 (100.0)	0.024	44 (72.0)	29 (97.0)	0.006
Fatigue	151 (81.2)	7 (50.0)	0.006	79 (82.3)	13 (100.0)	0.099	47 (77.0)	30 (100.0)	0.004
Myalgia (Muscle ache)	143 (76.9)	7 (50.0)	0.025	78 (81.3)	13(100.0)	0.088	53 (87.0)	30 (100.0)	0.038
Sore throat	109 (58.6)	3 (21.4)	0.007	61 (63.5)	13 (100.0)	0.008	33 (54.0)	29 (97.0)	0.000
Cough	126 (67.7)	5 (35.7)	0.015	72 (75.0)	13 (100.0)	0.041	5 (183.6)	29 (97.0)	0.072
Nasal obstruction	103 (55.4)	4 (28.6)	0.052	43 (44.8)	11 (84.6)	0.007	25 (41.0)	27 (60.0)	0.000
Rhinorrhea (runny nose	69 (37.1)	4 (28.6)	0.523	30 (31.3)	12 (92.3)	0.000	18 (29.5)	26 (86.7)	0.000
Dyspnea (shortness of breath)	88 (47.3)	4 (28.6)	0.175	93 (85.5)	13(100.0)	0.157	55 (90.2)	30 (100.0)	0.076
Ear pain	48 (25.8)	1 (7.1)	0.117	23 (24)	10 (76.9)	0.000	16 (26.2)	23 (76.7)	0.000
Chest pain	90 (48.4)	3 (21.4)	0.051	62 (64.6)	13 (100.0)	0.010	37 (60.7)	29 (96.7)	0.000
Arthralgia (Joints pain)	135 (72.6)	6 (42.9)	0.019	74 (77.1)	13(100.0)	0.053	42 (68.9)	28 (93.3)	0.009
Headache	144 (77.4)	11 (78.6)	0.921	56 (58.3)	12 (92.3)	0.018	35 (57.4)	27 (90.0)	0.002
Nausea /vomiting	52 (28.0)	2 (14.3)	0.267	39 (41.0)	8 (62.0)	0.153	25 (41.0)	27 (90.0)	0.000
Abdominal pain	77 (41.4)	3 (21.4)	0.141	43 (45.0)	7 (54.0)	0.539	25 (41.0)	26 (87.0)	0.000
Diarrhea	93 (50.0)	5 (35.7)	0.302	41 (43.0)	8 (62.0)	0.200	26 (43.0)	23 (77.0)	0.002
Loss of appetite	102 (54.8)	9 (64.3)	0.493	62 (65.0)	11 (85.0)	0.150	39 (64.0)	29 (97.0)	0.001
Face pain/ heaviness	53 (28.5)	3 (21.4)	0.570	30 (31)	11 (85.0)	0.000	21 (34.0)	25 (83.0)	0.000
Mental disorders	5 (2.7)	0 (0.0)	0.534	1 (1.0)	0 (0.0)	0.712	2 (3.3)	3 (10.0)	0.186
Retro-ocular headache	154 (82.8)	7 (50.0)	0.03	95 (99.0)	13 (100.0)	0.712	60 (98.4)	30 (100.0)	0.481

4.4 Association of COVID-19 disease severity with hematological markers

Analysis of the association between hematological biomarkers and disease severity revealed that there was a statistically significant association between most hematological markers and WBCs ratios and disease severity, except for the MCV, MCH, MCHC and WBCs. In particular, there is a gradual decrease in RBCs, Hb, Hct, lymphocytes, LY/Mon ratio, platelets and a gradual increase in monocytes, granulocytes, GR/Mon ratio, GR/Ly ratio, PT, INR and D-dimer levels that parallel the increase in disease severity (Table 4.8). The increase in granulocytes and monocytes reflects the reaction of the body to the viral infection. The decrease in lymphocytes reflects the dysfunction of the host to respond to the infection state. The increase in D-dimer reflects an activation of the coagulation process.

Table 4.8: Hematological characteristics of COVID-19 patients at time of diagnosis.

Parameter	Mild /moderate (n=200)			Severe (n=109)			Critical (n=91)			P- value
	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	
CBC										
RBC, x10E6/µL	5.0±0.6 [a,b]	3.4	6.3	4.8±0.6 [a,c]	2.7	6.1	4.3±0.8 [b,c]	2.4	5.7	0.000
Hb, g/dL	13.6±1.9 [b]	9.1	16.9	13.2±1.8	8.2	16.5	12.8±2.2 [b]	7.9	16.9	0.003
0Hct, %	41.5±5.1 [b]	27.8	51.3	40.5±5.3 [c]	25.2	48.8	36.3±6.9 [b,c]	20.5	51.3	0.000
MCV, fL	83.1±6.0	52.4	92.1	83.8±5.4	68.9	97.9	84.2±8.7	56.2	110	0.418
MCH, pg	27.3±2.3	19.6	31.6	27.5±2.0	22.7	31.5	27.5±3.0	17.6	32.2	0.495
MCHC, g/dL	32.8±1.3	24.0	36.1	32.9±1.1	30.2	38.4	32.6±1.3	25.2	35.7	0.510
RDW, %	12.3±1.3 [a,b]	10.5	16.4	13.4±1.7 [a,c]	11.1	20.6	14.3±2.5 [b,c]	11.5	25.2	0.000
WBC, k/µL	8.9±4.4	2.1	29.1	9.0±4.2	2.6	25.4	8.2±3.7	3.8	22.2	0.404
Lymphocytes, k/µL	2.2± 0.8 [a,b]	0.5	6.7	1.7±1.4 [a,c]	0.3	9.0	1.3±0.9 [b,c]	0.2	5.9	0.000
Monocytes, k/µL	0.3±0.2 [a,b]	0.1	1.3	0.5±0.5 [a,c]	0.1	2.6	0.7±0.6 [b,c]	0.1	2.8	0.000
Granulocytes, k/µL	5.0±2.3 [a,b]	1.3	14.5	7.6±4.5 [a,c]	2.2	27.9	9.2±5.7 [b,c]	1.5	28.2	0.000
LY/Mo ratio	8.6±3.7 [a,b]	1.3	22.0	5.5±5.2 [a,c]	0.3	35.0	3.9±3.6 [b,c]	0.2	16.0	0.000
GR/LY ratio	2.6±1.9[a,b]	0.5	17.3	7.0±5.8 [a,c]	0.6	30.7	10.0±8.5 [b,c]	1.0	56.5	0.000
GR/Mo ratio	20.1±12.5 [a,b]	4.1	103.0	27.7±27.5 [a]	3.3	115	27.9±31.3 [b]	3.6	194.0	0.003
Platelets, k/µL	256.6±76.4 [b]	53.0	501.0	239.0±97.9	37	499	221.8±103.9 [b]	20.0	513.0	0.000
Coagulation										
PT, seconds	13.3±2.1 [a,b]	10.0	22.0	14.4±2.5 [a]	10.1	25.0	14.8±3.1 [b]	11.0	29.5	0.000
INR	1.0±0.3 [a,b]	0.6	3.2	1.1±0.2 [a,c]	0.7	2.8	1.1±0.3 [b]	0.8	3.8	0.000
PTT, seconds	38.6±9.3	22.0	84.0	37.1±7.6 [a,c]	24.0	76.0	37.9±10.0 [b,c]	20.0	70.	0.666
D-Dimer, ng/mL	297.9±377.7 [a,b]	35.0	2808.0	2137.0±2729.0 [a]	86.0	10106	1747.0±2172.4 [b]	91.0	9966	0.000

ONE way ANOVA was used for mean comparisons among groups, and differences within each group was analyzed by LSD Post Hoc test: (a) mild/moderate vs. sever; (b) mild vs. critical; (c) sever vs. critical.

In addition, we analyzed the association between disease severity and hematological biomarkers based on sex (Table 4.9), age groups (Table 4.10) and SARS-CoV2 variants (Table 4.11). As shown in Table 4.9, there are statistically significant differences in the means of some markers between females and males within the mild/moderate, severe and critical groups. Most of these differences include the RBCs count and RBC indices, which normally show sex-based differences. However, males show a significantly higher lymphocyte count (p-value = 0.020) in the severe group compared to females. In addition, females in the mild/ moderate group showed significantly higher WBC count (p-vale = 0.015) compared to males. Otherwise, there were no significant differences observed between females and males in all other hematological biomarkers analyzed here.

Table 4.9: Hematological characteristics of COVID-19 patients based on disease severity and sex.

Parameter	Mild /moderate, mean ±SD			Severe, mean ±SD			Critical, mean ±SD		
	Female (n=106)	Male (n=94)	P-value	Female (n=41)	Male (n=68)	P-value	Female (n=49)	Male (n=42)	P-value
CBC									
RBC, x10E6/µL	4.8±0.5	5.3±0.5	0.000	4.5±0.5	5.0±0.7	0.000	4.1±0.8	4.4±0.8	0.142
Hb, g/dL	12.6±1.5	14.9±1.5	0.000	12.2±1.6	13.9±1.8	0.000	11.9±1.9	13.8±1.9	0.000
Hct, %	38.7±4.4	45.0±4.6	0.000	37.7±5.1	42.1±6.0	0.000	33.7±6.3	37.5±7.4	0.009
MCV, fL	81.0±7.7	84.9±4.2	0.000	83.1±6.7	84.3±5.4	0.327	81.6±9.8	85.8±5.5	0.016
MCH, pg	26.3±2.9	28.2±1.7	0.000	27.1±2.7	27.8±2.0	0.112	26.5±3.3	28.5±2.0	0.001
MCHC, g/dL	32.3±1.5	33.2±1.0	0.000	32.7±1.3	33.0±1.0	0.140	32.4±1.5	33.2±1.2	0.004
RDW, %	12.7±1.4	11.9±1.0	0.000	13.7±1.9	13.2±1.6	0.178	14.6±2.8	13.9±2.1	0.172
WBC, k/µL	9.6±5.0	8.1±3.4	0.015	9.2±4.7	8.9±4.1	0.714	8.2±3.9	8.3±3.5	0.835
Lymphocytes, k/µL	2.1±0.7	2.2±0.9	0.366	1.3±0.8	2.0±1.6	0.020	1.3±0.9	1.3±0.9	0.728
Monocytes k/µL	0.3±0.1	0.3±0.2	0.090	0.5±0.5	0.5±0.4	0.500	0.6±0.6	0.7±0.7	0.285
Granulocytes, k/µL	4.9±2.2	5.0±2.3	0.684	7.4±5.4	7.8±3.9	0.718	9.2±5.4	9.3±6.0	0.910
Ly/Mo ratio	8.8±3.8	8.3±3.5	0.370	4.2±3.7	6.3±5.8	0.039	4.3±4.0	3.3±3.1	0.091
GR/LY ratio	2.6±2.0	2.6±1.9	0.885	7.9±6.6	6.4±5.3	0.205	9.7±9.1	10.4±7.7	0.703
GR/Mo ratio	20.5±12.9	19.6±12.1	0.619	24.1±24.9	29.9±28.9	0.288	31.3±37.1	23.8±22.1	0.267
Platelets, k/µL	262.6±78.4	248.8±74.0	0.204	250.3±107.0	232.6±92.1	0.362	238.5±108.1	202.2±96.5	0.101
Coagulation									
PT, seconds	13.2±1.7	13.6±2.5	0.206	14.6±2.8	14.3±2.4	0.581	14.9±3.4	14.7±2.9	0.817
INR	1.0±0.3	1.1±0.3	0.548	1.1±0.3	1.1±0.3	0.736	1.3±0.6	1.2±0.3	0.300
PTT, seconds	38.2±9.8	39.0±8.7	0.216	39.1±8.8	36.9±8.1	0.187	39.6±12.2	37.6±9.4	0.367
D-Dimer, ng/mL	320.0±280.0	272.8±465.2	0.380	18560±2441	2327±2875	0.387	16665±1950	1850±2458 .	0.702

