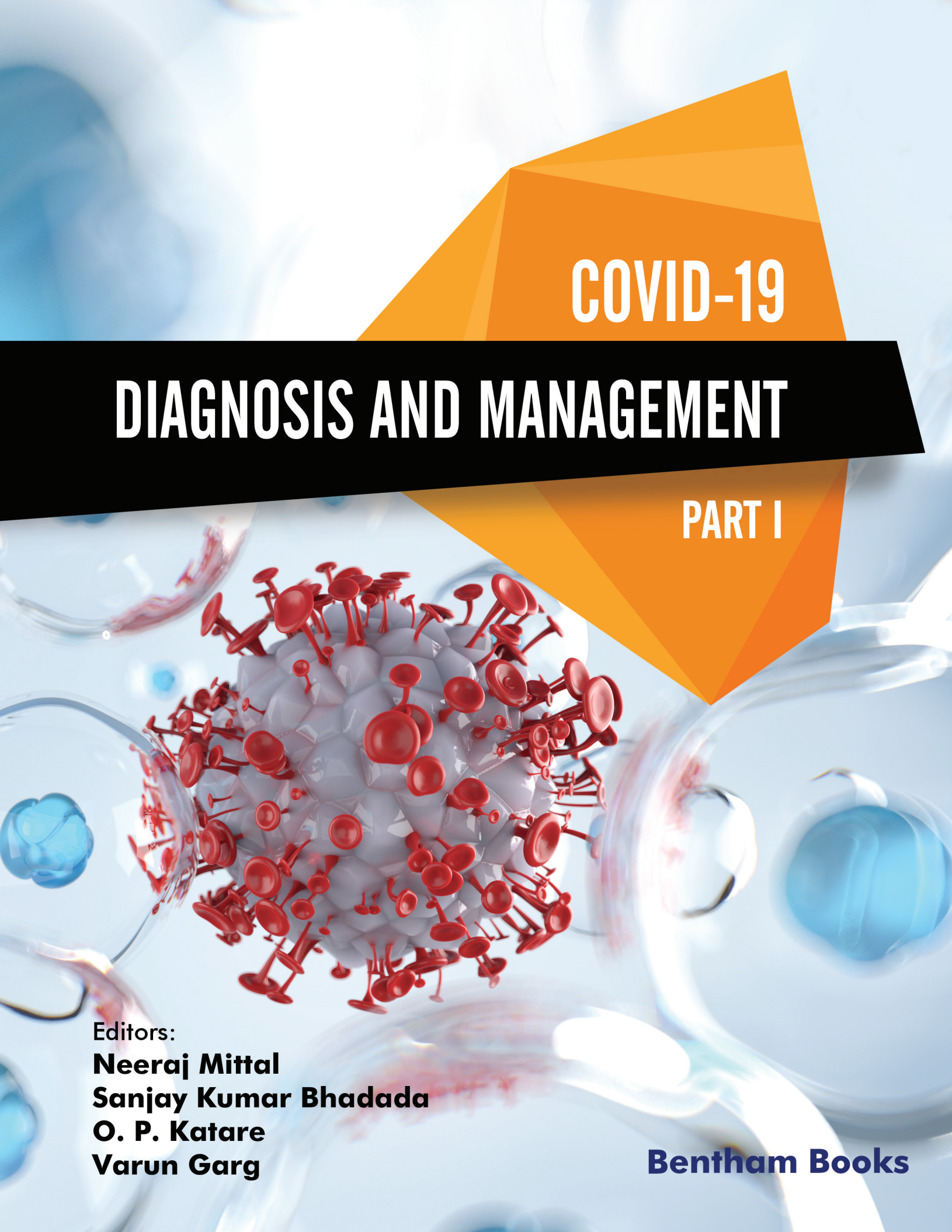
COVID-19

DIAGNOSIS AND MANAGEMENT

PART I



Editors:
Neeraj Mittal
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COVID-19: Diagnosis and Management-Part I

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FOREWORD

It is my proud privilege to introduce the book "COVID-19: Diagnosis and Management", which is authored by a group from PGIMER, Chandigarh. The timing of this monograph is very apt as it has been about 9 months since the start of the COVID-19 pandemic, and it is now that we are starting to unravel the various mechanisms of disease pathogenesis and treatment modalities for this viral infection which has infected 29 million people, out of which about 1 million have died till date globally.

It has been the need of the hour to come up with a treatment for this pandemic disease. Moreover, it is of utmost importance that all the information related to COVID -19 should be compiled in one place, a goal which this book will fulfill.

Though the tests for diagnosis of the infection have been developed in the start of the pandemic, there are still some issues in diagnosis, including the sensitivity of the best test available, *i.e*., Real-Time Polymerase Chain Reaction.

The book is very well organized and has been divided into two parts; each part is comprised of 6 chapters and covers all the aspects of COVID-19 from the history to the treatment of the disease. Based on the best scientific studies available, the editors and authors have used their vast professional experience to discuss the all clinical aspects of COVID-19, including clinical presentation to diagnosis in the first part and treatment of COVID-19 in the second part, and I am very sure that this compendium will become the benchmark to refer to for any information required on COVID-19.

Whenever we write books, we must have in our minds, as clearly as possible, the affirmation of Carlyle Guerra de Macedo, who was the Director of Pan American Health Organization, relative to the responsibility of what is being published: "It must be remembered that behind each table, every report or material examined, there are lives, there are people, there is suffering, waiting for our efforts and human solidarity." Both the parts of the book are very well organized, and the readers will get a mine of information available to date on COVID-19 in one place, and it would be helpful to both the clinicians and the lab professionals for day-to-day guidance in various matters. The monograph is comprehensive but is written in a lucid manner that is easy to grasp, and even complex topics are made simple for understanding.

I am also sure that as the knowledge of the virus evolves further, the authors will certainly keep updating the work from time to time, further adding to the importance of the book. I would like to congratulate the editors/authors for this tremendous effort, and I am very sure that this book will surely be of use to readers around the world and help them in the diagnosis and management of patients with COVID-19 and will also go a long way in the efforts to help fight the pandemic, which is being faced by the humanity now.

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PREFACE

The coronavirus disease 2019 (COVID-19) outbreak has spread throughout the globe and has been declared as a pandemic by the World Health Organization (WHO) on 11th March 2020. To date, *i.e.*, 1st September 2020, there are more than 25, 327, 098 confirmed cases of COVID-19 worldwide, and around 848, 255 deaths have been reported. Clinicians and scientists across the globe need all the information on this pandemic disease on one platform. This book, "COVID-19: Diagnosis and Management", is a concise and visual reference for this viral disease. This book has been divided into two parts I and II.

Part I will provide a comprehensive knowledge which will cover all the aspects related to COVID-19, such as: 1) History of coronaviruses, 2) epidemiology of COVID-19 3) clinical presentation of this viral disease, 4) how to diagnose it, whereas part II of the book covers the prevention and treatment methodology of this communicable disease.

Key Features:

1. Chapter wise description and segregation of all the areas from pathophysiology to diagnosis and management of COVID-19 in two different parts of the book.
2. Six chapters in the first part that begin with the history of the coronaviruses and their introduction.
3. Multiple tables and figures which summarize and highlight important points.
4. Covering all the aspects of COVID-19, making this a perfect textbook for virologists and medical students.
5. A summary of the current standards for the evaluation and diagnosis of COVID-19.
6. A detailed list of references, abbreviations, and symbols.

This book is an essential reference for practicing and training virologists, pulmonologists, medical students, scientists working in various research labs, pharmaceutical and biotechnology industries on COVID-19.

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CHAPTER 1

History of Pandemics

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Abstract: Pandemic is the term coined for the widespread of a disease or infection on a very large scale and across borders. COVID-19, an outcome of the spread of coronavirus, reportedly started from China and spread to almost all the countries of the world. Though it is not for the first time that there was an outbreak of a disease at such a high magnitude but the duration for which it has continued to grapple the world with its virulence and contagious nature, it has become important to take a peek into the history of other pandemics of the world too. Before COVID -19, about 20 major outbreaks of infectious diseases took place and claimed millions of lives in a sweep. The awareness of government bodies, WHO, and non-government organizations grew better with every pandemic. Understanding the role of basic hygiene, self-immunity, social distancing, living in coherence with other living and non-living components of the planet are some positive outcomes of these pandemics. These pandemics also necessitated the need for discovering new drugs and vaccines.

This chapter describes the major pandemics in the history of mankind, the origin and types of coronaviruses, the association of different types of coronaviruses with the ranges and severity of infections, and the origin of COVID-19.

Keywords: AIDS, Black Death, Contagious, Corona, COVID-19, Ebola, Epidemic, Flu, H1N1, Host, Outbreaks, Pandemic, Plague, SARS, Swine flu, Vaccine, Virulence, WHO, Yellow fever.

INTRODUCTION

The terms pandemic, epidemic, outbreak are primarily categorized based on the number of cases of a condition often used to describe infections. These terms have described the comparison of the expected number of cases in a particular time and how far-off cases have spread in the geographical area. Some conditions, such as cancer, hypertension, violence, beneficial behaviors, or even positive behaviors, can also be defined in the same way (Morens *et al*., 2009).

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The term ‘pandemic’, has its origin derived from the Greek words *pan* (meaning “all”) and *demos* (meaning “the people”). Pandemic refers to a spread of contagious illness that spreads across the countries or world, usually affecting a larger area than an ‘epidemic’. It is important to note that a disease that is affecting a large number of people or widespread cannot be said to be a pandemic till it is contagious (Fig. **1**). For example, cancer kills many people, but it is not a contagious disease, so not included as a pandemic (WHO, 2011).

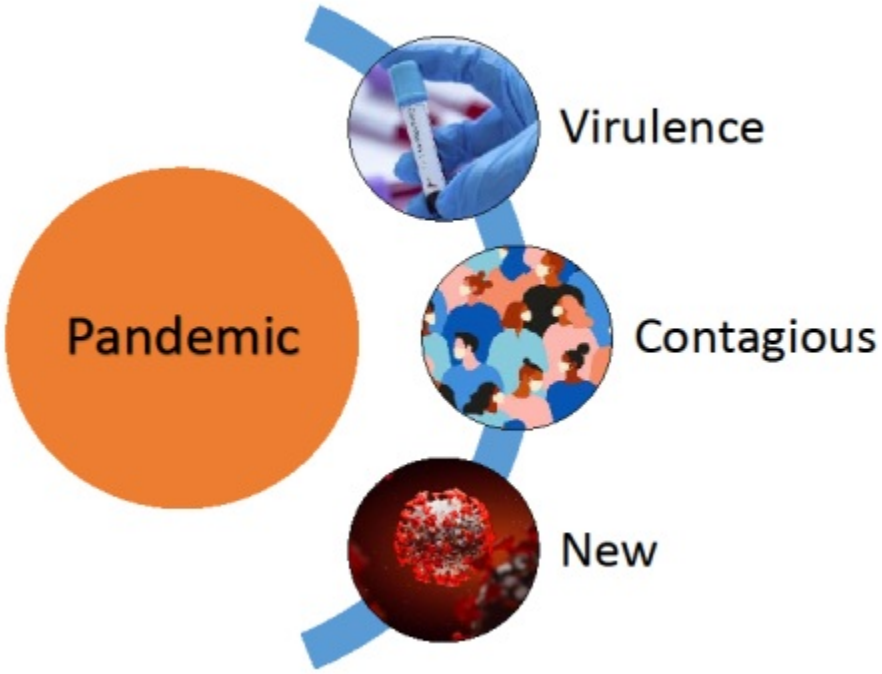


Fig. (1). The essentials of a pandemic.

The word “epidemic” is derived from the two Greek words *epi* meaning “upon or above”, and *demos* “the people”. An *epidemic* is an outbreak of disease that rapidly spread to a large population in a short period of time. For example, severe acute respiratory syndrome (SARS) took the lives of approx. 800 people worldwide during the epidemic of 2003 (Morens *et al*, 2009). An *outbreak* is an increase beyond expectation in the number of cases of a disease or condition occurring among a specified population in a limited geographic location and period of time (Gregg, 2002). The multi-state outbreak of *Salmonella Muenchen* in 1981 is an example of the outbreak.

Evidence suggested that the human population has suffered from many pandemics throughout history, be it the earlier form of smallpox or Spanish flu or the recent

incidence of Ebola or Covid. In world history, we can see a number of significant pandemics like cholera, dengue, plague, smallpox, AIDS, tuberculosis, influenza, West Nile disease, and severe acute respiratory syndrome (Rewar *et al*., 2015; Qiu *et al*., 2016). In 1999, WHO issued a printed paper on pandemic readiness overview, which was further revised in 2005 and 2009 while planning for an influenza pandemic. In this guidance, WHO defined different phases of pandemic and required appropriate actions for each phase. The revision includes the explanation of a pandemic and declaration of its leading phases (WHO, 2011). There have been many pandemics declared at different times as enlisted in Table **1**.

THE ATHENIAN PLAGUE

The plague has been responsible for three pandemics in history, including the 6th, 14th, and 19th centuries. The Athenian plague occurred during 430–26 B.C. It originated from Ethiopia, after that, it was distributed in Egypt and Greece. It is a well-known infectious disease primarily affecting rodents. *Yersinia pestis* bacteria is a causative agent of Athenian plaque that is related to the Enterobacteriaceae family. It is transferred in humans from rodents through skin-piercing by infected fleas. Transmission of bacteria in an uninfected person is possible by droplet contact, direct or indirect contact with infected material (Huremovic *et al*., 2017). Initial observed symptoms of the plague were headache, conjunctivitis, rashes on the whole body, and fever. After that patients showed severe symptoms like cough up blood, severe stomach cramps along with vomiting, and attacks of "ineffectual retching". Generally, on the seventh or eighth-day infected persons die (Thucydides, 2017). Approximately 75000 to 100000 people died due to the plague of Athens (Litteman *et al*., 2009).

Table 1. Chronology of pandemics.

Serial No.	Name of Pandemic Event	Year	Origin
1	Plague of Athens	430 B.C.	Ethiopia
2	Antonine Plague	165-190 A.D.	Italian peninsula
3	Justinian Plague	541-750 A.D.	Egypt
4	Japanese Smallpox	735-737 A.D.	Japan
5	Black Death	1347-1351	China
6	Aztecs Disease	1519-1520	Aztecs, America
7	London Plague	1665-1666	London
8	The Great Plague	1738	Central and Eastern Europe
9	Cholera 6 Outbreak	1817-1923	India

(Table 1) cont.....

Serial No.	Name of Pandemic Event	Year	Origin
10	The Third Plague	1885	Yunnan, China
11	Yellow Fever	1648-1800	Africa
12	Russian Flu	1889-1890	The first cases were observed at three separate and distant locations, Bukhara in Central Asia (Turkestan), Athabasca in northwestern Canada, and Greenland.
13	Spanish Flu	1918-1920	Europe
14	Asian Flu	1957-1958	The Chinese province of Guizhou
15	Hong Kong Flu	1968-1970	Hong Kong
16	HIV/AIDS	1980 – present	The Democratic Republic of the Congo
17	SARS	2003	China
18	Swine flu	2009-2010	Mexico
19	MERS	2012	Saudi Arabia
20	EBOLA	2014-2016	Zaire, Africa
21	COVID-19	2019 – present	Wuhan, China

PLAGUE OF JUSTINIAN

The Justinian plague is the first recorded pandemic that began in 541 AD, killed over 100 million people. The Justinian plague, the name given after the 6th century by Justinian I, Roman emperor of the Byzantine Empire at that time (Rosen, 2007). It was a “real plague” pandemic that originated in the mid-sixth century AD. The first recorded outbreak of this disease began in Egypt. Further, it spread in the capital of Turkey Constantinople caused the death of 10000 people per day, and killed around 40% of the city population. It reduced drop by drop European population by 50% between 550 AD and 700 AD (Horgan, 2014).

THE BLACK DEATH

The second pandemic was a global explosion of bubonic plague, widely known as the Great Plague, which originated from China in 1334, after that in 1347 it spread in Europe. After 50 years of its origin global population reduced from 450 million and caused approx 17 to 28 million deaths in Europe during the 14th century (Huremovic, 2016; DeWitte, 2014).

SPANISH FLU

The “Spanish” influenza pandemic of 1918-1919 first appeared in the USA. Dominating impression was mainly found in Europe, Asia, and North America, also appeared in New Zealand, India, and South Africa. It killed around 50

million people worldwide and remained a threat to public health. Around one-third of the world's population was infected and had clear symptoms of illness. This virus-derived in toto from an unknown source appeared as an avian-like influenza virus. It appeared quite ordinary, with mild symptoms. But it was not autopsies on victims that showed unusual hemorrhagic edema in the lungs, and it affected young adults more than others (Taubenberger, 2006). In 1918, this pandemic was caused by the venomous and pestilent influenza virus, a strain of the subtype H1N1 that mostly killed young adults. Examination of the virus proved it particularly deadly, as it activates overproduction, uncontrolled production of immune cells that destroy the immune system, and death of youngster. In 1918, the French bacteriologist Charles Nicole and his colleague Lebailly C concluded that the strain was found in mammals. The causative agent of Spanish flu is a virus of the "Orthomyxoviridae" family, which was recognized by Richard Edwin Scope (Tsoucalas *et al*., 2016).

AIDS

AIDS (acquired immunodeficiency syndrome) is an autoimmune disease caused by the human immunodeficiency virus (HIV). The causative virus was firstly identified in the early 1980s in the United States, after that scientists observed some cases in Africa and Europe, and at the same time all over the world. AIDS spreads through blood, vaginal secretions, semen, breast milk and mainly transmitted through unprotected mating with an HIV-positive partner. AIDS is a fatal disease, whereas a less number of infected people remain alive with AIDS around 20 years. It is the leading infectious cause of adult death around the world. AIDS was first recognized in the 1980s, public health officers try to prevent spreading disease by providing knowledge about the virus-like how the virus transferred from person to person and how could be protected ourselves from it (Farmer and Walton 2000; Lampatey and Merywen 2002).

SMALLPOX

Smallpox is one the most devastating diseases known to humankind, indigenous to Europe, Arabia, and Asia for centuries, responsible for the death of three infected individuals out of ten infectious patients, and the rest remain with pocky scars on the whole body. Evidence suggested that during the 20th century the global death toll from smallpox have been estimated at around 300 million. The native people of the United States and modern-day Mexico had nil natural immunity power against smallpox and the virus deducted them below by the tens of millions. Smallpox is the first virus epidemic cured by vaccination. During the 18th century, the vaccine of smallpox was discovered by a British doctor named Edward Jenner. Around 50 million people were infected by the smallpox virus in

one year- which was equal to the population of South Africa. After that WHO was established in 1948. In India, until the 1950s, annually more than a million people died due to smallpox. In 1980, WHO declared that smallpox had been eliminated from the Earth (Fenner and Henderson 1988; Gupta and Mahajan 2003; Roos, 2020).

SARS

The first case of Severe Acute Respiratory Syndrome (SARS) appeared in China in November 2002. After that, it was recognized as a global threat in March 2003. In the next few months, SARS disease spread over many continents such as North America, Europe, South America, and Asia. In the 21st century, it emerges as the first severe and readily transmissible disease. It is a viral respiratory illness caused by a coronavirus commonly known as SARS-associated coronavirus, having an incubation period of 2 to 10 days. The primary mode of transmission is respiratory droplets and direct contact. SARS-CoV has been detected mainly in stool, also detected in blood, urine, and conjunctival secretions but to a lesser extent (Christian and Poutanen 2004; Hui, 2005; WHO, 2013).

SWINE FLU OR H1N1 PANDEMIC

Swine flu is a respiratory disease of the pig, commonly known as Hog or Pig Flu. Infection caused by type A influenza virus subtype of H1N1that belongs to the Orthomyxoviridae family. The novel H1N1strain is the main reason for the explosion of swine flu, the origin of this new strain is unknown. It was first identified in April 2019 at the border region of the United States and Mexico. It emerged out as the first pandemic of the 21st century in a short term of two months, involving more than 170 countries and approximately 1.7 lakh infected individuals all over the world. Globally, the illness rate of swine flu was highest in children and young adults but the hospitalization rate was higher in children below one year of age (Mir *et al*., 2009; Sebastian *et al*., 2009).

EBOLA

It is defined as a neglected tropical disease (NTD), killed thousands of people. It is the largest and longest recorded pandemic in human history. The Ebola virus was the first point out in 1976 in Zaira, Yambuku, and Nzara, South Sudan as an outbreak of hemorrhagic fever. Ebola outburst started through zoonotic transmission, caused more than 30 outbreaks since 1976, with less than 1600 deaths before 2014, and since 2014 thousands of people killed in West Africa. The incubation period of the Ebola virus is 5 to 7 days. In most of the cases, it

started from rural background controlled by following regular community health measures like having a view on several infected people and the patient was isolated to break the chain of virus (Troncoso, 2015; Richardson *et al*., 2016).

COVID-19

Coronavirus disease (COVID-19) is a highly contagious respiratory illness that was quickly spread globally within the short term after finding the first case of COVID-19 (in Wuhan, China) in December 2019. WHO announced it as a life threat worldwide on 30 January and a pandemic on 11 March 2020, caused by the novel coronavirus, subsequently named SARS-CoV-2. In this respiratory disease, a person has mild symptoms like cold and cough after some time it emerged out as a critical illness with blockage of respiratory track and high fever. It has an overall low fatality rate in youngsters and a much higher rate in older persons. The causative agent of COVID-19 (SARS-CoV-2) spread very easily by respiratory droplets generated during talking, sneezing, and coughing by an infected person. It has 5 to 6 days incubation period (Balkhair, 2020).

ORIGIN OF CORONAVIRUS

"Coronavirus" word originated in 1968, given to the crown projections on their surfaces observed in the electron microscope. "Corona" in Latin means "halo" or "crown". Coronaviruses mainly attack the respiratory system and give rise to some manifestations like cough and difficulty in breathing. Some people, including older adults, are at risk of severe illness from these viruses. Coronavirus is a single positive-sense RNA virus; identified in 1960. Since 1970 pathogenesis and molecular mechanisms of replication of several coronaviruses have been actively studied. International Committee established the Coronaviridae family on Taxonomy of viruses in 1975. Coronaviruses have been described for more than 50 years, infect many species of animals including humans, and primarily affect the respiratory, gastroenteritis, and nervous system (Weiss and Navas-Martin, 2005).

Coronavirus species had been categorized into three groups. Group I and II infect mammals whereas group III is exclusively found in birds. SARS-CoV has been proposed as the first member of the fourth group of coronaviruses, separated from a child affected by bronchiolitis and conjunctivitis, and causes life-threatening pneumonia. HCoV-229E and HCoV-OC43 were identified to cause the common cold. There are only three types of coronaviruses have been studied that affect human, after addition of SARS-CoV; supplementary feature of HCoV-NL63 as the fourth member will supply a significant understanding of the variation among human coronaviruses. HCoV-NL63 is an arm of the group 1 coronaviruses

similarly to HCoV-229E, but the dissimilarities between them are important. These are as follows:

i. They have around 65% sequence similarities.
ii. In HCoV-NL63, a single gene ORF3 takes the place of the 4A and 4B genes in HCoV-229E.
iii. In HCoV-NL63, the 5′ region of the S gene contains a large in-frame insertion of 537 nucleotides.
iv. With a narrow host range, HCoV-229E is fastidious in cell culture. But, efficient replication of HCoV-NL63 in monkey kidney cells has been observed.
v. In infants and immunocompromised patients, HCoV-229E can cause severe respiratory disease whereas HCoV-NL63 causes acute respiratory disease (Hoek *et al*., 2004).

In December 2019, an extended number of pneumonia cases were found in China, caused by a newly recognized β-coronavirus, which is enveloped in a non-segmented positive-sense RNA virus. Initially, on 12 January 2020, WHO officially named this virus 2019- novel coronavirus (2019-nCoV) and disease as coronavirus disease 2019 (COVID-19). Coronavirus Study Group of the International Committee suggested the name to the new coronavirus as SARS-CoV-2 on February 11, 2020.

TYPES OF CORONAVIRUSES

All coronaviruses have a single-stranded RNA, size of genome up to 30kb in length and have similar morphology features. They have been united in the subfamily Coronavirinae within the family Coronaviridae which belongs to the Nidovirales order. The family consists of two subfamilies, Coronavirinae and Torovirinae. The Coronavirinae subfamily further divided into four genera, Alphacoronavirus, Betacoronavirus, Gammacoronavirus and Deltacoronavirus as summarized in Table **2**. Genera further classified into various species, containing various viruses that cause infections of cattle, horses, pigs, goats, and cats. Although, to date, it is not proven that the Torovirinae subfamily infects humans. Betacoronaviruses and Alphacoronaviruses are mainly originated from mammals, while Gammacoronaviruses and Deltacoronaviruses primarily began from avian (Wertheim *et al*., 2013).

Table 2. Types of coronaviruses.

