OVERVIEW OF THE RISK FACTORS, PREVENTION AND MANAGEMENT OF SARS-COV-2

By

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of
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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
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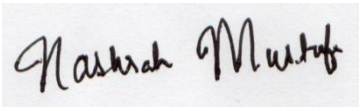

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Ethics Statement

This material is an original work, which has not been previously published elsewhere. It is my own research and analysis in a truthful and complete manner. The paper properly credits all the sources used (correct citation).

Abstract

SARS-CoV-2, the novel corona virus that originated in Wuhan, China in late December is a positive single stranded RNA virus. COVID-19, a disease caused by the SARS-CoV-2 is characterized by symptoms such as fever, sore throat, dry cough, loss of sense of taste and smell etc. The symptoms may range from asymptomatic, mild, moderate, severe and critical. The virus enters through its spike protein (S) to the ACE2 receptors. The understanding of the virus is crucial to devise treatment strategies and vaccines. COVID-19 disease has spread rapidly on a global scale within a short period of time. On January 30, 2020, World Health Organization (WHO) has identified it as a pandemic and declared it as an epidemic of international concern. Countries went into lockdown, and different countries have adopted various strategies to contain the disease. Non-pharmacological as well as pharmacological treatments are being widely practiced. Treatment or management of the disease depends on the severity of the disease. Currently, there is no single treatment, and the vaccines are still under clinical trials. Patients with comorbidities are at higher risk for developing secondary infections such as pneumonia. The study seeks to combine all the relevant published papers and articles to provide an overview about the virus, highlighting its similarities and differences with the previous variant (SARS-CoV), associated risk factors, symptoms, available treatment strategies and finally recommendations on how to improve the current situation. The current scenario of Bangladesh is described and three case studies are presented in this review.

Keywords: pandemic; COVID-19 disease; treatment strategies; vaccine

Dedicated to all those who have suffered from COVID-19.

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List of Acronyms

SARS	Severe Acute Respiratory Syndrome
MERS	Middle East Respiratory Syndrome
CoV	Coronavirus
HCoV	Human Coronavirus
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronaviruses
COVID-19	Coronavirus disease
RNA	Ribonucleic acid
cDNA	Complementary Deoxyribonucleic acid
S	Spike protein
NTD	N Terminal Domain
CTD	C Terminal Domain
ORF	Open Reading Frame
UTR	Untranslated Regions
RBD	Receptor Binding Domain
ACE2	Angiotensin Converting Enzyme-2
IL	Interleukin
M	Membrane Protein
WHO	World Health Organization
CDC	Centers for Disease Control and Prevention
IEDCR	Institute of Epidemiology, Disease Control and Research
DPP4	Dipeptidyl Peptidase-4
CSF	Cerebrospinal Fluid
ERGIC	Endoplasmic Reticulum-Golgi Intermediate Compartment

HD	Haemodialysis
DM	Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
CHD	Coronary Heart Disease
ARDS	Acute Respiratory Distress Syndrome
CARDS	COVID-induced Acute Respiratory Distress Syndrome
DILI	Drug-induced Liver Injury
AST	Aspartate Aminotransferase
ALT	Alanine Aminotransferase
MS	Microvascular Steatosis
HBV	Hepatitis B Virus
Ig	Immunoglobulin
Hb	Haemoglobin
WBC	White Blood Cells
ICU	Intensive Care Unit
PCR	Polymerase Chain Reaction
RT-PCR	Reverse Transcription Polymerase Chain Reaction
qRT-PCR	Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction
RT-qPCR	Quantitative Reverse Transcription Polymerase Chain Reaction
ddPCR	Droplet Digital Polymerase Chain Reaction
LAMP	Loop-mediated Isothermal Amplification
RT-LAMP	Reverse Transcription Loop-mediated Isothermal Amplification
ASOs	antisense oligonucleotides
AuNP	Gold Nanoparticles

LOD	Limit of Detection
RCT	Randomized Controlled Trials
ELISA	Enzyme-Linked Immunosorbent Assay
CLIA	Clinical Laboratory Improvement Amendments
LFA	Lateral Flow Assay
CQ	Chloroquine
HCQ	Hydroxychloroquine
HIV	Human Immunodeficiency Virus
CSS	Cytokine Storm Syndrome
FDA	Food and Drug Administration
Fab	antigen-binding fragment
Fc	fragment crystallizable region
JAK	Janus Kinase
RA	Rheumatoid Arthritis
AP2	Activating Protein-2
APA	Aegopdium podagraria agglutinin
UDA	Urtica dioica agglutinin
HHA	Hippeastrum hybrid (Amaryllis)

Chapter 1

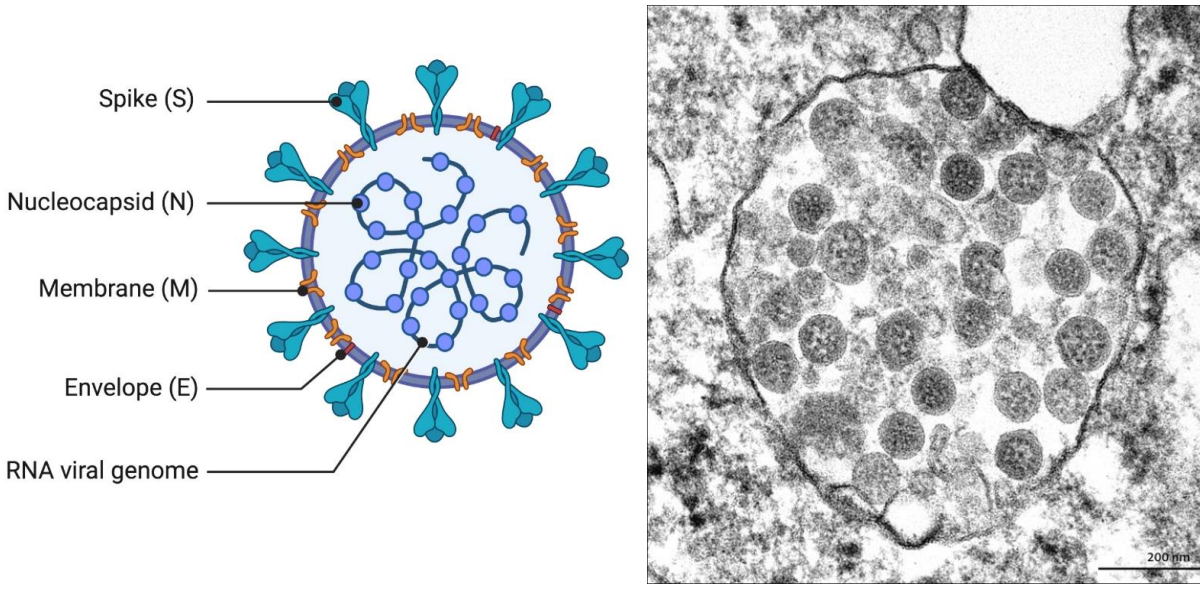
1. The rationale of the study

The Covid-19 pandemic, originating from China in late December 2019, has shaken the globe to its core, posing the world towards the worst health hazard of the century. Since its first case of infection, the COVID-19 virus has rapidly spread worldwide, impacting human health, lifestyle, global economy, and quality of life. As of now, confirmed cases of COVID-19 had exceeded 76.8 million globally, with the death toll of above 1.69 million according to the Johns Hopkins University map as on December 21, 2020 (*COVID-19 Map - Johns Hopkins Coronavirus Resource Center*, 2020) and the numbers are rising.

This study aims to collate all the relevant information from various distinguished publications and provide appropriate recommendations based on the available information. The review compiles information about the novel coronavirus, SARS-CoV-2 from journals endorsed by Elsevier, Nature, Springer and other distinguished reports, website articles and guidelines. The collected data were appropriately referenced providing a detailed understanding of the virus, mechanism of viral entry, comparison with the previous variant, risk factors, methods of diagnosis, symptoms, treatment/management options, the current scenario in Bangladesh and some recommendations. Initially, lack of data led to delayed decisions (Huq & Biswas, 2020) Attempts were taken to identify gaps or missing information in the available literature.

1.1 Introduction to Severe Acute Respiratory Syndrome (SARS) Coronaviruses

Coronaviruses (CoVs), thought to be of animal origin, consist of an enveloped positive single-stranded RNA genome wrapped in a helical nucleocapsid. Morphologically, they are characterized by spikes projecting from the surface (S). Each virus particle consists of a small set of genes enveloped in a lipid bilayer. In other words, the virus particles are enclosed by a sphere of fatty lipid molecules. They appear as a spiky ball or have a crown-like appearance under the microscope. The genome size ranges from approximately 26 to 32 kilobases, largest among the RNA viruses. Each nucleocapsid is composed of multiple copies of N protein, which is further surrounded by an envelope containing an S glycoprotein, an M glycoprotein, 3a glycoprotein of 180 to 190kDa, 23kDa and 30kDa respectively and a small E protein (Figure 1 a and b).



(a) **(b)**

Figure 1a: *Basic Stricture of a coronavirus*(Jonathan & Eric, 2020) ***b:*** *Viral isolate of the coronavirus, SARS-CoV-2 grown in cell culture* (Goldsmith et al., 2020)

The spike protein (S) has two regions- S1 and S2. The role of S1 and S2 is given below:

S1: mainly for host cell receptor binding. The S1 region also consists of an N-terminal domain (NTD) and three C-terminal domains (CTD1, CTD2, and CTD3).

S2: responsible for membrane fusion.

CoVs belong to the order, *Nidovirales* they include *Arteriviridae, Corinaviridae, Roniviridae and Mesoniviridae* families. The *Coronavirinae* comprises one of two subfamilies in the *Coronaviridae* family; the other is the *Torovirinae* (Figure 2).

The *Coronavirinae* family is further subdivided into four genera: the alpha CoV, beta CoV, gamma CoV and delta CoV. Members of this large family of viruses can cause respiratory, hepatic, enteric, and neurological diseases in different species of animal such as cattle, camels, bats, and cats. Seven human coronaviruses (HCoVs) have been identified till now.

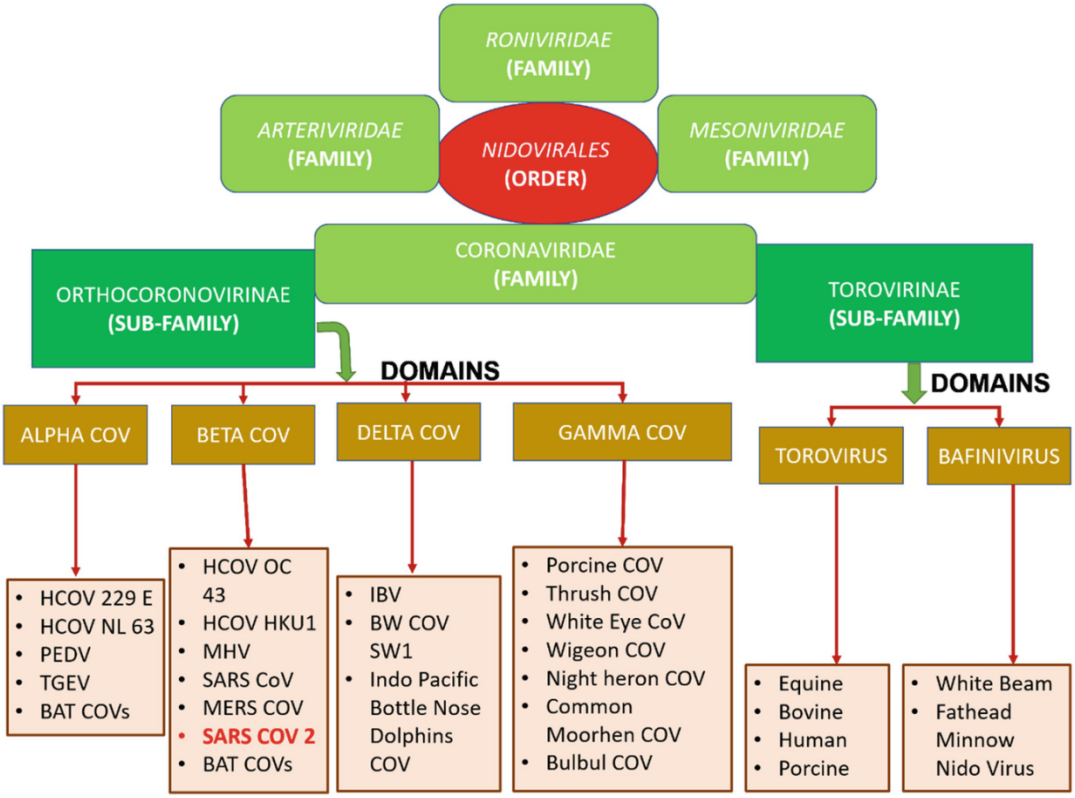


Figure 2: *The Coronavidae family*(Srivastava & Saxena, 2020)

The first two genera infect mammals, whereas the latter two infect birds (Wu et al., 2020). Seven kinds of HCoVs have been identified till now. These include HCoV- 229E and HCoV-NL63 from the Alpha CoV genus; HCoV-OC43, HCoVHKU1, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS CoV-2 from the Beta CoV genus (Liu et al., 2020). The viruses of 229E, OC43, NL63 and HKU1 cause mild common cold symptoms. However, the other three can cause severe illness (Veiga et al., 2020). MERS-CoV and SARS-CoV are highly pathogenic (Zhu et al., 2020), and it is highly likely that both SARS-CoV and MERS-CoV were transmitted from bats to palm civets (Guan et al., 2020) or dromedary camels, and finally to humans (Wu et al., 2020). The HCoVs did not attract attention worldwide until the SARS pandemic in 2003, followed by the MERS pandemic in 2012 and the recent 2019-nCoV outbreak

1.1.1 Genome composition of the coronavirus

SARS-CoV encodes for several unique group-specific open reading frames (ORFs) compared to other known coronaviruses. There are fourteen ORFs in coronaviruses which in some cases overlap. The coronavirus genome has the usual 5′ methylated cap and 3′ polyadenylated tail.

There are 265 nucleotides in the 5'UTR and 342 nucleotides in the 3'UTR. The 5' methylated cap and 3' polyadenylated tail enables the positive-sense RNA genome to be translated directly by the host cell's ribosome upon viral entry (Romano et al., 2020).

The viral gene order is similar in known coronaviruses: ORFs 1a and 1b encode the replicase/transcriptase polyprotein, and the ORFs 2, 4, 5, and 9a encode, respectively, the four major structural proteins: spike, envelope, membrane, and nucleocapsid. The downstream mRNAs encode structural proteins S, E, M, and N (Figure 3). These genes contain several accessory proteins that are not essential for *in vitro* replication (ORFs 3a, 3b, 6, 7a, 7b, 8a, 8b, and 9b). These accessory proteins, however, are not homologous to any reported proteins available in any database. The functions of these accessory proteins are of interest in order to understand the pathogenesis of the virus (Graham et al., 2008; Tan et al., 2005) .