Independent Sample t-test was used for mean comparisons between groups, and differences within each group was analyzed by LSD Post Hoc test: (a) mild/moderate vs. sever; (b) mild vs. critical; (c) sever vs. critical.

Analysis of the association between hematological biomarkers and disease severity based on age groups, revealed that there was a significantly gradual increase in RDW (p-value = 0.000) and D-dimer (p-value = 0.000) with increasing age within the mild/moderate group (Table 4.10). In addition, there were significant differences in WBC count and platelet count among age groups within the severe group (Table 4.10).

Table 4.10: Hematological characteristics of COVID-19 patients at time of diagnosis based on disease severity and age groups (years).

Parameter	Mild / moderate, mean ±SD				Severe, mean ±SD				Critical, mean ±SD			
	14-39 (n=108)	40-59 (n=69)	≥60 (n=23)	P-value	14-39 (n=17)	40-59 (n=47)	≥60 (n=45)	P-value	14-39 (n=9)	40-59 (n=17)	≥60 (n=65)	P-value
CBC												
RBC, x10E6/µL	5.0±0.5	5.0±0.5	4.8±0.6	0.217	4.8±0.8	5.0±0.6	4.7±0.6	0.075	4.2±0.6	4.6±0.8	4.3±0.7	0.465
Hb, g/dL	13.8±1.8	13.6±2.0	13.2±2.0	0.396	13.3±2.3	13.6±1.6	13.1±1.8	0.376	13.5±2.3	12.6±2.9	12.8±2.0	0.713
Hct, %	41.9±4.7	41.4±5.8	40.0±5.0	0.278	40.1±6.5	41.6±5.1	39.6±5.0	0.215	34.3±4.3	37.8±8.6	36.4±6.8	0.615
MCV, fL	83.1±5.9	83.3±6.5	82.6±5.1	0.908	84.7±4.1	83.0±5.7	84.3±5.5	0.448	82.5±4.2	81.4±8.6	85.0±8.7	0.386
MCH, pg	27.4±2.1	27.3±2.6	26.9±2.5	0.677	28.0±1.4	27.2±2.2	27.8±2.0	0.297	26.7±1.7	26.5±3.3	27.8±3.0	0.306
MCHC, g/dL	32.8±1.3	32.±1.3	32.8±1.5	0.984	33.1±0.9	32.9±1.3	33.0±1.0	0.826	32.4±1.1	32.6±1.1	32.7±1.4	0.844
RDW, %	11.9±1.0 [a,b]	12.6±1.5 [a]	13.0±1.2 [b]	0.000	13.1±1.4	13.2±1.6	13.5±1.8	0.636	13.2±0.8	14.2±2.4	13.7±2.0	0.718
WBC, k/µL	9.1±4.1	8.5±4.1	9.2±4.7	0.704	8.1±3.0	10.1±5.1 [c]	8.0±3.1 [c]	0.048	7.8±3.1	7.4±1.9	8.6±3.8	0.493
Lymphocytes, k/µL	2.3±0.9	2.0±0.6	2.2±0.7	0.063	1.5±1.0	1.8±1.3	1.8±1.7	0.804	1.3±0.4	1.6±0.9	1.2±0.91	0.441
Monocytes k/µL	0.3±0.2	0.3±0.2	0.3±0.2	0.865	0.6±0.5	0.5±0.4	0.5±0.5	0.726	0.4±0.5 [b]	1.0±0.9	0.6±0.0.6	0.068
Granulocytes, k/µL	4.8±2.2	5.2±2.3	5.2±2.0	0.441	7.2±4.0	7.4±4.0	8.3±5.4	0.587	11.3±9.0	11.4±7.3	8.1±4.3	0.091
Ly/Mo ratio	9.2±3.7	8.0±3.8	8.5±3.3	0.127	4.1±3.8	5.0±3.8	6.5±6.9	0.268	7.2±5.1	3.4±4.3	4.0±3.3	0.096
GR/LY ratio	2.3±1.7	3.0±2.3	2.5±1.0	0.115	7.3±7.4	6.9±5.9	7.1±5.9	0.972	8.8±8.0	9.5±8.0a,	9.5±7.0	0.973
GR/Mo ratio	19.5±12.6	21.7±14.2	20.2±8.9	0.539	19.5± 16.3	26.9±27.1	32.0±30. 2	0.324	46.8±31.7.	25.9±35.4	26.7±24.7	0.227
Platelet, k/µL	257.0± 76.4	254.0± 89.1	262.6± 76.4	0.903	239.4± 114.4	268.0± 86.8 [c]	209.3± 93.0 [c]	0.020	203.3± 64.4	227.8± 101.0	237.7± 102.5	0.718
Coagulation												
PT, seconds	13.5±2.3	13.2±2.1	13.1±1.5	0.455	14.0±3.4	14.2±2.0	14.2±2.4	0.947	13.8±1.5	15.8±2.9	14.6±3.5	0.420
INR	1.0±0.2	1.0±0.2	1.0±0.1	0.691	1.1±0.3	1.1±0.3	1.1±0.2	0.874	1.1±0.2	1.2±0.2	1.1±0.3	0.912
PTT, seconds	38.4±7.6	40.0±11.9	36.7±5.5	0.310	34.8±5.3	36.8±6.6	38.0±9.0	0.388	36.9± 10.0	37.1± 10.5	38.3±10.0	0.912
D-Dimer, ng/mL	189.± 148.7 [a,b]	403.1± 543 .1 [a]	525.7± 484 ·6 [b]	0.000	2023.± 2631.5	1816.5± 2671.5	2504.5± 2840.4	0.515	850.0± 712.6	2252.2± 3107.1	1734.9± 20 27.3	0.493

One way ANOVA was used for comparison of means between groups. For comparison of means within groups, the LSD Post Hoc test was used and statistically significant tests are labeled as follows: a: group 1 (14-39) vs group 2 (40-59); b: group 2 (40-59) vs group 3 (≥60); c: group 1 (14-39) vs. group 3 (≥60).

Investigation of the association of hematological markers and disease severity based on SARS-CoV2 variants (Table 4.11) revealed that there are significant differences in granulocytes and GR/Ly ratio within the mild/ moderate group, and in Hb levels within the critical group (Table 4.11).

Table 4.11: Hematological characteristics of COVID-19 patients at time of diagnosis based on disease severity and on SARS-CoV2 variants, Alpha versus Delta.

Parameter	Mild /moderate, mean ±SD			Severe, mean ±SD			Critical, mean ±SD		
	UK-mut (n=186)	Delta-mut (n=14)	P-value	UK-mut (n=96)	Delta-mut (n=13)	P-value	UK-mut (n=61)	Delta-mut (n=30)	P-value
CBC									
RBC, x10E6/µL	5.0±0.6	5.0±0.3	0.467	4.8±0.7	4.8±0.6	0.905	4.3±0.8	4.3±0.7	0.884
Hb, g/dL	13.7±1.9	13.5±1.9	0.829	13.3±1.8	13.4±1.9	0.878	13.3±2.1	11.9±2.1	0.005
Hct, %	41.5±5.2	40.8±4.4	0.623	40.4±5.3	40.±5.8	0.904	36.3±7.4	35.6±5.9	0.678
MCV, fL	83.1±6.0	83.2±5.7	0.936	83.9±5.3	84.3±6.8	0.809	84.2±7.1	83.5±10.6	0.748
MCH, pg	27.3±2.3	27.6±2.9	0.685	27.6±2.0	27.8±2.5	0.732	27.8±2.8	27.1±3.2	0.306
MCHC, g/dL	32.8±1.3	33.1±1.9	0.442	32.9±1.1	32.9±0.8	0.988	32.7±1.7	32.8±0.9	0.793
RDW	12.2±1.3	12.9±1.3	0.080	13.4±1.8	12.8±0.7	0.298	14.0±2.2	14.±2.0	0.854
WBC, k/µL	8.8±4.2	10.5±4.2	0.148	8.9±4.3	9.2±3.3	0.823	8.1±3.2	8.7±4.0	0.708
Lymphocytes, k/µL	1.9±1.1	1.5±1.1	0.695	1.8±1.5	1.1±0.8	0.109	1.2±0.7	1.4±1.1	0.355
Monocytes, k/µL	0.4±0.4	0.4±0.4	0.445	0.5±0.5	0.4±0.2	0.352	0.7±0.7	0.5±0.5	0.304
Granulocytes, k/µL	4.8±6.9	6.9±3.5	0.001	7.8±4.9	7.3±2.6	0.693	9.0±5.9	9.9±5.2	0.527
Ly/Mo ratio	8.8±3.7	7.4±3.6	0.202	5.7±5.5	3.7±3.8	0.219	3.5±3.1	5.1±4.5	0.076
GR/LY ratio	2.4±1.6	4.1±4.1	0.001	6.8±5.5	9.9±8.0	0.094	9.7±6.7	10.1±7.3	0.822
GR/Mo ratio	20.0±12.6	25.0±15.4	0.175	27.8±27.1	28.2±29.6	0.963	27.5±33.4	33.6±30.6	0.146
Platelets, k/µL	257.1±76.8	249.5±72.6	0.730	240.4±98.8	221.7±79.3	0.531	226.2±99.1	242.4±103.4	0.433
Coagulation									
PT, seconds	13.3±2.1	13.7±2.4	0.528	14.3±2.5	13.4±1.8	0.197	15.0±3.5	14.2±2.2	0.251
INR	1.0±0.2	1.1±0.3	0.802	1.1±0.2	1.0±0.3	0.139	1.2±0.3	1.2±0.1	0.061
PTT, seconds	38.6±8.7	40.6±14.0	0.459	36.7±7.6	39.7±6.8	0.188	38.0±10.1	36.9±9.4	0.639
D-Dimer, ng/mL	297.5±30.7	344.1±297.1	0.681	921.8±1669.6	1637.2±2388.9	0.029	1642.1±2106.9	1941.5±2320.9	0.583

Independent Sample t-test was used for mean comparisons.