Genus	Abbreviation used for the Virus	Species Affected	Disease Name or Organ System Affected
Alphacoronavirus	PEDV	Porcine	Porcine epidemic diarrhea virus
	TGEV	Porcine	Transmissible gastroenteritis virus
	FIPV	Feline	Feline coronavirus
	CCoV	Canine	Canine coronavirus
	PRCV	Porcine	Porcine respiratory coronavirus
	HCoV-229E	Human	Common cold
	HCoV-NL63	Human	Common cold
	Rh-BatCoV-HKU2	Bat	-
	Mi-BatCoV 1A	Bat	-
	Mi-BatCoV 1B	Bat	-
	Mi-BatCoV-HKU8	Bat	-
	Sc-BatCoV-512	Bat	-
Betacoronavirus	HCoV-OC43	Human	Common cold
	BCoV	Bovine	Calf diarrhea, winter dysentery
	PHEV	Porcine	Chronic wasting, encephalomyelitis
	AntelopeCoV	Antelope	-
	GiCoV	Giraffe	-
	ECoV	Equine	Enteric infection
	MHV	Mouse	Hepatic infection
	HCoV-HKU1	Human	Respiratory infections
	RCoV	Rat	-
	SARS CoV	Human	Severe acute respiratory syndrome
	SARSr-CiCoV	Human	Severe acute respiratory syndrome
	SARSr-Rh-BatCoV HKU3	Bat	-
	SARSrCoV CFB	Human	Severe acute respiratory syndrome
	Ty-BatCoV-HKU4	Bat	-
	Pi-BatCoV-HKU5	Bat	-
	Ro-Bat-CoV HKU9	Bat	-
Gammacoronavirus	IBV	Chickens	Avian infectious bronchitis virus
	TCoV	Turkey	Bluecomb, turkey coronavirus
	BWCoV-SW1	Whale	Beluga whale CoV

(Table 2) cont.....

Genus	Abbreviation used for the Virus	Species Affected	Disease Name or Organ System Affected
Deltacoronavirus	BuCoV HKU11	Avian	Bulbul coronavirus
	ThCoV HKU12	Avian	Thrush coronavirus
	MunCoV HKU13	Avian	Munia coronavirus
	PorCoV HKU15	Porcine	Porcine coronavirus
	WECoV HKU16	-	White eye coronavirus
	SpCoV HKU17	Avian	Sparrow coronavirus
	MRCoV HKU18	Avian	Magpie robin coronavirus
	NHCoV HKU19	Avian	Night heron coronavirus
	WiCoV HKU20	-	Wigeon coronavirus
	CMCoV HKU21	Avian	Common moorhen

The genus Alphacoronavirus includes several animal coronaviruses (PEDV, TGEV, FIPV) and human coronaviruses (HCoV-229E, HCoV-NL63). The genus Betacoronavirus comprise numerous animal coronaviruses BCoV, PHEV, GICoV, and three types of human coronaviruses *i.e.* HCoV-HKU1, HCoV-OC43, SARS-HCoV, and the MERS (Middle Eastern respiratory syndrome) coronavirus, SARS-related coronavirus.

Based on antigenic similarity Coronaviruses are classified into four groups, viruses in all groups can affect a large number of different host species. Several recognized human coronaviruses 229E, NL63, OC43, and HKU1 are common human viruses that easily infect immunocompromised and elder patients. These are responsible for approximately 10% mortality by causing common cold and self-limiting upper respiratory disease. MERS-CoV, SARS-CoV, and SAR-Co--2 are also human coronaviruses but these occur less frequently than common human viruses. These viruses cause severe respiratory disease and cause up to 35% of death (Cascella *et al*., 2020).

Alphacoronaviruses form a distinct monophyletic group in the Coronavirinae subfamily, having similar phylogenetic properties to other coronaviruses. General characteristics make it different among coronaviruses. Alphacoronavirus having an exclusive type of nsp1, specific size, and sequence from the nsp1 protein of betacoronavirus. Gammacoronavirus is similar to the other coronaviruses only in phylogenetic relation, there are no similarities in virion morphology, genome organization, and gene composition. These characteristics make it different from other coronaviruses. Viruses of the species avian coronavirus lack an nsp1 moiety. The genus Gammacoronavirus related to the third group of human coronavirus include viruses that were segregated from birds and whales. The genus

Deltacoronavirus is comprised of viruses isolated from pigs and birds. Until now various types of novel coronaviruses have been separated from bats. Many researchers suggested that human respiratory coronaviruses, MERS coronavirus, SARS coronavirus originated from an ancestor of bat viruses.

DISEASE ASSOCIATED WITH CORONAVIRUSES

Coronaviruses mainly strike on the respiratory system of mammals, birds, and humans. These are associated with pneumonia, common cold, bronchitis, severe acute respiratory syndrome (SARS), and coronavirus disease 2019 (COVID-19). From the last few years' studies, researchers concluded that these viruses can also infect rats, mice, dogs, cats, pigs, horses, and cattle. In most cases, animals transfer the viruses to humans.

Coronavirus is a significant pathogen that mainly affects the human respiratory tract and also attacks the gut of humans. The earlier outburst of coronaviruses having severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS) is earlier recognized as factors that are a major threat to public health. At the end of December 2019, numerous patients having pneumonia with unknown etiology entered hospitals.

MURINE HEPATITIS VIRUS (MHV)

Murine hepatitis virus is a commonly used laboratory strain that belongs to betacoronavirus genera; primarily infect the liver and brain. There are many strains of murine coronavirus exhibiting neurotropic and cause encephalitis with subsequent CNS demyelination. Although, the virus found in lasting infection of astrocytes, in most of the animals causes death by infecting oligodendrocytes and neurons. The animals that survive with acute infection develop a chronic progressive neurologic disease identified by outspread demyelinating lesions and CNS infiltration of lymphocytes and macrophages (Kyuwa and Stohlman 1990). It is one of the best animal models for encephalitis, hepatitis, and multiple sclerosis (Perlman, 1998).

Central Nervous System

The most frequently studied MHV strains are JHM and A59. The first time JHM was isolated from a paralytic mouse that was intensely neurovirulent producing encephalomyelitis with massive demyelination (Bailey *et al*., 1949; Cheever *et al*., 1949).

Hepatitis

MHV-induced hepatitis has been studied through several hepatovirulent strains including MHV-3, MHV-2, and the moderately hepatotropic A59. The most commonly used strain for the pathogenic study of MHV-induced hepatitis is the MHV-3 strain. It was separated from an offspring mouse that developed acute hepatitis following inoculation with the serum of an acute hepatitis patient (Dick *et al*., 1956).

Pneumonitis

MHV-1 strain is primarily pneumovirulent. MHV-1-induced pneumonitis is highly dependent on mouse strain; inbred albino strain, resistant to MHV-3 induced hepatitis is highly sensitive. Whereas other species of mice such as Balb/c and C57Bl/6 are resistible for MHV-1- induced pulmonary disease. MHV-1 infection of albino mice provides a mouse model for the pathogenic study of SARS-CoV in human beings (De Albuquerque *et al*., 2006).

BOVINE CORONAVIRUS

It is a non-segmented, positive-sense, single-stranded RNA virus that belongs to the Coronaviridae family and was first identified during an outbreak of diarrhea among neonatal calves in 1970 (Fauquet *et al*., 2005; Mebus *et al*., 1974). It was commonly identified in the respiratory system and gut of cattle where they used to shed fecal matter, nasal secretions and affect the lungs (Hasoksuz *et al*., 2002; Saif, 2010). It causes various diseases in cattle like winter dysentery, calf diarrhea in young cattle, and respiratory illness in the different age groups of cattle (Saif, 1990, 2007, 2010). It is also related to bovine respiratory disease in growing beef calves involved in the feedlots or grazing field (Fulton *et al*., 2011). To date, Bovine coronavirus monitoring programs might be included in the live attenuated virus or killed vaccines but there is no USFDA-approved bovine coronavirus vaccine in The United States to control bovine respiratory disease (North American Compendiums, 2010). Modified live vaccines can be used in newborn calf to prevent gastroenteric disease, also used for a pregnant cow. Antibodies of Bovine coronavirus transfer in neonates after calving to control BoCV (Fulton *et al*., 2011).

HUMAN CORONAVIRUS

In the mid-1960s, human coronaviruses were first recognised. To date, there are seven types of coronaviruses identified which can cause infections in the majority of people. There are seven mentioned types of human coronaviruses and these are

NL63 (alpha coronavirus), 229E (alpha coronavirus), HKU1 (beta coronavirus), OC43 (beta coronavirus), MERS-CoV (beta coronavirus that causes MERS), SARS-CoV (beta coronavirus that causes SARS), and SARS-CoV-2 (the new coronavirus which gives rise to COVID-19) (CDC, 2020).

Human coronaviruses are the respiratory pathogens that present an important group of coronaviruses linked with several respiratory diseases such as pneumonia, bronchitis, common cold, and respiratory disease (Pene *et al*., 2003). Evolution of HCoVs has been expedited through the regular combination of species by crossing of species barrier and genetic recombination of these viruses. Many of these are widely spread in the human population and globally hit one-third of common cold infections in human beings (Lim *et al*., 2016).

ABBREVIATIONS

AD	Anno Domini
AIDS	Acquired immunodeficiency syndrome
B.C	Before Christ
BoCV	Bovine coronavirus
CDC	Centers for Disease Control and prevention
CNS	Central nervous system
CoV	Coronavirus
COVID-19	Coronavirus disease 2019
HIV	Human immunodeficiency virus
MERS-CoV	Middle East respiratory syndrome coronavirus
MHV	Murine Hepatitis Virus
NTD	Neglected tropical disease
RNA	Ribonucleic acid
SARS-CoV	Severe acute respiratory syndrome coronavirus
USDA	United States Departments of Agriculture
WHO	World Health Organization

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 2

Introduction to COVID-19

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Abstract: In the mid 20th century, virologists identified a new category of the virus, which has a fringe of projections on its surface that appears like a crown and named coronavirus. Coronavirus belongs to the family of pleomorphic spherical viruses recognized by bulbous surface projection and ssRNA. As the virus belongs to the family of RNA-virus, the chances of mutation are very high, which further increases its pathogenicity. The coronavirus mainly attacks the respiratory tract and ultimately leads to respiratory failure. Recent outbreaks of coronaviruses are severe acute respiratory syndrome and the Middle East respiratory syndrome, which cause a great threat to human health with a high mortality rate. Later on, in late 2019, a new form of coronavirus appears in Wuhan, China where numbers of people are recognized with pneumonia-like symptoms. The condition was entitled with COVID-19 by WHO on Feb 2020, which was declared to be pandemic by the same agency in Mar 2020. The COVID-19 is considered to be originated from bats, which then transmit to humans due to the consumption of contaminated animal raw. The virus is highly contagious and spread at a very high rate, which produces global health risks. Further various existing treatment is used for treating the infection but still the precise and accurate treatment yet to be investigated.

Keywords: ACE-II, Air droplets, Breathlessness, China, Chronology, Coronavirus, COVID-19, Genome, Helicase, Membrane protein, MERS, Pneumocytes, Pneumonia, Replicase, Respiratory distress syndrome, SARS, Structural proteins, Virus, World, Transmission.

INTRODUCTION

The coronavirus belongs to a family of viruses that mainly affects the respiratory tract infection with prime symptoms, such as hyperthermia, pneumonia, breathlessness, and acute or chronic pulmonary infections. The coronavirus mainly affects animals, but recent investigations in the late 20th century reported some human cases. In 1930, the first coronavirus case was reported in chickens recognized by pneumonia-like symptoms and termed as avian infectious bronchitis virus. Further, in late 1960, the first human coronavirus case was

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reported (Pyrc *et al*., 2007). In 1968, electron microscopy of the virus was done and termed as **"Coronavirus"** due to its crown-like appearance, clearly depicted in Fig. **1**(**A** & **B**). Later on, in 1975, the International Committee on the Taxonomy of Viruses introduced a family Coronaviridae. In 2005, the family Coronaviridae was further split into two subfamilies, the Coronaviruses, and the Toroviruses (Tyrrel *et al*., 1968).

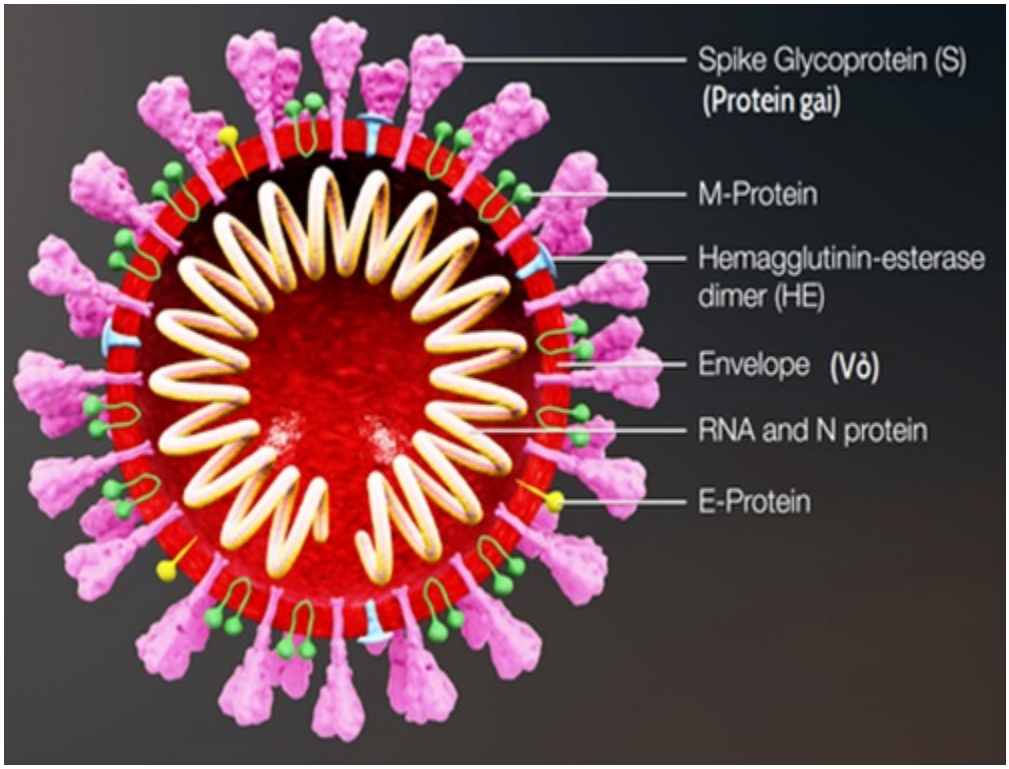


Fig. (1). Structure of Coronoavirus **(A)** Electron Microscopy and **(B)** Model of CoV.

Based on serological reactions, the coronavirus is divided into three groups or genera *i.e*. Group-I, Group-II, and Group-III (McIntosh *et al*., 1974). Group-I includes viruses that cause infection in animals only, like the Porcine epidemic diarrhea virus and feline infectious peritonitis virus. It also includes the viruses that produce respiratory infections in humans, such as Human Coronavirus 229E (HCoV-229E) and KHU1. The Group-II viruses are Porcine hemagglutinating encephalomyelitis virus, bovine coronavirus, and equine coronavirus, which shows pathogenicity in animals, while HCoV-OC43 and HCoV-NL63 produce pneumonia-like conditions in humans. Group-II viruses that cause infections in rodents are murine hepatitis virus (MHV) and Rat sialodacryoadenitis CoV, which produce enteritis, hepatitis, and encephalitis in addition to respiratory infections.

Lastly, infectious bronchitis virus (IBV), Turkey coronavirus, and pheasant coronavirus are classified under Group-III viruses.Table **1** summarizes the types of CoV (Cavanagh *et al*., 2020). All the CoV possess some common characteristics, which are as follows:

1. The shape of coronavirus is spherical with an average size of 80-220 nm.
2. The virus is covered with an envelope having club-shaped peplomers.
3. It all contains a tubular nucleocapsid structure with helical symmetry.
4. All are composed of single-stranded RNA with a genomic size of 27-32 kb.
5. The coronavirus contains numerous structural proteins, but some are present in all, such as nucleoprotein, peplomer glycoprotein, a transmembrane glycoprotein, and hemagglutinin esterase.
6. While some non-structural proteins are also present in CoV, such as RNA dependent RNA polymerase.
7. The newly-formed viruses *i.e*. virions are generally assembled in the endoplasmic reticulum and Golgi cisternae.
8. The mutation is very common in CoV and due to this reason, a diverse host range was exhibited (Enjuanes *et al*., 2000).

Table 1. Different types of coronavirus.

Group	Virus	Host	System Affected
I	229E	Human	Respiratory Infection
	TGEV	Pig	Respiratory & Enteric Infection
	PRCoV	Pig	Respiratory Infection
	Canine coronavirus	-	Enteric infection
	FeCoV	-	Enteric infection
	FIPV	Cat	Respiratory, enteric & neurologic infection
	NL-63	Human	Respiratory Infection
II	OC43	Human	Respiratory & enteric infection
	MHV	Mouse	Intestinal & neurological infection
	Sialodacryoadenitis CoV	Rat	Neurological infection
	Hemaagglutinating encephalomyocarditis virus	Pig	Respiratory, enteric & neurological infection
	BCoV	Cow	Enteric infection
	HKU1	Human	Respiratory infection
	SARS-CoV	Human	Life-threatening respiratory infection
III	IBV	Chicken	Respiratory infection, Hepatitis
	Turkey CoV	Turkey	Respiratory & Enteric infection

GENOMIC STRUCTURE OF CORONAVIRUS

Coronavirus is known to possess the largest genomic structure of around 30kb having single-stranded RNA. The genomes of CoV are arranged with replicase loci encoded within the 5'-end while within the 3'-end of the genome encoded the structural proteins arranged in the sequence hemagglutinin esterase, spike protein, small membrane protein, membrane, and nucleocapsid protein. The genomic RNA is generally present along with nucleocapsid protein to form a helical structure found within the virus-cell membrane. Apart from nucleocapsid protein, some other proteins are also found in the virus. For instance, the spike protein, which is a type-I glycoprotein, forms peplomer on the surface of virion which reveals the crown-like appearance on microscopic examination. Further another protein present in the virus is termed as membrane protein which spans three times the viral membrane. The membrane protein possesses a short N-terminal domain and a cytoplasmic tail. Apart from spike and membrane protein, a hydrophobic protein is commonly known as small membrane protein, also exists having a short domain, transmembrane domain, and cytoplasmic tail. The hydrophobic protein present as a bi-layered structure around the viral membrane with N-terminal and C-terminal in the inner face. Heamglutinin esterase protein is supposed to help the virus to gain entry in the cell but usually not present in each CoV.

The two-third of the genome is covered by 5'-end which encodes for a replicase gene that comprises open reading frame -1a (ORF) and open reading frame-1b. The gene is translated into two large proteins *i.e*. pp1a and pp1ab. The proteins are further split into small fragments of around 16 proteins having an enzymatic activity like proteases, RNA modification enzymes, polymerase, and helicase as depicted in Fig. (**2**). Additionally, certain non-structural genes are also present in the genome of the virus which encodes accessory proteins whose function, sequence, and number varies in each virus belongs to coronavirus (Brian *et* al., 2005).

STRUCTURAL PROTEINS

Structural proteins in the coronavirus, such as membrane-spanning proteins play a crucial role in the pathogenicity and replication as well as assembly of the virus. This section covers the different structural proteins.

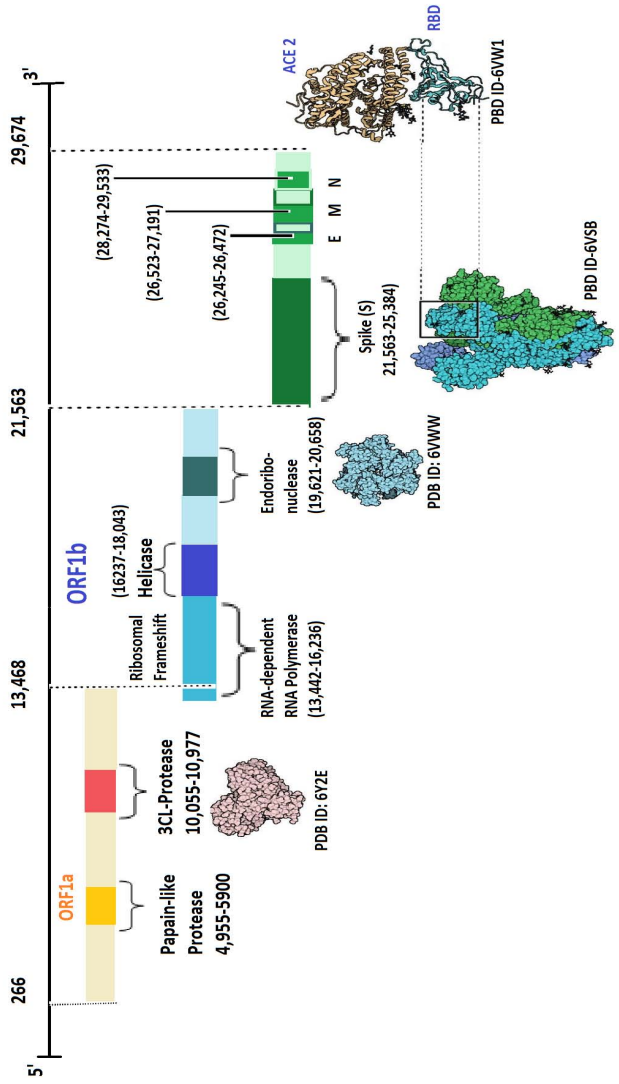


Fig. (2). Genomic Structure of COVID-19.

Spike Protein

The spike protein with a molecular weight of 180 kb belongs to Type-I glycoprotein enclosed in the outer membrane of the virus and form the peplomers. The arrangement of these peplomers is such that a crown-like morphology appears and also promotes the interaction of viral and host receptors to facilitate

the viral entry into the cell by inducing membrane diffusion. The protein may further cleave to form subunits, the C-terminal S2, and N-terminal S1, with an approximate weight of 90 kb (Frana *et al*., 1985). The reaction is catalyzed by a furan like a protease enzyme. The S1 subunit forms the globular head like structure while S2 subunit for a trans-membrane tail. At the time viral encounter to the host cell, the subunits of the protein interact with the host receptor and mediate the virus entry. The S1 subunit normally contains β-sandwich galectins similar to humans, which additionally promotes binding to the receptor. The spike protein influence mainly viral tropism and pathogenic phenotype (Sturman *et al*., 1980).

In the case of SARS-CoV apart from cellular receptors, the Angiotensin-Converting Enzyme-II (ACE-II) is another major determinant of infection. SARS-CoV is the only type in the category which infects nearly all animals and humans. Also, the receptor binding domain of SARS-virus contains a 192-amino acid spanning residue which is mainly responsible for the interaction with the human ACE-II receptor. Unlike all CoV, the SARS CoV spike protein does not cleaved into S1 and S2 subunits. However, the entry of the virus is mainly mediated through Cathepsin-L in acidic pH as in the case of other CoV. The spike protein also regulates the renin-angiotensin system by the down-regulation of ACE-II expression on the plasma membrane which ultimately results in SARS-CoV infection as ACE-II possesses pneumo-protective property (Li *et al*., 2006).

Small Membrane Protein

The small membrane proteins are generally integral transmembrane proteins and composed of 75-110 amino acid residues. The protein is composed of three domains, hydrophilic C-terminal domain, N-terminal domain, and a transmembrane domain. The palmitoylation and ubiquination of protein are necessary for proper assembly of the virus. Thus for efficient virus assembly, the small membrane protein, as well as the membrane protein, are the key proteins in every CoV. The interaction of both proteins is mediated through the cytoplasmic tails. In addition to assembly, this protein also aids in the release of the virion. Various studies demonstrated that the majority of the protein is localized near the nuclear membrane (Boscarino *et al*., 2008). But in certain cases like SARS-CoV, the protein is present along with Golgi complexes. The arrangement of this protein in the membrane varies in each type of CoV. For instance, in TGEV, the C-terminal is oriented towards the lumen of intracellular membranes with its N-terminal in the cytoplasm. While in the case of IBV, the N-terminal is in the lumen of the membrane while C-terminal exposes to the cytoplasm. Both C-terminal and N-terminal are exposed in the cytoplasm of MHV and forms a hairpin-like structure. In SARS, dual arrangement occurs, one hairpin loop

structure and second with a single membrane-spanning domain with C-terminal in the cytoplasm and N-terminal oriented towards the lumen. The over-expression of small membrane protein induces apoptosis through the activation of Bcl-2 and Bcl-xL and thus responsible for SARS virus-induced alveolar damage (Cohen *et al*., 2011).

Membrane Protein

The membrane protein is the most abundant protein found in a viral envelope, which normally has 221-262 amino acid residues. The protein composed of short hydrophilic N-terminal chain exposed on the outer surface of the virus, long C-terminal tail located inside the virus, and both connected by three transmembrane domains. The long C-terminal domain is further subdivided into two domains, one near to the third trans-membrane domain termed as amphipathic domain, and the second is the short hydrophilic domain. The N-terminal domain is either N-glycosylated or O-glycosylated, depending upon the type of CoV. The amphipathic is linked with the viral membrane and plays a significant role in virus assembly and budding (Armstrong *et al*., 1984). The membrane protein interacts with other proteins like spike proteins and small membrane proteins for enhancing the assembly and budding of virions. In the host, the membrane protein also stimulates the release of interferon-α and thus the protein is said to possess interferogenic property (Rottier *et al*., 1984).

Hemagglutinin-Esterase

In addition to spike protein, some viruses carry hemagglutinin esterase protein on the envelope. The protein is a glycosylated polypeptide with a molecular weight of 65 kDa and exists in the form of a homodimer linked by a disulfide bond. The major constituents of the protein are sialic acid and acetyl esterase which potentially play a role in viral entry or budding. In some viruses like MHV, the protein initiates the binding process of the virus with the host cell surface receptor while spike protein is responsible for binding to specific glycoprotein receptors. Additionally, the protein aids in the release of the virus. Hemagglutinin esterase suggested possessing the entero-pathogenic property and responsible for the spread of disease to non-infected cells. To cause infection in the gastrointestinal tract, the esterase component of the virus plays a significant role by allowing the movement of virions through the mucous of the infected cells (Brian *et al*., 1995).