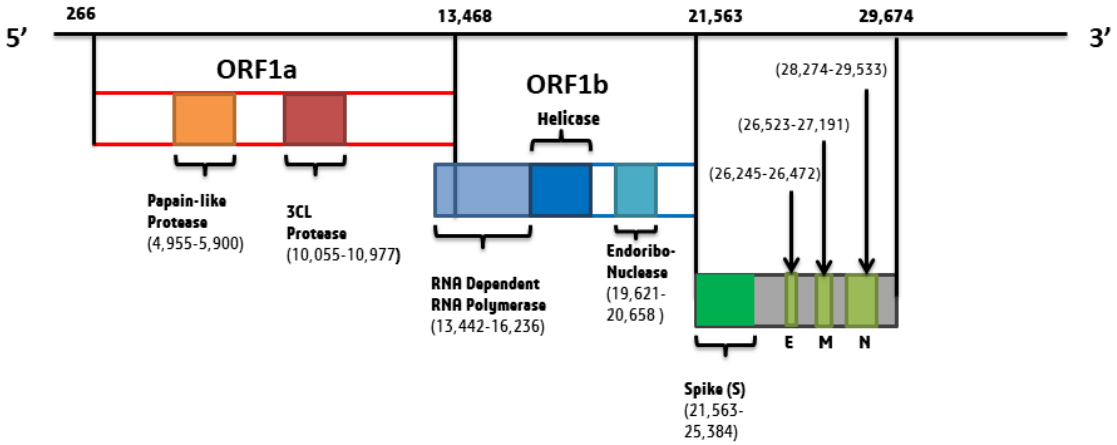


Figure 3: *Basic genomic organization of a coronavirus, SARS-CoV-2 [Adapted from* (Alanagreh et al., 2020)*]*

The SARS-CoV 2, for example, consists of an approximately 30 Kb RNA strand organized as follows (from left 5′ end to right 3’ end):

- Papain like protease (4,955-5,900 Kb)
- 3C-like serine protease (10,055 -10,977 Kb)
- RNA-dependent RNA polymerase (13,442-16,236 Kb)
- Helicase
- Endoribonuclease (19, 621-20,658 Kb)
- Spike protein (S) (21,563-25,384 Kb) containing RBD which binds to a human receptor, angiotensin converting enzyme 2 (ACE2)

- envelope protein (E) (26,245-26,472 Kb)
- membrane protein (M) (26523- 27,191 Kb)
- nucleocapsid protein (N) (28,274-29,533 Kb) (Pal et al., 2020)

Enzymes play an essential role in viral replication; the spike protein acts as a point for host cell entry. Despite a significant degree of phylogenetic divergence from previous coronaviruses, SARS-CoV and bat SARS-CoV are now under group 2b coronaviruses (Astuti & Ysrafil, 2020a)

1.2 Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2)

The emergence of SARS-CoV-2 causing the disease, COVID-19 in China in late December of 2019 led to a global outbreak and is currently a major public health concern (Lai et al., 2020). In late December 2019, a group of people who visited a seafood market in Wuhan had developed a mysterious respiratory syndrome. Unfortunately, the disease had already spread to quite an extent by the time Chinese authorities announced the outbreak (Cheng & Shan, 2020). By then COVID-19 had spread globally within a short period due to international travel to celebrate Chinese New Year.

The global pandemic has turned into an unprecedented global crisis in just a few weeks. The World Health Organization (WHO) declared the epidemic a 'public health emergency of international concern' on January 30, 2020. The confirmed cases of COVID-19 have exceeded 76.8 million globally, with the death toll of above 1.69 million according to the Johns Hopkins University map as on 23 November, 2020 (*COVID-19 Map - Johns Hopkins Coronavirus Resource Center*, 2020); the true numbers remain even higher. Initial data showed differences in severity of outbreaks between countries that adopted preventative measures quickly and decisively, e.g Taiwan and Vietnam took measures early compared to those that took measures late, e.g. Italy and the UK. Some countries had a more severe outbreak after that (Frowde et al., 2020).

WHO has declared the COVID-19 outbreak as a global health emergency and has identified it as a pandemic. To contain the epidemic, they had imposed travel restrictions, city lockdowns and other preventative measures (*Coronavirus (COVID-19) Events as They Happen*, 2020). Similar restrictions had then been adopted by other countries. COVID-19 cases in China had gone down. On the other hand, cases in other countries are rising. Scientists all over the world are working to address the global crisis (Anwar et al., 2020).

Health care professionals, doctors and nurses are working overtime to treat existing and new cases added each day to the COVID-19 pool. The transmission of SARS-CoV-2, which is a respiratory virus, could occur via droplets released through the infected person's sneezing and coughing within six feet. A new study indicated that SARS-CoV-2 can stay in the air for up to 3 hours after being aerosolized; on surfaces, it can stay from 4 hours to three days (Dhand & Li, 2020).

Few necessary precautions are recommended to stop spreading of the virus. These include maintaining a six feet distance, washing hands thoroughly, avoiding touching of face nose, eyes and mouth, sanitizing surfaces such as doorknobs before touching, covering nose while sneezing or coughing. It is advised to self-quarantine for a few days if sick rather than going out to work, putting others at risk. Similarly, it is also suggested to stay away from sick people (*Preventing the Spread of the Coronavirus - Harvard Health*, 2020). Children are equally prone to get infected as adults. However, the severity of symptoms appears to be comparatively less (Dhochak et al., 2020).

The disease is characterized by symptoms such as fever, lesions in lungs, sore throat difficulty in breathing, dry cough, fatigue, arrhythmia, lymphopenia, anorexia, and shock (Baj et al., 2020). The severity of the symptoms varies from person to person and depends on the overall health, age, comorbidities, extent of spread and virulence of the strain. The mechanism in which the virus enters host cell could be a key to curbing the virus. The treatment options are discussed in section 1.7.

The major differences in this virus with SARS-CoV are highlighted in Section 1.3.

1.2.1 Mechanism of viral entry

The overall lifecycle of coronavirus replication is similar to other positive-strand RNA viruses but the synthesis process of subgenomic mRNAs is unique for SARS-CoV-2.

SARS-CoV-2 and SARS-CoV use the human ACE2 receptor as entry point and human proteases as entry activators.

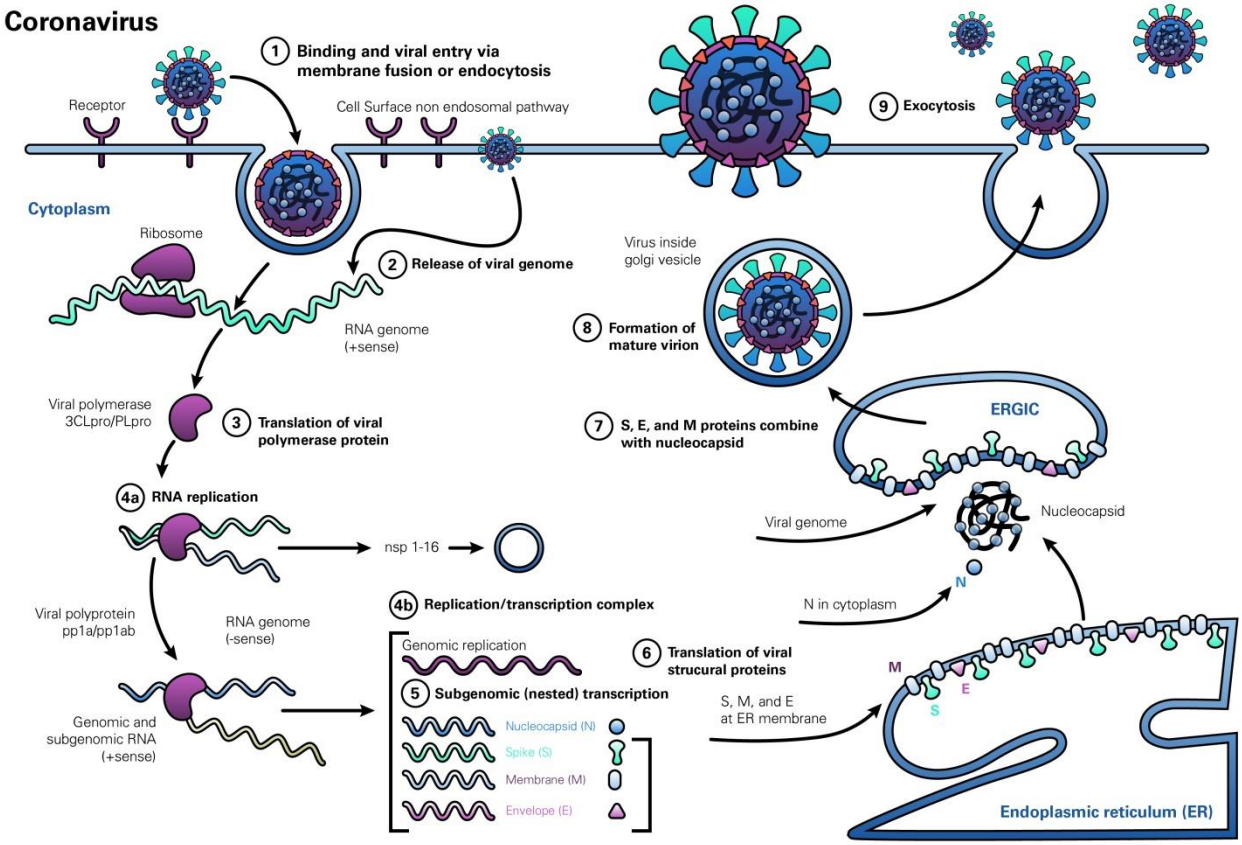


Figure 4: *Lifecycle of a typical coronavirus* (Burmer & Pabuwal, 2020)

The steps in the life-cycle of SARS-CoV-2 are explicitly discussed:

Step 1: The life cycle of SARS-CoV-2 commences as the virion binds to host cell RBD through its surface spike protein S1 subunit;

The interaction of S-protein-receptor determines:

- the host species range
- tissue tropism

For instance, SARS-CoV and HCoV-NL63 use ACE2 as a host receptor, whereas MERS-CoV uses DPP4 to enter human cells. The resulting disease profile depends greatly on the receptor distribution within tissues in the human body. SARS-COV-2 enters the human through its spike protein which attaches to the ACE2 receptor. ACE2 is found in the heart, vessels, lung, gut, testis, the brain and kidney, mostly bound to the cell membranes (Verdecchia et al., 2020). Although it has been clear that SARS-CoV-2 infects human cells through the binding of its RBD domain with the human ACE2 receptor, the molecular mechanism of the binding between the RBD protein and the ACE2 receptor is still unknown

(He et al., 2020). The brain has been reported to express ACE2 receptors, which makes them a potential target of COVID-19. Patients with acute SARS-CoV-2 illness have also demonstrated the presence of the virus in cerebrospinal fluid (CSF) (Mannan Baig et al., 2020)

Step 2: The coronavirus gains access to host cell cytosol after binding to the host ACE2 receptor; subsequently, an acid-dependent proteolytic cleavage of S protein occurs breaking it into S1 and S2 subunits by a cathepsin, TMPSSR2 (Figure 5), furin or another protease. This is followed by fusion, assisted by S2 of both the viral and cellular membranes, leading to release of the viral genome.

Step 3: The viral polymerase, replicase is translated from genomic RNA

Step 4a: Viral RNA is synthesized

Step 4b: Replication-transcription complexes form and assemble

Step 5: The viral structural proteins are translated from the RNA

Step 6: The viral structural proteins inserted into the endoplasmic reticulum (ER)

Step 7: They move to the endoplasmic reticulum-golgi intermediate compartment (ERGIC). Several copies of the nucleocapsid or N protein package the genomic RNA into helical structures, which form the ribonucleoprotein complexes in the cytoplasm. They then interact with hydrophobic M proteins (or envelope protein) in the ERGIC.

Step 8: Virions are budded from the ERGIC membranes

Step 9: Virions are then transported by the exocytic pathway out of the cell (Fung & Liu, 2014)

Viral entry into host cells is an essential determinant of coronavirus infectivity and pathogenesis. It is also an important target for host immune system surveillance and disease combating strategies.

Given the structures of SARS-CoV and SARS-CoV-2 are highly homologous, it is expected that the novel SARS-CoV-2 will also use the ACE2 receptor to enter human cells.

The SARS-CoV-2 has, however, undergone mutations that cause it to bind more strongly with the ACE2 receptor. Therefore, its infectivity has become relatively higher than the classical SARS-CoV (Wan et al., 2020).

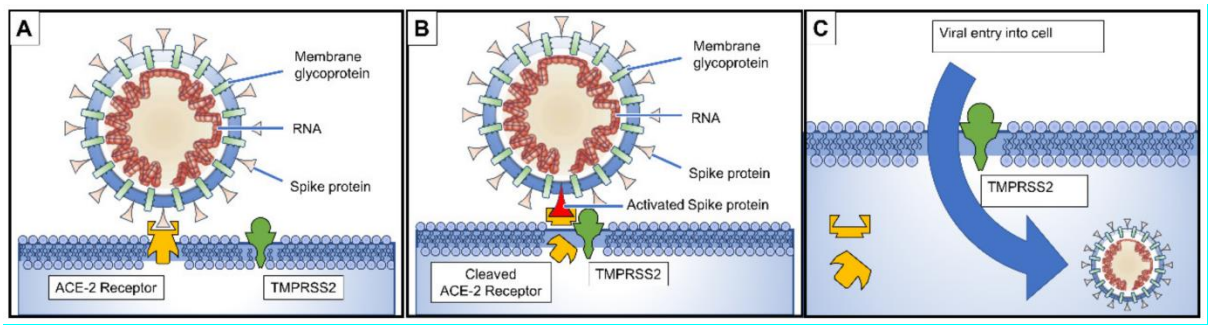


***Figure 5**: Mechanism of viral entry using TMPSSR2* (Hoffmann et al., 2020)

Current drug repurposing studies are targeting either or both of these two proteins, ACE2 and the serine protease TMPSSR2, by a clinically proven inhibitor to prevent and treat COVID-19 since the entry of the coronaviruses depends on the viral surface spike (S) protein binding to cellular receptors, followed by the S protein cleavage by host cell TMPSSR2 (Hoffmann et al., 2020).

For example, camostat methylate, which is a TMPSSR2 inhibitor, is being considered as an anti-COVID drug. In vitro human cell as well as animal studies has shown the drug inhibits virus-cell membrane fusion and, thus, viral replication. Camostat mesylate is an oral drug, which may be used in both outpatients and inpatients at all COVID-19 disease stages. It is currently under clinical trials to test whether the drug could be repurposed and used to combat the current pandemic (Breining et al., 2020).

1.3 Comparison between SARS-CoV and SARS-CoV-2

The following table illustrates a comparison between the previous variant, SARS-CoV with SARS-CoV-2.

Table 1: *Comparison of features between SARS-CoV and SARS-CoV-2*

Features	**SARS-CoV**	**SARS-CoV-2**	**Reference**
Entry	Uses human ACE2 as entry point	Uses human ACE2 as entry point	(Hoffmann et al., 2020)
Receptor Binding domain (RBD) and	Lower ACE-2 binding affinity (less efficient entry)	Higher ACE-2 binding affinity (more efficient entry)	(Wan et al., 2020)

ACE2 binding affinity			
ACE2-binding affinity of the entire virus S protein	comparable	Comparable or maybe even lower (less exposed)	(Rossi et al., 2020)
Receptor Binding Domain(RBD) position	Mostly RBD is in the "lying-down" position, a state associated with not only ineffective receptor binding, but also immune evasion	Mostly RBD is in "standing-up" position, a state associated with not only highly effective receptor binding but also immune recognition	(Shang et al., 2020)
Cleavage site in S protein	The S protein does not contain a furin-like cleavage site	S protein contains a furin-like cleavage site. Cleavage of S protein by furin at the S1/S2 site is an essential process for cell–cell fusion and SARS-CoV-2 entry into human lung cells	(Coutard et al., 2020)
TMPSSR2 activity	Spread depends on TMPSSR2 activity	Spread depends on TMPSSR2 activity	(Rossi et al., 2020)

1.4 Risk factors of coronavirus

COVID-19 is relatively a new disease; there is limited information backed up by evidence regarding risk factors for severe illness. Based on currently available data and clinical expertise, older adults and people with comorbidities tend to be at higher risk for severe illness from COVID-19. Other risk factors and conditions include cancer, chronic kidney disease, a patient treated with dialysis, or immunocompromised patients (weakened immune system) from an organ transplant, obesity, sickle cell anemia, Type 2 diabetes mellitus, chronic lung disease, diabetes, older people (65+ years), hemoglobin disorder, liver disease, serious heart conditions and people in long term care facilities (Sanyaolu et al., 2020).