The association between ABO and RhD phenotypes and COVID-19 disease severity was investigated and data are summarized in Table 4.12. Our results indicate that there were no significant association between disease severity and ABO or RhD phenotypes.

Table 4.12 ABO and RhD phenotypes of COVID-19 patients based on disease severity.

Parameter	**Mild /moderate (n=200)**		**Severe (n=109)**		**Critical (n=91)**		**P- value**
	n	**%**	**n**	**%**	**n**	**%**	
ABO blood group							0.053
• O type	65	32.5%	26	23.9%	21	23.1%	
• A type	86	43.0%	64	58.7%	56	61.5%	
• B type	34	17.0%	13	11.9%	8	8.8%	
• AB type	15	7.5%	6	5.5%	6	6.6%	
Rh(D) phenotype							0.144
• Positive	180	90.0%	99	90.0%	88	96.7%	
• Negative	20	10.0%	10	9.2%	3	3.3%	

Statistical analysis was performed using Chi-square test.

4.5 Association of COVID-19 disease severity with biochemical markers

Analysis of the association between COVID-19 disease severity and biochemical markers are shown in Table 4.13. There were statistically significant differences among patient groups and almost all biochemical markers except for total cholesterol and bilirubin. Serum total protein and albumin levels were deteriorating with increased disease severity. The CRP and serum ferritin both are positive acute phase reactant proteins and their levels increased parallel to the increase in disease severity and thus confirming a continued inflammation state as the disease severity increases. The fasting plasma glucose level also increased parallel to the increase in disease severity; and this confirms the findings in Table 3.2 that patients with other comorbidities and in particular, diabetes mellitus are more vulnerable to develop severe and critical COVID-19 disease. In addition, several serum enzymes increased parallel to the increase in disease severity indicating the progress of a general inflammation process (LDH) and involvement of the liver (ALT, AST, ALP), bone (ALP), pancreas (amylase), kidney (creatinine, BUN) and cardiac tissues (CK-total, AST) (Table 4.13). There were a decrease in TSH and an abnormal increase in PTH parallel to increased disease severity, indicating an involvement of these endocrine glands (Table 4.14). Although the decrease in TSH is still within the reference range (reference range for TSH in adults: 0.5-5.0 mIU/L) but it its decrease was associated with an increase in disease severity. However, the increase in PTH was above the reference range (reference range for adults for PTH: 10-55 pg/mL) and this increase was associated with disease severity. The changes in biochemical markers indicate deterioration of the organ function. Indeed, review of the medical records after data collection, revealed that most patients in the critical group, except two patients, have died. These findings indicate that the continued worsening of biochemical (Table 4.13) and hematological (Table 4.8) markers is a bad prognosis.

Table 4.13: Biochemical characteristics of COVID-19 patients at time of diagnosis based on disease severity.

Parameter	Mild / moderate (n=200)			Severe (n=109)			Critical (n=91)			P-Value
	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	
Total protein, g/dL	7.3±0.7 [a,b,]	4.23	8.26	6.9±0.7 [a]	4.36	8.3	7.0± 0.8 [b]	4.74	8.5	0.000
Albumin, g/dL	4.3±0.5	2.25	5.35	3.6±0.6 [a,c]	2.16	4.69	3.4±0.5 [b,c]	1.97	4.86	0.000
CRP, mg/L	10.3±26.9 [a,b,]	1.06	301.1	97.1±84.8 [a,c]	1.78	329	168.7±113.0 [b,c]	151	388	0.000
Fasting plasma glucose, mg/dL	98.6±33.5 [a,b,]	70	307.9	156.8±75.7 [a]	74.6	392.5	163.7±77.0 [b,c]	70	408	0.000
Total cholesterol, mg/dL	178.0±40.2	68.6	291.9	179.7±44.9	84.5	308	174.8±48.2	60	291	0.720
TG, mg/dL	162.0±94.3 [a,b,]	33	495	211.2± 90.9 [a]	54.9	477.7	192.2±72.8 [b]	70	420	0.000
LDH, mg/dL	175.0±59.2 [a,b,]	81	527	371.5±182.4 [a,c]	120	918	432.7± 179.9 [b,c]	99	913	0.000
ALT IU/L	23.1±22.4 [a,b,]	5.6	168.7	31.4±25.3 [a]	5.4	153	37.2±27.7 [b,c]	6.2	138	0.000
AST, IU/L	19.8±9.8 [a,b,]	8.3	75.9	32.7±18.6 [a]	9.3	99.00	34.2±18.8 [b]	10.0	109	0.000
Amylase, IU/L	69.2±23.4	22	149	64.6±20.0 [c]	27	131	73.0±27.9 [c]	20	147	0.042
ALP, IU/L	82.1±33.9 [a,b,]	33	245	99.8±48.6 [a]	20	275	109.8±59.6 [b]	27	290	0.000
CK-total, IU/L	93.9±87.0 [a,b,]	15	695	157.3±146.2 [a]	15	791	192.9±201.7 [b]	18	764	0.000
Creatinine, mg/dL	0.8±0.2 [a,b,]	0.58	1.78	1.0±0.5 [a]	0.56	2.78	1.1±0.5 [b]	0.55	2.59	0.000
BUN, mg/dL	16.4±5.7 [a,b,]	9.4	71.0	24.0±11.1 [a]	10.9	59.0	24.6± 13.5	10.5	64.0	0.000
Calcium, mg/dL	9.4±0.6 [a,b,]	7.35	10.49	9.1±0.6 [a,c]	7.51	10.9	8.84±0.68 [b,c]	7.59	10.43	0.000
Total bilirubin, mg/dL	0.54±0.28	0.12	1.62	0.5± 0.2	0.12	1.33	0.5±0.31	0.12	1.62	0.292
Direct bilirubin, mg/dL	0.17±0.11	0.12	1.62	0.2±0.1	0.10	0.82	0.2±0.1	0.10	0.9	0.056
Serum ferritin, ng/mL	149.1±199.3 [a,b,]	10.3	1702.2	669.6±586.1 [a,c]	14.3	2393	969.0±932.5 [b,c]	11.7	3389	0.000
Hormones										
TSH, mU/L	1.7±1.1 [a,b,]	0.1	7.59	1.3±1.0 [a,c]	0.1	4.9	0.9±0.9 [b,c]	0.11	4.5	0.000
PTH, pg/mL	69.0±32.5[b]	24	213	81.0±51.0 [c]	18	691	148.0± 110.0 [b,c]	32.1	691.1	0.000
Serology										
IgG (after 3 weeks of infection), IU/mL	115.2±105.3 [a,b,]	2.98	250	158.2±101.2 [b]	4.06	250	184.1±88.2 [b]	3.58	250	0.000

ONE way ANOVA was used for mean comparisons among groups, and differences within each group was analyzed by LSD Post Hoc test: (a) mild/moderate vs. sever; (b) mild vs. critical; (c) sever vs. critical.

Investigation of the immune response (IgG levels) in the study patients showed statistically significant differences (p-value = 0.000) among the three patients groups. Indeed patients who experienced severe or critical COVID-19 disease developed a higher immune response reflected by the increase in IgG levels at 3 weeks after disease onset (Table 4.13). However, this increase in immune response apparently was not able to limit the disease process or induce a clearance of the virus from the body.

Analysis of the association between biochemical markers and diseases severity based on sex (Table 4.14) showed that there were significant differences between females and males mainly in the mild/ moderate group and involved serum albumin, ALT, AST, amylase, CK-total, serum creatinine, bilirubin and ferritin. However, these differences did not extend to the severe and critical groups for the aforementioned markers, except for serum albumin and CK-total that showed sex-related differences in the mild/moderate (both CK-total and Albumin), severe (CK-total) and critical groups (Albumin). (Table 4.14).

Table 4.14: Biochemical characteristics of COVID-19 patients at time of diagnosis based on disease severity and sex. Data are shown as mean ± SD. Independent Sample t-test was used for mean comparisons.