Nucleocapsid and Internal Proteins

The nucleocapsid protein is an RNA binding protein that encodes in the 3'-end of the genome, having multiple roles in conjunction with other proteins. The nucleocapsid protein interacts with the membrane protein for the efficient

assembly of the virions. The protein is also associated with replication and transcription of the virus in infected host cells as these proteins are localized to the nucleus of infected cells. The nucleocapsid protein has a significant role in the pathogenesis of CNS disease and hepatitis. Nucleocapsid protein in the neurons interferes with the axonal transport mechanism. Furthermore, the protein also antagonizes the interferon in the host cells. The protein in the host induces the release of fibrinogen like protein-2, which possesses the pro-coagulant and immuno-suppressive activity and thus causes liver damage. The genome of group-II CoV contains internal ORF in the nucleocapsid gene. The internal protein possesses very little role in pathogenesis (Verheije *et al.,* 2010).

Replicase Protein

The replicase proteins are the key determinants of viral replication which may influence pathogenesis and tropism of the virus. It mainly interacts with non-coding 5' and 3' sequence in the viral genome *via* the cell specific or immune response factors. The enzymes that have been encoded in ORF 1a and 1b mainly concerned with subverting the host cell metabolism (Zhu *et al.,* 2004).

VIRAL CYCLE

The cycle of CoV is shown in Fig. (**3**). Coronavirus binds to specific host cell receptor by spike proteins, which initiates the structural change in the spike protein. As a result of conformational change in spike proteins, a fusion of the host cell membrane with viral envelope occurs, which causes the release of nucleocapsid protein into the cell. After gaining the entry of the virus into the host cell, the 5' end of the genome RNA is translated into pp1a and pp1ab. Further, ORF 1a encodes papain-like proteases and 3C-like protease that converts pp1a and pp1ab into replicase proteins. The X domain of ORF-1a encodes for ADP-ribose-1-phosphatase activity while ORF-1b encodes RNA dependent RNA polymerase, helicase, exonuclease, endoribonuclease, and S-adenosylmethionin--dependent ribose methyltransferase. Additionally, ORF-2a encodes for cyclic phosphodiesterase. The enzymes play a crucial role in the metabolism of CoV and interact with host cell mechanisms to induce pathogenicity (Bond *et al*., 1979).

As the virus gains entry in the host cell, replication of genome and transcription of mRNA initiates, which results in the synthesis of negative-strand and positive strand RNAs through unique discontinuous transcription phenomenon. The negative strand RNA serves as a template for full length genomic RNA and sub-genomic RNA. The multiple sub-genomic RNA so formed acts as an mRNA having leader sequence at 5’-end. The mRNA synthesis is regulated by transcription-regulating sequences, present at the transcriptional initiating sites in the genomic RNA. The sub-genomic negative strand RNA having anti-leader

sequences will be added onto the 3'-end of negative strand RNA that acts as a template for the synthesis of mRNA. Further, the viral proteins are translated from specific mRNA. For instance, replicase is translated from genomic RNA mainly at 5'-end. While in the case of spike protein, which is translated from ORF-5b on mRNA and subsequently translation of ORF-5b is mediated through the ribosome (Bredenbeek *et al.,* 1990).

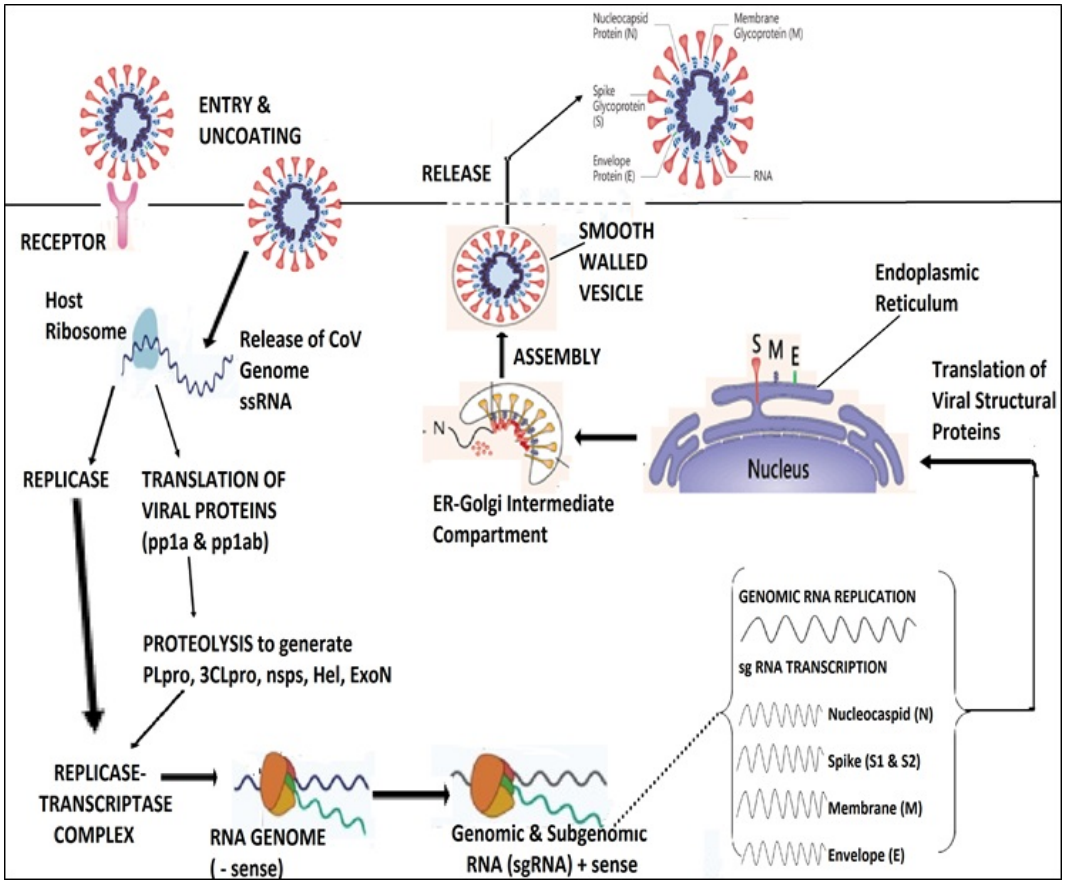


Fig. (3). Schematic Model of Viral Replication in Host Cell.

After the completion of the translation of membrane and spike proteins, the proteins are localized to the endoplasmic reticulum Golgi intermediate compartment and then to Golgi intracellular membranes from where actually budding takes place. The proteins and other viral factors determine the site of budding of virions. The spike proteins are uniformly present on plasma membranes as well as the intracellular membrane. At the time of assembly, the spike proteins interact with the transmembrane region of the membrane protein.

The spike protein sometimes mediates the fusion of cells and thus syncytium is formed and the virus is transmitted. Nucleocapsid protein forms a complex with RNA to form a helical structure. As a result of the interaction of nucleocapsid proteins with membrane proteins, the budding of virions occurs in the form of vesicles. Lastly, the virus arrives at the cell surface from where it exits the host cell (Lee *et al*., 1991).

OUTBREAKS OF CORONAVIRUS

Generally, coronavirus replicates in epithelial cells of the enteric or respiratory system of animals, including humans. Due to the presence of an envelope around the virus, the stability is very poor as compared to non-enveloped viruses. The SARSCoV is transmitted through direct contact and despite the envelope; it has high stability on environmental surfaces. The enveloped SARS CoV has the potential to cause infection even in the presence of proteolytic enzymes and bile in the gastric tract. The glycosylated glycoprotein present on the envelope makes them resistant to proteolytic degradation. Infections caused by flu CoV results in ciliostatsis *i.e*. loss of ciliary action and degeneration of cilia of the respiratory tract. SARS CoV generally targets type-I and type-II alveolar epithelial cells of the lower respiratory tract. The desquamation of alveolar epithelial cells causes the formation of the hyaline membrane in the alveoli, which ultimately results in acute respiratory distress syndrome.

The mechanism of infection and the immune reaction of the host cell would be demonstrated in the animals. For instance, MHV mainly affects the enteric system, lower respiratory organs, nervous system, and hepatic cells. The neurological manifestations mainly include multiple sclerosis, demyelination, neuronal degeneration and sometimes also involve oligodendrocytes, microglia, and astrocytes. The degree of neurological manifestation depends upon the strain and type of virus. Demyelination occurs due to virus-mediated immune reaction in the host through the activation of virus-specific T-cells. Further, due to demyelination, macrophages infiltrates and microglia cells start accumulated into the white matter. Moreover, the innate immune reaction is also a key determinant of viral replication in host cells. But certain CoV encounters the innate immune attack by producing certain proteins like ORF6 and ORF3b which inhibits interferon release mainly by preventing the nuclear translocation of proteins responsible for the production of interferons (Coleman *et al.,* 2014).

Porcine Coronavirus

In 1946, the first case was reported in porcine by the Transmissible gastroenteritis virus which majorly causes viral enteritis and diarrhea with a high mortality rate. The virus mainly attacks epithelial cells of the small intestine which leads to life-

threatening enteritis especially in neonates. In certain cases, the virus invades mainly the upper respiratory tract, which rarely reaches the lungs. Another virus that infects is the Porcine respiratory virus (PRCoV) which is an attenuated strain of TGEV. PRCoV mainly infects epithelial cells of lungs mainly in type-I and type-II pneumocytes, alveolar macrophages, and ultimately in interstitial fluid. The genome of both the virus is similar but differs in the 5' region of the spike gene which results in different pathogenic outcomes. In the late 1970s, group-I porcine coronavirus PEDV emerged in Europe, which later spreads to Asia. PEDV somewhat resembles human CoV mainly HCV-229E. Last but not least; another porcine CoV is hemagglutinating enteric CoV having an entirely different genomic structure than other porcine viruses (Saif 2004a and Saif 2004b).

Avian Coronavirus

IBV contributes to the majority of deaths in chickens whose mode of transmission is through air droplets and poses an economic burden on the poultry industry. IBV mainly multiplies in the upper respiratory tract and invades the entire bronchi tissue to cause severe and life-threatening ailment. In some cases, IBV also causes severe systemic infections such as nephritis, decreased egg production, and gastric disturbances. The B-cells mediated antibodies/immunoglobulins produced due to viral encounter in the respiratory tract prevents systemic manifestation in the host. Even maternal antibodies also possess the activity against the IBV (Saif 2004a and Saif 2004b).

Feline Coronavirus

There are two main types of feline CoV, FeCoV and FIPV. FeCoV (Feline Enteric Coronavirus) mainly transmitted from different components of the environment to produce an asymptomatic condition which, when converts into an active form in plasma produce virulent consequences. FIPV, on the other hand, concentrates and replicates in macrophages to cause a life-threatening condition. In the initial phase, FIPV replicates in pharyngeal or intestinal cells and further systemic outcomes appear when the virus starts invading the macrophages. The systemic manifestations are inflammation of the abdominal and thoracic cavity, ocular, and neurological disorders. One of the biggest challenges to treat FIVP is immune-mediated pathology. The FIVP induced immune system stimulation sometimes becomes the major cause of death (Addie, 2004).

Bovine Coronavirus

Bovine coronavirus attacks both respiratory as well as enteric systems and produces symptoms like diarrhea, winter dysentery, shipping fever, and respiratory infections (Saif, 2004a).

Murine Coronavirus

A vast variety of strains of coronavirus infects murine exhibits different characteristics and degrees of virulence. MHV mainly infects hepatic cells and neurons, where the prime manifestations of infection involve encephalitis, immune-mediated demyelination, multiple sclerosis, and hepatitis. MHV encounters the host immune system *via* variable viral gene products. The degree of virulence and tropism depends on the interaction of viral gene products and host immune response. The presentation of viral molecules activates both antibody and cell-mediated immunity. The CD8+ and CD4+ T-cells are the key immune cells responsible for encountering the virus during acute infection. Apart from T-cells, interferon-gamma is also responsible for the clearance of viruses from neuroglial cells of the nervous system. The CD8+ T-cells retard viral replication and thus limit the spread of the virus. It also decreases the amount of demyelination of neurons. On the other hand, B-cell mediated antibodies are required to prevent the re-occurrence of infection by the MHV in the CNS after the initial clearance by the T-cells (Barthold *et al*., 1993 and Glass *et al.,* 2002).

The second prominent infection in murine is caused by JHM, in which mass release of INF-α/β and chemotaxis agents like CCL3, CCL4, CXCL2, CXCL10, and CXCL5 occurs. The elevated levels of chemokines in the host result in the release of macrophages and neutrophils and thus represents acute infection. The neurovirulent JHM is unable to initiate significant T-cell activity and thus delays viral clearance, which accounts for a high mortality rate (Perlman, 1998).

Human Coronavirus

The first emerging CoV infection in humans is caused by OC43 and 229E, with a common sign of a common cold. Later on, SARS-CoV was identified, which likely to cause severe acute respiratory syndrome. Furthermore, two more strains were isolated from humans, one is HKUI and HCoV-NL63, with the prime symptom of pneumonia and bronchiolitis and conjunctivitis respectively. The main manifestations of HCoV-NL63 include respiratory syndrome, respiratory infections, bronchiolitis, and pneumonia. In Fig. (**4**), different human CoV are shown with their potential source of origin (Van der Hoek *et al*., 2005).

SARS-CoV

In 2003, a documentary was released from China which reports that new respiratory illness in the form of atypical pneumonia emerged in some patients. Later on, a detailed investigation was done by isolating the sample from the lungs of the patients, which demonstrates that a new coronavirus starts invading the society with a prime indication of respiratory distress (WHO, 2003). SARS is

characterized by hyperthermia, dyspnea, lymphopenia, and infections in the bronchioles. Later on, gastric symptoms such as epigastric distress, diarrhoea, and intestinal infection appear due to active replication of SARS-CoV. In a later stage, the virus and its products were also seen in the kidney, liver, and small intestine. Mainly the transmission occurs through air-droplets, blood, and fecal-oral route. SARS endemic ends with a toll death of around 778 out of 8000 infected persons. The SARS declared to be endemic in Singapore, Taiwan, and Beijing, with a mode of transmission is mainly *via* airborne droplets or through fomites (Nie *et al*., 2003).

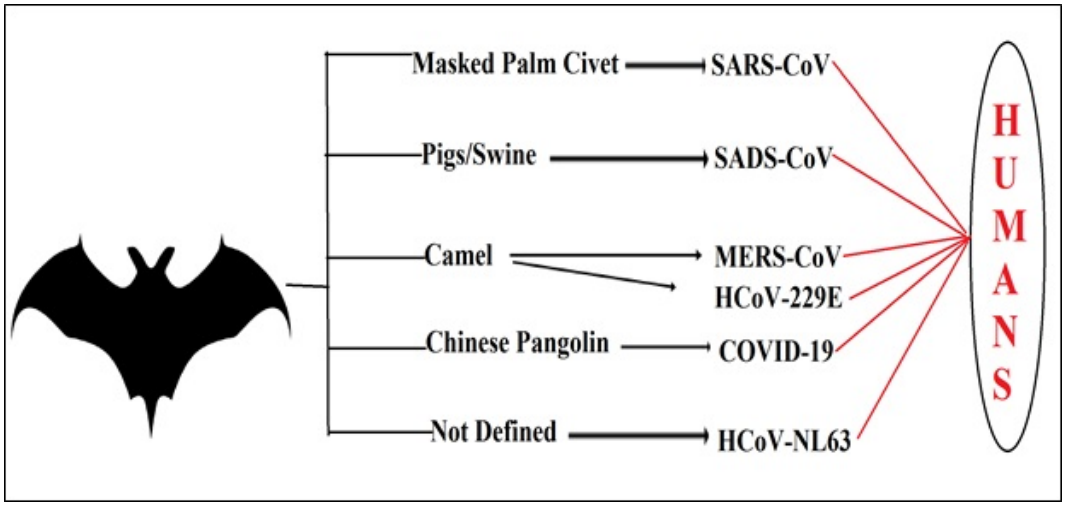


Fig. (4). Origin of Different Types of CoV.

The SARS patient normally exhibits a tri-phasic pattern of disease where patients initially have signs of flu such as hyperthermia, dry cough, sore throat, myalgia, and breathlessness, which persist for around 7-14 days. After around 14 days, the above signs become more severe, and additionally hypoxia and diarrhea also become prominent. The respiratory status of the patient deteriorates continuously and ultimately developed a condition termed as acute respiratory distress syndrome and maximum patients require an artificial respiratory system to overcome such conditions. By the end of approximately 21 days, the second phase ends up. The last phase involves systemic manifestations in which the virus sheds out from the respiratory tract and subsequently invades the gastrointestinal tract. The mortality rate is normally low but considered to be 50% for patients for more than 60 years. The histological examination revealed that the lungs contained a hyaline membrane, edema, fibrin exudates, thrombus in blood vessels, and sloughing of pneumocytes. Additionally, there is the infiltration of lymphocytes, macrophages, and polymorphonuclear leukocytes, and pneumocytes appear

abnormally as multinucleated giant cells. At the end of the second phase, the histological image indicates pneumonitis, which can be compensated by type-II pneumocyte hyperplasia, squamous metaplasia, and bronchiolitis. Further, the viral load decline as the immune system activates but it also worsens the disease due to the abrupt release of cytokines. Various pro-inflammatory cytokines released are MCP-1, MIP-1, RANTES, MCP-2, TNF-α, and IL-6 (Zhong *et al*., 2003).

The receptor binding domain (RBD) for SARS on the host cells is an angiotensin converting enzyme-II receptor (ACE2R). As the CoV binds to ACE2R, the structure of the receptor is transformed which enables the virus to initiates the course of infection. As the disease progresses, the expression of ACE2R on the cell surface is downregulated. The downregulation occurs due to two main reasons, the internalization of ACE2R during the viral entry and over expression of tumor necrosis factor converting enzyme commonly termed as metalloproteases. The metalloproteases cause cleavage of the ACE2 extracellular domain from its transmembrane domain and thus results in shedding of the domain. Since ACE2R possesses pneumoprotective activity, the disappearance of the receptor after CoV-RBD interaction additionally increases the severity of acute lung injury. Moreover, the downregulation of ACE2R also produces a detrimental effect on the myocardium, for instance, myocarditis and infection in the myocardium. Also, infection with the virus initiates a series of humoral and cellular immune responses in the host. In the plasma, high titers of immunoglobulins are found, mainly IgG and IgM, and also cytotoxic T-cells are also detected. The levels of immune cells in host plasma correlates with the infection period and recovery from the disease. It is believed that the more the count of immune cells in the host, the more will be recovery rate and thus immune cells are considered to be crucial for the clearance of the SARS-CoV. Till now, there is no treatment of the SARS-CoV, but the vaccine is supposed to be a suitable measure to eradicate the disease (Tsui *et al.,* 2003). Below mentioned Table **2** compares different characteristics of SARS-CoV and SARS-CoV-2.

MERS-CoV

Furthermore, another infection in humans caused by the member of CoV is the Middle East Respiratory Virus (MERS-CoV). The first case of MERS was reported in June 2012, in Jeddah, Saudi Arabia. The patients suffering from MERS-CoV present mainly acute pneumonia and renal failure. Later on WHO declared MERS-CoV as a pandemic as the disease affects around twenty-seven countries in Asia with a death rate of 36%. The preliminary manifestations include flu-like conditions or pneumonia, acute respiratory distress syndrome (ARDS), septicemia, shock-like condition, and lastly, organ failure, which lead to

death. Generally, the condition appears with hyperthermia, chills, dry cough, sore throat, arthralgia, myalgia, and breathlessness. The infection further progresses to life-threatening pneumonia, diarrhea, and vomiting. The progression rate of disease is very fast that within 7-11 days the infection presents with systemic manifestations. As in other cases, MERS-CoV invades the innate immune cells and thus, in immuno-compromised patients, the condition becomes life-threatening. Unlike SARS, MERS-CoV acts through CD26 receptors *i.e*. a dipeptyl peptidase (PLOS Currents Outbreaks 2013).

Table 2. Comparison of SARS-CoV and SARS-CoV-2.

Characteristic	SARS-CoV	SARS-CoV-2
Target receptor	ACE-2	ACE-2
Nucleocapsid protein	IFN-γ inhibitor	Unknown
Chest X-ray	Ground glass opacities	Bilateral, multilobar ground glass opacities
Chest CT-scan	Lobar consolidation Nodular opacities	No nodular opacities
Prevention	Hand hygiene, coughetiquette	Possibly hand hygiene, cough etiquette
Transmission	Droplets Contact with infected individuals	Droplets Contact with infected individuals, even asymptomatic ones
Case fatality rate (overall)	9.6%	2.3%

COVID-19

Introduction

As per the WHO, viral diseases continuously appear and produce a serious concern for societal health. In the last two decades, numerous epidemics or pandemics have been recorded due to viruses, for instance, SARS-CoV in 2002, H1N1 influenza in 2009, and MERS-CoV in 2012.

In a timeline that reaches the present day, an epidemic of cases with lower respiratory tract infection was detected in China. Initially, due to the unavailability of data and unidentified causative organism, the first case was designated as “pneumonia of unknown etiology” (Bogoch *et al.,* 2020). After that, the Chinese Center for Disease Control and Prevention (CDC) conducted a thorough investigation program. After completing the investigation, it was concluded that a new virus belongs to the coronavirus family responsible for such illness. Thus at the end of 2019, a cluster of pneumonia cases was reported in Wuhan, China, whose etiology was considered to be a new member of the β-

coronavirus subfamily. This member of the β-coronavirus subfamily was initially designated as a 2019-novel coronavirus (2019-nCoV) on 12 January 2020 by WHO. But on 11 February 2020, WHO re-designate the virus as COVID-19 (Coronavirus disease 2019), and Coronavirus Study Group of the International Committee termed it as SARS-CoV-2. The SARS-CoV-2 is highly contagious and spreads globally at a very high rate. International Health Regulation of WHO, in his meet on 30 January 2020, declared a report that the virus was spread to around 18 countries, with four countries reporting community transmission. Further, an alarming situation occurs; a case was reported on 26 February 2020 in the United States having no history of travelling from China (Zhao *et al.,* 2020).

The coronavirus has become one of the major pathogens which mainly attacks the respiratory tract and produces the pneumonia-like condition. Till now there is a big question on how these viruses can overcome the species barrier and successfully produce illness which ranges from common flu-like condition to severe respiratory distress syndrome as in the case of SARS and MERS. The former condition was possibly originated from bats virus and then enters into the mammalian hosts while the latter originates from dromedary camels.

Despite the origin of coronaviruses, these viruses have the potential to grow and transmit at a very high rate which causes a worldwide pandemic and seems to be a serious health issue. On 28 February 2020, the WHO declared the COVID-19 epidemic to a very high-level emergency. As the disease spread outside China to around 114 countries and the number of cases crosses one lakh, WHO declared the COVID-19 a pandemic.

The governments of all the countries try to build a plan of work to stem possible devastating effects. For this various international health, organizations flow out various information and guidelines used to lessen the impact of this virus. At the same time, further investigations are done to explore the mechanism of transmission, the clinical course of the disease, advanced diagnosis guidelines, prevention, and treatment strategies. Currently, no targeted drug is available to treat COVID-19. So at the moment, only supportive treatment is available and preventive measures are aimed to reduce community transmission. For instance, in China, aggressive isolation measures led to a great reduction in COVID-19 cases (Ren *et al.,* 2020).

Chronology of COVID-19

The chronology of COVID-19 infection is mentioned as follows. In December 2019, the first case of COVID-19 was reported. From 18 December 2019 till 29 December 2019 five cases were reported with ARDS and the number increases to 41 till 2 January 2020. Later on, it was diagnosed that less than half of the patients

have a history of diabetes, hypertension, and ischemic heart disease. Thus it was presumed that patients got infected mainly due to hospital-acquired infection. Further, till 22 January 2020, the number of cases increases to 571 in various cities of China, with a total death of 17 patients. But suddenly within the next three days, the total cases were jumped to 1975 with the death of around 56 infected patients. Whereas some reports suggest that the number of cases increases to 5502 till 24 January 2020 in China.

Furthermore, till 30 January 2020, 7734 cases were confirmed in China and a total of 90 cases reported from different countries such as Germany, France, Canada, Australia, India, United States, Japan, Singapore, Sri Lanka, Nepal, Malaysia, Thailand, Taiwan, and United Arab Emirates. The toll death rate is found to be 2.2% (170/7824). On 19 January 2020, the first case was reported in Washington, United States, who presents with a four-day history of cough and fever and recently returned from Wuhan, China (Du, 2020). On 30 January 2020, the first case of human-human transmission was reported in the US. Moreover, a report was issued on 7 February 2020, which declares a total of 31,161 cases with the death of more than 630 people. World health organization globally released a report on 16, February 2020 which confirmed 51,587 cases in around 25 countries.

Till the end of February 2020, the infected cases reach 88,000 worldwide (Holshue *et al.,* 2020). Further, the cases of COVID-19 increase exponentially which creates serious health issues, as mentioned in Fig. **5**(**A** & **B**).

In India, the first case was reported on 30 January 2020. While WHO released the first report on 14 Mar 2020, which said that a total of 84 COVID-19 patients are present in different territories of India, out of which two die. The first death was reported in Karnataka of a 76-year-old man and the second death occurs in Delhi of a 68-year-old female. By the end of May 2020, the total cases reported in India were around 191,000, with total death of 5,300 patients.