The risk for serious disease and death in COVID-19 cases increases with advancing age and the presence of comorbid health conditions. Since the emergence of the first case in Wuhan, China, in December 2019, tremendous research efforts have been carried out to understand the mechanisms of infectivity and transmissibility of SARS-CoV-2. To minimize the mortality rate, it is important to identify symptoms promptly and employ treatments appropriately. Even though no cure has been established, multiple clinical trials are underway to determine the most optimal strategy (Gosain et al., 2020).

People of any age with the following conditions are at increased risk of severe illness from COVID-19:

1.4.1. Cancer

Patients with cancer are at high risk of infections due to an overall immunocompromised status. The immune system protects the body against illness and infection caused by coronaviruses. Some cancer patients have a weak immune system, thus reducing their ability to fight infections. This is because cancer treatments, like chemotherapy, hinder the white blood cells' production from bone marrow. Some cancers can lower the ability to fight infections. However, this correlation is not proven for CoV infection, in which the host immune response is the main driver of tissue damage. A study on CoV pathogenesis and morbidity rate in cancer patients was conducted through the analysis of the previous CoV pandemics. Considering the interaction between CoV and the host immune system, cancer patients receiving immunotherapy might be more at risk for an aberrant immune response in case of infection and might therefore deserve additional precautions (Indini et al., 2020). The impact on COVID-19 in cancer is, however, controversial. Several studies reported an increased risk of death (Q. Li et al., 2020).

For example, the fatality rate for infected cancer patients (n=28) in China is 28.6%, compared to a 2.3% fatality rate for all COVID-19 patients (Zhang et al., 2020).

Managing cancer patients under these circumstances is rather challenging, given their vulnerable status and the aggressive nature of their underlying disease.

1.4.2 Chronic kidney disease (CKD)

CKD is associated with an increased risk of both inpatient and outpatient pneumonia. The pneumonia-associated mortality rate in CKD patients is 14–16 times higher than in the

general population (Henry & Lippi, 2020). COVID-19 infected patients with kidney disease and other severe chronic medical conditions are at higher risk for more severe illness.
People on dialysis tend to have weaker immune systems, making it harder to fight infections.
People with a kidney transplant need to take immunosuppressive medicines. These medicines work by keeping the immune system less active, which can make it harder to fight infections. Special precautions are thus recommended; it is advised to wash hands, maintain good hygiene, and follow the recommendations from their healthcare team.
Patients with chronic kidney and those who have received renal transplants are at increased risk of COVID-19 infection and severity. Moreover, there are frequent renal function abnormalities and an increased incidence of acute kidney injury in patients with COVID-19. It is not known yet whether this occurs from the effects of sepsis or is a direct nephrotoxic action of a virus. Patients with acute kidney injury have a higher mortality, and renal function monitoring should be a part of managing these patients (Gupta & Misra, 2020).

Notably, COVID-19 patients have demonstrated kidney damage through acute kidney injury, hematuria, mild proteinuria and a slight elevation in creatinine levels, possibly because of kidney tropism of the virus or multiorgan failure. The exact impact of COVID-19 on preexisting kidney impairment conditions such as CKD, recipients of a kidney transplant, patients on hemodialysis (HD) has not yet been clearly established (Benedetti et al., 2020).

1.4.3 Chronic lung disease

Clinical features of COVID-19 are non-specific and is not easily distinguished from other causes of severe community-acquired pneumonia (Phua et al., 2020). Chronic lung conditions include asthma, asbestosis, pneumonitis, COPD, and pulmonary fibrosis (Harber et al., 2016). Asthma is a major health issue worldwide. SARS-CoV-2 is a respiratory pathogen, and it is important to understand asthma associated with risks in COVID-19 patients. Currently, there is limited data to demonstrate any specific risk or exacerbation for COVID-19 patients from asthma or increased disease pathology in asthma patients. Previous pandemic coronaviruses have not caused asthma exacerbations. However, there are non-pandemic coronaviruses that have been reported to exacerbate asthma. It is imperative that asthma patients implement proper steps to keep their asthma under control to prevent chances of a serious exacerbation (X. Li et al., 2020).

On the other hand, the CDC states that 'patients with moderate-to-severe asthma are at a higher risk for severe respiratory illness when infected with SARS-CoV-2' (*People with Moderate to Severe Asthma | CDC*, 2020).

Theoretically, the coronavirus first infects the lining of the throat, lung, and trachea, transforming them into virus factories, producing large amounts of viruses that infect other surrounding cells. The high temperature is caused by the immune system response to the virus and sending signals for the release of cytokines. The virus, however, disrupts the immune response causing more inflammation. In a normal scenario, oxygen travels to the blood from the lungs, but in severe pneumonia, water begins to fill up the alveoli, causing shortness of breath and coughing with sputum (thick mucus that contains lung cells killed by the SARS-CoV-2) (Abd El-Aziz & Stockand, 2020).

Chronic obstructive pulmonary disease (COPD) is a growing healthcare concern that is expected to worsen by the increased worldwide use of tobacco products (Duncan, 2016). According to the WHO, "Tobacco smoking is a known risk factor for many respiratory infections and increases the severity of respiratory diseases. A review of studies by public health experts convened by WHO on 29 April 2020 found that smokers are more likely to develop severe disease with COVID-19, compared to non-smokers."(*WHO Statement: Tobacco Use and COVID-19*, 2020).

A recent meta-analysis revealed that smokers are vulnerable to severe COVID-19 with worse outcomes. Preventative and supportive strategies include reduction/withdrawal of smoking to reduce morbidity and mortality (Reddy et al., 2020).

1.4.4 Diabetes mellitus (DM)

Diabetes is among the leading causes of morbidity and mortality worldwide. It is associated with various macrovascular and microvascular complications that will ultimately impact the patient's survival. A correlation between diabetes and infection has been clinically recognized for a very long time. Infections, especially influenza and pneumonia, are usually more common and serious in elderly people having type 2 diabetes mellitus (T2DM). However, there is controversial evidence regarding whether diabetes itself increases the outcomes and susceptibility from infections, or the comorbidities (cardiovascular and renal)

that are associated with it are the major factors involved. During the 2009 influenza A(H1N1), SARS, and MERS CoVs pandemics, uncontrolled glycemia and diabetes were significant indicators of severity and deaths in infected patients. The current SARS-CoV-2 pandemic, however, still does not reflect a strong association between diabetes and the disease. Although reports from Italy and China demonstrated older patients having chronic diseases, such as diabetes, were at a higher risk for severe COVID-19 and mortality. There is insufficient data regarding glucose metabolism and acute complications of diabetes (ketoacidosis) in COVID-19 patients. It is being assumed SARS-CoV-2 infection in diabetes patients triggers higher than usual stress conditions, releasing a greater amount of hyperglycemic hormones, such as catecholamines and glucocorticoids; these hormones, in turn, lead to elevated blood glucose levels as well as abnormal glucose variability.

A retrospective study, on the other hand, reported that around 10% of the patients with T2DM and COVID-19 suffered at least an episode of hypoglycemia that is associated with elevated plasma glucose levels (Hussain et al., 2020). The high prevalence of CVD, hypertension, and obesity in DM patients triggers the question of whether DM contributes to this increased risk. Plasma glucose levels and DM, however, are independent indicators for morbidity and mortality and in SARS patients.

Possible mechanisms that may increase COVID-19 susceptibility in DM patients include:
a. high-affinity cellular binding leading to efficient viral entry,
b. decreased viral clearance,
c. decreased T cell function,
d. increased susceptibility to hyperinflammation resulting in cytokine storm syndrome
e. CVD (Muniyappa & Gubbi, 2020).

1.4.5 People aged 65 years and older

A literature review and meta-analysis by Zheng et. al. reported that male aged over 65 might have a greater risk of developing a critical or mortal condition; such patients usually tend to have underlying conditions/ comorbidities e.g., CHD, lung disease or DMT2 affecting the prognosis of COVID-19 (Z. Zheng et al., 2020). Older adults are at higher risk for developing more serious complications from COVID-19 illness(*Older Adults | CDC*, 2020).

1.4.6 Hypertension

Studies have reported that patients with hypertension have a tendency to develop acute respiratory distress syndrome (ARDS) and progress from ARDS to death in COVID-19 pneumonia (Fernández-Fernández, 2020). A meta-analysis by Roncon et al. suggested that hypertension patients with COVID-19 have a higher risk of ICU admission. A report from Italy suggested that COVID-19 patients in ICU are more likely to develop arterial hypertension, resulting in death (Roncon et al., 2020).

1.4.7 Liver disease

COVID-19-related liver injury is common in COVID patients, both with and without preexisting liver disease. Overall, hospitalized COVID-19 patients tend to demonstrate elevated serum liver biochemistries, primarily elevated levels of AST and ALT and a slight increase in bilirubin, ranging from 14% to 53%. Increased liver enzymes are more common in males and more severe cases than milder ones. Low albumin level is an indicator of severe infection or poor prognosis. To date, no report of acute or chronic liver failure has been published in COVID-19 patients.

Liver biopsy specimens of deceased severe COVID-19 patients showed moderate microvascular steatosis (MS) and mild lobular activity and portal activity, reflecting the injury may have been caused by SARS-CoV-2 infection or drug-induced liver injury.
Suggested mechanisms are listed below.

1. The severe inflammatory response could lead to immune-mediated damage following COVID-19 infection: the inflammation biomarkers include C reactive protein (CRP), elevated LDH, serum ferritin, D-dimer, IL-2, and IL-6, in severe COVID-19 patients.
2. Active viral replication can lead to direct cytotoxicity in hepatic cells: SARS-CoV-2 binds to target host cells through ACE2 receptors. ACE2 receptors are abundantly expressed in the liver particularly, on biliary epithelial cells. The liver is thought to be a potential target for direct infection,
3. The hallmark of COVID-19, anoxia is respiratory failure. In severe cases, anoxia frequently leads to hypoxic hepatitis
4. COVID-19 guidelines recommend antiviral drugs for COVID-19, such as favipiravir, remdesivir, lopinavir/ritonavir and other drugs such as chloroquine, tocilizumab and

Chinese traditional medicine, which are potentially hepatotoxic in some patients leading to Drug-induced liver injury (DILI).

5. Patients with pre-existing chronic liver disease might be more susceptible to liver damage from SARS-CoV-2. Drugs such as tocilizumab and baricitinib may also cause HBV reactivation, leading to the deterioration of liver function. It is still unknown whether infection exacerbates cholestasis in patients with underlying cholestatic liver disease (Sun et al., 2020).

1.4.8 Hemoglobin (Hb) disorder

A meta-analysis conducted by Lippi et al. showed that hemoglobin values are significantly reduced in severe COVID-19 patients compared to patients having a milder form of the disease, thus confirming the previous evidence from patients with pneumonia. Thus initial count and longitudinal monitoring of Hb values are recommended in patients with SARS-CoV-2 infection, where a significant decrease in the Hb concentration may indicate a worse clinical progression (Lippi & Mattiuzzi, 2020). Other laboratory changes in severe or fatal COVID-19 cases include elevated WBC and neutrophil counts and decreased Hb, platelet, and eosinophil count (Michael Henry et al., 2020).

1.4.9 Cardiovascular disease/ Serious heart condition

Patients with cardiovascular diseases are among the highest risk patients for severe COVID-19 disease and high mortality. Cardiac troponin I levels are tremendously increased in severe SARS-CoV-2 infection in comparison to milder forms. This is somewhat similar to what is observed in patients having acute respiratory illnesses; or it may be an indicator of myocardial injury as ACE-2 receptors are highly expressed on cardiomyocytes. American College of Cardiology suggests measuring troponin if the diagnosis of acute MI is being considered. Similarly, COVID-19 patients have increased natriuretic peptides, the significance of which is unknown. Hence an increased level of natriuretic peptides in COVID-19 should not be considered an indicator of heart failure(Gupta & Misra, 2020). SARS-CoV-2 infects host cells via ACE2 receptors, leading to COVID-19-associated pneumonia, also causing myocardial injury and chronic damage of the cardiovascular system (Y. Y. Zheng et al., 2020).

1.4.10 Neurological disorders

Headache has been reported as a COVID-19 symptom, which may call for neurology consultation. To date, only a few cases of COVID-19 meningitis have been reported; it must still remain in the differential diagnosis for patients with fever and headache (Arca & Starling, 2020). An increasing number of case reports describe a wide range of neurological manifestations in 901 patients, with insufficient detail, which is a major challenge of such studies. Encephalopathy in 93 patients has been reported in total. Among them were 16 (7%) of 214 hospitalized COVID-19 patients in China, and 40 (69%) out of 58 patients in ICU with COVID-19 in France. SARS-CoV-2 was detected in the CSF of some patients. Acute cerebrovascular disease is also evolving as an important complication, as cohort studies report stroke in 2–6% of COVID-19 hospitalized patients. As of now, 96 patients with myocardial infarction have been reported. They frequently had vascular events in pro-inflammatory hypercoagulable state with elevated levels of C-reactive protein, D-dimer, and ferritin (Suzannah et al., 2020).

1.5 Symptoms

COVID-19 seems to be very contagious and has quickly spread globally via human-to-human transmission, droplets, or direct contact. Moreover, data from reports provided by health policy agencies divide the clinical manifestations of the disease according to the severity of the clinical pictures in mild, moderate, or severe illness. Fever is the most common symptom, followed by cough, and the clinical course of the disease seems to predict a favorable trend in the majority of patients. Nevertheless, in a percentage of cases, which have not been defined yet, a sudden worsening of clinical conditions can be observed after about a week. In the most severe pictures, pneumonia and acute respiratory distress syndrome (ARDS) have been reported to worsen until sepsis and septic shock, a serious clinical condition characterized by a wide range of signs and symptoms related to multi-organ involvement (Silvestro et al., 2020).

According to the WHO, COVID-19 affects different people in different ways. Most infected people will develop mild to moderate illness and recover without hospitalization. The most common symptoms include fever, tiredness, and dry cough. Less common symptoms include sore throat, aches, and pains, conjunctivitis, diarrhea, loss of taste and smell, headache, rash on the skin, or discolouration of fingers or toes. The serious symptoms are shortness of breath, difficulty breathing, pain, or pressure in the chest, loss of speech, or inability to move.

On average, it takes 5–6 days from when someone is infected with the virus for symptoms to show; however, it can take up to 14 days (*Coronavirus*, 2020). Symptoms may vary from person to person and this depends on other factors such as age, comorbidities, genetic makeup and also the virus is mutating. Recently it has been reported to show dengue-like symptoms such as low platelet counts, kidney infections and shock syndrome along with the usual symptoms (Tajmim, 2020).