Parameter	Mild / moderate, mean ±SD			Severe, mean ±SD			Critical, mean ±SD		
	Female (n=106)	Male (n=94)	P-value	Female (n=41)	Male (n=68)	P- value	Female (n=49)	Male (n=42)	P-value
Total protein, g/dL	7.2±0.7	7.3±0.6	0.277	7.0±0.6	6.8±0.7	0.32	7.0±0.9	7.0±0.7	0.852
Albumin, g/dL	4.2±0.5	4.4±0.5	0.025	3.6±0.6	3.6±0.6	0.693	3.3±0.0.7	3.6±0.6	0.020
CRP, mg/L	8.0±12.7	12.9±36.9	0.202	111.4±86.6	88.5±83.2	0.178	159.3±105.6	180.1±121.4	0.391
Fasting plasma glucose, mg/dL	102.7±40.6	93.9±22.2	0.063	140.3±60.9	167.0±82.3	0.079	163.2±76.6	159.1±84.8	0.674
Total cholesterol, mg/dL	178.6±38.3	177.4±42.4	0.839	172.9±47.3	183.4±162.1	0.217	168.6±46.8	173.3±39.7	0.779
TG, mg/dL	161.9±97.4	162.2±91.0	0.979	215.7±92.4	208.5±90.5	0.695	202.8±78.6	188.5±79.7	0.639
LDH, mg/dL	170.0±41.6	180.8±74.1	0.200	371.0±173.9	371.7±188.6	0.985	431.4±182.8	434.6±178.6	0.941
ALT IU/L	19.9±181.0	26.7±25.2	0.032	30.7±25.1	41.2±28.6	0.057	28.5±23.2	34.9±28.0	0.238
AST, IU/L	18.2±8.8	21.6±10.6	0.016	32.6±20.6	32.7±17.5	0.972	34.4±18.4	33.9±19.5	0.909
Amylase, IU/L	63.7±22.4	75.2±23.1	0.000	61.0±21.5	66.7±18.9	0.152	71.0±24.7	75.7±31.8	0.445
ALP, IU/L	79.9±28.5	84.5±39.2	0.339	45.3±7.2	50.8±6.2	0.882	110.8±61.3	107.8±58.4	0.820
CK-total, IU/L	71.1±53.7	119.3±107.8	0.000	111.6±99.9	183.4±162.1	0.014	163.8±196.6	227.1±204.8	0.151
Creatinine, mg/dL	0.7±0.1	0.9±0.2	0.000	0.9±0.4	1.0±0.4	0.197	1.0±0.5	1.1±0.41	0.396
BUN, mg/dL	16.4±3.8	16.5±7.3	0.858	21.5±11.1	25.4±10.9	0.082	23.2±12.2	27.0±12.2	0.770
Calcium, mg/dL	9.3±0.6	9.4±0.6	0.126	9.2±0.7	9.0±0.6	0.34	8.8±0.8	8.9±0.6	0.559
Total bilirubin, mg/dL	0.4±0.2	0.52±0.31	0.000	0.5±0.3	0.5±0.2	0.57	0.5±0.3	0.6±0.3	0.214
Direct bilirubin, mg/dL	0.2±0.1	0.19±0.13	0.036	0.2±0.2	0.2±0.1	0.959	0.2±0.1	0.2±0.1	0.284
Serum ferritin, ng/mL	95.1±142.1	209.9±234.7	0.000	568.5±617.5	730.9±562.2	0.168	869.4±898.6	1086.0±969.4	0.83
Hormones									
TSH, mU/L	1.7±1.1	1.64±1.2	0.567	1.4±1.1	1.24±1.02	0.456	1.0±1.0	0.9±0.7	0.518
PTH, pg/mL	70.7±33.1	67.1±31.8	0.427	81.1±58.3	80.9±46.4	0.983	142.2±117.3	154.4±102.6	0.609
Serology									
IgG (after 3 weeks of infection), IU/mL	122.5±104.6	107.0±106.1	0.301	168.9±98.3	151.75±103.1	0.394	161.6±94.9	187.3±87.7	0.100

The association between biochemical markers and disease severity based on age groups (Table 4.15) showed statistically significant differences among age groups and in particular in the mild/ moderate group and involved the serum total proteins, albumin, FPG, total cholesterol, TG, LDH, ALT, AST, creatinine, BUN, serum ferritin and IgG levels. However, these differences did not extend to the severe and critical groups for most biochemical markers except for BUN. Whereas significant differences among age groups were observed for CRP in the severe group, CK-total in the critical group and BUN in the severe and critical groups.

Table 4.15: Biochemical characteristics of COVID-19 patients at time of diagnosis based on disease severity and age groups (year). Data are shown as mean ± SD.

Parameter	Mild/ moderate, mean ±SD				Severe, mean ±SD				Critical, mean ±SD			
	14-39 (n=108)	40-59 (n=69)	≥60 (n=23)	P-value	14-39 (n=17)	40-59 (n=47)	≥60 (n=45)	P-value	14-39 (n=9)	40-59 (n=17)	≥60 (n=65)	P-value
Total protein, g/dL	7.4±0.6 [a, b]	7.2±0.8 [a, b]	7.0±0.8 [b]	0.011	6.870.6	6.9±0.6	7.0±0.8	0.566	7.3±0.8	7.0±0.8	7.0±0.9	0.459
Albumin, g/dL	4.4±0.4 [a, b]	4.2±0.6 [a, c]	4.0±0.5 [b ,c]	0.000	3.7±0.6	3.7±0.5	3.5±0.7	0.383	3.3±0.5	3.5±0.7	3.4±0.7	0.408
CRP, mg/L	8.2±16.1	11.2±36.6	13.67±28.69	0.323	107.2±103.8	75.0±79.4	117.3±78.3	0.051	226.5±144.5	146.5±104.2	166.6±109.5	0.222
FPG, mg/dL	90.6±26.4.1 [, b]	107.2±38.0 [a]	111.0±39.8 [b]	0.001	119.2±26.9	162.6±0.5	165.7±67.6	0.079	117.0±27.0	192.1±81.7	162.7±77.8	0.058
Total cholesterol, mg/dL	170.9±35.2 [a]	188.4±44.8 [a]	180.3±42.2	0.017	194.3±31.9	176.8±44.9	177.2±49.1	0.346	182.8±62.5	180.1±38.9	171.92±48.71	0.737
TG, mg/dL	146.7±93.7 [a]	184.6±97.1 [a]	166.5±75.0	0.033	395.7±214.0	207.5±95.1	211.±88.3	0.884	217.9±66.75	187.0.1±90.1	190.10±68.45	0.575
LDH, mg/dL	165.4±41.1 [b]	179.4±62.0 [c]	207.7±100.0 [b, c]	0.005	395.7±214.0	336.5±189.5	398.0±158.9	0.231	550.3±130.7	421.4±184.6	418.3±180.8	0.116
ALT IU/L	19.4±15.3 [b]	26.2±22.28 [c]	32±42.2 [b, c]	0.020	45.7±24.7	37.2±32.6	33.5±22.9	0.311	40.6±30.1	40.9±38.7	27.64±19.5	0.084
AST, IU/L	17.6±6.2 [a, b]	21.1±10.4 [a, c]	26.1±16.5 [b,c]	0.000	37.4±17.9	31.3±16.2	32.3±21.1	0.509	43.4±15.5	31.4±12.6	33.5±20.3	0.279
Amylase, IU/L	65.8±20.9	72.5±25.0	74.6±28.0	0.095	63.5±19.3	64.6±21.6	65.0±19.0	0.966	78.9±20.4	75.9±33.0	71.6±27.5	0.719
ALP, IU/L	84.8±39.6	75.9±23.9	87.9±28.5	0.159	101.4±45.8	95.9±50.1	103.4±48.2	0.760	118.8±75.3	93.7±52.7	112.3±59.3	0.466
CK-total, IU/L	83.7±48.2	102.6±114.3	115.2±123.5	0.170	194.9±163.4	171.0±176.0	128.9±93.3	0.211	413.1±254.0 [a, b]	169.4±186.1 [a,]	174.1±187.3 [b]	0.009
Creatinine, mg/dL	0.8±0.2 [b]	0.8±0.1 [c]	0.9±0.2 [b, c]	0.027	0.8±0.1	0.9±0.4	1.1±0.5	0.125	0.8±0.3	0.9±0.3	1.2±0.5	0.055
BUN, mg/dL	15.6±4.0 [b,]	15.9±3.5 [c]	2.9±11. 8 [b, c]	0.000	17.5±4.1 [b]	22.0±8.8 [c]	28.8±13.2 [b, c]	0.000	14.7±4.1 [b]	19.2±8.2 [c]	27.5±14 .3 [b, c.]	0.006

Table 4.15: continued.

Parameter	Mild/ moderate, mean ±SD				Severe, mean ±SD				Critical, mean ±SD			
	14-39 (n=108)	40-59 (n=69)	≥60 (n=23)	P-value	14-39 (n=17)	40-59 (n=47)	≥60 (n=45)	P-value	14-39 (n=9)	40-59 (n=17)	≥60 (n=65)	P-value
Calcium, mg/dL	9.4±0.6	9.4±0.62	9.3±0.5	0.595	9.0±0.6	9.1±0.6	9.1±0.8	0.622	8.8±0.8	8.8±0.7	8.9±0.7	0.813
Total bilirubin, mg/dL	0.5±0.3	0.4±0.3	0.4±0.2	0.354	0.4±0.2	0.4±0.2	0.5±0.3	0.292	0.6±0.2	0.4±0.2	0.5±0.3	0.256
Direct bilirubin, mg/dL	0.2±0.1	0.2±0.1	0.2±0.1	0.781	0.2±0.1	0.2±0.1	0.2±0.1	0.186	0.3±0.1	0.2±0.1	0.21±0.1	0.062
Serum ferritin, ng/mL	106.1±118. 7 [a, b]	191.5±255.3 [a]	223.5±262.0 [b]	0.003	577.3±520.3	709.5±641.5	668.6±586.1	0.674	492.7±340.4	1012.1±1090.5	1020±934.4	0.319
Hormones												
TSH, mU/L	1.6±0.9	1.9±1.3	1.72±1.37	0.338	1.7±1.2	1.0±0.8	1.4±1.1	0.055	1.6±1.4	1.0±0.8	0.8±0.8	0.056
PTH, pg/mL	64.8±27.9	74.3±36.0	73.1±32.5	0.134	68.3±31.9	81.3±65.6	81.3±37.5	0.507	126.3±55.3	165.9±125.2	146.0±111.5	0.682
Serology												
IgG (after 3 weeks of infection), IU/mL	85.7±96.5 [a, b]	139.6±103.5 [a]	181.1±106.1 [b]	0.000	159.1±95.9	145.0±0.105.4	171.7±0.99.1	0.453	221.2±0.57.4	159.1±0.100.6	185.5±0.87.3	0.227

One way ANOVA was used for comparison of means between groups. For comparison of means within groups, the LSD Post Hoc test was used and statistically significant tests are labeled as follows: a: group 1 (14-39) vs group 2 (40-59); b: group 2 (40-59) vs group 3 (≥60); c: group 1 (14-39) vs. group 3 (≥60). FPG: fasting plasma glucose.

Analysis of the association between biochemical markers and disease severity based on SARS-CoV2 variants showed statistically significant differences for only few markers either in the mild/ moderate group (CRP, LDH, CK-total), severe group (amylase, BUN, calcium) and critical group (total protein, CK-total, direct bilirubin) (Table 4.16). However, none of these markers showed significant differences across the three patient groups. It is noteworthy that within the mild/moderate group patients infected with the Delta variant showed a statistically significant higher IgG levels (Table 4.16).