Symptoms

The signs of viral invasion come after an incubation period of around 4-6 days. Generally, the symptoms appear after 6 days of entry of the virus in the body and range to a maximum of 41 days. The period depends upon the patient's age and the immune system of the host. The commonly recognized symptoms (Fig. **6**) are excessive sputum production, hyperthermia, fatigue, cough, headache, breathlessness, lymphopenia, and hemoptysis. Computed tomography of the chest reveals unusual features such as pneumonia, RNAaemia, ARDS, cardiac injury, and multiple peripheral ground-glass opacities in the subpleural region, which is the main cause of death.

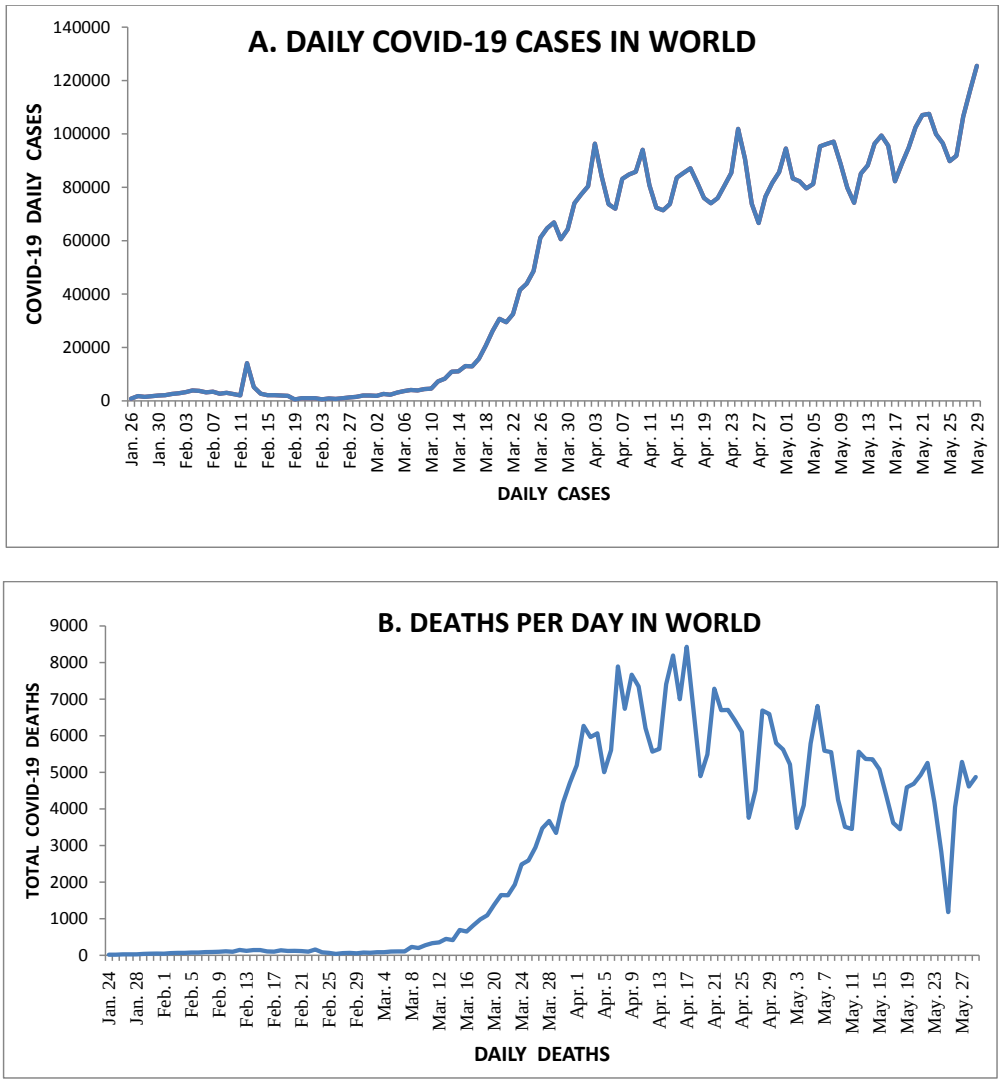


Fig. (5). Chronology of COVID-19 **(A)** Daily COVID-19 cases & **(B)** Deaths due to COVID-19.

The clinical features of COVID-19 and other human coronavirus are somewhat similar such as fever, cough, breathlessness, and ground-glass opacity in the subpleural region. However, certain unique clinical features of COVID-19 infection include rhinorrhoea, sneezing, and sore throat. The chest radiography reveals the infiltration in the upper lobe of the lungs that leads to profound dyspnoea and hypoxemia. Also, the chances of developing gastrointestinal manifestations such as diarrhea are more in COVID-19 as compared to MERS-

CoV and SARS-CoV. From this, a new diagnostic tool is recognized where fecal and urine samples to exclude a potential alternative route of transmission (Rothana *et al.,* 2020).

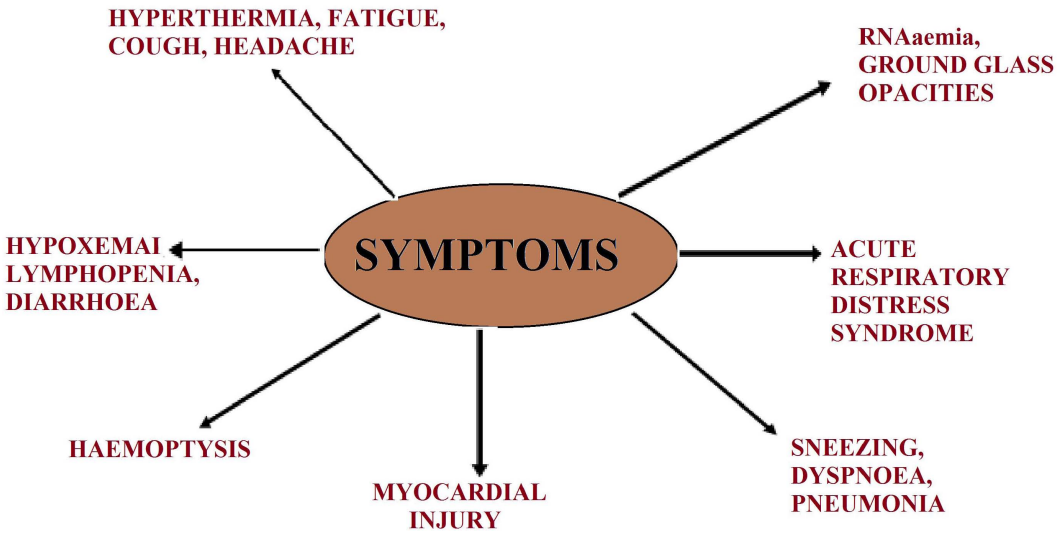


Fig. (6). Clinical features of COVID-19.

Transmission

As we know, around all the infected people were exposed to the animal market in Wuhan, it might be concluded that COVID-19 originates through the zoonotic source. Further investigation will be done to search out the reservoir host or intermediate carrier from which the infection may transfer to humans. The genomic analysis of COVID-19 showed approximately 90% similarity with SARS-like coronaviruses, which were derived from bats. This report indicates that only mammals are the connecting link between humans and COVID-19.

But human to human transmission is the fastest mode of transmission of infection. The transmission occurs either by direct contact or air droplets spread due to coughing or sneezing from a COVID-19 patient, as depicted in Fig. (**7**). Fortunately, it was confirmed that if pregnant women infected with COVID-19, no placental transmission occurs from infected mother to child (Rothana *et al.,* 2020).

Treatment

Currently, no antiviral drug is available for COVID-19, and no vaccine is introduced till now. The treatment recommended includes supportive therapy,

symptomatic therapy, and oxygen therapy. In case of respiratory failure, mechanical ventilation is required, whereas for managing septic shock, hemodynamic support is mandatory. The evidence-based precise guidelines were released by WHO on 28 January 2020 for sorting the patients based on their respiratory conditions; method of prevention and control; supportive therapy; diagnosis; management of respiratory distress and septic shock; prevention of complications; and treatment (Lu, 2020).

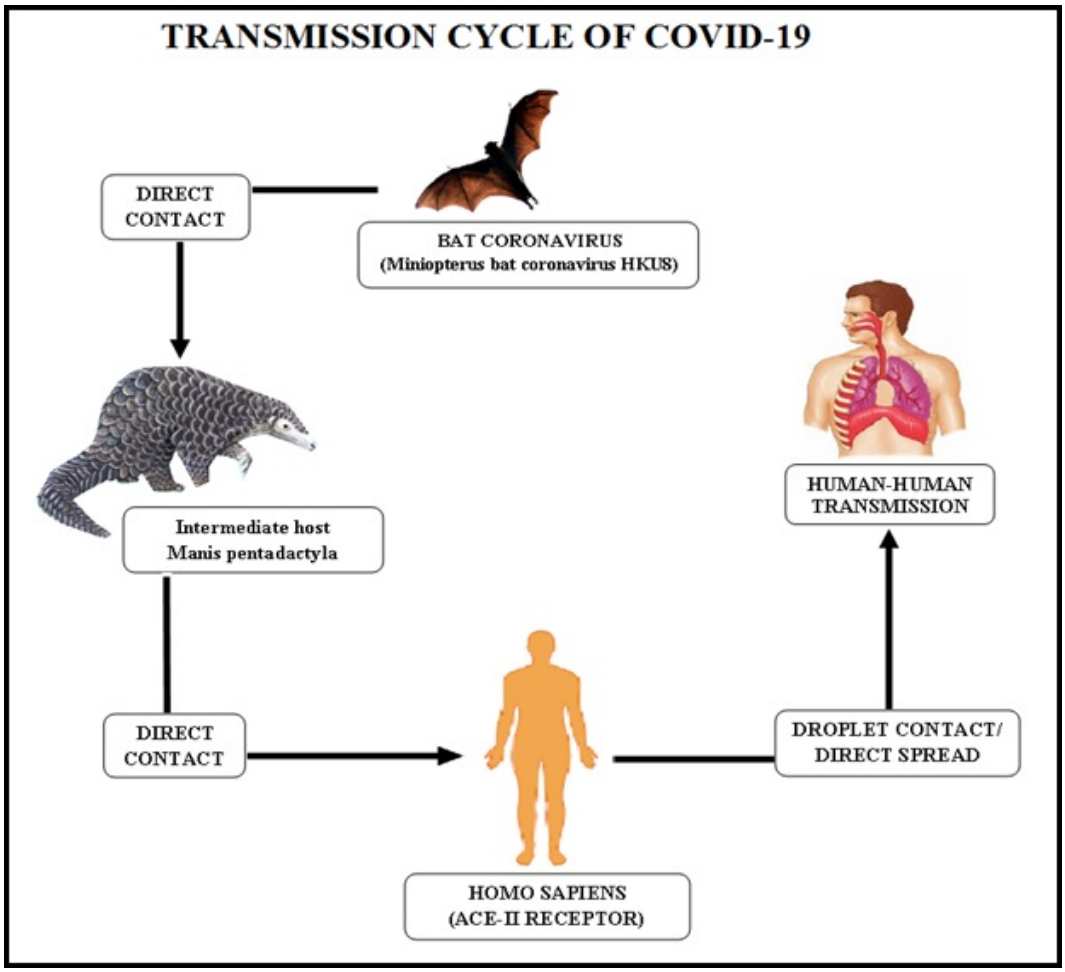


Fig. (7). Transmission Cycle of COVID-19.

LIST OF ABBREVIATIONS

2019-nCoV	Coronavirus
ACE-II	Angiotensin-Converting Enzyme-II

ACE2R	Angiotensin Converting Enzyme-II Receptor
ADP	Adenine diphosphate
ARDS	Acute respiratory Distress Syndrome
Bcl	B-Cell Lymphoma
CCL	Chemokine Ligand
CD	Cluster of Differentiation
CDC	Chinese Center for Disease Control and Prevention
CNS	Central nervous system
CoV	Coronavirus
COVID-19	Coronavirus disease 2019
FeCoV	Feline Enteric Coronavirus
FIPV	Feline infectious peritonitis Virus
HCoV-229E	Human Coronavirus 229E
HCoV-KHU1	Human Coronavirus KHU1
HCoV-NL63	Human Coronavirus NL63
HCoV-OC43	Human Coronavirus OC43
IBV	Infectious bronchitis virus
IgG	Immunoglobulin gamma
IgM	Immunoglobulin mu
IFN	Interferon
IL	Interleukins
kDa	Kilo Dalton
MCP	Monocyte chemoattractant protein
MERS	Middle East Respiratory Virus
MHV	Murine Hepatitis Virus
MIP	Macrophage inflammatory protein
ORF	Open Reading Frame
PEDV	Porcine Epidemic Disease Virus
pp	Polyproteins
PRCoV	Porcine respiratory virus
RANTES	Regulated upon Activation, Normal T cell Expressed, and Secreted
RBD	Receptor Binding Domain
RNA	Ribonucleic acid
SARS-CoV	Subacute Respiratory Syndrome Coronavirus
TGEV	Transmissible Gastroenteritis Virus

TNF	Tumor Necrosis Factor
WHO	World Health Organization

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 3

COVID-19: Epidemiology

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Abstract: In the history, the year 2019 will be remembered as the year that has witnessed the beginning of a pandemic, primarily affecting the respiratory tract and then, spreading from human to human. A total of 25.18 million reported cases and 0.84 million deaths, as of 30th August 2020, and still counting, were caused by a novel coronavirus named COVID-19 that originated in Wuhan, China. By the beginning of the year 2020, this virus spread to several countries like Singapore, South Korea, Japan, Italy, Spain, Germany, the United Kingdom, and the United States of America. Between January 2020 and March 2020, the disease took a paradigm shift and started to affect the majority of European countries like Italy, Spain, France, Germany and UK. In the majority of the patients with a competent immune system, this disease goes unnoticed or without symptoms, thus making them highly susceptible to spread this disease to whoever comes in their contact. Aged patients (>60 years) or patients with chronic health issues like heart diseases, cancer, diabetes, and weak immunity are at greater risk of developing the symptoms. In severe conditions, patients need hospitalization and respiratory support (respirators/ventilators), thus causing an overload on the health system of the world. This initiated the movement of "flattening the curve" by social distancing and isolation to decrease the burden on the health system and to decrease the spread of the disease.

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Keywords: China, Confirmed Cases, *Cordon sanitaire*, Coronavirus, COVID-19, Curfews, Epidemiology, Geographical distribution, Global Epidemiology, Hotspots, Lockdown, National Responses, Non-essential, Outbreak, Pandemic, Person-to-person Transmission, Quarantines, SARS-CoV-2, WHO Region, Wuhan.

INTRODUCTION

The year 2019 has witnessed the beginning of a pandemic majorly affecting the respiratory tract; however, effects on the other body organs have also been reported (Singh *et al*., 2020a). The pandemic has affected 25,182,329 people across the globe, with a total death toll of 846,936 registered up to 30th August 2020. The total recovered cases from the COVID-19 pandemic are 17,515,059 and the active cases till 30th August 2020 are 6,820,334. Half of the total affected population of the world resides in the USA, Brazil, and India. This disease spreads from human to human *via* air-borne droplets. This pandemic is not the first pandemic world has ever seen; however, it is one of the biggest of our times and the most vulnerable after World War II. The severity of this can be estimated from the fact that it is as destructive as the influenza pandemic in 1918, which emerged after World War I and caused deaths of tens of millions of people due to lack of antibiotics. Similarly, Acquired Immune Deficiency Syndrome caused by a retrovirus was equally lethal before it was controlled by using antiretroviral drugs (DiMaio *et al.*, 2020).

Broadly, pandemics are the epidemics that spread worldwide and thus are not confined to any particular geographical region. This current world epidemic originated in Wuhan, China is caused by a novel coronavirus and has been named coronavirus disease 2019 or COVID-19. Due to its similarity with SARS (Severe Acute Respiratory Syndrome), ICTV (International Committee on Taxonomy of Viruses) coined it as SARS-CoV-2 virus. COVID-19 was officially proclaimed as PHEIC Public Health Emergency of International Concern by WHO on 30th January 2020 with various countries starting to impose travel restrictions, issuing traveling warnings, and also exercising travel bans (Malviya *et al.,* 2020; Tang *et al.*, 2020). However, the lag between identification of the first case of COVID-19 infection to realizing its person to person transmission and to declare it as a world pandemic led it to spread to various countries and as of now at least 208 countries are infected with this infection (Hamid *et al.*, 2020).

Fortunately, due to previous exposure and understanding of coronavirus infections, such as SARS (Severe Acute Respiratory Syndrome) and Middle Eastern Respiratory Syndrome (MERS), this causative agent of SARS-CoV2 was quickly identified and its genome was rapidly sequenced to help researchers around the globe to develop potential drugs and/or vaccines. Fig. (**1**) shows various events and developments happened so far related to SARS-CoV-2.

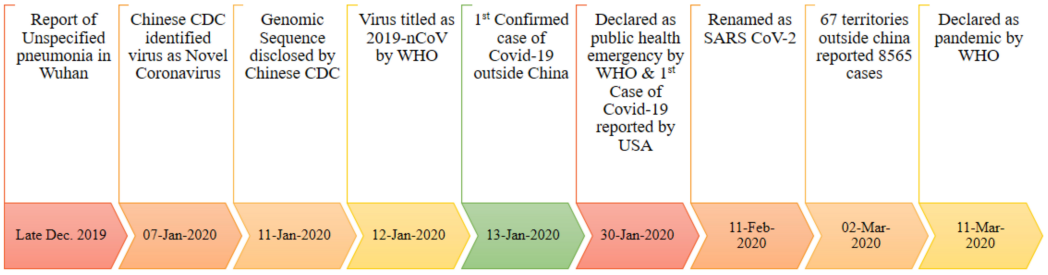


Fig. (1). Timeline of various developments in SARS-CoV-2 (Gennaro *et al.*, 2020; Park, 2020; Sun *et al.*, 2020; Srivastava *et al.*, 2020; Hamid *et al.*, 2020; Tang *et al.*, 2020).

In this chapter, we shall discuss the epidemiology of the COVID-19 pandemic. Basically, epidemiology is the study of various determinant factors of health and disease-related conditions or events in a sample population or globally, and the purpose of this study is to control the various factors related to disease and to control the spread of the disease. Therefore, from studying the epidemiology of COVID-19, one can conclude the frequency and pattern of this disease. In this chapter, the data has been collected and reported up to 12th June 2020. We shall discuss various determinants and factors like geographical distribution of the disease, patterns in the global spread of the pandemic, *etc.* Further, we shall discuss the comparison of the number of cases of COVID-19 in different WHO regions along with the effect of lockdown on the spread of the pandemic. Besides, the condition of the COVID-19 pandemic in India along with some published reports by clinicians/researchers will be discussed in the latter part of the chapter.

GEOGRAPHICAL DISTRIBUTION

On 13th January 2020, the first confirmed case of COVID-19 outside China was reported in Thailand. It made the situation clear that this virus is no longer confined to China or the provenances nearby Wuhan. Soon after, reports of confirmed cases started to emerge from different countries in Asia. In January 2020, this virus spread to many Asian countries like Thailand, South Korea, and Japan and also cases of patients affected by COVID-19 started to appear in the western part of the world, including Italy, Spain, Germany, the UK, and the USA. In early February, WHO realized that the occurrence of COVID-19 is very high and ranges between 2.24 to 3.38. From the end of January 2020 to March 2020, it took a paradigm shift and disease started to affect the majority of European

countries like Italy, Spain, France and, Germany, and to UK and USA, surpassing China in the number of affected patients. As of 12th June 2020, the five countries with the most number of cases worldwide were: US, Brazil, Russia, India, and the UK. The majority of the patients with competent immune systems act as carriers of the COVID-19 as it goes un-noticed/without any symptoms in these patients thus making them highly susceptible to spread this disease far and near. Elderly patients (>60 years) or patients with underlying chronic health problems, like heart diseases, cancer, diabetes, and weak immune system, *etc*., are at higher risk of developing severe symptoms of this novel coronavirus. Development of silent hypoxia in severely affected patients demanded hospitalization with respiratory support (respirators/ventilators), thus causing an overload on the health system. This initiated the movement of "flattening the curve" and effective parameters like social distancing and isolation were enforced to decrease the burden on the health care system and to decrease the person-to-person spread of the diseases. For more details, refer to the 'national responses of COVID-19' part mentioned later in this chapter. Fig. (**2**) enlists series of events during the initial spread and geographical distribution of the COVID-19 pandemic.

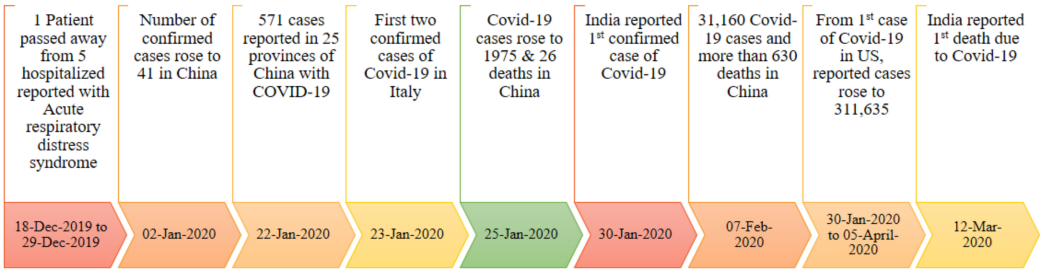


Fig. (2). Epidemiological events and geographical distribution of novel coronavirus (Giovanetti *et al.*, 2020; Hamid *et al.*, 2020).

GLOBAL EPIDEMIOLOGY OF COVID-19

Since the 1st confirmed case of Coronavirus (COVID-19) in Wuhan, China, the virus has widely and rapidly spread in various countries across the globe despite the efforts done by various counties to stop the spread (Guan *et al.*, 2020; Lai *et al.*, 2020). According to a WHO report, COVID-19 has affected 7,724,615 people in more than 210 countries and caused 427,675 mortalities till 12th of June 2020. The incidences of COVID-19 and number of affected cases globally explained are explained in Fig. (**3**).

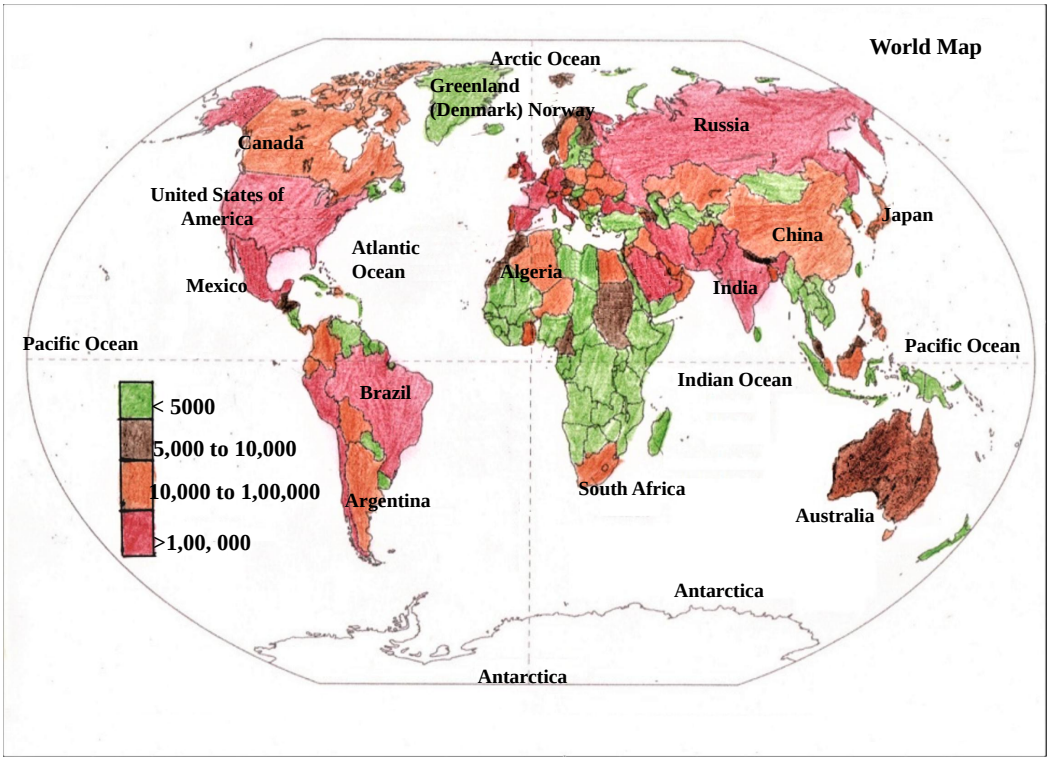


Fig. (3). World map showing COVID-19 cases as of 12th June 2020.

According to the data given in Table **1**, the countries with most cases of COVID-19 up to 12th June 2020 are:

1. USA, with total number of 2,116,211 patients and 116,814 total deaths along with 839,781 recovered cases and 1,159,616 active cases.
2. Brazil, with total number of 829,902 patients and 41,901 total deaths along with 396,692 recovered cases and 391,309 active cases.
3. Russia, with total number of 511,423 patients and 6,715 total deaths along with 269,370 recovered cases and 235,338 active cases.
4. India, with total number of 309,603 patients and 8,890 total deaths along with 154,231 recovered cases and 146,482 active cases.
5. Italy, with total number of 236,305 patients and with 34,223 total deaths along with 173,085 recovered cases and 28,997 active cases.
6. Peru, with total number of 220,749 patients and with 6,308 total deaths along with 107,133 recovered cases and 107,308 active cases.
7. Germany, with total number of 187,251 patients and with 8,863 total deaths

along with 171,600 recovered cases and 6,788 active cases.