Table 2: Characteristics according to Severity of the Disease

Symptoms	**Characteristics**	**References**
Asymptomatic	Having the infection and showing no symptoms at all.	(Christiano, 2020)
Mild	Mild respiratory illness symptoms e.g. runny nose, nasal congestion, sore throat. Other symptoms include: • Fever not more than 100 degrees Fahrenheit • Fatigue (feeling tired all the time) • Headache • Gastrointestinal (GI) upset, vomiting and diarrhea • Muscle pain • Dry cough • Malaise • Redness of the eye • Diarrhea • Skin rash • New loss of taste or smell • COVID patches: itchy, painful patches on toes. This is more common in young people • No shortness of breath • No radiological findings of pneumonia in CXR or HRCT chest	
Moderate	• Mild clinical symptoms (Fever, sore throat cough, malaise, headache, malaise, muscle pain, diarrhea, loss of taste &smell sensation, redness of eye, skin rash etc.)	

	• Consistent fever of about 101-102℉ • Deep cough • Chills, with repeated shaking • Muscle pain • Fatigue and body aches • General feeling of being unwell • No shortness of breath • No radiological findings of pneumonia in CXR or HRCT chest	
Severe case	Severe case includes all the symptoms mentioned above. Some other symptoms that may be observed are as follow: • Shortness of breath, • Chest discomfort • Trouble staying awake • Eye problems, e.g. watery eyes or swollen eyelids • Confusion/unresponsiveness • Bluish face or lips **Moderate case and** a**ny one of the following criteria:** • Respiratory distress (≧30 breaths/ min) • O_2 saturation (SpO_2) ≤93% at rest on pulse oximeter • Arterial partial pressure of oxygen (PaO2)/fraction of inspired oxygen (FiO2)<300mmHg (1 mmHg=0.133kPa) in ABG or SpO_2/FiO_2 < 315 mmHg	
Critical case	**Any of the following features:** • Respiratory failure &requiring mechanical ventilation • Shock • ARDS • With other organ failure that requires ICU care	

1.6 Available diagnostic techniques

1.6.1. Clinical specimens for SARS-CoV-2 testing

Acceptable specimens include upper respiratory tract specimens, lower respiratory tract specimens, whole blood specimens, and serum specimens, and the respiratory secretions are the most frequently sample for diagnosis. Currently, SARS-CoV-2 has been detected in nasopharyngeal swabs, oropharyngeal swabs, throat swabs, sputum, bronchoalveolar lavage fluid, whole blood, serum, stool, urine, saliva, rectal swabs and conjunctival swabs (C. Li et al., 2020a).

With limited understanding of COVID-19, it is difficult to exclude SARS-CoV-2 infection based on a single negative PCR result, especially when testing was used for upper respiratory tract specimens. Collection and detection of lower respiratory tract specimens are strongly recommended even if the upper respiratory tract specimens are negative, especially in patients with severe or progressive conditions. ACE2 is mainly distributed in alveolar type II epithelial cells, suggesting lower respiratory tract specimens) may contain high viral RNA loads (Dheda et al., 2020) .

1.6. 2 Manual laboratory-based nucleic acid tests

a. Real-time quantification RT-PCR

With the recent advancement in medical diagnosis, laboratory-based nucleic acid detection approaches have become rapid and reliable for viral detection. Among the known nucleic acid tests, the method polymerase chain reaction (PCR) is considered the ‘gold standard for virus detection. The qualitative assay is characterized by quick detection, high sensitivity, as well as high specificity. As such, RT-PCR appears promising today for the detection of SARS-CoV-2. Moreover, it offers early diagnosis and is a primary method to detect the causative virus of COVID-19, SARS-CoV-2. However, the real-time RT-PCR test demonstrates the risk of eliciting false-negative as well as false-positive results. Therefore, a negative result alone does not exclude a patient from the possibility of COVID-19 infection. It is suggested that a combination of real-time RT-PCR with the patients’ clinical features should be considered (Tahamtan & Ardebili, 2020). There are different protocols for qRT-PCR for detecting SARS-CoV-2 on the WHO website for different countries. As for

example, the gene targets for detection are ORF1ab and N genes in China, N gene in Thailand, and RdRp, E, and N genes in Germany. The CDC established an RT-PCR panel for specific detection SARS-CoV-2 and universal detection of SARS like beta-CoVs. Three sets of different primers were designed for the N gene; one set of primers/probes was used universally to detect all beta- CoVs, while the other two were specific to identify SARS-CoV-2. The confirmation of COVID-19 must be positive for all three targets (C. Li et al., 2020a).

Konrad et al. compared qRT-PCR tests in different PCR systems and a commercial reagent using a nasopharyngeal swab or sputum samples from COVID-19 patients in Germany (Konrad et al., 2020). When the same primers and probes were used, the distinctions in analytical sensitivities between different PCR systems were also observed. They found that the E gene target was more sensitive than the RdRp target when using a one-step qRT-PCR system. However, the high background of E gene target hindered the clear evaluation of the test, and further optimization of E gene assay may improve the sensitivity (Toptan et al., 2020)

b. Nested RT-PCR

Real-time nested RT-PCR assay has proven to be quite suitable for detecting low-copy-number viruses present in the early stages of the disease. It connects the time-saving real-time instruments with the high sensitivity of nested PCR. Initially, the detection of SARS-CoV-2 by nested RT-PCR approach was verified in Japan. The method developed has identified twenty-five positive patients successfully in Japan. More recently, Ji et al. designed a one-step nested real-time RT-PCR (OSN-qRT-PCR) assay that targets SARS-CoV-2 ORF1ab and N genes. The sensitivity of the assay was 1 copy per test and was 10-folds higher than that of a commercial qRT-PCR assay, which is about 10 copies per test. In comparison to the qRT-PCR kit, nested RT-PCR analysis demonstrated both higher sensitivity and specificity, thus indicating that it is a better choice for clinical application, especially in cases with low viral content. However, laboratory cross-contamination associated with the method could lead to false-positive results (El-Tholoth et al., 2020; Rnas et al., 2020)

All the RT-qPCR-based methods analyze SARS-CoV-2 nucleic acids starting from rhino-pharyngeal swab samples obtained from subjects with suspected COVID-19 infection. However, the sensitivity of such a technique may be very low (depending on the platform

used, sample impurities, low amount of viral cDNA, etc.), leading to a high percentage of false-negative results and failing to assess the viral load during the follow-up of quarantined patients (Falzone et al., 2020).

c. Droplet Digital PCR

Droplet digital PCR (ddPCR) has been shown to improve the lower limit of detection (LOD), sensitivity, and accuracy in detecting SARS-CoV-2. A study by Suo et al. analyzed the feasibility of the method for SARS-CoV-2 RNA detection as compared with qRT-PCR (Suo et al., 2020). The same primer or probe sets by China CDC that targets ORF1ab or N gene were used in the study. Twenty-six patients with negative RT-PCR results were confirmed positive by ddPCR. The sensitivity and accuracy were improved from 40% and 47% for RT-PCR to 94% and 95% for ddPCR, respectively. The study showed that ddPCR could reduce the false negatives results largely that were caused by qRT-PCR. The overall performance of ddPCR was significantly better than qRT-PCR, especially for low viral load samples. However, ddPCR also has some limitations. To ensure commutability between molecular diagnostic laboratories, gold standards still need to be defined precisely. It is more costly than qRTPCR for each test performed as dedicated instruments and consumables are used (Rnas et al., 2020).

Different studies with ddPCR have demonstrated both higher sensitivity and robustness compared to other employed molecular techniques. The technology is based on the absolute quantification of targets. It uses oil-water emulsion and is based on the principles of dilution and partition of the reaction mix in 20,000 nano-droplets. This improves the accuracy as well as detection of targets at a low-cost and greater sensitivity. At present, ddPCR is effectively being used for the absolute quantification of viral content, for analysis of microRNA and gene expression, analysis of the circulating DNA and of gene copy number variation (Falzone et al., 2020).

1.6.3 Rapid and point-of-care nucleic acid tests

a. Loop-mediated isothermal amplification (LAMP)

LAMP has the advantage of quick amplification at a single temperature; this method is efficient, quick, and reliable in the diagnosis of coronaviruses. Zhang et al., in their study, designed full LAMP primers that targeted the 5′ region of the ORF1a and N genes of SARS-CoV- 2. They detected the virus by a colourimetric RT-LAMP alongside a commercial RT-

PCR assay. 6 out of 7 samples showed visible color change, indicating positive amplification. On the other hand, the single sample remained pink color was confirmed negative. The analysis was 100% consistent with the RT-PCR results across a range of Cq values. The method does not use sophisticated instrumentation. This method is suitable for use at home, in the clinic, and point-of-care and does not require trained personnel or sophisticated instrumentation. Additionally, it reduces false-negative results from routine nucleic acid tests (Mautner et al., 2020; Wang et al., 2020a).

b. Nanoparticles based amplification

In order to improve the specificity and sensitivity of SARS-CoV-2 detection, nanoparticles have been added to the nucleic acid amplification system. Recently, a naked-eye colorimetric test was designed by Parikshit et al., which consisted of gold nanoparticles (AuNPs) with thiol-modified antisense oligonucleotides (ASOs) to target the SARS-CoV-2 N-gene (Moitra et al., 2020). The SARS-CoV-2 viral load had a limit of detection (LOD) of 0.18 ng/μL. In addition, a LOD of 12 copies/test was observed in a one-step nanoparticles-based biosensor (NBS) coupled with RT-LAMP. The analytical sensitivity of SARS-CoV-2 was found to be 100% (33/33) in COVID-19 patients. When RNA templates of non-COVID-19 patients were analyzed, the specificity of the assay was again 100% (96/96). Nanoparticles have certain unique properties that are more beneficial over traditional methods, which may be quite expensive and laborious. The nanoparticles-based amplification could be a promising approach to test for SARS-CoV-2 infection, especially in areas that lack proper medical resources. However, this technique is more complex and expensive than the currently used qRT-PCR. Besides, systems using traditional organic carriers have a possibility of photobleaching that could lead to reduced sensitivity and a false-negative result (C. Li et al., 2020b).

c. Portable benchtop sized analyzers

The automated molecular diagnostic platform demonstrates a highly sensitive, powerful and accurate method for the rapid identification of SARS-CoV-2. The assay does requires neither PCR training nor point-of-care testing to achieve rapid decisions and technological innovation. The inconsistent performance of the different portable benchtop-sized analyzers for SARS-CoV-2 detection was reported (La Marca et al., 2020; C. Li et al., 2020a).

1.6.4 Antigen tests for COVID-19

SARS-CoV-2 is composed of multiple virus-encoded proteins such as S, N, E, and M. Among these proteins, S and N were the two main antigenic targets of SARS-CoV-2 antibodies. The S protein is cut into two separate polypeptides in the presence of host cell furin-like protease (Astuti & Ysrafil, 2020b). S protein is present on the surface of the virus and is crucial for its entry; however, N protein is the most expressed immune dominant protein that primarily interacts with RNA and is more conserved than S protein (Robson, 2020). S protein consists of two subunits, of which the S1 submit is more specific to SARS-CoV-2 and less conserved, whereas S2 submit acts as an antigen for COVID-19 serologic detection (Tian et al., 2020). In addition, the RBD domain of S1 protein is more conserved than S1 or full-length S, and has much less cross-reactivity with other CoVs (Ou et al., 2020). Multiple forms of N protein or S protein (full-length S, S1 domain, S2 domain, or receptor-binding domain [RBD]) — are used as targets. The immunochromatographic assay is the most commonly used method for the detection of SARS-CoV-2 antigens (Huang et al., 2020; Ou et al., 2020). The four lateral flow antigen-detection kits (RapiGEN, Savant, Liming bio, and Bioeasy) employed for the detection of SARS-CoV-2 was compared by Thomas et al., they all exhibited test performances with noticeable differences. Among the listed tests, Bioeasy demonstrated the highest accuracy (89.2%) and Kappa coefficient (0.8), while Liming biotest was discontinued due to poor performance. On the other hand, sensitivities of other kits were ranging from 16.7% to 85%, for Savant assay and Bioeasy test, respectively (Weitzel et al., 2020). Due to the low sensitivity of immunochromatography, highly sensitive biosensors-based tests are used in the detection of SARS-CoV-2 (C. Li et al., 2020c). For the detection of SARS-CoV-2 S1 protein, Sophie et al. constructed a portable, rapid cell-based biosensor with a human chimeric spike S1 antibody. The biosensor can complete tests in 3 minutes with a detection limit of 1 fg/mL and a semilinear response range of 10 fg to 1 μg/mL. Similarly, a biosensor device (eCovSens) was developed by Subhasis et al. to target the SARS-CoV-2 S1 protein. In comparison to commercial potentiostat sensors (with LOD of 120 fM), the LOD of eCovSens was found to be 90 fM in saliva samples (Mavrikou et al., 2020). These platforms could be used to monitor SARS-CoV-2 antigen on a larger scale, thus proposing a promising scheme for routine monitoring and control of the global pandemic.

1.6.5 Antibody tests for COVID-19 diagnosis

Individual antibody types, such as IgG, IgM, and IgA, can be determined with specific reagents. After infection by SARS-CoV-2, IgM antibody can be produced within 5–7 days and is most suitable for determining recent infection, while IgG antibody can be produced within 10–15 days and may remain detectable for months or years (Hou et al., 2020). IgA can be detected in mucous secretions within 6–8 days (Y. Li et al., 2020). In cases where RT-PCR assays are negative, and there is a strong epidemiological link to SARS-CoV-2 infection, paired serum samples could support diagnosis once validated serology tests are available with the initial samples collected in the first week of COVID-19 and the second collected after 2–4 weeks (C. Li et al., 2020c). Tests that detect binding antibodies can be either laboratory tests or point-of-care tests.

Manual laboratory-based antibody tests include enzyme-linked immunosorbent assay. A higher sensitivity was observed in S protein-based ELISA compared to N protein-based ELISA (Kilic et al., 2020). Others include immunofluorescence assay, Chemiluminescence immunoassay, which is one of the most popular immunology assays in identifying infectious diseases with the advantage of quantitative detection. Both assays exhibited very similar specificities of IgG, which were 99% and 100% for ELISA and CLIA assays, respectively (Padoan et al., 2020).

Rapid and point-of-care (POC) antibody tests include lateral flow assay. The qualitative or semi-quantitative detection for SARS-CoV-2 IgM and IgG antibodies in serum, plasma, and venous blood samples in vitro were proved by Lateral flow assay (LFA) (Ragnesola et al., 2020). According to studies, the positivity of IgM antibody for LFA yielded a decrease in specificity compared to IgG antibody alone and does not improve diagnostic performance (Whitman et al., 2020). The studies also indicated that measuring only LFA IgG can avoid false-positive results of IgM. Other tests include microarray and microfluidic chip, in which platforms can be easily converted to point-of-care settings by portability, miniaturization, automation, and integration of multiple functions onto chips. With small size, high sensitivity, and high throughput analysis, microarray and microfluidic chips are powerful tools for pathogen identification. This platform has the advantages of low-cost, high-throughput, and can handle more than 100,000 samples, potentially valuable for serosurveillance in COVID-19 patients. In addition, Tan et al. designed a microfluidic ELISA test for quantitative detection of SARS-CoV-2 S1 protein and anti-SARS-CoV-2 S1 IgG

using humanized SARS-CoV-2 IgG and recombinant S1 protein, respectively. These studies demonstrated high performance in analyzing SARS-CoV-2 specific antibodies and antigens in COVID-19 patients by microarray and microfluidic chip. However, some proteins on the microarray were observed not expressed in mammalian cell systems and need to be further optimized (C. Li et al., 2020c).