Table 4.16: Biochemical characteristics of COVID-19 patients at time of diagnosis based on disease severity and SARS-CoV2 variants, Alpha versus Delta. Data are shown as mean ± SD.

Parameter	Mild / moderate, mean ±SD			Severe, mean ±SD			Critical, mean ±SD		
	UK- mut (n=186)	Delta- mut (n=14)	P-value	UK- mut (n=96)	Delta- mut (n=13)	P-value	UK- mut (n=61)	Delta- mut (n=30)	P-value
Total protein, g/dL	7.3±0.7	7.2±0.5	0.543	6.9±0.5	7.2±0.4	0.177	6.8±0.8	7.7±0.5	0.001
Albumin, g/dL	4.3±0.5	4.1±0.6	0.126	3.7±0.6	3.7±0.4	0.968	3.5±0.6	3.5±0.5	0.795
CRP, mg/L	8.3±17.1	19.3±27.5	0.033	88.0±82.6	145.4±119.3	0.080	146.7±113.2	182.3±102.8	0.350
FPG, mg/dL	97.6±33.2	111.6±50.5	0.178	151.0±73.0	129.3±22.3	0.409	156.9±76.6	178.2±91.8	0.436
Total cholesterol, mg/dL	177.4±39.2	178.1±47.3	0.956	178.6±47.0	189.0±17.0	0.54	168.81±43.0	177.6±46.4	0.556
TG, mg/dL	156.9±93.5	173.6±62.8	0.545	211.5±88.8	206.2±61.6	0.869	205.1±83.2	164.2±48.9	0.127
LDH, mg/dL	172.5±59.6	216.2±70.0	0.016	337.5±177.3	441.0±220.8	0.131	394.3±181.2	512.3±138.6	0.051
ALT IU/L	22.1±21.2	25.3±14.9	0.616	37.3±29.4	57.0±22.3	0.069	23.1±11.9	24.3±17.9	0.785
AST, IU/L	18.8±8.5	23.8±12.2	0.060	30.9±16.6	38.2±15.0	0.239	28.7±14.1	37.4±17.1	0.088
Amylase, IU/L	69.4±23.3	71.7±16.7	0.503	62.7±19.2	83.3±13.4	0.044	67.8±27.2	73.5±26.6	0.543
ALP, IU/L	81.1±35.2	23.7±12.2	0.403	105.2±52.0	90.5±42.1	0.443	92.8±47.2	123.0±70.6	0.098
CK-total, IU/L	88.3±67.5	168.7±154.9	0.000	158.5±161.5	144.4±75.9	0.808	145.6±167.6	277.9±252.94	0.043
Creatinine, mg/dL	0.8±0.2	0.8±0.1	0.458	1.0±0.5	0.8±0.1	0.364	1.1±0.5	1.1±0.5	0.940
BUN, mg/dL	16.3±5.8	17.4±7.1	0.528	24.9±10.8	15.6±4.0	0.019	25.5±13.0	22.4±11.5	0.474
Calcium, mg/dL	9.4±0.6	9.4±0.7	0.931	9.2±0.6	8.7±0.3	0.014	8.9±0.6	8.5±0.4	0.474
Total bilirubin, mg/dL	0.5±0.3	0.3±0.1	0.086	0.5±0.2	0.4±0.1	0.557	0.5±0.3	0.46±0.21	0.872
Direct bilirubin, mg/dL	0.2±0.1	0.1±0.1	0.286	0.2±0.1	0.2±0.1	0.145	0.2±0.1	0.28±0.18	0.007
Serum ferritin, ng/mL	145.5±196.7	161.1±164.2	0.790	685.1±634.9	960.0±473.0	0.223	864.6±869.8	819.1±691.0	0.873
Hormones									
TSH, mU/L	1.7±1.2	1.6±1.0	0.829	1.3±0.9	1.0±1.2	0.437	1.1±0.9	1.3±1.3	0.463
PTH, pg/mL	68.8±31.9	78.4±41.7	0.323	81.1±57.6	75.4±36.0	0.571	128.2±80.7	149.6±105.18	0.467
Serology									
IgG (after 3 weeks of infection), IU/mL	108.5±103.3	217.0±62.0	0.000	166.7±98.9	116.8±92.0	0.177	166.1±95.3	198.7±75.7	0.300

Independent sample t-test was used for mean comparisons.

Chapter Five

Discussion

5.1 General characteristics

Since its declaration in March 2020, the COVID-19 pandemic has overburdened the healthcare system worldwide and affected all aspects of life. The majority of COVID-19 patients either showed mild symptoms or even remained asymptomatic. However, some patients developed severe COVID-19 disease that required hospitalization and another group of patients showed progressively worsening and life-threatening disease. Thus understanding the factors associated with disease severity will aid the management of patients and reduce mortality associated with this disease.

In this study, 400 COVID-19 patients were included in this study. Based on disease severity, patients were grouped into three groups: mild/moderate (n=200), severe (n=109) and critical (n=91) per the recommendations of the Palestinian MoH (MoH, 2020) and Chinese CDC (Wu and McGoogan, 2020). From the study population, 49% (n=196) were females and 51% (n=204) were males (Table 4.1). Patients in the mild/moderate group were not hospitalized, while patients in the severe group were hospitalized and all of them have recovered. Patients in the critical group required admission to intensive care unit due to worsening of disease symptoms and all of them died, except three patients.

Analysis of patients' age, showed a statistically significant difference in the average age (p-value = 0.000), where the mild/moderate group was the youngest and the critical group were the oldest. In addition, older patents (≥60 years) are more represented in the critical group (71.4%) compared to the severe (41.3%) or mild/moderate (11.5%) groups (p-value= 0.000) (Table 4.1). These data indicate that older age was associated with increased disease severity. Elderly people are usually more vulnerable due to several factors including reduced immunity and presence of other comorbidities (Marin et al., 2021; Poruondo-Jimenez et al., 2023). The immune system has been shown to change during aging in two ways: one includes reduced function of immune system called immunesenescence that impairs pathogen recognition, while the second way includes a chronic increase in systemic inflammation called inflammaging associated with over-reactive but ineffective system

(Mueller et al., 2020). Our findings are consistent with previous report that showed older age as a risk for adverse evolution of COVID-19 (Portuondo-Jimenez et al., 2023)

In the present study, 196 patients were females (49%) and 204 were males (51%). Our findings showed that COVID-19 was more common among females (53% of mild/moderate group are females) and that progression of the disease toward the critical stage (53.8% females vs. 46.2% males) was more common among females too. However, females were less represented among the severe group (37.6% females vs. 62.4% males). Our findings concerning the severe group are consistent with previous reports (Jin et al., 2020, Abdul Barek et al., 2020). While our findings in the mild/moderate and critical group are in contrary to previous reports that showed that men with COVID-19 are at increased risk for developing severe disease and death, regardless of age compared to women (Jin et al., 2020; Abdul Barek et al., 2020). Where, Jin et al (2020) analyzed a cohort of Chinese COVID-19 patients including 139 deaths and found that the number of men who died from COVID-19 were 2.4 times that of women (70.3 vs. 29.7%, P = 0.016). Investigation of an Italian cohort of COVID-19 patents by Fortunato et al. (2021) showed that male sex, age ≥55 years and the presence of at least one comorbidity increases the risk of hospitalization and death. Further explanation for the differences in COVID-19 disease outcome and severity between adult males and females may include biological factors such as hormonal differences that influence their susceptibility to infection and immune response (Fortunato et al., 2021). The disease outcome can also be affected by gender-related differences between sexes that influence their psychological, social and behavioral differences, exposure to the virus, initiation of treatment and compliance (Fortunato et al., 2021).

There were significant differences among the three patient groups concerning BMI (p=0.000). Where patients in the severe and critical group tend to have a high BMI values compared to mild/moderate group. Also patients with BMI≥30 represented the highest percentage among severe (42.2%) and critical (40.7%)) groups. A meta-analysis study by Yang et al. (2021) and a single center study from Wuhan city in China (Zhang et al., 2021) found that high BMI was associated with increased risk for developing severe and critical disease. Wu et al. (2021) found a U-shaped relationship between BMI and COVID-19

disease severity, where both obesity and underweight were associated with increased risk of developing severe COVID-19 disease.

Our data showed that there were no association between disease severity and smoking (p-value = 0.785) (Table 4.1). Although, smokers represented the highest percentage of patients among our cohort, where they represented 79%, 80.7% and 82.4% among the mild/moderate, severe and critical group, respectively. In confirmation of our findings, previous reports observed that smoking was not associated with enhanced risk of developing severe disease among Chinese (Lippi et al. 2020) and Malaysian COVID-19 patients (Ismail et al., 2022). In contrary, a meta-analysis study (Mahamat-Saleh et al., 2021) found that the absolute risk of death due to smoking increased by 7% among COVID-19 patients. However, the controversial results of the effect of smoking can be explained by the inaccuracy in quantifying the smoking habits and behavior, its duration and its interaction with other comorbidities.

Vaccination against SARS-CoV2 virus cannot prevent re-infection but can ameliorate the course of the disease. Our results showed only 22 out of 400 patients received COVID-19 vaccine because at time of sample collection, the local vaccination campaign was still limited. There was no significant difference between those who were vaccinated and those who were not. Previous studies have showed that vaccination cannot prevent re-infection but can prevent COVID-19 associated hospitalization and death compared to non-vaccinated people (Al-Hatamleh et al., 2023). In addition, some people who were partially vaccinated got infection before developing immunity against SARS-CoV2, and some vaccinated people may be non-responders to SARS-CoV2 vaccines (Bajpai et al., 2022).

Several SARS-CoV2 variants have been reported since the emergence of COVID-19 disease and these variants showed different virulence capacity (Funk et al., 2021; Lin et al., 2021). In the present study, most patients (n=343/400; 85.8%) were infected with Alpha variant (British variant) of SARS-CoV2 virus as this variant was the most prevalent at time of sample collection, and by the end of sample collection, the Delta variant (Indian variant) emerged and was detected in (n=57/400; 14.3%) of patients. Remarkably, 30 patients out of 57 who were infected with the Delta variant were in the critical group (Table 4.1). Also

the number of Delta infections in the critical group (n=30) is almost 2-folds of that observed in the mild/moderate (n=14) or severe group (n=13). These data indicate that the Delta variant was more virulent than the Alpha variant. Our findings confirm previous reports that showed that the Alpha, Beta, Gamma and Delta variants had a higher risk of hospitalization, ICU admission and mortality compared to wild type virus (Lin et al., 2021) and that the Beta and Delta variants have a higher risk than the Alpha and Gamma variants (Lin et al., 2021; Varea-Jimenez et al., 2022).