8. Mainland China, with total number of 83,064 patients and with 4,634 total deaths along with 78,365 recovered cases and 65 active cases.

Table **1** enlists the global data of COVID-19 until 12th June 2020 indicating total number of COVID-19 cases worldwide & country-wise along with the total number of mortalities, recoveries and active cases. However, according to WHO these statistics will change in coming months as the spread of COVID-19 is expected to increase. The resurgence of corona cases in China has also supported this claim.

Table 1. Global Data of COVID-19 until 12th of June 2020 (Worldometer 2020).

S. No.	Country/ Other	Total Cases	Total Deaths	Total id="c3t Recovered	Active Cases	Population	1 Case Every X ppl
-	**World**	7,724,615	427,675	3,916,489	3,380,451	-	-
1	**USA**	2,116,211	116,814	839,781	1,159,616	330,901,704	156
2	**Brazil**	829,902	41,901	396,692	391,309	212,479,860	256
3	**Russia**	511,423	6,715	269,370	235,338	145,931,382	285
4	**India**	309,603	8,890	154,231	146,482	1,379,270,740	4,455
5	**UK**	292,950	41,481	N/A	N/A	67,867,577	232
6	**Spain**	290,289	27,136	N/A	N/A	46,753,887	161
7	**Italy**	236,305	34,223	173,085	28,997	60,466,117	256
8	**Peru**	220,749	6,308	107,133	107,308	32,946,025	149
9	**Germany**	187,251	8,863	171,600	6,788	83,770,376	447
10	**Iran**	182,525	8,659	144,649	29,217	83,933,136	460
11	**Turkey**	175,218	4,778	149,102	21,338	84,289,552	481
12	**Chile**	160,846	2,870	131,358	26,618	19,107,435	119
13	**France**	156,287	29,374	72,572	54,341	65,266,270	418
14	**Mexico**	133,974	15,944	98,064	19,966	128,859,025	962
15	**Pakistan**	125,933	2,463	40,247	83,223	220,638,507	1,752
16	**Saudi Arabia**	119,942	893	81,029	38,020	34,782,906	290
17	**Canada**	97,943	8,049	58,523	31,371	37,724,444	385
18	**China**	83,064	4,634	78,365	65	1,439,323,776	17,328
19	**Bangladesh**	81,523	1,095	17,249	63,179	164,600,549	2,019
20	**Qatar**	76,588	70	53,296	23,222	2,807,805	37
21	**South Africa**	61,927	1,354	35,006	25,567	59,267,163	957

(Table 1) cont.....

S. No.	Country/ Other	Total Cases	Total Deaths	Total id="c3t Recovered	Active Cases	Population	1 Case Every X ppl
22	**Belgium**	59,819	9,646	16,498	33,675	11,587,039	194
23	**Belarus**	52,520	298	27,760	24,462	9,449,475	180
24	**Sweden**	49,684	4,854	N/A	N/A	10,095,978	203
25	**Netherlands**	48,461	6,053	N/A	N/A	17,132,971	354
26	**Colombia**	46,858	1,545	18,715	26,598	50,853,330	1,085
27	**Ecuador**	45,778	3,828	22,679	19,271	17,627,809	385
28	**UAE**	41,499	287	25,946	15,266	9,883,799	238
29	**Egypt**	41,303	1,422	11,108	28,773	102,220,760	2,475
30	**Singapore**	39,850	25	28,040	11,785	5,847,901	147
31	**Indonesia**	36,406	2,048	13,213	21,145	273,366,090	7,509
32	**Portugal**	36,180	1,505	22,200	12,475	10,198,122	282
33	**Kuwait**	34,952	285	25,048	9,619	4,266,990	122
34	**Switzerland**	31,063	1,938	28,800	325	8,651,283	279
35	**Ukraine**	29,753	870	13,567	15,316	43,745,847	1,470
36	**Argentina**	28,764	785	8,743	19,236	45,173,491	1,570
37	**Poland**	28,577	1,222	13,805	13,550	37,848,619	1,324
38	**Ireland**	25,250	1,705	22,698	847	4,934,765	195
39	**Philippines**	24,787	1,052	5,454	18,281	109,499,539	4,418
40	**Afghanistan**	23,546	446	3,928	19,172	38,874,978	1,651
41	**Dominican Republic**	22,008	568	12,754	8,686	10,842,017	493
42	**Romania**	21,404	1,380	15,445	4,579	19,243,552	899
43	**Oman**	21,071	96	7,489	13,486	5,098,511	242
44	**Israel**	18,795	300	15,288	3,207	9,197,590	489
45	**Panama**	18,586	418	10,977	7,191	4,310,882	232
46	**Iraq**	17,770	496	6,868	10,406	40,167,590	2,260
47	**Japan**	17,332	922	15,493	917	126,494,838	7,298
48	**Bahrain**	17,269	36	11,903	5,330	1,697,568	98
49	**Austria**	17,064	675	15,985	404	9,003,730	528
50	**Bolivia**	16,165	533	2,372	13,260	11,664,091	722
51	**Armenia**	15,281	258	5,639	9,384	2,962,966	194
52	**Nigeria**	15,181	399	4,891	9,891	205,822,203	13,558
53	**Kazakhstan**	13,872	70	8,829	4,973	18,764,311	1,353
54	**Serbia**	12,175	252	11,348	575	8,739,024	718

(Table 1) cont.....

S. No.	Country/ Other	Total Cases	Total Deaths	Total id="c3t Recovered	Active Cases	Population	1 Case Every X ppl
55	**Denmark**	12,099	594	10,993	512	5,791,166	479
56	**S. Korea**	12,003	277	10,669	1,057	51,267,003	4,271
57	**Moldova**	11,093	385	6,229	4,479	4,034,411	364
58	**Ghana**	10,856	48	3,921	6,887	31,034,041	2,859
59	**Algeria**	10,698	751	7,322	2,625	43,804,765	4,095
60	**Czechia**	9,938	329	7,215	2,394	10,707,989	1,077
61	**Azerbaijan**	9,218	113	5,116	3,989	10,134,274	1,099
62	**Cameroon**	8,681	212	4,836	3,633	26,504,775	3,053
63	**Norway**	8,620	242	8,138	240	5,418,993	629
64	**Morocco**	8,610	212	7,618	780	36,886,438	4,284
65	**Guatemala**	8,561	334	1,567	6,660	17,896,120	2,090
66	**Malaysia**	8,402	119	7,168	1,115	32,342,925	3,849
67	**Honduras**	7,669	294	837	6,538	9,895,582	1,290
68	**Australia**	7,290	102	6,783	405	25,483,610	3,496
69	**Finland**	7,073	325	6,200	548	5,540,291	783
70	**Sudan**	6,879	433	2,416	4,030	43,786,472	6,365
71	**Nepal**	5,062	16	877	4,169	29,106,200	5,750
72	**Tajikistan**	4,902	49	3,158	1,695	9,524,612	1,943
73	**Uzbekistan**	4,869	19	3,758	1,092	33,441,780	6,868
74	**Senegal**	4,851	56	3,100	1,695	16,716,133	3,446
75	**Ivory Coast**	4,684	45	2,263	2,376	26,337,705	5,623
76	**DRC**	4,637	101	580	3,956	89,383,696	19,276
77	**Djibouti**	4,441	38	2,730	1,673	987,187	222
78	**Guinea**	4,426	24	3,106	1,296	13,110,203	2,962
79	**Luxembourg**	4,055	110	3,918	27	625,393	154
80	**Hungary**	4,053	555	2,447	1,051	9,661,522	2,384
81	**Haiti**	3,941	64	24	3,853	11,394,839	2,891
82	**North Macedonia**	3,701	171	1,694	1,836	2,083,378	563
83	**El Salvador**	3,481	68	1,587	1,826	6,484,517	1,863
84	**Gabon**	3,463	23	1,024	2,416	2,222,506	642
85	**Kenya**	3,305	96	1,164	2,045	53,699,517	16,248
86	**Bulgaria**	3,191	172	1,716	1,303	6,950,812	2,178
87	**Thailand**	3,129	58	2,987	84	69,791,171	22,305

(Table 1) cont.....

S. No.	Country/ Other	Total Cases	Total Deaths	Total id="c3t Recovered	Active Cases	Population	1 Case Every X ppl
88	**Greece**	3,108	183	1,374	1,551	10,425,424	3,354
89	**Ethiopia**	2,915	47	451	2,417	114,786,713	39,378
90	**Bosnia and Herzegovina**	2,893	163	2,119	611	3,281,756	1,134
91	**Venezuela**	2,814	23	487	2,304	28,439,773	10,107
92	**Somalia**	2,513	85	532	1,896	15,864,908	6,313
93	**Mayotte**	2,268	28	1,790	450	272,408	120
94	**Croatia**	2,249	107	2,133	9	4,106,429	1,826
95	**Cuba**	2,233	84	1,902	247	11,326,953	5,073
96	**Kyrgyzstan**	2,166	26	1,668	472	6,517,994	3,009
97	**CAR**	2,044	7	360	1,677	4,824,890	2,361
98	**Maldives**	2,003	8	1,193	802	539,990	270
99	**Estonia**	1,970	69	1,703	198	1,326,491	673
100	**Sri Lanka**	1,880	11	1,196	673	21,408,657	11,388
101	**Iceland**	1,807	10	1,794	3	341,127	189
102	**Lithuania**	1,756	74	1,400	282	2,723,889	1,551
103	**Mali**	1,752	101	1,023	628	20,213,291	11,537
104	**South Sudan**	1,670	24	48	1,598	11,186,498	6,699
105	**Costa Rica**	1,612	12	731	869	5,091,620	3,159
106	**Mauritania**	1,572	81	278	1,213	4,641,964	2,953
107	**Slovakia**	1,542	28	1,409	105	5,459,512	3,541
108	**New Zealand**	1,504	22	1,482	0	5,002,100	3,326
109	**Slovenia**	1,490	109	1,359	22	2,078,924	1,395
110	**Nicaragua**	1,464	55	953	456	6,620,207	4,522
111	**Guinea-Bissau**	1,460	15	153	1,292	1,965,141	1,346
112	**Lebanon**	1,422	31	853	538	6,826,874	4,801
113	**Albania**	1,416	36	1,034	346	2,877,949	2,032
114	**Zambia**	1,321	10	1,104	207	18,351,048	13,892
115	**Equatorial Guinea**	1,306	12	200	1,094	1,399,912	1,072
116	**Paraguay**	1,254	11	633	610	7,127,687	5,684
117	**Madagascar**	1,240	10	344	886	27,646,431	22,296
118	**Hong Kong**	1,109	4	1,060	45	7,493,747	6,757
119	**Sierra Leone**	1,103	51	648	404	7,967,302	7,223

(Table 1) cont.....

S. No.	Country/ Other	Total Cases	Total Deaths	Total id="c3t Recovered	Active Cases	Population	1 Case Every X ppl
120	**Latvia**	1,096	27	818	251	1,887,105	1,722
121	**Tunisia**	1,093	49	995	49	11,811,891	10,807
122	**French Guiana**	1,043	2	489	552	298,196	286
123	**Cyprus**	980	18	807	155	1,206,895	1,232
124	**Niger**	978	65	881	32	24,146,544	24,690
125	**Jordan**	915	9	671	235	10,197,652	11,145
126	**Burkina Faso**	892	53	791	48	20,866,826	23,393
127	**Andorra**	853	51	781	21	77,259	91
128	**Chad**	848	72	711	65	16,395,556	19,334
129	**Uruguay**	847	23	780	44	3,473,119	4,100
130	**Georgia**	843	13	697	133	3,989,535	4,733
131	**Congo**	728	24	221	483	5,509,663	7,568
132	***Diamond Princess***	712	13	651	48	-	-
133	**Cabo Verde**	697	6	294	397	555,657	797
134	**San Marino**	694	42	520	132	33,927	49
135	**Uganda**	686	-	161	525	45,645,761	66,539
136	**Malta**	645	9	600	36	441,484	684
137	**Sao Tome and Principe**	639	12	156	471	218,920	343
138	**Yemen**	632	139	28	465	29,786,156	47,130
139	**Jamaica**	611	10	408	193	2,960,505	4,845
140	**Channel Islands**	565	48	512	5	173,777	308
141	**Togo**	525	13	279	233	8,266,815	15,746
142	**Rwanda**	510	2	321	187	12,932,272	25,357
143	**Tanzania**	509	21	183	305	59,625,015	117,141
144	**Mozambique**	509	2	145	362	31,199,461	61,296
145	**Palestine**	489	3	414	72	5,094,148	10,417
146	**Réunion**	488	1	460	27	894,975	1,834
147	**Malawi**	481	4	65	412	19,098,955	39,707
148	**Eswatini**	472	3	246	223	1,159,511	2,457
149	**Taiwan**	443	7	431	5	23,814,623	53,758
150	**Liberia**	421	32	210	179	5,050,381	11,996
151	**Libya**	409	6	59	344	6,866,054	16,787

(Table 1) cont.....

S. No.	Country/ Other	Total Cases	Total Deaths	Total id="c3t Recovered	Active Cases	Population	1 Case Every X ppl
152	**Benin**	388	5	217	166	12,103,226	31,194
153	**Zimbabwe**	343	4	51	288	14,850,685	43,296
154	**Mauritius**	337	10	325	2	1,271,663	3,773
155	**Isle of Man**	336	24	312	0	85,010	253
156	**Vietnam**	333	-	323	10	97,291,603	292,167
157	**Montenegro**	324	9	315	0	628,062	1,938
158	**Myanmar**	261	6	165	90	54,390,671	208,393
159	**Martinique**	202	14	98	90	375,279	1,858
160	**Mongolia**	197	-	95	102	3,275,260	16,626
161	**Suriname**	187	3	9	175	586,350	3,136
162	**Cayman Islands**	187	1	115	71	65,679	351
163	**Faeroe Islands**	187	-	187	0	48,854	261
164	**Gibraltar**	176	-	172	4	33,691	191
165	**Guadeloupe**	171	14	157	0	400,121	2,340
166	**Syria**	164	6	68	90	17,474,364	106,551
167	**Comoros**	163	2	97	64	868,486	5,328
168	**Guyana**	159	12	95	52	786,357	4,946
169	**Bermuda**	141	9	127	5	62,289	442
170	**Brunei**	141	2	138	1	437,253	3,101
171	**Angola**	130	5	42	83	32,799,138	252,301
172	**Cambodia**	126	-	125	1	16,705,964	132,587
173	**Trinidad and Tobago**	117	8	109	0	1,399,258	11,959
174	**Bahamas**	103	11	68	24	393,041	3,816
175	**Aruba**	101	3	98	0	106,743	1,057
176	**Monaco**	99	4	93	2	39,227	396
177	**Barbados**	96	7	83	6	287,358	2,993
178	**Burundi**	85	1	45	39	11,867,795	139,621
179	**Liechtenstein**	82	1	55	26	38,122	465
180	**Sint Maarten**	77	15	61	1	42,849	556
181	**Bhutan**	62	-	19	43	771,143	12,438
182	**French Polynesia**	60	-	60	0	280,824	4,680
183	**Botswana**	48	1	24	23	2,348,797	48,933

(Table 1) cont.....

S. No.	Country/ Other	Total Cases	Total Deaths	Total id="c3t Recovered	Active Cases	Population	1 Case Every X ppl
184	**Macao**	45	-	45	0	648,839	14,419
185	**Saint Martin**	42	3	36	3	38,628	920
186	**Eritrea**	41	-	39	2	3,543,663	86,431
187	**Namibia**	31	-	17	14	2,538,214	81,878
188	**Gambia**	28	1	22	5	2,412,325	86,154
189	**St. Vincent Grenadines**	27	-	25	2	110,922	4,108
190	**Antigua and Barbuda**	26	3	20	3	97,886	3,765
191	**Timor-Leste**	24	-	24	0	1,316,964	54,874
192	**Grenada**	23	-	22	1	112,496	4,891
193	**Curaçao**	22	1	15	6	164,059	7,457
194	**New Caledonia**	21	-	20	1	285,350	13,588
195	**Belize**	20	2	16	2	397,206	19,860
196	**Laos**	19	-	19	0	7,269,590	382,610
197	**Saint Lucia**	19	-	18	1	183,584	9,662
198	**Dominica**	18	-	16	2	71,977	3,999
199	**Fiji**	18	-	18	0	896,103	49,784
200	**Saint Kitts and Nevis**	15	-	15	0	53,179	3,545
201	**Falkland Islands**	13	-	13	0	3,473	267
202	**Greenland**	13	-	13	0	56,765	4,367
203	**Turks and Caicos**	12	1	11	0	38,688	3,224
204	**Vatican City**	12	-	12	0	801	67
205	**Montserrat**	11	1	10	0	4,992	454
206	**Seychelles**	11	-	11	0	98,315	8,938
207	***MS Zaandam***	9	2	-	7	-	-
208	**Western Sahara**	9	1	6	2	596,428	66,270
209	**British Virgin Islands**	8	1	7	0	30,220	3,778
210	**Papua New Guinea**	8	-	8	0	8,937,038	1,117,130
211	**Caribbean Netherlands**	7	-	7	0	26,210	3,744
212	**St. Barth**	6	-	6	0	9,875	1,646

(Table 1) cont.....

S. No.	Country/ Other	Total Cases	Total Deaths	Total id="c3t Recovered	Active Cases	Population	1 Case Every X ppl
213	**Lesotho**	4	-	2	2	2,141,348	535,337
214	**Anguilla**	3	-	3	0	14,996	4,999
215	**Saint Pierre Miquelon**	1	-	1	0	5,795	5,795
	Total:	**7,724,615**	**427,675**	**3,916,489**	**3,380,451**	-	-

X: Population Number.

As given in Table **1**, as of 12th June 2020 according to population density, Qatar has become the most affected country globally as it has one COVID-19 positive patient per every 37 people. San Marino occupied the second place with one COVID-19 positive patient per every 49 people followed by Bahrain having one COVID-19 positive patient per every 98 people.

Similarly, according to population density, Lesotho is the least affected country in the world as it has one COVID-19 positive patient per every 535,337 people followed by Vietnam, which has one COVID-19 positive patient per every 292,167 people. Myanmar stood third in this having one COVID-19 positive patient per every 208,393 people.

Effect of Age, Sex and other Factors on Covid-19-Related Deaths

Williamson *et al.* studied the various factors associated with COVID-19-death by creating an OpenSAFELY analytical platform in the field of health sciences. This study, which is related to the factors associated with COVID-19 mortality up to 6th May 2020, was carried on the behalf of NHS, England. The study included a total of 17,278,392 adults and reported that age and gender were entrenched risk factors for dreadful COVID-19 outcomes. The study revealed that about 90% of COVID-19-related deaths in the UK have been reported in the population over the age of 60 years with the majority of them being males (60%). The Chinese Center for Disease Control and Prevention revealed in an investigation including 44,672 individuals (1,023 deaths) that other diseases like hypertension, cardiovascular disease, respiratory disease, diabetes, and cancers were associated with an elevated risk of mortality; whereas, the establishment of a relationship with age was not possible. A UK cross-sectional survey including hospitalized 16,749 patients revealed that the death risk was greater for patients with pulmonary, cardiac, and renal disease, dementia, cancer, and obesity. In a recent communication, Sharma *et al.,* reported severity and risk factors of COVID-19 in cancer patients-based on various clinical evidence (Sharma *et al*., 2020). Clinical evidence indicated that the patients with ophthalmic manifestations are also at

high risk of COVID-19 infection (Singh *et al*., 2020b). According to this study, the death rate increased with age as shown in Fig. (**4**). Among different sexes, males were more prone to COVID-19 as compared to females. (Williamson *et al.,* 2020).

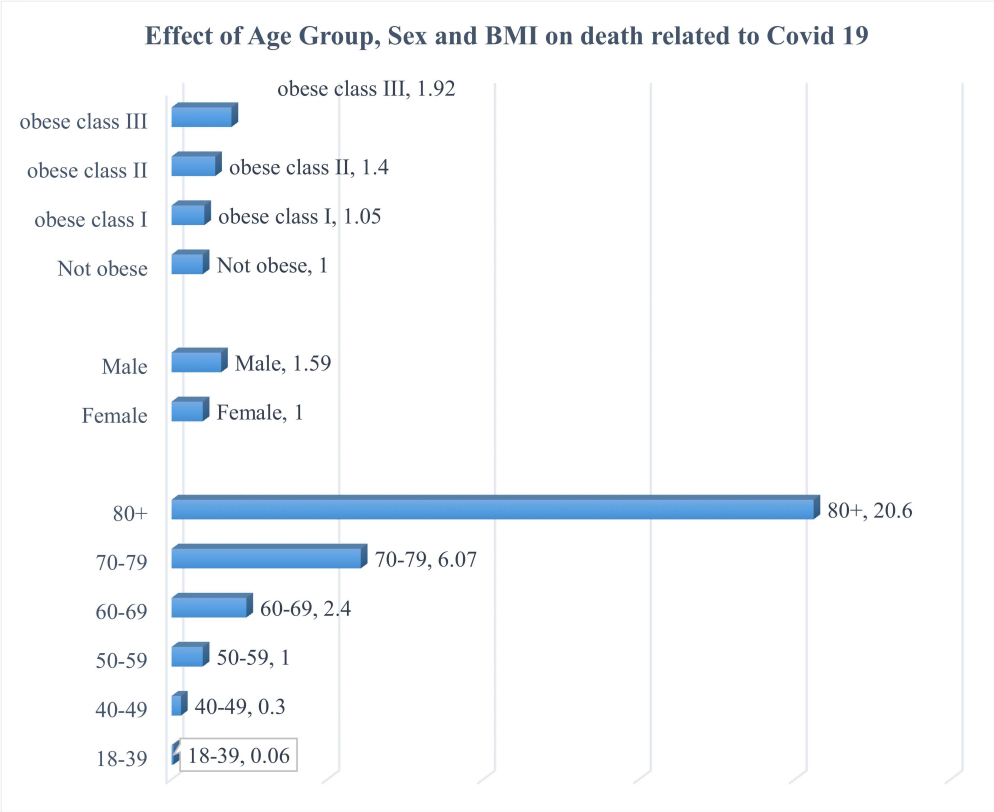


Fig. (4). Effect of Age, Sex and BMI on death related to COVID-19 (data represents hazard ratios in percentage for COVID-19).

COVID-19 CASE COMPARISONS IN DIFFERENT REGION OF WORLD (WORLD HEALTH ORGANIZATION, 2020)

WHO has divided the world into six regions, first being, Americas, which include various countries like Barbuda and Argentina, Barbados, Colombia, Grenada, Antigua, Costa Rica, Bahamas, Brazil, Jamaica, Bolivia, Canada, Chile, Cuba, Dominica, Dominican Republic, El Salvador, Saint Vincent, Guyana, Mexico, Nicaragua, United States, Paraguay, Ecuador, Guatemala, Haiti, The Grenadines, Honduras, Panama, Saint Kitts and Nevis, Saint Lucia, Belize, Suriname, Peru, Trinidad and Tobago, Uruguay, Venezuela, and Azerbaijan.

2nd region is European Region having Albania, Hungary, Armenia, Iceland, Austria, Belarus, Belgium, Bosnia, Herzegovina, Estonia, Bulgaria, Croatia, Andorra, Cyprus, Czech Republic, Denmark, Slovenia, Spain, Kyrgyzstan, San Marino, North Macedonia, Poland, Latvia, Finland, France, Germany, Georgia, Greece, Israel, Turkmenistan, Ukraine, United Kingdom, Slovakia, Netherlands, Ireland, Kazakhstan, Lithuania, Luxembourg, Italy, Malta Montenegro, Monaco, Norway, Portugal, Moldova, Romania, Russia, Serbia, Sweden, Switzerland, Tajikistan, Turkey, and Uzbekistan.

3rd region is Eastern Mediterranean Region having Afghanistan, Libya, Qatar, Jordan, Djibouti, Egypt, Somalia, Iran, Kuwait, United Arab Emirates, Saudi Arabia, Lebanon, Morocco, Sudan, Oman, Pakistan, Bahrain, Palestine, Syria, Tunisia, and Yemen.

4th region is South East Asia Region having North Korea, Maldives, Sri Lanka, Myanmar, Bhutan, Indonesia, Bangladesh, India, Nepal, Thailand, and Timor-Leste.

5th region is Western Pacific Region which includes South Korea, Australia, China, Laos, Cambodia, Marshall Islands, Nauru, Cook Islands, Fiji, Japan, Kiribati, Malaysia, Brunei, Micronesia, Mongolia, Niue, Papua, Taiwan, New Zealand, Palau, Vanuatu, New Guinea, Samoa Tonga, Solomon Islands, Singapore, Tuvalu, Philippines, and Vietnam.

6th region in the African Region which includes South Africa, Central African Republic, Ethiopia, Algeria, Botswana, Nigeria, Burundi, Madagascar, Burkina Faso, Cameroon, Equatorial Guinea, Cape Verde, Chad, Angola, Uganda, Comoros, Benin, Ivory Coast, Democratic Republic of Congo, Eritrea, Gabon, Gambia, Ghana, Guinea, Guinea- Bissau, Kenya, Lesotho, Liberia, Malawi, Mali, Mozambique, Mauritius, Niger, Namibia, Republic of Congo, Somalia, Rwanda, Mauritania, Tanzania, São Tomé and Príncipe, Senegal, Seychelles, Sierra Leone, Swaziland, Togo, Zambia, and Zimbabwe countries.