1.7. Treatment/Management options

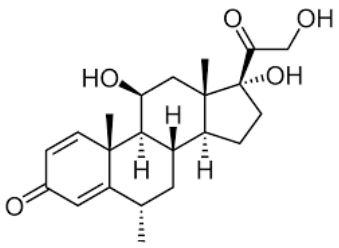
Non-pharmacological intervention approaches and strict regulations are recommended in addition to the current treatment options available (Alvi et al., 2020). Currently, there is no single antiviral treatment that is recommended, and there is no approved vaccine available (Cascella et al., 2020). The treatment is usually symptomatic. For example, oxygen therapy is given for addressing respiratory issues. In case of respiratory failure, non-invasive and invasive mechanical ventilation may be needed. In order to deal with more complicated cases of the disease, intensive care is required (Table 4 and Table 5).

A study by Gattinni et al. suggested that COVID-induced Acute Respiratory Distress Syndrome (CARDS) is different from the typical ARDS (Gattinoni et al., 2020). This has negatively affected the treatment approach strategy at the onset of the pandemic. Non-invasive mechanical ventilation has an important role in case of CARDS therapy.

In cases of asymptomatic or mild cases, antibiotic is not recommended as it poses a risk of resistance. Antipyretics are indicated to reduce fever; diclofenac for body aches. Additionally, minerals, such as zinc and other supplements are given to boost the immune system. In severe cases, particularly in patients with comorbidities such as hypertension or diabetes, anticoagulants are given as prophylaxis. Studies have shown increased glucose concentrations in patients following COVID-19. However, the use of anticoagulants remains controversial, owing to insufficient data.

In critically ill patients, several factors need to be considered before giving medication. In such cases, antibiotics, interferons such as tocilizumab are given. The following subsections list some drugs that are currently being indicated in COVID-19 patients:

1.7.1 Drugs currently used in COVID-19 treatment



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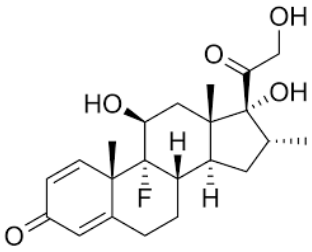

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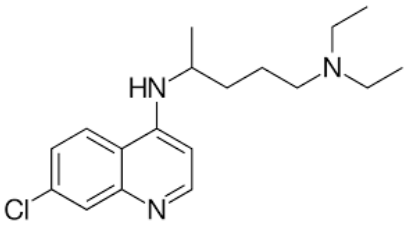

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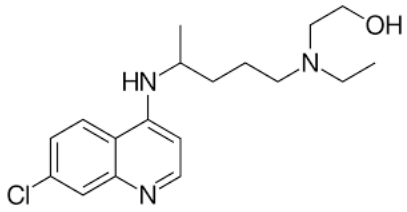

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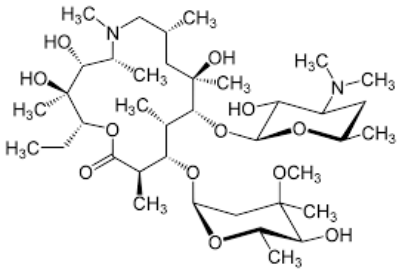

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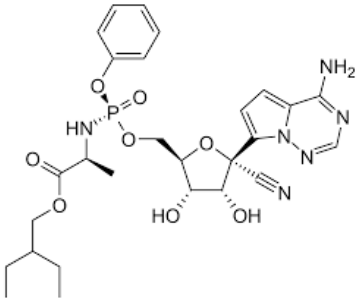

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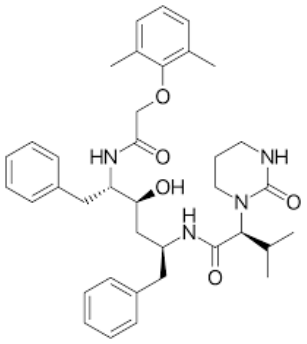

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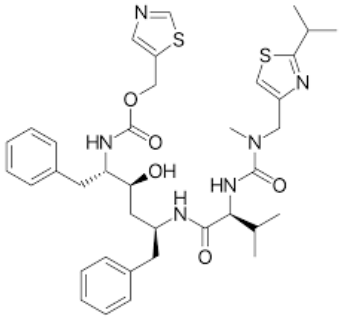

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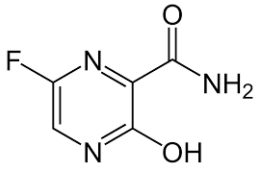

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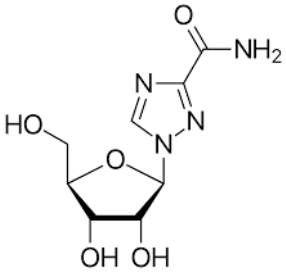

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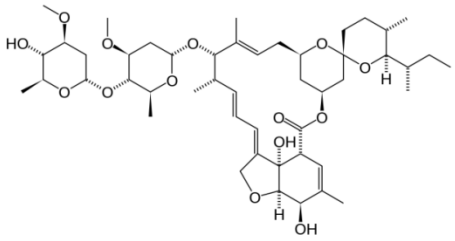
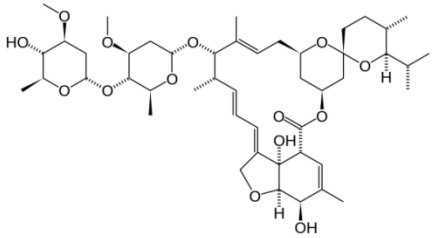
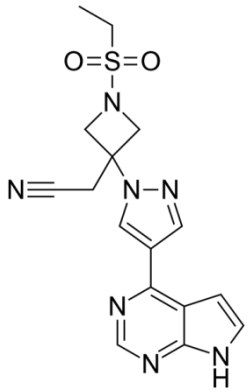




(k)



(l)

Figure 6:Some drugs currently used to treat moderate to severe COVID-19

a: Methyl prednisolone(corticosteroid); b: Dexamethasone (corticosteroid); c: Chloroquine (anti-malarial); d: Hydrochloroquine (anti-malarial); e:Azithromycin (antibiotic); f: Remdesivir (anti-viral); g: Lopanavir (anti-viral); h: Ritonavir (anti-viral); i: Favipiravir

(anti-viral); j: Ribavarin (anti-viral); k: Ivermectin (anti-parasitic); l: Baciritinib (monoclonal antibody)

a. Corticosteroids

Among the therapeutic strategies, though systemic corticosteroids in the treatment of viral pneumonia /ARDS were usually not recommended, in severe CARDS therapy, drugs such as methylprednisolone (1mg/kg/day) are being used. Recently a large-sized RCT showed that dexamethasone reduced the number of deaths by one-third in critically ill patients. In the study group, 2,100 patients received 6 mg dexamethasone/day for 10 days. On the other hand, the control group patients consisting of 4,300 patients received standard care (Cascella et al., 2020). Cytokine storm syndrome (CSS) characterized by an upsurge of inflammatory cytokine, is a critical condition induced by COVID-19 disease (Gao et al., 2020). These drugs show anti-inflammatory and immunosuppressive effects (Coutinho & Chapman, 2011). Thus, they are used in moderate to severe COVID-19 cases.

b. Chloroquine (CQ)/Hydroxychloroquine (HCQ)

Antimalarial drugs have been used for viral infections for the past 70 years. They act on the different pathways of viral entry and exit through host cells. They work by causing disruption of the viral protein synthesis. Previously, in vitro activity against other viruses, such as Ebola, HIV, the dengue virus, influenza virus, and the chikungunya virus, has been reported (Ali et al., 2020). Considering these, studies are being performed to investigate the effect of CQ and HCQ in COVID-19. The antimalarial drugs CQ and HCQ demonstrated an inhibitory effect on the mRNA production of SARS-CoV-2 in vitro. HCQ shows greater efficacy in inhibiting protein synthesis than CQ. The in vitro activity, however, is not an indicator of clinical activity and thus, clinical trials have to be performed (Ali et al., 2020).

In a non-randomized trial (NRT), 36 patients with COVID-19 were given HCQ alone or in combination with azithromycin. After 6 days of treatment, all the patients treated with the combination had no detectable viral load present in nasopharyngeal swabs in comparison to 57.1% of patients treated with HCQ alone and 12.5% of the control group. Another report from China showed 100 patients with COVID-19, treated with HCQ showed better clinical outcomes than the control patients. However, HCQ and CQ have a narrow therapeutic index and are associated with side effects such as torsade de pointes, QT interval prolongation, bone marrow suppression, retinopathy, myopathy, and seizure. If the safety, ethical use and clinical efficacy of CQ/HCQ are properly established by trials, it would be an important advancement in the treatment of COVID-19. The collection of accurate data regarding the safety and clinical efficacy of the drugs for the treatment of COVID-19 is a strategy that would allow robust available data for the near future (Rismanbaf, 2020).

c. Azithromycin

Azithromycin, a bacteriostatic antibiotic that belongs to the macrolides class, inhibits protein synthesis in bacteria. It interferes with bacterial growth and is considered a potential drug for COVID-19 patients. In addition to its antibacterial effects, it also demonstrates antiviral properties. It has been indicated in respiratory viral infection. In a study conducted by Gautret et al., it was demonstrated that azithromycin, in combination with HCQ has a substantial antiviral effect against SARS-CoV-2. However, evidence on azithromycin alone in the treatment of COVID-19 is rare, and it is ambiguous whether macrolides should be used alone or in combination with HCQ (Gautret et al., 2020). Masashi et al. stated that macrolides alone or in combination with other drugs are effective against SARS-CoV-2 (Ohe et al., 2020). Clinical trials to check the safety efficacy of the combination of azithromycin-HCQ are being conducted for SARS-CoV-2.

d. Remdesivir

Remdesivir, an adenosine analogue interferes with the new viral RNA synthesis by chain termination. Though it was developed for use against Marburg and Ebola virus infections, it demonstrated antiviral effects against several other RNA viruses such as respiratory syncytial virus, and other coronaviruses, as for example, MERS-CoV and SARS-CoV (Warren et al., 2016). Since the drug demonstrates antiviral activity against MERS-CoV and SARS-CoV, it has been tested against SARS-CoV-2. Remdesivir showed satisfactory results in mouse models. It was observed that it significantly decreased the virus load in the lungs, thus improving the pulmonary function when given one day after the onset of the disease ($p<0.0001$). However, although the virus titers decreased discernibly, it resulted in high mortality rates of mice when it was administered for 2 days after the onset of the disease. It was found from the study, reducing the virus titers simply cannot suppress the immune responses in mice when the lung injury reaches a peak value. When remdesivir was administered at an early stage, it was observed that it significantly improved the symptoms and mortality rates ($p=0.0037$) (Sheahan et al., 2020). It was reported that a SARS-CoV-2 patient showed a drastic improvement within a day of remdesivir administration. An in vitro study demonstrated that remdesivir inhibited the growth of coronaviruses such as the human-CoV and bat CoV. In a randomized clinical randomized placebo-controlled clinical trial with COVID patients, it was observed that remdesivir showed a shorter recovery time.

Another randomized study of 1062 patients underwent intravenous remdesivir treatment with a loading dose of 200 mg on day 1, and 100mg for up to 9 days daily). In the study 541 patients were assigned to remdesivir and 521 to placebo. It was observed that patients receiving remdesivir had a median recovery time of 10 days (95% CI, 9 to 11 days), as compared to placebo with 15 days (95% CI, 13 to 18 days). The study indicated that remdesivir demonstrated a short-recovery time in comparison to placebo to treat COVID-19

patients. Another finding of the study was it reduced the chances of respiratory tract infections (Beigel et al., 2020).

Remdesivir is however associated with toxicities such as hepatotoxicity, elevated level of transaminases and kidney injury (Ali et al., 2020).

e. Doxycycline

Doxycycline, a broad spectrum antibiotic of the tetracycline family exhibits good intra-cellular penetration. Doxycyclin also demonstrates anti-inflammatory activity, through numerous pathways. It binds to the 30S ribosomal subunit, also to the 50S ribosomal subunit, thereby interfering with the binding of aminoacyl-tRNA to the mRNA-ribosome complex, thus inhibiting protein synthesis. It also inhibits collagenase activity(Chukwudi, 2016; Nguyen et al., 2014)

Doxycycline has many potential mechanisms through which it may prevent or improve the effects of COVID-19. Doxycycline is known to inhibit metalloproteinases (MMPs), particularly MMP-9, which is possibly required for viral entry into the cell. It also inhibits IL-6. Both IL-6 and MMPs are critical regulators of the 'cytokine storm' often associated with severe pneumonia (Malek et al., 2020).

Doxycycline, an established ionophore, helps transport zinc intracellularly, with increased concentrations of zinc *in vitro* to inhibit coronavirus replication. Doxycycline inhibits nuclear factor (NF)-κB, thus may lower viral entry; this is because they directly inhibit DPP4 cell surface receptor and reduce hyperactive immune response after infection (Y. Zhou et al., 2015). Non-antimicrobial, low-dose doxycycline *in vivo* inhibited expression of CD147/EMMPRIN. CD147/EMMPRIN may be necessary for entry of SARS-CoV-2 into T lymphocytes. Structural analysis shows that it can inhibit PLpro and 3CLpro viral proteins that are essential to viral replication and lifecycle (Figure 3 and 4) (Malek et al., 2020)

A randomized control study on 70 COVID patients were treated with a combination of ivermectin and doxycycline. The combination reduced the recovery time and percentage of patients progressing to a more advanced disease stages; the combination also reduced mortality rate in severe COVID-19 patients from 22.72% to 0%; 18.2% of critically-ill patients died (Hashim et al., 2020). Doxycycline is being used in COVID-19 patients as it reduces the recovery time.

f. **Lopinavir/Ritonavir**

Lopinavir, an HIV type 1 protease (HIV-1) inhibitor, halts the maturation of HIV-1 and, therefore, its infectivity. Ritonavir, also a protease inhibitor, is given in combination with lopinavir. The combination, considered an effective antiretroviral agent, enhances the bioavailability by inhibiting the metabolic inactivation (Cvetkovic & Goa, 2003)(Chandwani & Shuter, 2008). It was found that lopinavir, along with other drugs such as loperamide, CQ and chlorpromazine, inhibited the in vitro replication of SARS-CoV and MERS-CoV (De Wilde et al., 2014). In patients with SARS-CoV infection, the aforementioned combination, lopinavir/ritonavir, and ribavirin caused a lower rate of ARDS and death at day 21, compared to the control group treated with only ribavirin ($p < 0.001$). The combination with ribavirin allowed a decline in steroid dosages and decreased nosocomial infection among patients (Chu et al., 2004). It was reported that out of four patients treated with lopinavir/ritonavir in addition to umifenovir, and Shufeng Jiedu Capsule (a traditional Chinese medicine), showed significant improvements (Wang et al., 2020b). Another study showed the administration of lopinavir/ritonavir to a patient with SARS-CoV-2 mild pneumonia caused a decrease in the viral load from the second day. It was also observed that the viral titers were undetectable later (Lim et al., 2020). However, the author also pointed out the decline in titers could also be due to the natural course. Hence, further studies are needed.

g. Favipiravir

Favipiravir, an antiviral drug, inhibits RdRp of RNA viruses. However, they do not inhibit cellular RNA and DNA synthesis. The drug shows a broad-spectrum antiviral activity (Furuta et al., 2005). Favipiravir (T-705) has the ability to induce mutations in the genome of influenza viruses that result in the reduction of in vitro infectivity of the virus. This mechanism is proposed to be the main antiviral mechanism of the drug (Baranovich et al., 2013). The drug had shown effectiveness against the influenza virus, Ebola virus, and rabies virus in vitro. However, it was observed to be ineffective in vivo, particularly after the neuroinvasion (Ali et al., 2020).