5.2 Chronic diseases and clinical symptoms of COVID-19 patients

Comorbidities like hypertension, diabetes, heart disease and obesity are associated with increased risk of fatal disease among COVID-19 patients (Mueller et al., 2020; Magadum and Kishore, 2020; Abdul Barek et al., 2020; Altuntas et al., 2021; Marin et al., 2021; Mahamat-Saleh et al., 2022). Explanations for these findings include immune system related changes resulting from over-reactive but ineffective immune system alert system (Mueller et al., 2020). In confirmation of these results, our findings depict a significant association of chronic diseases such as diabetes mellitus (p-value= 0.000), lung diseases (p-value= 0.004), hypertension (p-value= 0.000), heart diseases (p-value= 0.000) and COVID-19 disease severity (Table 4.2). In contrast, there was an inverse association between allergy/asthma (p-value= 0.039) and COVID-19 disease severity. This inverse association is probably due to the fact that allergic patients are aware of their disease difficulties and thus are very cautious and adopt strict preventive measures that reduce their risk of exposure to SARS-CoV2 virus. In this context, Singh et al. (2023) investigated 5 morbidities among an Indian cohort and reported that older age (>50 years) and preexisting comorbidities are predictors of severe COVID-19 disease outcomes. In the later report, the strongest risk factors were diabetes (OR 2.39), hypertension (OR 2.31) and heart diseases (OR 2.19). There was no statistically significant differences between the rest of chronic diseases analyzed in this study and COVID-19 disease severity. Some of these morbidities were found at low frequency among the study population. However, others like immune suppressive conditions and depression showed an increasing percentage from mild/moderate to critical group (Table 4.2).

Patients presented with a range of clinical symptoms and some of these symptoms namely fever, myalgia, sore throat, cough, dyspnea, ear pain, chest pain, headache, nausea/vomiting, abdominal pain, loss of appetite, face pain and retro-ocular pain showed a statistically significant association with disease severity, i.e., they proportionally increased with disease severity (Table 4.4). While other symptoms analyzed in this study showed no significant association with disease severity. A meta-analysis study that included 55 studies and 10014 cases of COVID-19, found that several disease symptoms (fever, dyspnea, chest pain, abdominal pain, fatigue, anorexia, hemoptysis and diarrhea) significantly affect disease severity and prognosis (Abdul Barek et al., 2020). The latter study also reported no association between other symptoms (myalgia, nausea/ vomiting, headache, sore throat dizziness and pharyngalgia) and disease severity (Abdul Barek et al., 2020). The findings of the latter study are consistent with our findings concerning some but not all symptoms, reflecting the differences in the homogeneity of study populations. Furthermore, our finding showed a significant variation of symptoms based on sex, but no variation was observed based on age groups. A study of 1420 mild-moderate cases from different European countries showed that disease symptoms varied based on age and sex (Lechien et al., 2020).

When reporting the disease symptoms at three weeks after disease onset, a significantly higher proportion of patients in severe and critical groups compared to mild/moderate group were still suffering from some disease symptoms (Table 4.4). A set of symptoms were found in more than 80% (fatigue, myalgia, dyspnea and retro-orbital headache), while other were found in more than 50% cough, chest pain, arthralgia and mental disorders) or in more than 40% (loss of smell, taste dysfunction and headache) of patients severe and critical patients. Notably, dyspnea was found in 32.5%, 85.3% and 92.3% of mild/ moderate, severe and critical patients, respectively. Thus these symptoms can be used for disease prognosis. Additionally, these findings suggest that some disease symptoms or disease sequelae may last for at least three weeks after onset of the disease. Even in the mild/ moderate group, more than half of patients were still suffering from some symptoms three weeks after disease onset, namely fatigue, myalgia, arthralgia and retro-orbital

headache. A study by Huang et al. (2021) reported the COVID-19 consequences at 6 months after symptom onset in 1733 cases in Wuhan city (China) and found that fatigue or muscle weakness, sleep difficulties and anxiety or depression were present in 63%, 26% and 23% of cases, respectively. In the latter study, a variable degree of diffusion impairment among a proportion of cases was also reported (Huang et al., 2021). A recent meta-analysis and systemic review that included 47,910 patients, aged 17-84 years and have long COVID-19 that ranged from 14 to 110 days post-viral infection, reported that approximately 80% of COVID-19 patients developed one or more long-term symptoms (Lopez-Leon, et al., 2021). The later report found that the most common long-term symptoms of COVID-19 disease were fatigue (58%), headache (44%), attention disorder (27%), hair loss (25%) and dyspnea (24%) (Lopez-Leon, et al., 2021). Dyspnea or breathlessness is a frequent finding among patients with severe or advanced disease. Hentsch et al. (2021) proposed that SARS-CoV2 virus affects the higher cortical structures involved in breathlessness perception either directly by interrupting the interpretation of afferent signaling or by interrupting the top-down regulation of breathlessness. Additionally, the COVID-19 induced damage to the lungs contributes to breathlessness (Hentsch, et al., 2021). Furthermore, Grewal et al. (2023) reported that post-COVID-19 dyspnea is common and it negatively affects the patients' quality of life and proposed that post-COVID-19 dyspnea could be attributed to mood abnormalities, and cardiorespiratory abnormalities. It also has been emphasized that COVID-19 patients should be assessed for dyspnea and mood abnormalities in order to identify patients at high risk of dyspnea and facilitate early and efficient treatment (Grewal, et al., 2023).

5.3 Association of COVID-19 disease severity with hematological markers

Our findings concerning the CBC, showed that there is a gradual decrease in RBCs, Hb, Hct, lymphocytes, LY/Mon ratio, platelets and a gradual increase in monocytes, granulocytes, GR/Mon ratio, GR/Ly ratio, PT, INR and D-dimer levels that parallel the increase in disease severity (Table 4.8). These findings indicate that the increase in disease severity was associated with continued reaction of the body to viral infection (increased granulocytes and monocytes), dysfunction of the host to respond to the infection

(lymphocytopenia), development of anemia of inflammation (reduced RBCs, Hb, Hct and increased serum ferritin) and activation of the coagulation process (thrombocytopenia, prolonged PT, increased D-dimer). In confirmation of our results, earlier reports showed that COVID-19 patients with severe and fatal disease had increased WBC count, lymphocytopenia, thrombocytopenia, elevated D-dimer levels (Terpos et al., 2020; Henry et al., 2020; Hashem et al., 2021), decreased Hb (Bhowmik et al., 2022), and prolongation of PT and APTT, and increased fibrin degradation products that lead to disseminated intravascular coagulation (DIC) (Terpos et al., 2020). Also anemia has been reported as a risk factor for developing severe COVID-19 disease (Hashem et al., 2021).

Analysis of the association of ABO blood group and RhD status revealed no significant association with COVID-19 disease severity. In the present study, blood group A was present in a higher proportion of patients in the critical (61.5%) and severe (58.7%) groups compared to mild/moderate group (43.0%; p=.053); while O and B groups have the opposite distribution. Several studies have tackled this point and some studies were in agreement with our study (Barnkob et al., 2020; Kim et al., 2021) while other studies were in disagreement with our findings and reported that A and AB blood groups have increased risk of developing severe COVID19 illness compared to O or B blood groups (Hoiland et al., 2020). Zietz et al. (2020) reported that risk of death from COVID-19 as was slightly increased among AB patients and decreased among A, B, O and Rh-negative patients. Taken altogether, the ABO and Rh type may play a role in susceptibility or affect the outcome of COVID-19 alongside other factors, however, the different findings may be due to different study settings as these studies used different patients groups and analyzed different disease outcomes (Kim et al., 2021) as well as the epidemiology of ABO types in different ethnicities.

5.4 Association of COVID-19 disease severity with biochemical markers

A statistically significant difference was observed between patient groups and almost all biochemical markers analyzed in this study, except for total cholesterol and bilirubin. Serum total protein and albumin levels were deteriorating with increased disease severity.

The CRP and serum ferritin both are positive acute phase reactant proteins and their levels increased parallel to the increase in disease severity and thus confirming a continued inflammation state as the disease severity increases. The fasting plasma glucose level also increased parallel to the increase in disease severity; and this confirms our findings that patients with other comorbidities (Table 4.2) and in particular, diabetes mellitus are more vulnerable to develop severe and critical COVID-19 disease. In addition, several serum enzymes increased parallel to the increase in disease severity indicating the progress of an inflammation process (LDH) and involvement of the liver (ALT, AST, ALP), bone (ALP), pancreas (amylase), kidney (creatinine, BUN) and cardiac tissues (CK-total, AST) (Table 4.13). In confirmation with our findings, a recent meta-analysis reporting about Bangladeshi COVID-19 patients (n=1542) found an elevated levels of CRP, serum ferritin, D-dimer, LDH, random blood sugar and serum creatinine in severe compared to non-severe cases (Bhowmik et al., 2022). Another report analyzing a cohort of Indian COVID-19 patients and found that elevated serum CRP, LDH, D-dimer and serum ferritin were elevated in severe compared to non-severe cases and thus can be used to triage cases at time of admission and identify cases that require intensive care (Sana and Avneesh, 2022).

Our findings indicate a significant association between high PTH levels and disease severity, and mean PTH level was above the reference value in all three patients groups and was highest in the critical group, i.e., PTH was 69, 81 and 148 pg/mL in mild/moderate, severe and critical group, respectively. Patients with hyperparathyroidism can experience symptoms of hypercalcemia like vomiting, nausea and abdominal pain and dehydration (Aojula et al., 2021) and thus these patients are more likely to develop a severe disease. As for the TSH, our findings indicated its serum levels were significantly decreased with increasing disease severity. In confirmation of our results, an Italian study reported that low TSH levels in COVID-19 patients were associated with prolonged hospitalization and increased mortality (Lania et al., 2020). Another two studies have reported low TSH in COVID-19 patients and showed that thyroiditis in COVID-19 patients is attributed to direct viral injury and post-inflammatory reaction (Aemaz Ur Rehman et al., 2021; Lui et al., 2021). However, the latter findings were questioned by Chen et al. (2022) who indicated

that available evidences are still insufficient to confirm the role of thyroid changes in progression of disease severity in COVID-19 cases.