All the regions of the WHO have active COVID-19 cases. The most affected region is the Americas with 3,638,525 cases as of 12th June 2020. Whereas Europe stood 2nd with 2,378,958 cases and least affected is Africa with 161,254 cases as of 12th June 2020. In the Americas, the most affected countries are the USA, Brazil, and Canada. In Europe, the most affected countries are the United Kingdom, Germany, and Italy. Eastern Mediterranean is a third most affected region with 737,641 cases and Iran being the most affected country in this region. Southeast Asia is having 4,393,348 cases with India being the most affected country in the region. Western Pacific and Africa are also the affected regions as shown in Table **2** and Fig. (**5**).

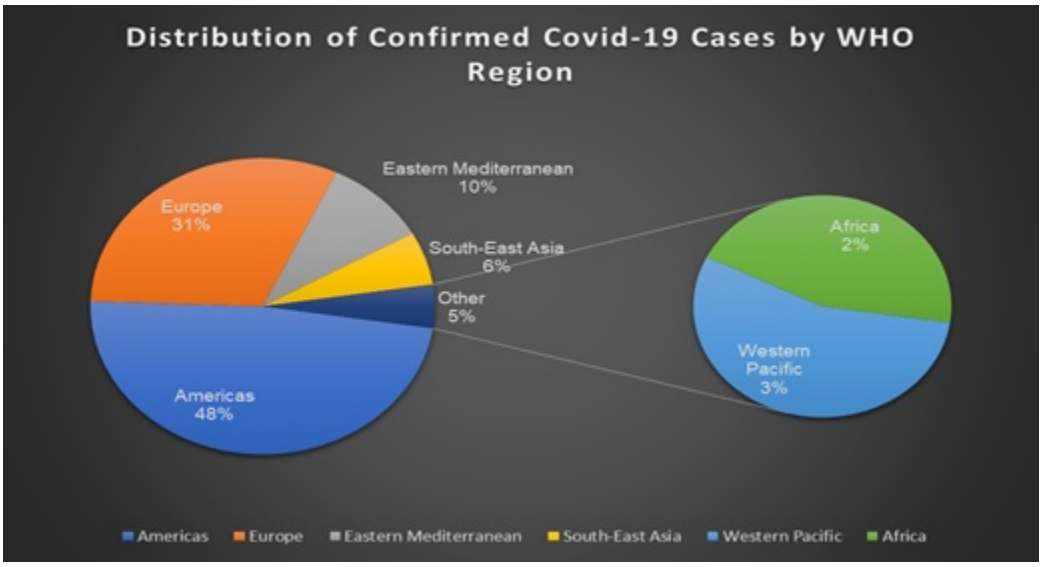


Fig. (5). Region-wise distribution of corona cases (as of 12 th June 2020).

Table 2. Total number of COVID-19 confirmed cases as per WHO regions.

WHO Region	No. of Confirmed Cases
Americas	3,638,525
Europe	2,378,958
Eastern Mediterranean	737,641
South-East Asia	439,348
Western Pacific	196,715
Africa	161,254

On comparing COVID-19 cases among the WHO regions, it is found that the Americas, South East Asia, Africa, and Eastern Mediterranean are still growing wrt number of patients, whereas, there is a steady decrease in corona cases in Europe and Western Pacific as shown in Table **3** and Fig. (**6**).

Table 3. Date wise comparison of COVID-19 cases in different WHO region.

Region	**31st Dec. 2019**	**10th Jan. 2020**	**30th Jan**	**9th Feb.**	**29th Feb.**	**10th Mar.**	**30th Mar.**	**9th April**	**29th April**	**9th May**	**29th May**	**8th June**
Americas	0	0	8	11	62	826	146,850	331,997	385,273	437,293	563,221	668,199
Europe	0	0	10	27	1,104	15,215	292,038	360,199	285,400	268,728	193,655	184,276

(Table 3) cont.....

Region	31st Dec. 2019	10th Jan. 2020	30th Jan	9th Feb.	29th Feb.	10th Mar.	30th Mar.	9th April	29th April	9th May	29th May	8th June
Eastern Mediterranean	0	0	4	3	500	7,069	24,983	39,355	52,344	69,353	125,770	166,580
South-East Asia	0	0	15	20	7	10	3,240	7,628	24,055	39,354	89,310	126,128
Western Pacific	27	32	7,547	29,604	8,194	6,628	10,475	11,314	15,327	12,064	9,321	12,746
Africa	0	0	0	0	2	7	2,833	4,547	9,156	16,611	28,363	42,216

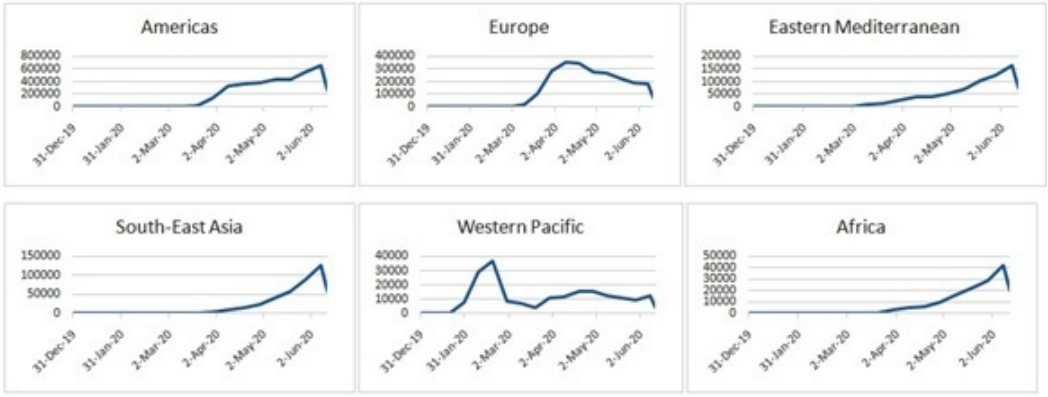


Fig. (6). Date-wise comparison of COVID-19 cases in different WHO regions.

The data shown in Table **3** and Fig. (**6**) indicates that some countries have witnessed a decline in the number of COVID-19 positive cases after strict lockdown restrictions (for more details regarding lockdown effects, refer to the next part of this chapter).

COVID-19 IN INDIA

India registered its first COVID-19 confirmed case at the end of January 2020. As of 12th June 2020, a total of 309,603 confirmed cases and 8,890 mortalities have been reported in India. The prevalence of confirmed COVID-19 cases with the number of cases in various states of India is shown in Fig. (**7**) (Worldometer, 2020). The figure represents states with more than 25,000 cases such as Maharashtra, Tamilnadu, and Gujrat. Various states had confirmed cases in the range of 10,000 to 25,000.

There were only three epidemiological surveys done in India till 12th June 2020:

- Twenty-one COVID-19 confirmed cases from the hospital of New Delhi.
- By Government of Maharashtra, with 12,296 cases data of the state.
- 41 sentinel sites of the country: data were taken and analyzed for Severe Acute Respiratory Infection (SARI) cases.

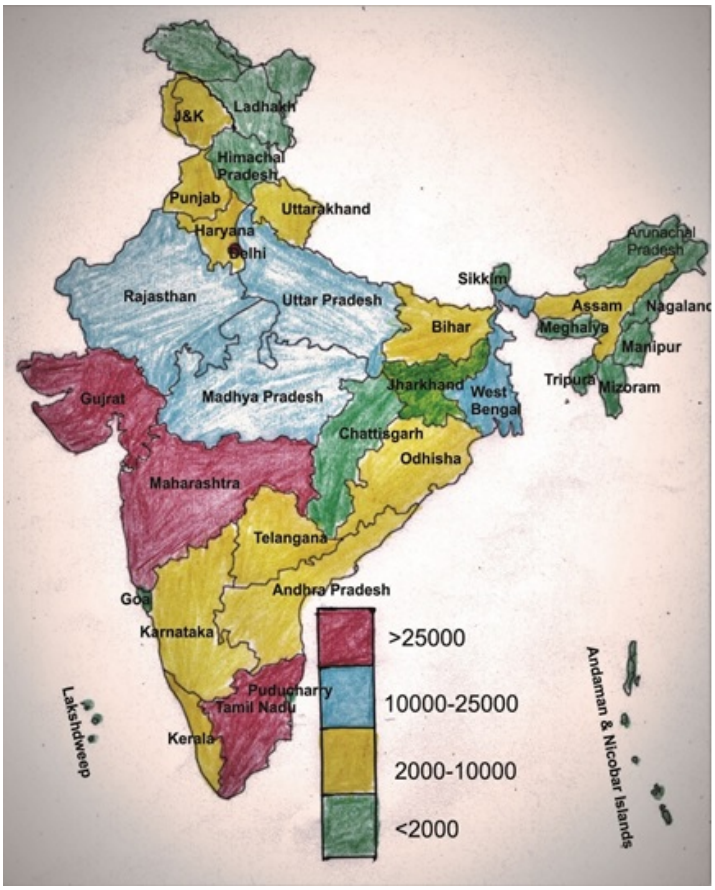


Fig. (7). Confirmed cases of COVID-19 in India as of 12th June 2020.

As of 3rd May 2020, 168,374 tests were completed in 41 sentry sites of Maharashtra for COVID-19. About 7.3%, approximately 12,300 patients were found positive in contrast with approximately 100 patients for SARI infections. Among the tested individuals, 84% were males and higher cases of infection also represented the poor unhygienic habits of the sanitary population. (Government of Maharashtra, 2020; Gupta *et al.*, 2020).

In another study conducted in Delhi, clinical data of the sample population showed that in all the tested individuals for COVID-19, 43% of patients were found to have cough and fever like symptoms, 24% patients with a sore throat, 14% with headache, and 5% were having breathlessness symptoms. This study revealed that symptomatic patients have a cough and fever-like symptoms (Gandhi *et al.*, 2020).

According to the government of Maharashtra, the case fatality rate was found to be 4.8% in males as compared to 4% in females and there was no correlation between gender and the death rate ($p>0.05$). This study further reported that the death rate is higher, *i.e*. 19.2% in the age group of 61-70 years as compared to 18 years old individuals who showed a 14% death rate. This study can be related to the clinical and physical conditions of the patients with their age (Government of Maharashtra, 2020; Gupta *et al.*, 2020).

Gupta *et al.,* studied 102 COVID-19 patients and found that the positive patients from sentinel sites having SARI infection had no abroad travel history or any kind of history of close contact with COVID-19 patients, and the source of infection was not known (Gupta *et al.*, 2020). Another study revealed that in India, community transmission had occurred very early in Pune and other cities, which is why the sources of infection were not traceable (Pulla, 2020).

All of these studies reported and concluded the basic demography of COVID-19 positive cases in India. These studies did not interpret the exact clinical outcomes; therefore, further studies were required to properly understand the epidemiology of corona cases in India.

More epidemiological information of the infection like co-morbidities, risk factors, mode of transmission and socio-demography, and their dynamics is needed in India. All these factors and their details are required to access the exact epidemiology of the COVID-19 pandemic in India.

REPORT BY ICMR ON COVID-19 ABOUT CONTAINMENT ZONE IN INDIA

Indian Council of Medical Research (ICMR) conducted a population-based survey on COVID-19 in India and found that 15-30 percent of the people living in the containment zones *i.e*. in main affected or hotspot cities were infected by the COVID-19 and were already recovered. These findings of ICMR have been shared with the union cabinet secretary and the Prime Minister's Office.

For this survey, ICMR collected the blood samples with the help of the National Centre for Disease Control, the World Health Organization's India office, and the

state governments. Around 24,000 samples were collected from the 70 districts of Pune, Delhi, Indore, Kolkata, Chennai, Surat, Mumbai, and Jaipur where there is 70 percent of total COVID-19 cases of India. As many as 500 samples were collected from 10 randomly chosen containment areas in each of these cities. 400 samples each from the other 60 districts across 21 states, categorized based on the low, medium, and high caseloads, were also collected.

The report suggested that all the containment areas, except Surat and Kolkata, are the worst hit and have 100 to 200 times higher number of cases than the reported cases in the cities like Mumbai, Pune, Delhi, Ahmadabad, and Indore.

NATIONAL RESPONSES ON COVID-19 WORLDWIDE

Almost every country or territory in the world has reported at least one COVID-19 positive case. Different countries have taken different measures to contain viral infections. For instance, Schengen Area countries (Europe) have restricted any kind of movement and control the setup of borders (Schengen Visa Information, 2020). National responses include containment actions like curfews and quarantines, shelter-in-place, and stay-at-home orders.

In the last week of March 2020, globally 1.7 billion persons were under lockdown which got elevated to 3.9 billion people on the first week of April, which represents half of the world's population (Kaplan, 2020).

At the end of April, about 300 million people were under lockdown in different countries of Europe whereas about 200 million people were under lockdown in Latin America. Almost 90 percent of the United States population, around 100 million people in the Philippines (Buchholz, 2020), around 1.3 billion people in India, and 59 million people in South Africa (Nair, 2020) have been under lockdown. Ever since the beginning of the pandemic, over 100,000 new infections were reported on one single day worldwide on 21st May. Below are some detailed examples of corona-related developments in various countries and various measures taken to contain the COVID-19 virus.

Asia

All the countries present in the Asia continent reported COVID-19 positive cases by 19th May 2020 except North Korea and Turkmenistan.

Asia was the first in the world which got hit by the outbreak; however, South Korea, Vietnam, and Taiwan responded to the pandemic very well and were able to control the spread of the pandemic in their respective countries in the beginning, except Taiwan, which maintained to have very low infection rate.

China

As we discussed earlier, the first confirmed case of COVID-19 was reported in China in December 2019 (Chen *et al.*, 2020; Lu *et al.*, 2020) yet one unconfirmed report suggests that the cases were started to appear as early as 17th November 2019 (Ma, 2020). On 27th December 2019, patient samples reported the existence of a SARS-like coronavirus in original genetic testing. Wuhan Municipal Health Commission issued a public notice on 31st December 2019, confirmed 27 cases, and advised to wear masks (Zhu *et al.*, 2020), and on the very same day, WHO was also informed. The Chinese National Health Commission initially stated that there was no clear proof of person-to-person transmission; however, in a conference held on 14th January 2020, Chinese officials confidentially stated that there was a possibility of person-to-person transmission and preparations for the pandemic situation where required.

On 20th January, the Chinese National Health Commission confirmed person-to-person transmission of the virus. A lockdown in the city of Wuhan was enforced during Chinese New Year 2020 (Kuo, 2020). On 10th February, the Chinese government instigated a radical campaign called a "people's war" to contain the viral spread. On 23rd January, the "largest quarantine in human history", a *cordon sanitaire*, was declared with travel restrictions inside and outside of the city of Wuhan which was then extended to 15 cities of Hubei province affecting about 57 million people. Temporary hospitals were established including Huoshenshan Hospital (completed in ten days). Another hospital named Leishenshan Hospital was created to handle the load of additional patients. Facilities in Wuhan, like convention centers and stadiums, were converted into temporary hospitals (Ni *et al.*, 2020; Cai *et al.*, 2020).

On 26th January, the Chinese government instituted further measures as well as mandatory health declarations by travelers and postponing the Spring Festival season holidays. Institutes, schools, museums, universities, and other crowded places throughout China were temporarily closed and work from home was initiated in several regions. Controlled movement of the public was initiated in many cities and around 760 million people had restricted all kinds of outdoor activities. During the peak of the epidemic in Wuhan, in January-February 2020, about 5 million people lost their jobs. Almost 300 million migrants, belonging to rural areas, got trapped at their respective places during travel restrictions (Cai *et al.*, 2020; Ni *et al.*, 2020).

By the time pandemic reached its global phase in February-March 2020, it came under control in China, and therefore, strict measures were taken to prevent the re-entering of the virus in China from other countries. A 14-day compulsory

quarantine was imposed for all international travelers entering China as enforced by Beijing. Regretfully, a strong anti-foreigner fury quickly took hold, and discrimination against foreigners such as forced evictions from hotels; lodges, and apartments were reported (Li *et al.*, 2020).

On 24th March, the official announcement was made by Chinese Premier Li Keqiang that the outbreak has been contained in China. Except for Wuhan, travel restrictions were eased in Hubei from 24-26th March, after two months of lockdown. The Chinese Ministry of Foreign Affairs announced the suspension of visas or residence permits from 28th March onwards, with no specifications regarding its resumption. Re-application for visas in Chinese embassies or consulates had become mandatory for people who wish to enter China. Monetary stimulus packages for firms and factories were given to boost the local economy and to re-open businesses by the Chinese government on 30th March (Lau *et al.*, 2020).

Coinciding with the Qingming Festival, a day of grief with a countrywide three-minute silence moment on 4th April was observed by the State Council. However, to avoid a transformed virus outbreak, the Chinese government suggested families pay their admirations online by observing physical distancing (Zhang, 2020). On 25th April, the last patients got discharged from hospitals in Wuhan; however, on 13th May, the city of Jilin was put on partial lockdown, by considering the fear of the second wave of COVID-19 infection (The Hindu, 2020).

South Korea

On 20th January 2020, COVID-19 was confirmed to have spread in South Korea as observed by the nation's health agency due to a substantial increase in the number of confirmed positive cases majorly attributed to an assembly of the Shincheonji Church of Jesus in Daegu. Shincheonji devotees who visited Daegu from Wuhan were supposed to be infected with the virus before entering S. Korea; and therefore, they were the carriers of the infection. By 22nd February, 1,261 supporters of the church (approx. 13%) amongst 9,336 in total reported viral symptoms following which, South Korea stated the highest level of alert on 23rd February 2020.

The number of confirmed cases in South Korea reached more than 2,000 on 28th February to 3,150 on 29th February. Furthermore, after examinations, three soldiers came positive for the COVID-19 virus and all of the South Korean military bases were sent for quarantine. Timetables of airline carriers were also changed.

A national-level drive to contain the virus was put into force by S. Korea which included screening the S. Korean population for the virus, isolating sick people, if any, and trace and quarantine people who had direct or indirect contact with the infected individuals. This drive is considered the largest and best-organized program in the world. Effective screening methods included were:

1. Compulsory self-reporting of symptoms through a mobile application by all new international arrivals.
2. Rapid testing for the virus was done through Drive-through testing resulting in next day.
3. 20,000 people were tested every day.

South Korea's program was successful in controlling the outbreak without imposing lockdown among all the cities of South Korea (Shim *et al.*, 2020).

On 23rd March, South Korea reported to had reported the lowest one-day case toll over the last four weeks. On 29th March, two weeks quarantine for all new overseas arrivals was announced to be implemented from the beginning of April. As per some reports, by early April, 121 different countries were reported to reach out to South Korea for assistance in virus testing. On 15th May, after a group of a hundred infected individuals was discovered, almost two thousand businesses were again closed briefly (Inocencio, 2020).

Middle Eastern

Iran

Iran recorded its first confirmed case of COVID-19 on 19th February in Qom. However, on the same day, two people had died due to COVID-19 as reported by the Ministry of Health and Medical Education. Primary actions were taken by the government involved:

1. Termination of cultural events, sports events, concerts, Friday prayers, and other similar actions.
2. Closings of schools, universities, and other higher education institutions.
3. Allocation of 5 trillion Rials to combat the virus.

However, on 26th February, President Hassan Rouhani declared that only infected individuals would be quarantined and that there were no plans to quarantine the affected areas. Despite travel restrictions announced in March, heavy traffic between cities was observed ahead of ‘Nowruz’. Shrines were continually open to Shia pilgrims until 16th March in Qom.

After China, Iran became the center of the virus outbreak in February 2020 and over ten countries had outlined their cases related to Iran. On 3rd March, the Iranian Parliament had a blackout because twenty-three parliamentary members had tested positive out of 290 parliamentarians. Twelve government officials and various Iranian politicians succumbed to death due to the COVID-19 infection. On 15th March, over one hundred deaths were reported in one single day, the most recorded in Iran since the beginning of the outbreak. (Takian *et al.*, 2020). By 23rd March, there were fifty new cases per hour of corona infection, and one new death per ten minutes was also reported due to COVID-19. However, according to an official working with WHO, cases in Iran may be at least five times more than what is been reported. Furthermore, it was inferred that U.S. sanctions on Iran might be creating a financial crisis and affecting its ability to respond to the viral outbreak. Ease in economic sanctions was demanded by The UN High Commissioner for Human Rights, including Iran. On 20th April, Iran reopened various shopping malls and other areas with a high number of gatherings across the country; however, it sparked the risk of a second wave of infection. A new peak of the COVID-19 case was reported on 4th June, raising suspicions of the second wave (BBC, 2020).

Europe

The number of corona infected people in Europe increased very quickly and when on 13th March 2020, the number of infected people in Europe crossed China, WHO announced Europe as a new dynamic area of the pandemic. Infection cases in various countries in Europe started to double within 3 to 4 days, with some countries showing replication every 2 days.

By 17th March, there was at least one confirmed case of COVID-19 in all countries within Europe except Vatican City. Till 18th March, approximately 250 million people were restricted through lockdown in Europe (Henley, 2020).

Spain

After having the first confirmed positive case at the end of January 2020, Spain experienced community transmission starting from mid-February. Genetic examination of the samples from infected individuals revealed at least 15 strains of the virus indicating the adaptive nature of coronavirus. COVID-19 positive cases were confirmed in all of the 50 provinces of the country by 13th March (Ansede, 2020).

Lockdown was imposed on 14th March 2020. It was reported that all healthcare professionals and those living in old age homes were having greater rates of infection. Madrid recorded the greatest number of cases and deaths connected to

COVID-19 in the country by late March. On 25th March, the total number of death in Spain crossed 9,000 people approximately. On 2nd April, it was recorded that 950 people in a single day died due to corona infection (the highest number in any country within 24 hours) (Regencia, 2020).

There have been approximately 245,000 confirmed cases with COVID-19 infection and more than 27,000 deaths were reported till 14th June 2020. On 4th June, results of the nation-wide seroprevalence study directed by the Spanish Government showed that around 5.2% (roughly 2 million people) were infected during the pandemic and this figure was ten times more than the number of confirmed cases on that date. This study was based on a sample size of greater than 63,000 people and revealed that several provinces of Castilla–La Mancha, Castilla y León, and Madrid were amongst the most affected areas. (Andrino, 2020).

France

Originally, it was found that the first case of the COVID-19 pandemic in France was on 24th January 2020, with a confirmed case in Bordeaux. Christian Open Door annual assembly Church was organized during 17-24th February. This assembly was attended by about 2,500 people in Mulhouse and was suspected to be the main reason for the spread of the disease in the country. It was predicted that at least half of the attendees to have contracted the virus during this assembly (Point, 2020).

As of 16th March, the government of France announced a mandatory lockdown which was extended until 11th May. As of 23rd April, France reported more than 21,856 deaths, 42,088 recoveries, and 120,804 confirmed cases ranking fourth highest in the number of confirmed positive cases in Europe. Amidst the corona pandemic, when the situation appeared to be under control, the government decided to re-open schools; however, they were soon shut down due to COVID-19 cases flared up amongst children (CBS News, 2020).

North America

The cases of viral infection started to appear in the United States of the North American continent in January 2020. All North American countries had at least one COVID-19 positive case as of 25th March. With Bonaire confirming a case on 16th April, all North American territories had at least one positive case (Davis, 2020).

With over 82,000 cases on 26th March 2020, U.S. became the highest COVID-19 infected country surpassing China, and is still the country with the highest number

of cases as of 12th June 2020. On 11th April 2020, U.S. reported the highest, more than 20,000 deaths, due to COVID-19 infection. As of 12th June 2020, the US confirmed 2,116,211 with 116,814 total deaths.

Canada reported 97,943 confirmed cases and 8,049 deaths on 12th June. Mexico has reported 133,974 COVID-19 cases and 15,944 mortalities till 12th June. Cuba, Dominican Republic, and Haiti are the only Caribbean countries that reported more than 1,000 confirmed cases; while Honduras and Panama led Central America with 7,669 and 18,586 cases respectively (Worldometer, 2020).

United States

As of 20th January, the first known confirmed case of COVID-19 was found in Washington in a man who had returned from China in January mid (Holshue *et al.*, 2020). On 31st January, government officials announced a public health emergency, limiting the entry of tourists and travelers, especially from China.

With a slow start in the testing of samples, the United States on 28th January developed its testing kit as announced by the Centers for Disease Control and Prevention (CDC). However, testing was impaired due to defective test kits; moreover, there was a lack of federal approval for the test kits approved by non-government bodies. Restrictive criteria for the inclusion of people for a test further impaired the effective testing. (Selsky, 2020).

By 2nd March, 80 confirmed cases were reported in the US with, a maximum of cases (almost half) in California. By that time, New York and Florida also stated their first two cases with Washington reporting many suspected cases along with the first death; however, Vice President stressed a small chance of the virus spreading all over the US (Heeb, 2020).

To deal with the rising number of cases in the States, on 6th March, Coronavirus Preparedness and Response Supplemental Appropriations Act were signed by President Trump, enabling federal agencies with $8.3 billion as emergency funding for the pandemic. Work from home was put into practice by various corporations and various sports events and related activities got cancelled (Heeb, 2020).

Despite ongoing efforts, the numbers of infected cases keep on increasing which led President Trump to declare a national emergency on 13th March. Beginning from 15th March, many businesses, schools, universities, stores, *etc*. across the country were either closed or had reduced working hours. By 17th March, all fifty states of the US had confirmed cases. In a survey of 323 hospitals across the U.S in late March, federal health inspectors reported severe shortages of test supplies,

widespread shortages of Personal Protective Equipment (PPE), and other standard healthcare facilities due to the high load of patients.