A study in China designed to compare the efficacy of favipiravir and umifenovir showed that favipiravir had a higher recovery rate. The time for cough relief and fever reduction was also shorter with favipiravir. Clinical trials of favipiravir had been approved in China (Du & Chen, 2020). Research is also being conducted in other places such as Harvard University and Japan.

h. Ribavarin

Ribavirin, a guanosine analog acts as a chain terminator. It does so by inhibiting RNA polymerase (Maag et al., 2001). Other potential mechanisms include incorporation into Hepatitis C virus genome. It then has the capability to induce mutations. As a result, defective viral progeny is produced by a process termed ‘error catastrophe’. It can even cause the inhibition of inosine monophosphate dehydrogenase (S. Zhou et al., 2003). The drug demonstrates good results with the Hepatitis C virus when given in combination with interferon. The combination showed improvement in immunocompromised patients with a respiratory syncytial virus (Marcelin et al., 2014).

Due to its antiviral properties, studies were conducted with COVID-19 patients. The studies revealed that when the drug was given in combination with corticosteroids, it led to fever resolution and caused improvement in lung infections within 14 days (Lee et al., 2003). Another study demonstrated that the drug lowered the viral titer in five out of eight patients (F. Chen et al., 2004).

Previously the drug showed significant improvement when given with interferon a2a to MERS CoV patients, which is a cousin of the SARS-CoV-2 virus (Elfiky, 2020).

The drug showed in vitro antiviral property against the SARS-CoV-2 and thus be used in COVID-19 patients. The most recommended method to use the drug is through intravenous infusion, mainly because it binds with the viral RdRp tightly, inhibiting the polymerase function (Elfiky, 2020). More trials are required to come to a conclusion regarding its potential to treat the disease. Furthermore, it is recommended to be used in combination with lopinavir/ritonavir or interferon in order to enhance its activity against SARS-CoV-2.

i. Ivermectin

Ivermectin, an antiparasitic FDA-approved drug, has been able to inhibit the in vitro viral replication of SARS-CoV-2. This was indicated by a significant fold reduction in viral RNA in ivermectin-treated samples (Caly et al., 2020). The drug demonstrates a broad-spectrum antiviral activity. Available data indicates it can inhibit the replication of the yellow fever virus by targeting NS3 helicase activity. In inhibits, other viruses such as the dengue virus and HIV-1 viruses (Mastrangelo et al., 2012; Wagstaff et al., 2012). Thus, anti-SARS-CoV-2 properties are being investigated.

j. Immunoglobulin

IgG antibodies contain two functional components: Fab fragments and the Fc fragment. Fab fragment helps in antigen recognition, and Fc is responsible for immune system activation (Galeotti et al., 2017). Intravenous immunoglobin is used effectively for chronic inflammatory diseases and autoimmune diseases. In addition, they have been used to treat bacterial, fungal and viral infections.

Similarly, SARS-CoV-2 infections could possibly be treated using extracted polyclonal antibodies from recovered patients. It is, however, recommended to take from the same area or city, from the same environment, having similar lifestyles and diet patterns as this increases the likelihood of developing the same specific antibodies. For instance, immune IgG collected from Europe will be most likely to be different from that collected from Bangladesh.

This passive antibody therapy method may provide an effective treatment against the rising number of COVID patients. Even though serum antibodies have been used in treatment for a long time, more trials are necessary to support the use of serum antibodies to treat COVID-19.

k. Interferons

Interferons are proteins that occur naturally and are secreted by cells of the immune system, for example, fibroblasts, white blood cells, and epithelial cells. There are three main interferons- alpha, beta, and gamma, each having a different function. They boost the immune system against antigens such as bacteria and viruses; they affect both stimulated cells as well as neighboring cells. They have been used for years against new viruses before any treatment options were available. They actively boost the immune system, acting on both the stimulated cell as well as the neighbouring cells. They are being used for several years

against emerging viral diseases before any approved treatment for the disease is available. Previously, they have shown promising results when given in combination with lopinavir/ritonavir or ribavirin in both MERS-CoV and SARS- CoV. In vitro and in vivo studies demonstrated they decreased the viral replication. However, the benefits did not meet the desired expectations. A possible reason could be because they were given at a post-infectious or a later stage of the disease. Since it showed promising results against the previous coronaviruses, they could potentially be used against the SARS-CoV-2 virus. The aforementioned viruses could interfere with the proteins directly involved in interferon expression (Orf6 and Orf3b), disrupting interferon signalling pathways. The in vitro sensitivity of the virus to interferons may be because it might have lost its anti-interferon activity due to the truncated proteins, Orf6 and Orf3b. This gives interferons better potential as a treatment option compared to the other coronaviruses. As they are more effective in the earlier stages, they can be used as a prophylaxis against SARS-CoV-2. This can be further supported by a study by Shen et. al stating interferon-2a could effectively decrease the infection rate of the SARS-CoV-2 virus.

In China, oral ribavirin is given in combination with 5M units of interferon via inhaler twice a day as a recommended guideline. The inhalation therapy acts directly on the respiratory tract. But the pharmacodynamics and pharmacokinetics of this route are not yet precisely known. The effectiveness of lopinavir/ritonavir or ribavirin for COVID-19 patients is under clinical trial. The results of the clinical trials are necessary to determine the efficacy of interferons in COVID-19.

i. Tocilizumab

Tocilizumab, indicated in rheumatoid arthritis (RA) and juvenile idiopathic arthritis, is a recombinant humanized anti-human monoclonal antibody that inhibits interleukin (IL)-6

(both membrane-bound and soluble). The binding of tocilizumab to the (IL)-6 receptors inhibits further inflammatory cascades. Critical COVID-19 patients have an excess immune response as a result of multi-organ failure in some patients or respiratory distress, leading to a surge of inflammatory cytokines known as the cytokine storm (Anthony L. Komaroff, 2020). This cytokine storm was observed in SARS, MERS, and influenza viruses; high levels of interleukin (IL)-6 was observed in patients (L. Y. C. Chen et al., 2020). In CoVID-19 patients, the inflammatory markers were high, contributing to systemic inflammation. Among them, IL-6 and IL-2 predict the severity of COVID-19 induced pneumonia. Thus, blocking IL-6R with tocilizumab can improve and save patients with severe COVID-19. Many cases in which tocilizumab was used, reported significant improvement in COVID-19 patients. It is recommended in severe to critical COVID-19 pneumonia (Costela-Ruiz et al., 2020). However, there is a lack of safety and efficacy data which is being investigated.

ii. Baricitinib

Baricitinib, an inhibitor of cytokine-release, is an anti-inflammatory drug. It is a Janus kinase inhibitor (anti-JAK) approved for the treatment of rheumatoid arthritis (RA) with good efficacy and safety records. The drug is shown to have anti-viral effects due to its affinity for AP2-associated protein AAK1; it thus reduces SARS-CoV-2 endocytosis. In a pilot study, baricitinib, 4 mg/day/orally was administered to 12 patients with moderate COVID-19. It was observed that the clinical and respiratory conditions improved significantly in baricitinib-treated patients and no adverse effects were recorded after 2 weeks; additionally, none of the patients needed ICU admission (Cantini et al., 2020).

Data on its clinical use for COVID-19 patients are very limited. Some COVID-19 cases showed a promising efficacy of baricitinib. Currently, up to 14 clinical trials globally are recruiting patients (Cingolani et al., 2020).

l. Natural products

Medicinal plants and some pure natural molecules isolated from plants have demonstrated significant inhibitory and antiviral activity against SARS-CoV and other coronaviruses (Orhan & Senol Deniz, 2020). Natural products are bioactive substances; some phytochemical classes such as alkaloids, peptides, and flavonoids are known antiviral bioproducts. They have been tested virtually, showing activity against COVID-19. However, their bioavailability and true efficacy need to be investigated *in vivo*. These natural products can act as potential candidates against COVID-19, considering their mechanisms of action, sources, and previously reported pharmacological usages (Antonio et al., 2020).

The presence of secondary metabolites such as terpenoids, flavonoids, polyphenols, alkaloids, curcumin, catechin, kaempferol, quercetin, naringenin, luteolin-7-glucoside, demethoxycurcumin, apigenin-7-glucoside, epigallocatechin and oleuropein can fight coronaviruses including COVID-19. Plants such as *Houttuynia cordata*, litchi seeds, beta-sitosterol from *Isatis indigotica* root extract, and Chinese Rhubarb extracts have the capacity to block the enzymatic activity of the virus (Singh et al., 2020).

Herbal medicines and plant-based natural compounds with medicinal properties are a rich resource for antiviral drug development. Some natural medicines have demonstrated antiviral activities against different virus strains such as coronavirus, influenza virus, herpes simplex virus, human immunodeficiency virus, hepatitis viruses (Hepatisis B and C), MERS and SARS viruses. Many Chinese herbs and natural compounds possess antiviral activities. The antiviral action mechanisms of these natural products on the viral life cycle: viral entry,

replication and assembly, release, and virus-host-specific interactions have been reported (Xian et al., 2020). Some of them have shown antiviral activity at a nanomolar concentration (e.g., homoharringtonine, lycorine, silvestrol, ouabain, 7-methoxycryptopleurine and tylophorine). In addition, a good number of natural products with anti-corona virus activity are the major constituents of some common dietary supplements, which can be exploited to improve the immunity of the general population in certain epidemics (Muhammad T. Islam et al., 2020).

Most of the active natural compounds belonging to polyphenols and flavonoids (luteolin, quercetin, hesperetin, tetra-O-gallyl-β-D-glucose, amentoflavone, forsythoside A, broussochalcone, sinigrin, psoralidin, tomentin B, papyriflavonol A and terrestrimine). Additionally, some alkaloids (tylophorine, lycorine, 7-methoxycryptopleurine, nummularine B and jubanine H), anthraquinones (emodin, aloe-emodin,) saponins (glycryrrhizin, saikosaponin B2 and escinidin), terpenes (curcumin, savinin, betulinic acid, dihydrotanshinone I, iguesterin, cryptotanshinone, chrysanthemumin B and 3β-friedelanol), coumarins (leptodactylone and xanthoangelol E), lectins (APA, UDA, HHA, alstotide 1) and diarylheptanoids (hirsutenone) proved to be promising against the previous SARS-CoV. Considering the structures of SARS-CoV and SARS-CoV-2 are homologous, they might act as potential candidates to combat COVID-19. However, more studies need to be conducted to determine the efficacy of such traditional products.

Recently, in silico studies revealed that molecules like myricitrin, 5,7,3′,4′-tetrahydroxy-2′-(3,3-dimethylallyl) isoflavone, methyl rosmarinate, 3,5,7,3′,4′,5′-hexahydroxy flavanone-3-O-β-D-glucopyranoside, calceolarioside B, (2S)-eriodictyol 7-O-(6″-O-galloyl)-β-D-glucopyranoside, myricetin 3-O-β-D-glucopyranoside, amaranthin and licoleafol could be potential leads against novel anti-SARS-CoV-2 drugs. The natural products open the door for further COVID-19 drug research (Muhammad T. Islam et al., 2020). They could be given as

prophylaxis, to strengthen the immune system as well an antiviral agent to combat the disease. In vivo studies need to be conducted in this regard.

1.7.2 COVID-19 Vaccine

There is currently no approved vaccines as of yet.

Currently, 236 vaccines are under development; 40 are under clinical trials. The usual vaccine development process takes around a decade, but the COVID-19 timelines are being compressed due to global urgency.

Globally, almost all people have agreed that the invention of a promising SARS-CoV-2 vaccine can possibly be proven as the most efficient line of defense. Through numerous researches, scientists have managed to develop several candidates of vaccine technology and of which the clinical trials have already been initiated (*What We Know about COVID-19 Vaccine Development*).

However, the main challenge of establishing and mass production of an effective vaccine treatment involves the collection of essential resources and the enhanced financial risks. Since the earliest 21st century, scientists, in order to fight against viral infection, have evolved two major vaccination techniques, and these are:

a. **Recombinant vaccines/Viral vectors:** One or more genes are delivered into the host cell that can eventually encode a specific target antigen inside an engineered attenuated virus.
b. **Nucleic Acid Vaccines:** An antigen encoding plasmid DNA or RNA is inserted into the host cell, which initiates essential viral protein synthesis, eventually triggering a humoral and cell-mediated immune response (Nascimento & Leite, 2012).

Various institutions are striving to make vaccines. Moderna, an American biotechnology company, based in Cambridge, Massachusetts is fixated on drug discovery, drug development, and vaccine technologies based solely on messenger RNA. They are trying to make progress with mRNA-1273, which is their main vaccine candidate against novel COVID-19. The RNA mRNA-1273 is an mRNA vaccine candidate against the novel coronavirus SARS-CoV-2 encoding for perfusion stabilized form of the Spike (S) protein. The S protein complex is necessary for membrane fusion and host cell infection and has been the target of vaccines against the coronavirus responsible for Middle Eastern Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). With the Phase 3 dose being finalized at 100 μg, the Company said it remains on track to be able to deliver approximately 500 million doses per year, and possibly up to 1 billion doses per year, beginning in 2021 from the Company's internal U.S. manufacturing site and strategic collaboration with Lonza. On November 16, 2020, Moderna announced the trial had met the statistical criteria pre-specified in the study protocol for efficacy, with a vaccine efficacy of 94.5% (*Moderna's COVID-19 Vaccine Candidate Meets Its Primary Efficacy Endpoint in the First Interim Analysis of the Phase 3 COVE Study | Moderna, Inc.*, 2020.).

The Oxford COVID-19 team, led by Prof Sarah Gilbert, Prof Andrew Pollard, Prof Teresa Lambe, Dr Sandy Douglas, Prof Catherine Green and Prof Adrian Hill includes scientists from both the Jenner Institute and the Oxford Vaccine Group. As soon as the genetic sequence was available, they began work on a trial. The ChAdOx1 vaccine is a chimpanzee adenovirus vaccine vector. It is a harmless, weakened adenovirus that usually causes the common cold in chimpanzees. ChAdOx1 was chosen as the most suitable vaccine technology for a SARS-CoV-2 vaccine as it has been shown to generate a strong immune response from one dose in other vaccines. It has been genetically changed so that it is impossible for it to grow in humans. This also makes it safer to give to children, the elderly, and anyone with a

pre-existing condition such as diabetes. Chimpanzee adenoviral vectors are a very well-studied vaccine type, having been used safely in thousands of subjects. Coronaviruses have club-shaped spikes on their outer coats, which form a *corona* – Latin for the crown – on the virus surface. Immune responses from other coronavirus studies suggest that these spikes are a good target for a vaccine (*The Oxford Vaccine | Research | University of Oxford*, 2020.).