In this study patients in the severe or critical COVID-19 disease developed a higher immune response reflected by the increase in IgG levels at 3 weeks after disease onset (Table 4.13). But, this increase in immune response apparently was not able to limit disease process or induce a virus clearance. In agreement with our findings, it has been shown that severe COVID-19 cases had a higher level of IgG against N and lower IgM against RBD compared to non-severe cases (Xie et al., 2020; Takamatsu et al., 2022; Zhang et al., 2023). However, poor and delayed anti-SARS-CoV2 IgM and IgG responses have been linked to poor outcomes in children with COVID-19 (Zhang et al., 2023). Natural immunity induced by Delta variant generated a longer and stronger protection against infection and hospitalization compared to vaccination (Gazit et al., 2022).

5.5 Conclusions

This is the first study in Palestine that provides a comprehensive analysis of the anthropometric, symptoms, comorbidities, hematological and biochemical markers of 400 COVID-19 patients and their association with disease severity. Diseases severity is a multifactorial and our findings indicate a significant association between disease severity and older age, female sex, high BMI, SARS-CoV2 variant and presence of comorbidities such as diabetes mellitus, hypertension, lung and heart diseases.

Significant alterations in most hematological markers investigated were observed parallel to the progression of disease severity, either by showing a gradual decrease in RBCs, Hb, Hct, lymphopenia, LY/Mon ratio, platelets; or a gradual increase in monocytes, granulocytes, GR/Mon ratio, GR/Ly ratio, PT, INR and D-dimer levels that parallel the increase in disease severity. ABO type is not associated with disease severity.

Significant alterations in biochemical markers were also observed parallel to the progression of disease severity, either by showing gradual increase in markers of

inflammation (serum ferritin, CRP, LDH), markers of liver function (AST, ALT, ALP), pancreatic function (amylase), kidney function (creatinine, BUN), cardiac function tests (CK total, AST) and parathyroid gland (PTH); or a decrease in serum albumin and thyroid function (TSH).

The continued inflammation in patients with worsening disease severity was significantly associated with increased immune response (IgG level) that was not sufficient to limit the progression of disease severity reflecting that other factor play a role in determining the fate of COVID-19 disease.

Continued worsening of hematological and biochemical markers should be used to triage patients who need intensive care.

5.6 Recommendations

- Further studies are needed to study hematological and biochemical markers at weekly intervals during hospitalization to evaluate and develop an algorithm for triage of patients who need intensive care and develop a treatment plan.
- Adopt and enhance the infection prevention and control standards by the MoH and other healthcare institutes.
- Raise awareness of healthcare professionals about the transmission and spread of COVID-19 disease.
- Raise awareness of healthcare professionals about hematological and biochemical markers associated with COVID-19 disease.
- Develop a guideline or information kit for healthcare professionals at risk containing information about COVID-19 disease, prevention measures and immunization.
- Develop a guideline or information kit for the management of patients with chronic diseases to protect this group of patients from contacting COVID-19; and probably provide them with home therapy.

5.7 Study limitations

- Samples were collected from few referral hospitals that were running at their upper limit. Thus severe and critical patients may have been differently treated or managed if hospitalized in a different hospital and their disease outcome may be affected.
- Sample size of the three groups was not matched.
- Symptoms of thromobosis were not included in the study questionnaire and biochemical markers of thrombosis (other than PT, APTT and D-dimer) were not measured. Thus conclusions about the thrombotic state of patients cannot be interpreted.
- The number of cases infected with delta variant of SARS-CoV2 was low compared to alpha variant and thus analysis of the effect of virus variant on disease severity was limited.
- Smoking history of patients before infection with SARS-CoV2 virus was not known.
- The number of vaccinated patients was very limited because at time of sample collection, COVID-19 vaccines were not available widely available for the general population.

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Appendices

Appendix 1: Study questionnaire and consent Form of the study

استبانة لبحث بعنوان:

البحث في العلامات الدموية والكيميائية الحيوية والمناعية لمرض كوفيد-19

فريق البحث: السيدة كفى الريماوي، الدكتور محمود سرور

برنامج ماجستير العلوم الطبية المخبرية – جامعة بيرزيت

آذار 2021

مقدمة:

ان مرض كوفيد-19 يمثل وباءا عالميا والعلامات الدموية والكيميائية الحيوية للمرضى متباينة بشكل كبير وقد تختلف بعض العلامات من منطقة جغرافية الى أخرى. إضافة لذلك فإن الاستجابة المناعية لمرضى كوفيد-19 متباينة وليست مدروسة بشكل جيد. ولذلك تهدف هذه الدراسة للبحث في العلامات الدموية والكيميائية الحيوية للمرض وعلاقتها بالاستجابة المناعية لفيروس كورونا بين مرضى كوفيد-19 في الضفة الغربية.

تتضمن هذه الدراسة ثلاثة اجزاء:

الجزء الأول عبارة عن استبانة تتطلب ان يقوم المريض بتقديم معلومات عامة واخرى عن التاريخ المرضي للشخص، من اجل تعبئة الاستبانة.

الجزء الثاني يتطلب تبرع المريض بعينة دم لأغراض الدراسة عند تعبئة الاستبانة، والموافقة على تقديم عينة ثانية بعد 3 اسابيع.

الجزء الثالث يتضمن اقرار من المريض بالمشاركة في الدراسة.

تنويه:

• المشاركة في هذه الدراسة اختيارية ويمكنك الانسحاب في أي وقت من المشاركة في الدراسة. قرارك بعدم المشاركة لن يؤثر في الرعاية الصحية التي تتلقاها.

• مشاركتك في هذه الدراسة تساعدنا على توصيف أفضل لمرض كوفيد-19 بين المرض الفلسطينيين ويساهم في تحسين التشخيص والعلاج لهذا المرض.

• المعلومات التي يتم جمعها ضمن هذه الدراسة سرية ولن تتم مشاركة أية معلومات شخصية للمشاركين خارج طاقم البحث حيث سيتم استبدالها بأرقام وحفظها في مكان آمن.

• لقد تمت مراجعة الاجراءات البحثية والأخلاقية للدراسة من قبل لجنة أخلاقيات البحث العلمي في جامعة بيرزيت

تصريح الموافقة على المشاركة

لقد قمت بالاطلاع على المعلومات الخاصة بالدراسة وحظيت بالفرصة للاستفسار وطرح الاسئلة وتمت الاجابة على استفساراتي بشكل مناسب. وبناء على ذلك، أمنح موافقتي على اشتراكي في هذا البحث.

وأبدي موافقتي على تقديم عينة دم للدراسة بعد 3 أسابيع من تاريخ اليوم.

اسم المشارك:_____

التوقيع: _____

التاريخ: _____

(اليوم/الشهر/السنة)

رقم التلفون أو الجوال للاتصال: .....

الاستبانة لبحث بعنوان:

البحث في العلامات الدموية والكيميائية الحيوية والمناعية لمرض كوفيد-19

فريق البحث: السيدة كفى الريماوي، ، الدكتور محمود سرور

برنامج ماجستير العلوم الطبية المخبرية – جامعة بيرزيت،

الجزء الأول من الاستبانة \ المقابلة الأولى:

1	رقم العينة:			
2	اسم الشخص الذي أجرى المقابلة			
3	اسم المركز الصحي			
4	تاريخ اجراء المقابلة			
	المعلومات الشخصية			
5	الاسم (الاسم الأول والعائلة)			
6	رقم الهوية			
7	الجنس	ذكر \ انثى		
8	الوزن \ الطول	..... كغم \ ..... سم		
9	تاريخ الميلاد (يوم \ شهر \ سنة)			
10	المهنة			
11	رقم الجوال أو التلفون			
	عنوان السكن			
12	فصيلة الدم (ABO/Rh)			
	التاريخ الطبي	يرجى وضع دائرة حول الإجابة الصحيحة		
13	خلال ال 12 سنة أخيرة، هل دخنت بشكل مستمر؟		نعم	لا
14	هل تعاني من أي من الأمراض التالية؟	1. أمراض الأوعية الدموية	نعم	لا
		2. مرض السكري	نعم	لا
		3. الربو أو الحساسية	نعم	لا
		4. مرض رئوي مزمن مثل الانسداد الرئوي المزمن أو التهاب الشعب الهوائية أو انتفاخ الرئة	نعم	لا
		5. السرطان (حالي \ تم علاجه)	نعم	لا
		6. ارتفاع ضغط الدم	نعم	لا
		7. التهاب الكبد الوبائي المزمن نوع (أ) أو (ب)	نعم	لا
		8. فشل كلوي مزمن	نعم	لا

		9. ضعف في جهاز المناعة أو أمراض نقص المناعة	نعم	لا
		10. السمنة او زيادة الوزن (BMI>=25)	نعم	لا
		11. نوبة قلبية أو مرض قلبي أو غيرها من امراض القلب	نعم	لا
		12. نقص في عمل الغدة الدرقية (حالي \ تم علاجه)	نعم	لا
		13. أمراض في الأعصاب	نعم	لا
		14. الاكتئاب	نعم	لا
15	منذ بدء انتشار وباء كورونا المستجد في اذار 2020، هل عانيت من اي من الاعراض التالية:	1. حمى ≤ 38 درجة مئوية	نعم	لا
		2. فقدان حاسة الشم	نعم	لا
		3. فقدان حاسة الذوق	نعم	لا
		4. قشعريرة أو ارتجاف	نعم	لا
		5. الإرهاق	نعم	لا
		6. وجع أو الم في العضلات	نعم	لا
		7. التهاب في الحلق	نعم	لا
		8. السعال	نعم	لا
		9. زكام في الأنف	نعم	لا
		10. سيلان الأنف	نعم	لا
		11. ضيق في التنفس	نعم	لا
		12. ألم في الأذن	نعم	لا
		13. ألم في الصدر	نعم	لا
		14. ألم في المفاصل	نعم	لا
		15. صداع في الرأس	نعم	لا
		16. الغثيان أو التقيؤ	نعم	لا
		17. ألم في البطن	نعم	لا
		18. إسهال	نعم	لا
		19. فقدان الشهية	نعم	لا
		20. ألم أو ثقل في الوجه	نعم	لا
		21. ألم أو ثقل في العين	نعم	لا
16	هل إصابتك بأي من هذه الاعراض (في البند السابق 14) أدى الى:	1. طلب العناية او العلاج الطبي؟	نعم	لا
		2. التغيب عن العمل \ الدراسة \ المدرسة؟	نعم	لا
		3. دخول المستشفى؟	نعم	لا
17	منذ بداية شعر آذار \ مارس لعام 2020، هل سبق أن:	1. خضعت للحجر المنزلي نتيجة كوفيد-19؟	نعم	لا
		2. عملت في مجال تقديم الرعاية الصحية؟	نعم	لا
		3. أجريت فحص ال PCR للكشف عن مرض كوفيد-	نعم	لا