On 22nd April, two Californians who were previously thought to have died (on 6th and 17th February respectively) from the influenza were reported to be dead because of the COVID-19 virus; however, it was three weeks later when first COVID-19 death officially occurred in U.S. New York Times database revealed over 1,474,600 patients had infected with COVID-19 in the US with at least 88,600 had died as of 17th May.

South America

South America reported its first case on 26th February with a confirmed case in São Paulo, Brazil. Soon after, by 3rd April, all the countries and territories of South America had reported at least one confirmed case.

As of April 17th, the highest number of COVID-19 infections and COVID-19 related mortalities were reported in Brazil followed by Chile and Peru.

On May 13th, Latin America and the Caribbean described over 400,000 COVID-19 positive cases with 23,091 deaths. With the rapid increase of patients having COVID-19 infection in Brazil, WHO stated South America as the new center of the coronavirus pandemic on May 22nd. Due to the lack of proper testing kits and medical facilities, it is considered that the actual numbers of affected people were far more than those reported.

Africa

Africa is of one particular concern by the experts as indicated by Michael Yao, Head of Emergency Operations of WHO in Africa. According to him, early detection would be vital in tackling the COVID-19 situation in Africa as the island's health systems are already overloaded due to other ongoing disease outbursts. To minimize lockdowns in African countries, an approach based on effective testing could be of particular use thus enabling those who depend on daily wages to be able to feed themselves and their families. However, as per a report of the United Nations, even in the best scenario, at least 74 million test kits and at least 30,000 ventilators will be needed for 1.3 billion people of African countries.

Maximum COVID-19 infected cases are reported from the six African countries, which are: South Africa, Ghana, Nigeria, Egypt, Morocco, and Algeria. Though, it is supposed that in African countries with poorer health care systems there is widespread under-reporting. As of 13th May 2020, all African countries reported

COVID-19 confirmed cases and Lesotho was the last country to report its 1st confirmed case on the same date.

Oceania

1st case of COVID-19 in Oceania was reported by the end of January 2020 with the first confirmed case reported in Melbourne, Australia. However, several small Pacific island countries had so far restricted the outbreak by closing their international borders.

On 19th May 2020, for an inquiry into the origins of the virus and the response of governments and the UN, Australia filed a motion in the UN. With the support from over 100 countries motion was passed unanimously in the UN.

EFFECT OF LOCKDOWN ON COVID-19 CASES IN TOP TEN MOST AFFECTED COUNTRIES OF WORLD

As discussed earlier, the most affected countries with the highest COVID-19 positive cases in the world include; USA, Russia, Brazil, Spain, India, UK, Peru, Germany, Italy, and Iran. All of these countries have imposed lockdowns in their respective areas. There is a huge influence of lockdown on the spread of this disease as shown in Table **4** (Worldometer 2020). During the lockdown, the numbers of corona cases were less as compared to cases before the lockdown. The possible reason behind this is the control of the movement of people, which is the main reason for the spread of the infection and thus preventing community spread of the infection.

Table 4. Corona cases from pre- to post lockdown (as of 12th June 2020).

Country	Pre-Lockdown				During Lockdown				Post-Lockdown			
	Period	No. of Confirmed Cases	Death	Average Confirmed Cases per Day	Period	No. of Confirmed Cases	Death	Average Confirmed Cases per Day	Period	No. of Confirmed Cases	Death	Average Confirmed Cases per Day
Brazil	25th Feb. to 23rd Mar.'20	45,775	25	1,635	24th Mar. to 20th Apr.'20	37,108	2,437	1,325	20th Apr. to till now	798,964	38,457	4,796
Russia	31st Jan. to 27th Mar.'20	840	02	15	28th Mar. to 15th May	251,405	2,303	5,130	15th May to till now	250,191	4,227	8,936
India	30th Jan. to 22nd Mar.'20	439	07	09	23rd Mar. to 31st May	181,704	5,157	2,634	31st May to till now	215,392	3,334	6,568

(Table 4) cont.....

Country	Pre-Lockdown				During Lockdown				Post-Lockdown			
	Period	**No. of Confirmed Cases**	**Death**	**Average Confirmed Cases per Day**	**Period**	**No. of Confirmed Cases**	**Death**	**Average Confirmed Cases per Day**	**Period**	**No. of Confirmed Cases**	**Death**	**Average Confirmed Cases per Day**
UK	22nd Jan. to 22nd Mar.'20	5,018	250	84	23rd Mar. to 10th May	210,242	31,337	4,280	11th May to till Now	76,149	9,692	2,380
Spain	31st Jan. to 13th Mar.'20	5,958	84	139	14th Mar. to 02nd May'20	211,846	25,016	4,237	3rd May to till now	25,405	2,036	620
Italy	30th Jan. to 21st Feb.	59,138	5,476	1,115	21st Feb. (partially) and 23rd Mar.'20 to 3rd May'20	150,198	23,234	3,664	3rd May to till now	26,804	5,457	682
Peru	6th Mar. to 14th Mar.'20	38	--	5	15th Mar. to till now	214,750	6,109	2,413	---	---	---	---
Germany	27th Jan. to 22nd Mar.'20	21,463	67	358	22nd Mar. to 19th Apr.'20	118,434	4,469	4,084	20th April to till now	45,777	4,469	848
Iran	19th Feb. to 12th Mar.'20	9,000	354	360	13th Mar. to 10th Apr.'20	57,220	3,756	1,974	11th April to till now	113,956	4,474	1,808

In the USA, there was lockdown executed only in some areas thus the infection throughout the period is the same. Moreover, the USA was dealing with a gigantic number of COVID-19 cases. New York reported 4,000 on 7th April, the highest number of cases in a day, and thus topped the country ever since the pandemic started to affect the country. Various states of the USA forced the restrictions; however, it was impossible to lock down the whole nation due to the federalist state system of the country and the independent nature of the states. In the mid of March'20, California imposed restrictions to avoid the non-essential outdoor movement & stay at home orders were also issued. Chicago executed the lockdown on 21st March 2020 being the 3rd most populated area of the country. There was no national strategy on lockdown; therefore, many US citizens are free to go anywhere throughout the country (Holshue *et al.*, 2020).

Besides, many states of the USA had workplaces, educational institutes, and businesses to shut their services and imposed social distancing. Amongst them West Virginia, Washington, California, Indiana, Illinois, Wisconsin Michigan, and Ohio, adopted the policies of state-wide quarantine (Train, 2020). States such as Oklahoma and Mississippi, which were less-affected, had shut their educational institutes; however, other restrictions were not imposed as can be seen from the

fact that, they permitted public gatherings of up to 10 people.

On 25th February 2020 Brazil reported the first positive case of COVID-19 in Sao Paulo. Until 12th June 2020, 802,828 cases were reported in the country and 40,919 deaths were reported. Globally, Brazil stood 2nd in the terms of highest number of confirmed cases of COVID-19. Brazilian government imposed lockdown from 24th March 2020 till 20th April 2020. Statistical data as given in Table **4** revealed that during the lockdown period the average number of confirmed cases was 1,325/day as compared to 14,796/day after lockdown indicating the effectiveness of restricted movement and home quarantine during the lockdown in tackling the spread of coronavirus (Schwartz, 2020; Painel Coronavírus, 2020; Shailesh, 2020).

In Peru, the first case was reported on 6th March 2020. A 25 years old man with a history of travel to France, the Czech Republic, and Spain tested positive. As of 15th of March, President Martín Vizcarra broadcast country-wide lockdown, limiting domestic travel, closing borders, and forbidding non-essential businesses except pharmacies, food vendors, health facilities, and institutions related to financial activities. With an extended state of emergency and a national lockdown until the end of June, Peru witnessed one of the world's longest compulsory isolation periods aimed at containing the coronavirus outbreak. Peru's confirmed COVID-19 positive cases were 111,698 the second-highest total in the American continent with total death of 3,244 as of 12th June'20.

In terms of Covid cases across the globe, Russia was on the 3rd spot as of 12th June 2020. According to official data, Russia had 511,423 confirmed cases till 12th June 2020. During treatment 269,370 cases recovered while 6,715 died. Pandemic started in country on 31st January 2020, when two citizens from China were found positive. Russia immediately imposed preventive measures and started testing every person crossing the Russia-China border. However, new cases of the infection were found on 2nd March 2020, with a history of travel to Italy, which led to an imposition of additional preventive actions such as cancelation of various events, closing theatres, educational institutes, and museums. Lockdown lasted until 11th May in most parts of the federal; including Moscow which was under lockdown until the end of March 2020. On 27th April, the total number of COVID-19 confirmed cases in Russia surpassed the number of cases in China. A similar trend in pre-and post-lockdown periods, like that of Brazil, was also observed in Russia with an average number of 8,936 cases per day after the lockdown was lifted compared to 5,130/day during the lockdown period (Table **4**) (Elsevier, 2020; Rainsford, 2020).

The first case of COVID-19 in India was reported on 30th January 2020. The Ministry of Health and Family Welfare (MoHFW) confirmed 309,603 numbers of cases, 154,231 recoveries, and 8,890 deaths in the country as of 12th June 2020. As of 12th June'20, India had the highest number of confirmed cases in Asia and was in the fourth number amongst the countries with the highest number of corona cases worldwide. However, India's fatality rate, 2.80%, was relatively lower than the global fatality rate of 6.13%. On 22nd March, India imposed 14 hours of Janta Curfew, which was followed by mandatory lockdown in all the major cities and states of India. On 24th March, a nationwide lockdown for 21 days was imposed, followed by extensions. From 1st June onwards, the government started unlocking the country with restrictions at places of containment zones. A similar trend, like that of Brazil and Russia, in the pre-and post- lockdown period, was observed in India.

In the UK, as of 12th June 2020, there were 292,950 confirmed cases and 41,481 confirmed deaths. This was the second-highest mortality rate per capita in the World. In the UK, among all the cities, London topped the country with cases of infections; but the rate of infection was found to be highest in North-East England.

In March, the UK government imposed a lockdown and barred all "non-essential" travel. The government also banned making contact with people outside one's domicile (including family and partners), closed businesses, schools, and other places of public gatherings. People with symptoms were asked to self-isolate, while the high-risk people (those over the 60s and with other diseases) were told to isolate and protect themselves. Social distancing was put into practice. Government efforts to contain the virus by lockdown resulted in a decrease in the rate at which people were getting infected before lockdown.

The first case of SARS-CoV-2 infection in Spain was reported on 31st January 2020, with COVID-19 positive tourists in La Gomera, Canary Islands, who came from Germany. Soon after, different provinces started to report corona cases and by 13th March, all 50 provinces of the country had COVID-19 positive cases. The lockdown was imposed on 14th March 2020. At the end of March, all government authorities ordered non-essential workers to stay at home for two weeks. In late March, Madrid recorded the highest number of infected persons and deaths in the whole country. Healthcare professionals and people living in old age homes were found to have higher rates of infection. As of 12th June 2020, there were 290,289 confirmed positive cases and 27,136 mortalities.

In Italy, the government implemented a nationwide quarantine on 9th March 2020, thus restricting the movement of the people except when required in case of work

and health circumstances, to contain the mounting COVID-19 pandemic in the country. The temporary closure of unimportant shops and businesses was authorized by additional lockdown restrictions. Although approved by the public, these lockdown measures were the largest destruction of constitutional rights in the history of the republic. Nonetheless, lockdown effectively reduced the frequency and spread of the infection in the country. After the lockdown, the average per day corona cases were lowered to 682 as compared to the highest 3,664/day before lockdown.

The first case of the COVID-19 pandemic in Germany was a worker working in an automobile-parts producer and was confirmed on 27th January 2020. By the end of February 2020, multiple cases related to the coronavirus infection were detected in Baden-Württemberg named as 'Italian Outbreak'. Germany imposed a lockdown which lasted until 19th April 2020 with a decrease in the rate of infection transmission after lockdown. (Amante and Pollina, 2020; Seckin, 2020).

The coronavirus infection started in Iran on 19th February 2020. Iran reported its first confirmed case of infections in Qom. It was suspected that the virus may have entered the country from a merchant in Qom with travel history to China. Lockdown in Iran was started on 13th March 2020. To contain the infection, the government cancelled Friday prayers, public events, closed all educational institutes, markets, shopping centers, and holy shrines, and banned festival celebrations. Lockdown which ended on 10th April 2020 had successfully brought down the number of infected cases (Amante and Pollina, 2020; Seckin, 2020).

As can be seen, lockdown had a reasonable effect on containing the spread of the disease. Also, various countries have taken various other measures along with lockdown which was discussed in earlier parts of this chapter.

THE ROLE OF WHO AND INGOS FOR PROVIDING DATA IN COVID-19

To facilitate the wellbeing of public health worldwide, in 1948 World Health Organization (WHO) was established by United Nations with the main objective of obtaining reliable information and pieces of advice in the field of human health through publication. WHO supports national health strategies of various nations and addresses the alarming public health concerns by its publications. In the current pandemic situation, WHO is working actively in providing funding, information regarding disease progress, and giving statistical analysis on the COVID-19 to help control the spread of the disease in various part of the world and to help the researchers to find out the possible treatments of this disease; thus, helping to promote and protect health worldwide and to limit the spread of the disease throughout the world. Information provided by WHO contributes to

achieving the organization's principal objective – the attainment by all people of the highest possible level of health. Similarly, various other International Non-Government Organizations (INGOs) are working day and night in parallel to various national agencies in and around their working area to keep updated information about COVID-19 by collecting samples, conducting studies, applying statistics, and publishing high impact papers on the spread, control, and management of the disease.

OUTCOMES FROM SOME PUBLISHED REPORTS ON COVID-19 EPIDEMIOLOGY

- Hamid *et al*. reported that the persons with age group 35-55 years got less infections as compared to children and infants. The study found that the median age of patients was 59 years and the majority were males (59%). Furthermore, the study reported that old age people with various problems were a high-risk group. They also found that transmissibility and pandemic risk of SARS-CoV-2 was at high rates as compared to that of SARS-CoV. The COVID-19 average incubation duration was estimated to be 4.8 ± 2.6 ranging from 2-11 days (Hamid *et al.*, 2020).
- Chen *et al.*, studied and analyzed the cases for demographic, epidemiological, radiological, and clinical features and laboratory data. The study reported that 49% of cases had exposure to the seafood market. They observed that the patients had clinical illustrations of fever (83%), cough (82%), breath shortness (31%), muscle ache (11%), confusion (9%), headache (8%), sore throat (5%), rhinorrhoea (4%), chest pain (2%), diarrhoea (2%) and nausea and vomiting (1%). They illustrated that according to imaging examination, 75% of patients manifested bilateral pneumonia, 14% patients showed multiple mottling and ground-glass opacity, and 1% of patients had observed pneumothorax. 17% of patients developed acute respiratory distress syndrome and 11% of patients had their conditions exacerbated in a short period of time and died of multiple organ failure (Chen *et al.*, 2020).
- Gandhi *et al.*, reported the epidemiological study on India which was based on literature from PubMed, WHO, GoI, and Google Scholar. They evaluated the study based on a test performed in various metro cities of India till 2nd April 2020 and found out that 1.8% cases were found positive with a corona in Maharashtra of which 39.7% were males with a mean age of 40.3. The median age of the patients in sentinel Delhi areas was 54. Furthermore, in the report, they revealed that 43.8% of cases had a travel history, and 31.3% of cases had direct contact with COVID-19 cases (Gandhi *et al.*, 2020).
- Ahn *et al.* reported epidemiology on COVID-19. They found that the death rate of SARS-CoV-2 (3.8%) is lesser as compared to SARS-CoV (10%) or MERS-

CoV (37.1%), but infection rates are 10 times more as compared to the other two. Reports suggested that SARS CoV-2 can be transmitted from patients which are having mild infections or are asymptomatic. The unexpected and immediate epidemic outspreading of the virus could be explained by these findings (Ahn *et al.*, 2020).

- Zhai *et al.*, reported in their study that the incubation period of COVID-19 was 5.2 days to 6.4 days. Authors also reported that the reproduction number in the majority of studies was found to be 2.24 to 3.58 which is higher than that of SARS (Zhai *et al.*, 2020).
- Bulut, C *et al.*, reported that the COVID-19 pandemic has R0 value of 2.3, which can be as high as up to 5.7, and the case fatality rate was found to be 6.3 which was different in various age groups amongst different countries. The study also suggested that 10-12 weeks are required for the control of pandemics in the community (Bulut *et al.*, 2020).
- Sun *et al.* reported that the spread of the virus in the 2nd phase occurred in the hospitals and within the family starting from 13th January in Wuhan to other areas. The third phase was started on 26th January 2020. 1,716 staff members from 422 medical institutions were infected in February 2020 in China. In the initial evaluation, it was found that the reproductive number of COVID-19 was 1.4 to 3.9 (Sun *et al.*, 2020).
- Lai *et al.*, reported that there is a difference in the epidemiological data among the different countries whereas China registered a high number of deaths and illnesses than other sites of the world. The Daily Cumulative Index was calculated for COVID-19 as cumulative cases/no. of days between the first reported case and this number was highest in China (1,320.85) on 29th February 2020 followed by the Republic of Korea (78.78) and further followed by Iran (43.11) and Italy (30.62). In other countries/territories, the Daily Cumulative Index was not more than 10 per day (Lai *et al.*, 2020).
- She *et al.*, reported the epidemiology study on children infected with COVID-19. They found that around 56% (34/61) of children infected with COVID-19 were infected from their families. They reported that in China 17 days old baby was found COVID-19 positive. After that, a neonatal, 5 days old infant, was infected with corona as his mother was found corona positive. The report further revealed that the incubation period in the children was approximately 6.5 days as compared to 5.4 days in adults (She *et al.*, 2020).
- Yoo *et al.* examined the epidemiological reports on the Republic of Korea and reported that as of March 2nd, 2020, the Republic of Korea had the second-highest number of confirmed cases (n = 4,212) after China (n = 80,026). The main cause of augmented elevation in CoV-2 patients in Korea appeared by the exposure between the Daegu Church gathering attending members with additional hospital and community transmission. Mainly, the detonation of

transmission occurs in Daegu and adjoining areas (Yoo *et al.*, 2020).

- In a hypothesis Singh *et al*., reported the scope of lung biofabrication tool, 3D bio-printing technology in reducing the chances of clinical trial failures in COVID-19 treatments. However, this area needs to explore further to establish the hypothesis (Singh *et al*., 2020c).

Herd Immunity-COVID-19

Immunity can be attained by recovering from a pre-infection or vaccination. Not every individual can attain immunity due to the medical affliction *i.e*. immunosuppression, immunodeficiency, hence for such a group, herd immunity is a crucial way of protection (Coughlin *et al.,* 2017). Herd immunity happens when the herd *i.e*. large portion of a community achieves immunity to a disease which results in an unlikely spread of disease from person to person. Hence, the whole community can be protected, not just those who are immune.

If the population proportion which is immune to the disease is more than the threshold, the disease spread will decline. This is referred to as the herd immunity threshold (Kwok *et al.,* 2020).

There are two ways to get herd immunity for COVID-19 *i.e*. vaccines and infection. The vaccine against the COVID-19 virus would be an ideal approach to achieve herd immunity. Utilizing the herd immunity concept, many deadly contagious diseases such as diphtheria, smallpox, polio, have been successfully controlled (Fine *et al.,* 2011). Obtaining herd immunity down vaccination has downsides, though *i.e*. re-vaccination, skepticism, religious objections, *etc.*

Considering the current scenario, even if infection with the COVID-19 virus generates enduring immunity, a large percentage of the population would have to get infected to gain the herd immunity threshold. Recent update estimates that in the U.S.A, >70% of the population (200 million) would have to recover to stop the epidemic (Poland, 2020). Under such circumstances, the amount of infection could also lead to millions of deaths, serious complications. Until the vaccine is developed against the COVID-19 virus, its critical decelerates the viral spread. Hence, to lower down the risk of infection (Giubilini 2019, Kwok *et al.,* 2020):

- Avoid mass gatherings, close contact with people.
- Stay home if you are feeling sick and avoid public transportation and ride-sharing.
- Keep the distance between yourself and others in public transportations.
- Avoid public transportation, taxis, and ride-sharing if you're sick.
- Cover your mouth and nose, wear masks.

- Disinfect the most used surfaces.
- Eat and drink healthy to boost your immune system.

CONCLUSION

COVID-19 has emerged as one of the most infectious pandemics of recent times. It has changed the world once and for all. With no vaccine and no medicine in the market as of 30th Aug. 2020, measures like quarantine, isolation, and social distancing are the only choices humans had. With the number of corona positive patients increasing every day, it appears that we are still far away from getting rid of this problem. With already reported mutations of this virus, it might not be an easy task to develop vaccines, but efforts are underway. However, measures like lockdown, wearing masks, and social distancing have proved the effectiveness and should be practiced. High-risk group people should be extra cautious. COVID-19 is probably one of those diseases with which the human race might need to learn how to co-exist!' However, continuous efforts of the researchers in finding vaccines and treatment drugs, health care providers, various NGOs, awareness groups, *etc*. are commendable and are surely a silver lining in clouds of the COVID-19 pandemic.

LIST OF ABBREVIATIONS

WHO	World Health Organization
ICTV	International Committee on Taxonomy of Viruses
SARS	Severe Acute Respiratory Syndrome
MERS	Middle Eastern Respiratory Syndrome
MoHFW	The Ministry of Health and Family Welfare
SARI	Severe Acute Respiratory Infections
NGO	Non-Government Organization

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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CHAPTER 4

Pathophysiology

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Abstract: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causal pathogen of the novel coronavirus disease 2019. This novel Covid-19 has created a serious public health crisis throughout the world. The primary symptoms of coronavirus infection are common cold and influenza-like illness and with time it causes pneumonia. Although various studies are going on throughout the world, its actual pathophysiology is not very well clear to date. The Coronavirus is a positively charged single-stranded RNA virus. This virus gets easily transmitted from human to human. Numerous investigations have been found that the virus enters into the human body *via* its spike (S) proteins. The S-protein binds to ACE2 receptors and silently comes in contact with alveoli *via* blood. This entry hypersensitizes various receptors, epithelial cells, macrophages, T-cells, dendritic cells and thus implants pro-inflammatory cytokines and chemokines, resulting in stressful conditions. Studies found that Hemagglutinin-Esterase protein, Spike protein, Nucleocapsid protein, small envelope protein, internal proteins, group-specific proteins take part in viral pathogenesis, whereas, replication proteins (eIF4A, Cyclophilin, 3CLpro, RdRp) participates in Coronaviruses (CoVs) replication and translation phases, influencing both pathogenesis and pathophysiological conditions. In this chapter, we elaborate on viral pathogenesis, the various functions of proteins, structural, enzymatic, and accessory that are linked with the pathological conditions and will also highlight the correlation causing physiological alteration associated with this infection.

Keywords: ACE2 receptors, Covid-19, Hemagglutinin-Esterase protein, Life cycle, Membrane protein, Nsp1 protein, Nsp3 protein, Nsp8 primase, Nsp12 polymerase, Nsp13 helicase, Nsp14 protein, Nsp15 protein, Nsp16 protein, Nucleocapsid protein, Orf3b, Orf6, Orf7a, Pathogenesis, Pathophysiology, Spike proteins, Thrombosis, Transmission.

INTRODUCTION

Coronavirus (CoV) is a member of the Coronavirinae subfamily, which comprises a single positive-stranded RNA virus. The various endogenous proteins present in

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it are integral membrane (M), spike (S), nucleocapsid (N), envelope (E), and other accessory proteins, which not only facilitate its entry into the cells but also helps in replication (Fig. **1**) (Garoff *et al*., 1998). There are 3 different categories of CoVs- category 1, which includes human coronavirus 229e and transmissible gastric enteritis virus; category 2 includes human CoV-OC43, murine hepatitis virus, and bovine CoVs; class 3 comprehends avian infectious bronchitis virus (Fehr and Perlman, 2015). On 31st Dec 2019, China alerted WHO of a huge number of pneumonia-like cases in Wuhan city. After continuous investigations, Chinese scientists and WHO claimed the presence of a new strand of CoVs causing this pneumonia-like problem. CoV symptoms include- dry cough, shortness of breath, and respiratory distress. This virus has spread to almost every part of the world and costs about 469,587 lives worldwide till 23rd June 2020. From various investigations, WHO had declared that these CoVs are spreading through contacts by infected persons or patients. The S-protein of CoVs binds with ACE2 receptors and silently comes in contact with alveoli *via* blood. Initially, at the time of infection, the CoVs contaminates the epithelial cells, macrophages, T-cells, dendritic cells and execute pro-inflammatory cytokines and chemokines takes place, initiating stressful condition. In this chapter, we are discussing the viral pathology associated with proteins, enzymes, and accessory along with their roles in pathogenesis.

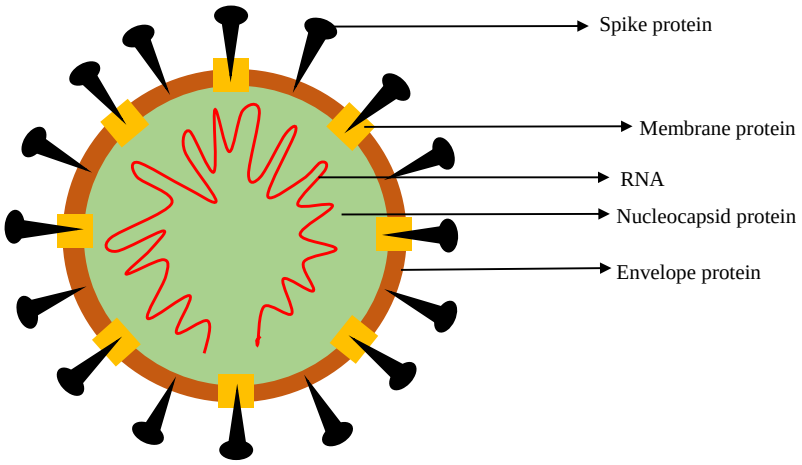


Fig. (1). Structure of CoV.