The vaccine comprises two vector components, recombinant adenovirus type 26 (rAd26) and recombinant adenovirus type 5 (rAd5), both of which carry the gene for SARS-CoV-2 full-length glycoprotein S (rAd26-S and rAd5-S). A full dose of the vaccine was 1011 viral particles per dose for both recombinant adenoviruses, and all participants received full doses. The dose was set based on findings of preclinical studies (unpublished data). The vaccine was manufactured as two formulations, frozen (Gam-COVID-Vac) and lyophilised (Gam-COVID-Vac-Lyo). The frozen vaccine has a volume of 0.5 mL (per dose), and the lyophilized vaccine needs to be reconstituted in 1.0 mL of sterile water for injection (per dose).

In all cases, vaccines were administered intramuscularly into the deltoid muscle. During phase 1 of both studies, participants received one dose intramuscularly of either rAd26-S or rAd5-S and were assessed for safety over 28 days. Phase 2 of both studies began no earlier than 5 days after phase 1 vaccination after an interim safety assessment had been done. During phase 2, participants received prime-boost vaccination, with one dose of rAd26-S administered intramuscularly on day 0 and one dose of rAd5-S administered intramuscularly on day 21. Injection-site reactions, systemic reactogenicity, and medication use to alleviate such symptoms were monitored for 28 days after the first injection (in phases 1 and 2) and at day 42 (phase 2 only).

No randomization or special selection was done for phases 1 and 2. Participants were included as soon as informed consent was signed. Participants underwent clinical and laboratory assessments on days 0, 2, and 14 in phase 1 and on days 0, 14, 28, and 42 in phase 2. Laboratory analyses included complete blood and urine counts, alanine aminotransferase, aspartate aminotransferase, protein, bilirubin, total cholesterol, lactate dehydrogenase, alkaline phosphatase, prothrombin index, glucose, urea, and creatinine. Immune status was analyzed on days 0 and 28 in phase 1 and on days 0, 28, and 42 in phase 2. Volunteers were in the hospital for 28 days from the start of vaccination. Information on adverse events was recorded daily. Based on the encouraging phase 2 results, phase 3 trials are being conducted (Logunov et al., 2020).

Table 3 lists the leading candidates as vaccines against COVID-19.

Table 3: *Leading Candidate vaccines against COVID-19 (https://www.covid-19vaccinetracker.org/)*

Company/ Institution	Type	Phase	Status
BioNTech/Fosun/Pfizer	mRNA	Regulatory Review	Approved in Canada and other countries. Emergency use is US and other countries
Moderna	mRNA	Regulatory Review	Under F.D.A Review
University of Oxford/AstraZeneca	Adenovirus	Regulatory Review	
Sinovac/ Butantan Institute	Inactivated	III	Limited use in China
Wuhan Institute /Sinopharm	Inactivated	III	Limited use in China, U.A.E.
Beijing Inst./Sinopharm	Inactivated	III	Approved in U.A.E, Bahrain. Limited use in China
CanSino Biologics	Adenovirus	III	Limited use in China

***Table 4**: General Management of COVID-19*

General Management of COVID-19
1. Complete bed rest and strengthening support therapy
2. Administering antipyretic if temperature rises above 100 degrees Fahrenheit
3. Ensuring healthy food and proper calorie intake
4. Anti-histamine and bronchodilators when necessary
5. Monitoring important signs and O_2 saturation regularly
6. Providing effective O_2 support timely
7. Monitoring water & electrolyte balance
8. Having supplements: zinc, vitamin C, D

***Table 5:** Management of COVID according to severity of the disease*

Mild Case Management	**Moderate Case Management**	**Severe Case Management**	**Critical Case Management**
General Management in addition to: ➢ Thromboprophylaxis: LMWH-enoxaparin 40 mg S/C daily or if D-dimer can be done ➢ Antibiotics: Not	General Management in addition to: 1. Proning- At least 4-6 hours/day 2. Thromboprophylaxis: ➢ Enoxaparin 0.5 mg/kg SC twice	General management in addition to: 1. O_2 therapy Proning- At least 4-6 hours/day Judicious fluid management: Crystalloids are preferred with	Should be managed in ICU/Critical care medicine Steroids: Can be used in following dosage: ARDS Inj.Methylprednisone 1-2 mg/kg q12 or

recommended empirically ➢ Monitor important signs and O_2 saturation closely	daily (dose adjustment with CrCl< 30ml/min) ➢ If LMWH can't be given or contraindicated, Inj.Unfractionated heparin (UFH): 60U/kg bolus+12units/kg/hr infusion or ➢ If D-dimer can be done follow *Guideline for Thromboprophylaxis ➢ Continue until symptom resolves & followed by Rivaroxaban 10 mg once daily - 1 month 3. Oral steroids- Indicated for other	conservative approach Early Norepinephrine for hypotension 5. Thromboprophylaxis: ➢ Enoxaparin 1 mg/kg SC twice daily/ day (dose adjustment with CrCl< 30ml/min) ➢ If LMWH can't be given or contraindicated, Inj.Unfractionated heparin (UFH): 60U/kg bolus+12units/kg/hr infusion or ➢ Continue until symptom resolves & followed by Rivaroxaban 10 mg once daily - 1 month	Inj.Dexamethasone 20 mg IV daily for 5 days & then 10 mg IV daily for 5 days &then 5 mg IV daily for 5 days as per Refractory Sepsis Hydrocortisone 50 mg IV 6 hourly Cytokine Release Syndrome(CRS) Inj.Methylprednisone 1-2 mg/kg q12 or Inj.Dexamethasone 10 mg q6 For Cytokine Storm /CRS: Consider steroid or Tocilizumab if any of the following occurs: a. Clinical deterioration

	reason e.g. acute exacerbation of asthma & COPD or patient is getting steroids beforehand 4. Empirical Antibiotics: Only required if- ➢ Elderly > 50 years or children <5 years ➢ Productive cough with purulent sputum ➢ Unilateral lobar consolidation ➢ High CRP &Pro-calcitonin or high pro-calcitonin	6. Steroids: Oral/IV.Dexamethasone 6 mgdaily for 10 days 7. Antibiotics: Broad spectrum antibiotic coverage	b. ↑O_2 demand requiring >6 L/min O_2 over 24-48 hours or on mechanical ventilation c. Supported by elevated inflammatory markers (e.g., ferritin >1000 ug/mL; CRP> 50 mg/dl, D-dimer > 1mg/L) & clinical decline Convalescent plasma therapy: Inclusion Criteria: Respiratory rate > 30 breaths/min PLUS Severe respiratory distress; OR SpO2 ≤ 88% on room air OR PaO2/FiO2≤ 300 mm of Hg

			PLUS Radiological evidence of bilateral lung infiltrate AND/OR Systolic BP < 90 mm of Hg or diastolic BP <60 mm of Hg

Chapter 2

2. Current COVID-19 scenario in Bangladesh

COVID-19 has paralyzed the whole world in less than three months; Bangladesh is not far behind in the list of most affected nations, ranking 24th in the world, with nearly 501,000 positive cases and 7,280 deaths (21 December, 2020; DGHS) that accounts for 0.8% of the global disease burden. Bangladesh confirmed its first COVID-19 case on March 8, 2020. However, many experts speculate that the virus has entered the country earlier, but due to inadequate monitoring, had not been detected.

About 67.4% of the confirmed cases using RT-PCR were from the capital, Dhaka, 12.2% from Chittagong and the remaining were reported from other divisions including Sylhet (3.1%), Khulna (5.4%), Rangpur (3.1%), Rajshahi (5.0%), Barishal (2.2%) and Mymensingh (1.6%) (Anwar et al., 2020).

Dhaka, the capital city, is among the fastest-growing cities in the world. It supports around15 million people in approximately 325 square kilometres area, making it very densely populated. Moreover, it has most of the industries, including tanneries, textiles, fertilizer plants, cement factories, pulp and paper factories, pharmaceutical companies, and many government and non-government offices, suggesting a possible reason for higher disease prevalence (Md. Taimur Islam et al., 2020).

7% of the population of Bangladesh consists of senior citizens. The majority of these citizens and many mid-aged people suffer from non-communicable diseases such as COPD (11.9%), asthma (5.2%), diabetes (9.7%), and cardiac disorders (4.5%), making them more vulnerable to COVID-19. Notably, the number of cancer patients is around 1.5 million in the country. Also, among the South Asian countries, Bangladesh has the highest number of smokers. Studies reported smokers, and cancer patients demonstrate a higher risk of developing serious complications, although the understanding of the association is not so clear. Owing to the upsurge of people belonging to the vulnerable groups, there is an urgent need to develop more hospitalization facilities and intensive care units. Therefore, ventilation supports are a must in every hospital and medical centre. This pandemic has been an eye-opener for all, as it uncovered a shortage of personal protective equipment (PPE) like gloves, masks, and gowns, lack of medical doctors, limited number of ICU beds, shortage of ventilators, and most

importantly government's ignorance towards healthcare services. Initially, after the first COVID-19 case was detected in Bangladesh, IEDCR (Institute of Epidemiology Disease Control And Research) was the sole diagnostic facility for a country of 180 million (Anwar et al., 2020).

There are about 11,453 general beds (GB) and 564 ICU throughout the country, of which only 4,213 GB and 357 ICU are dedicated to COVID-19 patients. Bangladesh admitted that there was a severe shortage of testing kits, so the country had received some testing kits, masks, infrared thermometers, and PPE from China to manage the crisis. After much criticism, the health authorities decided to expand its testing facilities (Anwar et al., 2020). As of December 21, 116 laboratories around the country have conducted 3,091,349 COVID-19 tests. Among the reported COVID positive cases, 438,610 of the patients recovered, 7312 died and 56,717 people are still infected (Hasan Mohiuddin, Surveillance and Health Informatics).

There has been a sharp rise in the number of positive cases as the number of testing facilities increased. However, the recovery rate (57.67%) reported was lower than the neighboring countries as of 11 August, 2020. The situation is better in other countries such as India, Pakistan, Russia, Italy, and Brazil (70.35%, 91.60%, 78.34%, 70.53%, 80.59 and 70.53%). The possible reason could be the lack of health facilities in Bangladesh. Also, a large population is facing a double burden of the disease as they suffer from other non-communicable diseases (Md. Taimur Islam et al., 2020). The current recovery rate has increased significantly and is now 76.5 %.

So far, the young and working group (21-50 years) has mostly been infected according to IEDCR (68%), followed by patients aged over 50 years (21%) and children and youths below 20 years (11%). A similar trend has been observed in India (Md. Taimur Islam et al., 2020).

Like other countries, Bangladesh has also adopted non-therapeutic measures to control the spread of COVID-19. These include:

i. the formation of a COVID-19 response committee led the Health Minister
ii. closure of educational institutions
iii. 'Rice for TK. 10 per KG' program is being operated for the needy people
iv. extension of social safety net to the poor and distressed people
v. deployment of the law enforcement such as police and army to make sure people maintain social distancing

Coronavirus has taken the whole by surprise and Bangladesh is not prepared for this massive shock. Even though institutions are implementing cost cutting measures to overcome the financial imbalances temporarily, we must focus on the long-term scenario. To emerge successfully, hospital authorities must provide adequate treatment for their current patients as well as plan to expand their pool of services. The government should also provide assistantship in this regard and help set up new hospitals.

Chapter 3

3. Need for public awareness

A survey including 21 406 adult participants in the Indian society with age groups between 18 and 80 years was carried out to determine the relationship between public awareness with cause, spread, prevention and treatment of COVID-19. The results showed an urgent need to expand the knowledge-base among people and encourage active participation of the public in the prevention mechanisms. This would minimize the spread of the pandemic (Kaushik et al., 2020).

Public participation is essential and is the most effective approach for controlling the spread of the novel coronavirus. However, its novel nature urges to create awareness among the public to take preventive measures timely. A study conducted in Saudi Arabia indicated a lack of understanding could result in severe outcomes concerning COVID-19. Effective awareness campaigns, including relevant information from reliable sources, can improve people's knowledge. They must develop positive attitudes towards adopting preventive measures (Alanezi et al., 2020).

To address the outbreak of COVID-19, the Bangladesh government, the Directorate General of Health Services (DGHS), and the Institute of Epidemiology, Disease Control and Research (IEDCR), have raised a national-level alert. They have implemented a wide range of public health measures according to the WHO and CDC guidelines. However, the lack of public awareness, wide-spread anxiety, and panic due to an unknown illness with limited health facilities have various challenges and threats to the population. Because COVID-19 is transmitted from person to person via respiratory droplets, interaction or close contact, and fomites in the environment around the infected person, a public campaign was initiated by the government highlighting the significance of practicing respiratory and hand hygiene. It emphasized on use of necessary personal protective equipment (PPE): mask and gloves, with evidence of minimal adoption by the public at large. Early detection and isolation of cases have been the bedrock for curbing the rapid spread of communicable diseases such as COVID-19, and extra precautions need to be taken to promptly identify asymptomatic viral carriers (Banik et al., 2020).

The following lists the necessary precautions from CDC, WHO and other relevant reports.

1. The CDC recommends wearing a mask when in contact with people and to maintain a minimum distance of 6 feet; avoiding crowded places/social gatherings. WHO recommends wearing a fabric mask unless an individual is in a specific risk group; a medical/surgical mask if a person is above 60 years of age, has underlying medical conditions, feels unwell or is looking after a family member who is ill. For health workers, medical masks are essential; personal protective equipment should be used when dealing with patients with suspected or confirmed COVID-19. Respirator masks (such as FFP2, FFP3, N95, N99) are advised in settings where aerosol-generating procedures are performed. It is also essential to make sure the correct size is worn.
2. Immune strengthening strategies should be adopted- supplements such as vitamin C, D, A, zinc, selenium, raw honey, garlic, black cumin seeds, turmeric incorporated in the diet, regular exercise, getting quality sleep could help improve lives.
3. Cleaning and washing hands frequently are advised using soap and water or sanitizers (alcohol-based hand rubs). Washing hands for 20 seconds is recommended.

   Touching eyes, nose, or mouth is strongly discouraged. In addition, one should cover his/her nose and mouth with bent elbow/ tissue when coughing or sneezing.

4. WHO recommends staying at home if a person feels ill. If a person shows symptoms such as fever, cough, or difficulty breathing, it is advised to immediately seek medical attention. Taking steps initially allows healthcare providers to quickly direct to the right health facility. This not only protects the individual but also prevents the spread of the virus.
5. Cleaning and disinfecting surfaces particularly, those which are touched regularly, for example, faucets, door handles, and phone screen, is advised frequently.
6. Home isolation no longer depends on a test-based strategy except in certain situations/circumstances. The symptom-based criteria were modified. The noteworthy ones being “at least 24 hours” from “at least 72 hours” since last fever without the use of medications, “improvement in symptoms” to “improvement in respiratory symptoms” to address the list of associated symptoms. For severely ill patients, isolation duration is up to 20 days after symptom onset. In the case of asymptomatic patients, isolation, and other necessary precautions can be discontinued after 10 days of their first positive test for SARS-CoV-2.