		؟19 إذا كانت الإجابة نعم، ماذا كانت النتيجة؟ إيجابي \ سلبي تاريخ اجراء الفحص		
18	هل تعرف شخصيا أي شخص في مجتمعك المحلي مصاب بالحمى بالإضافة الى سعال أو صعوبة في التنفس أو لديه فحص إيجابي(PCR) إيجابي ل كوفيد-19؟ أذا كان الجواب بنعم، يرجى تحديد علاقتك بهذا الشخص أو الأشخاص:	نعم		لا
19	هل تلقيت اللقاح او التطعيم ضد فيروس كورونا (المسبب لمرض كوفيد-19)؟ إذا كانت الإجابة نعم، كم عدد الجرعات التي تلقيتها؟ جرعة واحدة بتاريخ ..... جرعتين وتاريخ الجرعة الثانية هو	نعم		لا

الاستبانة لبحث بعنوان:

البحث في العلامات الدموية والكيميائية الحيوية والمناعية لمرض كوفيد-19

فريق البحث: السيدة كفى الريماوي، الدكتور محمود سرور

برنامج ماجستير العلوم الطبية المخبرية – جامعة بيرزيت

آذار 2021

الجزء الثاني من الاستبانة \ المقابلة الثانية بعد 3-4 أسابيع:

1	رقم العينة:				
2	اسم الشخص الذي أجرى المقابلة				
3	اسم المركز الصحي				
4	تاريخ اجراء المقابلة				
	المعلومات الشخصية				
5	الاسم (الاسم الأول والعائلة)				
6	رقم الهوية				
7	الجنس	ذكر \ انثى			
8	الوزن \ الطول	..... كغم \ ..... سم			
9	تاريخ الميلاد (يوم \ شهر \ سنة)				
10	المهنة				
11	رقم الجوال أو التلفون				
	عنوان السكن				
12	فصيلة الدم (ABO/Rh)				
	التاريخ الطبي	يرجى وضع دائرة حول الإجابة الصحيحة			
15	خلال الثلاث الأسابيع الماضية أي بعد اعلامك بإصابتك بفيروس كورونا، هل عانيت من اي من الاعراض التالية: يرجى التوضيح إن كانت أي من هذه الأعراض اختفت او زادة حدتها وذلك بملء الخانة الأخيرة بأحد العبارات التالية: ظهرت حديثا\ ازدادت حدة\ اختفت.	1. حمى ≥ 38 درجة مئوية	نعم	لا	
		2. فقدان حاسة الشم	نعم	لا	
		3. فقدان حاسة الذوق	نعم	لا	
		4. قشعريرة أو ارتجاف	نعم	لا	
		5. الإرهاق	نعم	لا	
		6. وجع أو الم في العضلات	نعم	لا	
		7. التهاب في الحلق	نعم	لا	
		8. السعال	نعم	لا	
		9. زكام في الأنف	نعم	لا	

		10.سيلان الأنف	نعم	لا	
		11.ضيق في التنفس	نعم	لا	
		12.ألم في الأذن	نعم	لا	
		13.ألم في الصدر	نعم	لا	
		14.ألم في المفاصل	نعم	لا	
		15.صداع في الرأس	نعم	لا	
		16.الغثيان أو التقيؤ	نعم	لا	
		17.ألم في البطن	نعم	لا	
		18.إسهال	نعم	لا	
		19.فقدان الشهية	نعم	لا	
		20.ألم أو ثقل في الوجه	نعم	لا	
		21.ألم أو ثقل في العين	نعم	لا	
16	هل إصابتك بأي من هذه الاعراض (في البند السابق 14) أدى الى: في حال كانت الإجابة لا في المقابلة الأولى، أما اذا كانت الإجابة نعم في المقابلة ألأولى فلا داعي لإجابه هذا الجزء)	1. طلب العناية او العلاج الطبي؟	نعم	لا	
		2. التغيب عن العمل \ الدراسة \ المدرسة؟	نعم	لا	
		3. دخول المستشفى؟	نعم	لا	
18	هل تعرف شخصيا أي شخص في مجتمعك المحلي مصاب بالحمى بالإضافة الى سعال أو صعوبة في التنفس أو لديه فحص ايجابي(PCR) إيجابي ل كوفيد-19؟ (يرجى الإجابة فقط إذا ظهرت إصابة جديد في مجتمعك بعد المقابلة الأولى) أذا كان الجواب بنعم، يرجى تحديد علاقتك بهذا الشخص أو الأشخاص:		نعم	لا	
19	هل تلقيت اللقاح او التطعيم ضد فيروس كورونا (المسبب لمرض كوفيد-19) بعد المقابلة الأولى؟ إذا كانت الإجابة نعم، كم عدد الجرعات التي تلقيتها؟ جرعة واحدة بتاريخ ..... جرعتين وتاريخ الجرعة الثانية هو		نعم	لا	

Appendix 2: Ethical approval

جامعة بيرزيت
كلية الصيدلة والتمريض والمهن الصحية

ص.ب 14، بيرزيت، فلسطين، هاتف 02-2 2982093 فاكس 02-2982017

Faculty of Pharmacy, Nursing & Health Professions

Research Ethics Committee Report

Reference number: BZUPNH2103

Date: 16/03/2021

Dear Dr Mahmoud Srour,

Thank you for submitting your application for research ethics approval. After reviewing your research proposal entitled "**Investigation of the hematological, biochemical and immunological markers of COVID-19 patients**", the Research Ethics committee confirms that your application is in accordance with the research guidelines of Birzeit University at the date of submission (available at: https://ritaj.birzeit.edu/university-laws/).

Please note that you must notify the committee of any changes in the research methodology/ subjects/plan.

We would appreciate receiving a copy of your final research project. Thank you again and wish you a productive research that serves the best interest of your subjects and the community.

On behalf of the Research ethical committee,

Dr. Abdullah Rabba

Arabic abstract

التحقيق في مؤشرات الدم والكيمياء الحيوية لدى مرضى كوفيد-19 في الضفة الغربية – فلسطين

إعداد الطالبة: كفى راتب أديب الريماوي

إشراف: الدكتور محمود عبد الرحمن سرور

المُلَخَّص

المقدمة: طغى مرض كوفيد-19 (COVID-19) مؤخرًا على أنظمة الرعاية الصحية بما فيها الأكثر كفاءة وتسبب في حدوث ملايين الوفيات في جميع أنحاء العالم. يتسبب المرض في اختلال العديد من المؤشرات الحيوية في مصل لدم، وبالتالي يتم البحث عن طريقة بسيطة وسريعة للتقسيم الطبقي للمرضى لتحديد شدة المرض وبالتالي إدارة العناية الفورية للمريض.

الأهداف: التحقيق في ارتباط القياسات البشرية والأمراض المصاحبة وكذلك مؤشرات الدم والكيميائية الحيوية مع شدة مرض كوفيد-19 في فلسطين.

طُرُق العمل: هذه دراسة مقطعية مستقبلية شملت 400 مريض كوفيد -19 تم تصنيفهم بناءً على شدَّة المرض إلى خفيف / معتدل (ن = 200)، شديد (ن = 109) وحَرِج (ن = 91). المجموعة الشديدة تتطلب الاستشفاء والمجموعة الحَرِجة تتطلب العناية المركزة. تم استخدام استبيان منظم لجمع بيانات القياسات البشرية وأعراض المرض والبيانات الصحية العامة في وقت التسجيل وبعد 3 أسابيع. تم قياس تعداد الدم الكامل، التخثر والعلامات الكيميائية الحيوية، هرمونات الغدة جار درقية (PTH) وهرمونات الغدة الدرقية (TSH) ومستويات IgG في الدم.

النتائج: تعافى جميع المرضى الحَرِجين من العدوى بينما توفي جميع المرضى الحَرِجين باستثناء ثلاثة مرضى. لوحظ ارتباط ذو دلالة إحصائية بين شدة المرض وكبر السن، وجنس الإناث، وارتفاع مؤشر كتلة الجسم ، ووجود الأمراض المزمنة (السكري ، ارتفاع ضغط الدم ، أمراض الرئة والقلب) ومتغير دلتا. لوحظ وجود علاقة عكسية بين شدة المرض والحساسية / الربو بينما لوحظ عدم وجود ارتباط مع التدخين. كانت نسب كبيرة من المرضى الحرجين والحرجين يعانون من بعض أعراض المرض بعد ثلاثة أسابيع من المرض مقارنًة بالمعتدل / المعتدل. انخفاض معنوي في الهيموجلوبين والصفائح الدموية والخلايا الليمفاوية ونسبة الخلايا الليمفاوية / الوحيدات وزيادة الخلايا المحببة ووقت البروثرومبين و D-dimer. لم يلاحظ أي ارتباط كبير بين نوع ABO

أو Rh (D) وشدة المرض. أظهرت النتائج التي توصلنا إليها وجود ارتباط كبير بين العلامات البيوكيميائية وشدة المرض بما في ذلك المستويات المرتفعة من CRP ، الفيريتين ، IgG، PTH وانخفاض TSH.

الخُلاصة: تقدم الدراسة تحليلاً شاملاً لخصائص المريض والعلامات المختبرية لمرضى COVID-19 مجمعة على أساس شدة المرض. ارتبط تقدم العمر ، وزيادة الوزن / السمنة ، ووجود الأمراض المصاحبة والعلامات الدموية والكيميائية الحيوية غير الطبيعية مع شدة المرض وتوفر طريقة بسيطة وسريعة لفرز المرضى المصابين بمرض شديد والذين يحتاجون إلى العلاج في المستشفى أو العناية المركزة.