Chapter 4

4. Recommendations

The systematic review gives information on SARS-CoV-2 virus, viral entry and infection mechanism, lifecycle, symptoms, risk factors, diagnosis, current treatment and management options, vaccines under development, and the need for public awareness. However, there is no available concrete data on after COVID effects/complications; there is no doubt it has affected our mental health. More studies need to be carried out to determine the after effects, physical and biological complications associated with the disease.

Management of COVID-19 requires facilities- hospital beds, ICU beds with ventilators, general OT, entry flu corner, suspected isolation unit, confirmed isolation unit, general ward, dialysis unit for kidney patients, two-step triage system/ protocol at the emergency room. The same building should have a quarantine facility (accommodation): the required food and entertainment for patients. There should be a dedicated COVID-19 management team consisting of COVID-trained doctors, clinical pharmacists, nurses, respiratory therapists for the building.

Clinical Pharmacists (CP) have a very crucial role to play here. Some of them are listed below:

Doctors: They can support doctors by providing clinical drug information, recommend the accurate dosages of drugs, assist in the selection of the right drug, help in decision-making in critical cases; they can monitor drug-drug (DD) interactions, anticoagulation/steroid therapies, adverse drug events and also monitor the use of HAM and provide feedback.

Nurses: They can support nurses by providing proper guidance on the handling and administration of the given drugs.

Patients: They can provide proper counseling and resolve conflicts about drugs.

Currently, there is no one specific guideline recommended for treating COVID-19. The treatment decisions depend on the patient's situation, co-morbidities, and clinical condition. Considering the aforementioned parameters, a risk-benefit ratio should be calculated, prioritizing the patients' lives. The coherence among causative factors, for instance, mouth sore due to anti-inflammatory and steroid drugs, should be looked upon. This could be done using correlations based on time, for example, when the therapy started.

Justification of the given therapies should be highlighted. Remdesivir, which is an inhibitor of RdRp of the virus, is associated with a major side effect, hepatotoxicity. This calls for monitoring of patients after drug administration. This can be done by measuring ALT/ AST.

Tocilizumab, an inhibitor of IL6 is used in severe to critical COVID-19 pneumonia. Cytokine storm in patients elevates IL-6 significantly among the other 17 interleukins. Tocilizumab, directly inhibits IL-6. However, there is insufficient efficacy and safety data on this Mab. It is associated with some adverse effects such as abdominal perforation and infections; Monitoring of patients after drug administration can be done by measuring the IL6 levels.

Baricitinib, JAK inhibitor, can be used as an alternative to treat moderate to severe COVID-19 pneumonia.

From a Bangladeshi perspective, owing to the high population density, it is vital to reduce the disease burden. This can be ensured by maintaining the distance between the beds of patients in hospital wards. The triage protocol should be strictly followed. There should be separate protocols for identifying asymptomatic patients to separate COVID patients from Non-COVID patients; a flu corner should be maintained. Symptom analysis can be carried out to determine the severity of the disease so that the patient can be transferred to a separate facility for COVID patients. Patients with mild symptoms should be recommended to take the treatment at home since there are still an insufficient number of beds to address the population's needs. Proper patient consultation is the key to managing resources efficiently.

After recovery of COVID patients, follow up treatment/care is recommended.

Control of antimicrobial use and consumption is important. Management of superinfections is a major challenge; decisions may hamper the rational use of antibiotics, so it is important to focus on issues simultaneously. Antibiotics are not recommended in asymptomatic or patients with a mild form of the disease.

Drug supply indicator is an important factor to guide drug consumption, place a new purchase order and for prioritization set-up for supply chain management. It prevents wastage/drug dumping or overstock of unnecessary drugs.

On the other hand, the safety of staff and personnel should be ensured; basic rules of hygiene and monitoring should be strictly followed. Most importantly, COVID management guidelines should be developed in hospitals under WHO and government protocol for the

improvement of pharmacotherapies; it is also important to incorporate issues from global guidelines.

Three case reports from Bangladesh are discussed below.

Case Study 1:

A 34y/o man without any comorbidities or significant medical history developed a fever and had shortness of breath. He was admitted to the emergency department (ED) of a tertiary care hospital in Dhaka, Bangladesh. He did not have a history of traveling to any COVID-prone area. He also did not have any direct contact with COVID-positive patients. His chest X-ray reports showed ground-glass opacity in the middle right and lower region of the lung. The first PCR test on the throat and nasal swabs showed negative results for COVID. On the basis of his chest X-ray result, RT-PCR was again done, and this time the result was positive. Primarily he was treated with chloroquine (CQ) and azithromycin. After recovery, on day 12, he was discharged from the hospital after two throat swab samples tested negative (24 hours apart). He was advised to maintain home quarantine for the next 14 days. SARS-CoV-2 RNA by swab remained negative. After 7 days of discharge, his blood sample showed antibodies, IgM, and IgG (Jahan et al., 2020).

Inference from Case 1: A person should test immediately after he/she experiences COVID symptoms. Chest X-ray could also be an indicator to determine whether the person is infected. It is essential to perform tests for the second time if he/she exhibits COVID-like symptoms for confirmation.

After recovery from symptoms, two tests (24 hours apart) should be performed to confirm the person is no longer infected. Routine check-ups and follow-up care is recommended to check for the long-lasting effects of the virus on the patient's body.

Case Study 2:

A 56 y/o male was admitted to hospital (PMCH) with fever and dry cough for 7 days; the highest temperature recorded was 102° F. Initially, he was suffering from dry cough, which later progressed in terms of intensity and frequency. He did not complain of sore throat, altered smell sense, vomiting, nasal congestion, diarrhea, or headache. He did not have a contact history with COVID patients. He had bronchial asthma (comorbidity), which was maintained with a combination of salmeterol and fluticasone inhaler. He had been a smoker

for 20 years. On the 4th day of symptoms, RT-PCR results for COVID-19 from Nasopharyngeal swab was negative.

The pulse oximeter readings showed he was desaturating (SPO_2 89-91% at room air). CBC results were normal, but CRP, D-dimer, s.ferritin levels were raised. CxR results showed bilateral lung infiltrate at the periphery; and HRCT chest showed typical bilateral pneumonia suggestive of COVD-19. The patient was labeled as a case of COVID 19. Initially, he was managed with oxygen (3L/M), Injection Enoxaparin (60IU S/C 12 hourly), Oral Moxifloxacin (400 mg once daily), and other supportive management. He became afebrile after this. However, after admission, he developed an intractable cough producing sputum.

A second RT PCR for COVID-19 was performed on 12th day. The results were again negative. The patient started having restlessness and increased breathlessness. He needed a Rebreather Mask (15LPM of oxygen). His CRP (inflammatory blood parameter) increased from 69 to127mg/L and s ferritin from 512 to 1069 ng/L. However, his D-dimer, CBC, S.creatinine and S.procalcitonin were normal; CxR showed an increased bilateral shadow.

Based on test reports, he was treated with Tocilizumab injection (2 doses on 12 hours apart) according to his body weight and considering cytokine storm syndrome.

Additionally, the patient was given corticosteroid support throughout the illness. Initially, Dexamethasone injection (10 mg 12 hourly) was given. He was put on Meropenam injection (1gm IV 8 hourly).

Despite the mentioned measures, the patient became progressively dyspnoic. An urgent chest X-ray showed right-sided pneumothorax. The HRCT film showed extensive B/L lung involvement, more organized shadows, development of tension pneumothorax on the right side. Immediately, water seal drainage was inserted. The symptoms relieved as evidenced by oxygen demand of only 5-6 litres/min with an SPO_2 of 95%.

His clinical features, blood parameters, radiology were improving, maintaining greater than 95% saturation with 2 liter O_2/min (Tuli et al., 2020).

Inference from Case Study 2: All the symptoms (SPO_2 89-91%, CBC CRP, D-dimer, s.ferritin levels were raised, bilateral lung infiltrate, pneumonia) were suggestive of COVID-19 even though the RT-PCR showed negative results two times subsequently (12 days apart). The report suggests COVID-19 treatment protocol was followed. After 12 days, when he was tested negative again, the CRP and s ferritin was high which indicates a secondary infection

as a result of COVID. He may have developed pneumonia as a secondary infection. His D-dimer levels were normal, indicating he was recovering from COVID.

The CRP value was increasing, which is an indicator of the inflammatory response (cytokine storm) in the body. To suppress that, Tocilizumab and corticosteroids were given. Meropenem was assigned to manage pneumonia. Water seal drainage was done to manage parapneumonic effusion, resulting in improvement of the patient's condition.

Initially, moxifloxacin was given, which is a fourth-generation, expanded spectrum fluoroquinolone. Ceftriaxone or amoxiclav could have been given. Routine check-ups and follow-up care is recommended to check for the long lasting effects of the virus on the patient's body.

Case Study 3:

A 59 y/o diabetic, non-smoker male, with no other medical history was presented in Labaid Specialized Hospital with the complaints of fever, diarrhea and breathing difficulty for eight days. Fever was persistent, low-grade, and occasionally higher. On examination, his temperature was 102° F and pulse oximeter readings showed oxygen saturation above SPO_2 97% at room air. He did not have any other symptoms like sore throat, loss of smell and taste.

On the 5th day of symptom, he was advised to perform dengue and typhoid tests, the results of which were negative. On the 6th day of symptoms, RT-PCR results for COVID-19 from nasopharyngeal swab was positive. His CT scan results showed 70% lung involvement with mild thickening of the interlobular septum and prominence of regional vessels. Besides that, diffuse infiltration, centrilobular emphysema, and subpleural reticulation were seen at both lungs, along with consolidations at bilateral lower lung fields. The report was suggestive of bilateral pleural effusion. The D-dimer level was within the normal range; however, his serum ferritin level (586 ng/ml) was significantly higher. The hematology report showed elevated Neutrophil count and lower lymphocyte count. Serum electrolyte and creatinine reports showed sodium, potassium, and chloride levels were markedly reduced.

After being diagnosed as a COVID-positive patient initially, he consulted a physician who advised him to have 2 ivermectin tablets, and started doxycycline according to the prescription. No improvement was observed. To manage shortness of breath/ breathing difficulty 2-3 liters oxygen was given. Along with the conventional therapies of COVID-19

treatment guideline (Montelukast, Vitamin B, C, D, zinc sulphate, etc), he was treated with Favipiravir and Remdesivir (IV), meropenem 1 gm, IV 8 hourly was given. He was also given dexamethasone IV 1 amp. 8 hourly, enoxaparin 40 mg subcutaneous around the umbilicus and aspirin (75mg) tablet and NaCl (600 mg). Rostil was given to treat diarrhea.

After taking the medicines for 4 days, the patient was discharged from the hospital as he became afebrile and no longer had breathing difficulties. However, he was told to stay in isolation for 7 days. The patient had to continue tablet dexamethasone (0.5 mg) for 12 days and rivaroxaban (10 mg) once a day for two months as an antithrombotic agent.

After 21 days of infection, the RT-PCR test of the nasopharyngeal swab gave a negative result. Two weeks after being discharged from the hospital the patient had performed a chest CT scan. His lung condition improved and was almost as normal.

After three weeks, the patient started to feel normal; one month after release from the hospital, the patient was found to have a high titer of antibody against COVID-19, and his plasma could improve the clinical condition of a 72 years old COVID patient who was released from the hospital within a week after getting plasma therapy.

Inference from Case 3: The patient did not have any COVID symptoms other than fever and diarrhea initially as he developed the fever. 3 days after he was tested positive (8^{th} day), he started having breathing difficulties and was admitted to a hospital. The breathing difficulty was managed using external oxygen support. Even though he had COVID, his D-dimer range was normal. The chest CT scan report indicated he had developed pneumonia as secondary infection. He was given ivermectin after he was diagnosed with COVID, but no improvement was observed. This was possibly because he was too late in starting medication; the disease has already progressed. Two antiviral drugs, favipiravir and remdesivir were given as antiviral therapies to prevent viral replication. As his lab reports showed bacterial infection, prophylaxis (meropenem 1 gm, IV 8 hourly) was given. Doxycycline and meropenem (IV) were given for lung infection detected from the chest CT scan. The corticosteroid, dexamethasone was given to prevent lung damage due to hypersensitivity reaction resulting from cytokine storm associated with COVID. High neutrophil and lower lymphocyte counts indicated the body was fighting infection. Enoxaparin and aspirin were given to prevent blood clot. Antithrombotic agents are usually given to patients with comorbidities and as prophylaxis for VTE (Venous Thromboembolism). In this case the patient was diabetic. NaCl was given to compensate for the low electrolyte levels. After being treated with the

above mentioned medications in addition to the conventional therapies, the patient's condition improved. His temperature was under control and he no longer had breathing difficulties. As his condition improved, he was discharged from the hospital. He was given aspirin and edoxaban initially and later rivaroxaban as antithrombotic agents. Rostil was given to manage diarrhea. After 21 days he was tested negative and his lung condition improved as per chest CT scan. His antibody titers were high making him suitable for plasma therapy. it is recommended that the patients who were recovered from COVID19 should be tested for antibody titre so that they can help other patients by donating plasma. Therefore it is recommended recovered COVID-19 patients should test for antibody titers so that they can help other patients by donating plasma.

Chapter 5

5. Conclusion

The pandemic has taken a toll on both the physical and mental health of people across the globe. Starting from getting affected, social lockdown, loss of loved ones and jobs, altogether has affected the mental health of the general mass throughout their attempt to adapt to the new normal. COVID-19, suspected to be of bat origin, is a disease caused by the SARS-CoV-2 virus. The virus attaches to the human host cells by binding to the angiotensin-converting enzyme II (ACE2), mostly to the cell membranes. Viral membrane and host cell membrane fuse causing infection. This is a known mechanism of coronavirus entry into the human body. These two proteins could thus be targets for inhibiting viral entry and thus be used as an effective strategy. Camostat mesylate, an inhibitor of TMPSSR2 is under clinical trial. The disease produces symptoms like fever, sore throat, loss of smell and taste, lesions in the lungs, and difficulty breathing. Other experiences include dry cough, lymphopenia, fatigue, anorexia, arrhythmia, and shock. The effects are more pronounced in elderly people (65+years) with comorbid conditions, such as diabetes, cardiovascular diseases, lung diseases, chronic kidney disease, cancer, and neurological disorders. There is no approved treatment or clear guidelines for COVID-19; the risk benefit ratio of the patient is considered. Medicines currently being used include combination of ivermectin and azithromycin, doxycycline, corticosteroids, such as prednisolone, remdesivir, favipiravir, lopinavir/ritonavir, ribavirin and anticoagulants. Interferons and immunoglobulins are also being employed to boost immunity. Monoclonal antibodies, tocilizumab, and baricitinib. Natural molecules such as epigallocatechin, curcumin, black cumin seeds, vitamin C, and zinc boost immunity. Currently, treatment involves addressing the symptoms, e.g., antipyretics to reduce fever, diclofenac to treat body ache, inhalers for the difficulty in breathing and ventilators for more severe cases. Moderate to severe cases require antibiotics, but the selection of antibiotics is crucial and depends on the severity of the infection. The recovery rate from COVID-19 has improved over the months.

However, there is an urgent need to develop, approve, and manufacture COVID-19 vaccine. There are currently 236 vaccines under development; 40 are under clinical trials. The usual vaccine development process takes around a decade, but the COVID-19 timelines are being compressed due to global urgency. The Oxford University/Aztrazeneca vaccine, which

demonstrates very promising results till now, is under phase 3 clinical. Other noteworthy vaccines have been tabulated in the review (Table 3).

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