TRANSMISSION OF COV

Available reports have found that the CoVs are conveyed from animals to humans through close contact with animals like pigs, camels, and bats, are more susceptible to CoV infection, creating a reservoir (Mackay and Arden, 2015). At the early stage of CoV infection, the primary symptoms are not well expressed but as it gets matured and replication occurs CoVs slowly start to show their

symptoms. The incubation time of CoV infection is 2 to 14 days. It has been found that Covid-19 gets transmitted from an individual to another *via* cough, sneezing, hands shaking and thus found to get settled themselves at the respiratory tract (Gunalan, 2011) (Fig. **2**).

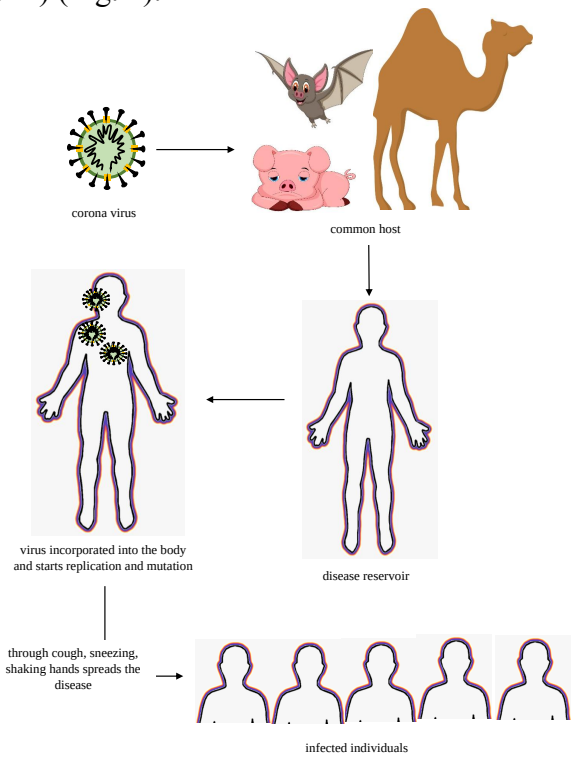


Fig. (2). Schematic representation of transmission of CoV.

Virus Life Cycle

The CoV life cycle has been summarized in this portion by discussing the various functions of viral proteins. It has been found that CoV with the help of the S-proteins gets attached with specific cellular receptors and thus initiates a conformational structural alteration of spike proteins, resulting in the liberation of the nucleocapsid into cells. After entering inside the cells, the 5' end of RNA, orf1a, and 1b gets translated to form pp1a and pp1ab. The orf1a represents the papain-like protease (PLpro) and a 3C-like protease (3CLpro), which acts to progress the pp1a and pp1ab to mature replicase protein (Lee *et al*., 1991; Ziebuhr *et al*., 2001). The orf1a X domain encodes the ADP-ribose1”-phosphatase activity (Ziebuhr, 2005; Snijder *et al*., 2003), while the orf1b encodes a helicase and an RNA-dependent RNA polymerase (RdRp) which is processed by pp1ab (Gorbalenya, 2001). This orf1b also encodes various other enzymatic activities-

3'-to 5'- exonuclease (ExoN), poly (U)- specific endoribonuclease (XendoU), and S-adenosyl methionine- dependent ribose 2'-O- methyltransferase (Ziebuhr, 2005; Snijder *et al*., 2003; Ivanov *et al*., 2004). The downstream orf2a encodes the enzymatic activity of cyclic phosphodiesterase.

The infection of CoV involves replication and transcription of genomic mRNA. During replication, few long negative strands of RNA are being synthesized which acts as a template for genetic RNA. Several overlapping 3'- coterminal sub-genetic RNA acts as mRNA (75- to 78- nucleotide). These mRNA has a common initial sequence which is present at the 5' end (Lai *et al*., 1984; Shieh *et al*., 1987). With recent advancement and immense research, it has been found that interrupted transcription takes place during negative-stranded sub-genomic RNA synthesis, with an additional attachment of 3' end of negative RNA strands that acts as a template for mRNA synthesis (Enjuanes *et al*., 2005). It has been found that the envelope (E) protein gets translated by a downstream or f5b of mRNA5. This translation of orf5b is intervened by the internal entry site of the ribosome (Jendrach *et al*., 1999). The M and E protein gets immediately localized over the Golgi intracellular membrane just after translation. These M and E proteins are capable of producing virus-like particles without any viral protein and viral RNA (Corse and Machamer, 2003; Corse and Machamer, 2000; Klumperman *et al*., 1994; Krijnse-Locker *et al*., 1994). The distribution of spike (S) protein over intracellular membranes and also over the plasma membrane was found to take place. During assembly, this S-protein then interacts with M-protein's transmembrane region. The helical structure is found to occur due to interaction with genetic RNA and with nucleocapsid protein complexes. The interaction between N- and M-proteins (Kuo and Masters, 2002) leads to budding within the vesicles. Immediately, after budding, the viruses travel towards the cell surface and gradually leave the cell.

Role of Structural Proteins in The Pathogenesis

A. Spike Protein

The CoVs spike (S) are of type I glycoprotein which produces a peplomer-like structure on its envelope. During processing in the Golgi body, these spikes with the help of furin-like enzyme activities get cleaved into two distinct sub-units. The S1 subunits have an amino-terminal which forms the spherical head of the protein and also holds the receptor-binding domain (RBD) at its first 330 amino acids (Kubo *et al*., 1994). The S2 subunits contain the carboxylic terminal which is supposed to form the stalk-like assembly which helps in anchorage over the membrane (Fig. **3A**). It comprehends two parts- 2 heptads repeat (HR) domains and a fusion peptide (La Monica *et al*., 1991; Luo and Weiss, 1988; Parker *et al*.,

1989; Taguchi, 1995). A bridge-like connecting loop is formed by a cysteine enriched domain in between the anchor and the cytoplasmic tail which is essential for its fusion (Chang *et al*., 2000). The RBD of CoVs is 192 amino acid regions reside over 319 to 510. The central part of CoV S-protein contains a loop which is also known as a receptor-binding motif (RBM); its role is to create direct connections with ACE2 receptors (Angiotensin-converting enzyme).

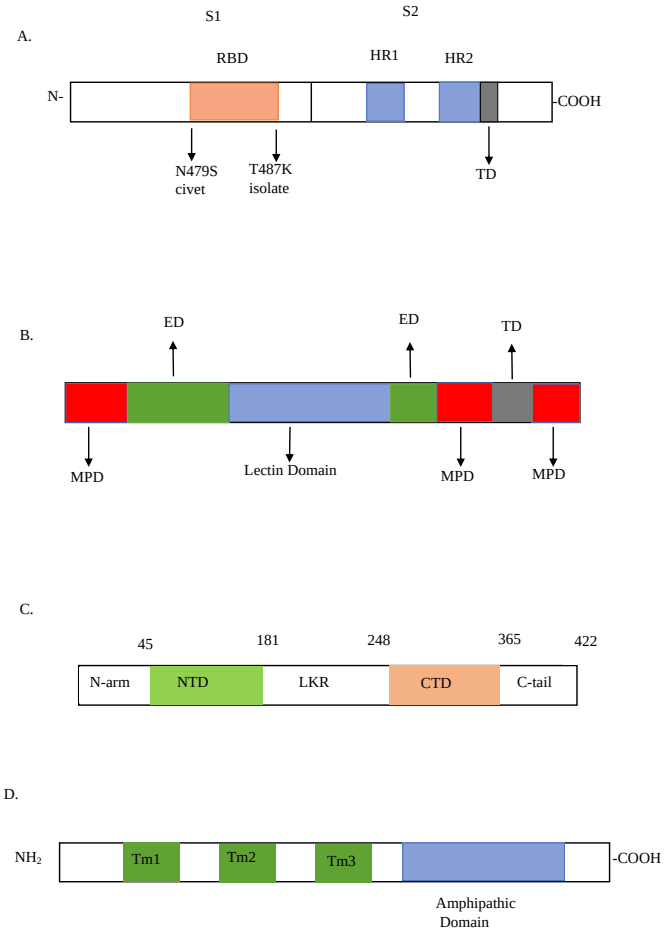


Fig. (3). Schematic representation of Spike Protein, Hemagglutinin-Esterase Protein, Nucleocapsid Protein, Membrane Protein structural proteins Where, RBD, Receptor binding domain; HR, Heptad repeats; TD, transmembrane domain; MPD, membrane-proximal domain; ED, Esterase domain.

Various researches have clarified that S-protein in addition to substitution of one or two amino acid groups interacts largely with human ACE-2 receptors. The S-RBM contains two residues – N479 and T487. These two residues are mostly

preferred by human ACE-2 receptors and cause more pathogenicity. The CoVs S during synthesis does not get cleaved into S1 and S2 subunits. During viral entry, the cathepsin-L helps to facilitate the endosomal low pH requiring cleavage. In a study, it has been found that during viral entry, cathepsin facilitates the fusion, while for triggering the cell to cell fusion, the cellular protease facilitates the leupeptin-sensitive like cleavage (Simmons *et al*., 2011). The S1/S1 boundary and in the S2 (R797) are the two cleavage events for mediating membrane fusion and for viral infection (Belouzard *et al*., 2009; Belouzard *et al*., 2010). It has been found that colocalization of ACE2 with transmembrane protease/subfamily of serine membrane 2 (TMPRSS2) increases the CoVs entry (Matsuyama *et al*., 2010; Shulla *et al*., 2011). This protease family TMPRSS2 is mainly found in the lungs and thus determines the viral pathogenesis. It has also been found that the virus after its entry regulates the renin-angiotensin system by downregulating the ACE2 in the plasma membrane and thus slowly initiates the CoVs infection (Haga *et al*., 2008; Rockx *et al*., 2009; Wang *et al*., 2008). It is also known that the resin-angiotensin system maintains blood pressure and fluid balance. From various models of lung injury, it has been determined that ACE2 acts as a pneumo-protective agent and thus degrading the angiotensin II, proinflammatory mediators which are synthesized by ACE-1 (Kuba *et al*., 2005; Hamming *et al*., 2007; Wösten-van Asperen *et al*., 2008). It has also been described that the S-protein activates the interleukin-8 (IL-8) by initiating MAPK and AP-1 in the lungs (Chang *et al*., 2004).

B. Hemagglutinin-Esterase (HE) Protein

HE is a type of glycoprotein that forms a secondary type of S-protein, these types of S-proteins are smaller than the S-protein peplomer and are found on type 2 CoVs envelopes (Kienzle *et al*., 1990; Yokomori *et al*., 1989). This HE gets synthesized as an apoprotein of 42kDa which glycosylated to form 65kDa and homodimers are also formed due to disulfide links (Fig. **3B**). This HE is found to yield the activities hemagglutinating and esterase (Kienzle *et al*., 1990; Brian *et al*., 1995). It has also been noted that HE is found to acquire the group II CoVs homologous RNA recombination that contains no HE proteins (Snijder *et al*., 2003). This HE protein was found to have sialic acid-binding and acetyl esterase activities, which activate the viral entry and its release from the cell surface by interconnecting the domains of sialic acid. It has been found that HE acts as a non-essential protein that gets activated by isolation of JHM (Yokomori *et al*., 1991). It has been also found that HE acts as a binding molecule, where the spike acts by attaching to specified glycoprotein receptors over the cell. It has been notified to have a destructive activity towards the supplying receptors during virus removal from non-susceptible cells. After immense investigation, it has been

found that the S-protein gets attached with sialic acid residues that produce the main role of HE for viruses that want to get released from glycans with the help of esterase activity (Wurzer *et al*., 2002). It has also been noted that these S-protein increase its receptor specificity towards the residues of glycan (Langereis *et al*., 2010).

Till now the proper role for pathogenesis of HE is not well clarified. From various investigations, it has been found that the A59 strain adapted from the genome of tissue culture shows not only a variety of HE mutations but also shows transcriptional regulatory sequences (TRS), which causes a negative impact over HE pseudogene and also over its lost expression on chimeric A59 during the *in-vitro* passage. HE also shows an immense impact in causing viral infection in CNS. When observed by comparing the isogenic recombinant viruses that express, HE wild type protein, the esterase activity of HE has been found eliminated whereas, short HE proteins were found to be synthesized by the virus (Kazi *et al*., 2005).

In a mice study, it has been found that the virus having large polypeptides of HE shows more virulent activity and spreads vigorously in CNS. This result does not directly correlate the virulence with esterase activity but suggests the HE entertains the attachment or spread of the virus by forming bridges with molecules containing sialic acid. From various studies, it has been found that the communication of HE with molecules residing on the cellular surface increases the binding of neural cell types, whereas the isogenic recombinant JHM strains do not show any discrimination in the case of neurovirulence; cumulatively suggesting that HE does not always aim for neuropathogenesis. The esterase activity may not be an important event for neurons but is a vital event for the gastrointestinal tract which allows the virus to pass through mucous or may have the power to get detached from cells and are believed to cause neuraminidase. During the influenza neuraminidase, the sialic acids specified enzymatic activity which may cause cell subtype infection in the respiratory tract and cause pathogenic severity (Matrosovich *et al*., 2004).

C. Membrane (M) Protein

Earlier the M-protein was known as envelop-1 (E1) protein. It is a several-layered bridge protein consisting of 221 to 262 amino acids and is found to be adequate in viral envelopes (Escors *et al*., 2001). It is a short hydrophilic protein and its glycosylated N-terminal motif is present on the outer surface of the virion. The inner surface of the virus contains 3 transmembrane motifs, positioned at its long C-terminal end. The two motifs are present at the C-terminal ends, an amphipathic motif is also present just next to the third transmembrane motif which contains a

small hydrophilic portion (Fig. **3D**). It has been found that both alpha and gamma CoVs contain an N-glycosylated N-terminal motif. This domain is very sensitive to protease and is in contact with the viral surface.

In an *in-vitro* study, it has also been found that this domain gets translocated into the lumen of ER during cDNA translation, containing the M-protein in the presence of microsome. It has also been found that the N-terminal domain is capable of neutralizing the viral infectivity by recognizing the monoclonal antibodies. The C-terminal end contains maximum amphipathic motifs which are linked through the viral envelop protein or by the vesicular part of the cytoplasmic region where viral assembly and budding takes place depending upon the sensitivity of protease during *in-vitro* translation of cDNA that encodes the M-protein in presence of microsome. It has been found that when the m-protein is expressed alone it gets localized on the Golgi apparatus (Nal *et al*., 2005). A fact has been found that the first transmembrane motif of M-protein possesses an indicator that can keep the membrane protein into cis-Golgi apparatus, but in some cases, it has been found that due to deletion of first two transmembrane, or the tail of cytoplasm may cause loss of membrane protein in the Golgi apparatus. The M-protein is present inside the intracellular region of the ER-Golgi intermediate compartment (ERGIC). In infected cells, it has been found that the position of ERGIC depends on the expression of E-protein (Lim and Liu, 2001). It has been found that this M-protein has a vital role in assembling and budding viral particles (de Haan and Rottier, 2005). This M-protein has been found to interconnect with themselves and also with N, E, S, HE proteins (Kuo and Masters, 2002; de Haan *et al*., 2000). Various reverse genetic, biochemical, and two-hybrid studies have been conducted using virus-like particles (VLP) or by other protein interactions to monitor the role of M-protein for CoVs assembly (Corse and Machamer, 2003; Arndt *et al*., 2010; Verma *et al*., 2007; Nakauchi *et al*., 2008; He *et al*., 2004; Hsieh *et al*., 2008; McBride and Machamer, 2010). The M-M protein interactions are mostly umpired by transmembrane motifs (de Haan *et al*., 2000) and also by SWWSFNPETNNL a classified protected belt of 12 amino acids that continues up to the third transmembrane motif (Arndt *et al*., 2010). The M-S protein interactions are controlled through the remnant of cytoplasmic tail whereas it can be proved that half of the N-terminal residues participate in this event (McBride and Machamer, 2010; Voß *et al*., 2009). It has been found that the M-N protein interactions are also umpired by the M-cytoplasmic motif (Kuo and Masters, 2002; Verma *et al*., 2007; Hsieh *et al*., 2008; Fang *et al*., 2005).

Besides, all these interactions it has also been found that the M-protein also interacts directly with the signal of RNA packaging in N-protein independent ways to process the genomic packing into the virions (Narayanan and Makino,

2001; Narayanan *et al*., 2003). It has been notified that for assembly and binding of the virus, the interaction between M-cytoplasmic domain protein and b-actin is important (Wang *et al*., 2009). With the initiation of interferon responses as a role of M-protein for CoV infection has been found, whereas, with the initiation of interferon-α in peripheral blood mononuclear cells (PBMCs) by the help of glutaraldehyde-fixed purified virus or the cells infected by viruses, that can be inhibited by monoclonal antibodies which are sensitive for N-terminal domains of M-protein. Various studies have claimed that the VLPs consisting of M-proteins of CoVs (α, β, γ) confirmed the interferogenic actions of M-protein are not restricted in only a few CoVs. Some genetic studies have been performed using recombinant A59 mutants, in which alteration of M-protein was done for stoppage of glycosylation or for altering N-glycosylation site in place of o-glycosylation site. In this case, this N-glycosylated M-protein modulates the interferogenic activity and can cause a better inducer than in O-glycosylated M-protein (de Haan *et al*., 2003). It has been found that the unglycosylated M-protein mutation does not cause any conformational induction of interferon. During *in-vivo* studies, it has been found that these viruses can replicate in hepatic surroundings but not in the brain which correlates their *in-vitro* interferogenic capacity (de Haan *et al*., 2003). This study can also explain the interaction of the virus with lectins, a mannose receptor, present in the liver, which induces interferon-α in dendritic cells. The overexpression of M-protein correlates with RIG-I, TBK-1, IKKe, and TRAF3 and can inhibit the IRF3 and IRF7, which results in inhibition of interferon-β, advocated by dsRNA (Siu *et al*., 2009).

D. Small Envelop (E) Protein

The envelope (E) proteins are endogenous membrane proteins (Yu *et al*., 1994). This E-protein along with M-proteins takes part in viral assembly (Vennema *et al*., 1996). The expression of E-protein in the presence or absence of M-protein is capable of producing viral particles. This E-protein has been found to play an important role in causing infection (Kuo and Masters, 2003). It has been found that disarrangement of the E gene in TGEV proteins causes lethal effects (Curtis *et al*., 2002; Ortego *et al*., 2002). It has been found that the E-protein has significant specific ion channel activity (Wilson *et al*., 2004), although this needs to be further excavated. This E-protein ion channel acts as a site for budding and increases the morphogenesis and assembly of viruses. During *in-vitro* studies, it has been found that E-protein induces apoptosis in A59 infected 17Cl-1 cells by modulating the caspase-dependent pathways which get suppressed by excessive Bcl-2 expression. It has also been found that inhibition of apoptosis may increase viral production during the terminal section of infection, which suggests that this apoptosis may be the host response that checks the expression of viral production

(An *et al*., 1999), but this data needs to be verified for an *in-vivo* condition also. At the time of Jurkat-T cell, it has been found that the E-protein could result in apoptosis, but their activity can be suppressed by the expression of anti-apoptotic protein Bcl-xL (Yang *et al*., 2005), thus suggesting that this T-cell apoptosis may lead to activation of lymphopenia, which is observed in most patients.

E. Nucleocapsid (N) Protein and Internal (I) Protein

The N-protein acts as a primary RNA binding protein, which is encoded in 30 portions of the CoV genome (Fig. **3C**). It has been found to act both as structural as well as non-structural roles during infection. This N-protein binds with genomic RNA, resulting in virus capsid and during assembly, it interacts with M-protein (Hurst *et al*., 2005). In addition to all these, the N-protein binds to both genomic and sub-genomic mRNA and gets placed specifically over TRS (Grossoehme *et al*., 2009) and thus preferentially increases the recovery rate of infectious virus from transfected genome length synthetic RNA (Yount *et al*., 2002). From various studies, it has been found that the N-protein gets attached with replication transcription complexes in infected cells and thus causes the attachment of N-protein to them which requires the C-terminal N2b domain and thus helps to interact with other N-proteins (Verheije *et al*., 2010). This N-protein is quite well distinguishable from other structural proteins and is present in the nucleus of the infectious cells (Wurm *et al*., 2001).

Few available data have already claimed that N-protein has a prominent role in CNS pathogenesis and also for hepatitis. After observing the viruses that contain the N-genes have explained that the N-protein of A-59 causes increased neurovirulence and also increases the viral antigen spread within the brain (Cowley *et al*., 2010). It has been found that this N-protein is linked with microtubules and thus takes part in axonal transport and trafficking in neurons, and does not participate in spreading viral infection in an artificially prepared primary hippocampal neuronal cell (Cowley *et al*., 2010). It has been reported during investigation in 17Cl-1 cells, the N-protein acts as an antagonist of type-I interferon by inhibiting the activity of RNase L. In these 17Cl-1 cells, type-I INF does not get activated as the N-protein binds with RNA which causes hiding from deletion of recognized receptors and also induces type-I interferon (Ye *et al*., 2007). In a study, it has been found that N-protein is also likely to induce the fibrinogen-link protein 2 (fgl2), which acts as a procoagulant and immunosuppressant and thus causes drastic liver damage during the infectious case. During *in-vitro* studies conducted upon 293J cells, the over-expressed N-protein causes inhibition of production of IFN by blocking IRF3 and suppresses the expression of NF-kB responsive promoter (Kopecky-Bromberg *et al*., 2007).

The internal (I) protein is about 23kDa, containing hydrophobic structural protein of unknown functions that are not precise. This I-gene gets encoded inside the +1 N orf reading frame. During both *in-vivo* and *in-vitro* studies, no differences in replication have been found to take place in any of the systems-brain and liver, when compared between the negative recombinant I-gene of A59 virus with its isogenic control wild type. It has been found that loss of expression of I-protein leads to hepatitis, however, further investigations need to be done.

F. Replicase Proteins

The site of CoV replication has been demonstrated by pp1a and pp1ab that has been exercised into 16 non-structural proteins that can provide some essential functions- RNA-dependent RNA polymerases (RdRp). This RdRp acts as an enzyme that can alter the genomic 5' end with a methylated cap and also by some proteases for processing precursor proteins. There is another replicase protein that shows non-essential functions during the interaction between virus and host. These proteins are expressed as below:

1. **Nsp12 polymerase and Nsp8 primase:** Nsp12 is embedded within orf1b, featuring the translational frameshift domain orf1a/1b. It comprises the central action of RdRp, which is liable for amplification of the viral genome through a negative intermediate strand. It also causes transcription of several sub-genomic mRNAs which contain 5' termini, originating from genomic 5' end even *via* intermediate negative segment. Depending on frameworks the RdRp character has been noted previously. It has been found that the catalytic domain forms a three-dimensional model and with located conserved motifs which get shared by all RdRps (Xu *et al*., 2003). This behavior has been little defined as a consequence of protein expression difficulties. During the *in-vitro* study, the expression of nsp12 has been found in E. coli along with its ordinary N-terminus, showing primer-dependent activity on RNA substrates like poliovirus and Hepatitis C enzymes (TeVelthuis *et al*., 2010). It has also been found that Nsp8 provides the operation of RdRp, which recommends internal 5'-(G/U) CC-3' sequences to induce oligonucleotide replications for not more than six residues. Consequently, the nsp8 C-terminal end has similarities with the RNA viral polymeric catalytic palm domain. These studies indicate that nsp8 may represent a primase for the production of nsp12-dependent coronavirus RNA synthesis primers (Imbert *et al*., 2006).

2. **Nsp15 protein:** The CoV orf1b encodes the nsp15 protein which synthesizes the part of precursor polyprotein (pp1ab), it establishes 38kDa of nsp15 protein to get released by 3CLpro protease (Hegyi and Ziebuhr, 2002; Prentice *et al*.,

2004). This nsp15 has been found to contain a motif that suggests being familiar to Xenopus laevis U representing endonuclease, which helps the processing of small nucleolar RNA (Snijder *et al.*, 2003). Various studies claimed that true recombinant nsp15 contains an endo-ribosomal property which gets facilitated by the presence of divalent cations along with strong Mn^{2+} and thus focused over cleaving at uridine nucleotide in S or dsRNA substrates by cutting its 2'-3' cyclic phosphate end (Ivanov *et al*., 2004; Bhardwaj *et al*., 2004). It has been found that cleavage of enzyme takes place at 3' site of specified uridylate residues but it is well preferred by the U-site and has very little interaction towards the substrates containing C (Bhardwaj *et al*., 2006). After conducting the mutagenic studies, it has been found that the structure of nsp15 towards the Xenopus sequence claims to have two histidines and a lysin component that helps to conduct various functions of enzymes (Guarino *et al*., 2005). It has been found that the nsp15 structure containing catalytic residues of RNase A claims to conduct enzymatic-like mechanistic action (Bhardwaj *et al.*, 2008). After studying the nsp15 amino acid sequence of β-CoV it has been found to contain retinoblastoma (pRb)- binding domain which is present on the surface of protein near to the active site of Nendo U. During the *in-vitro* study when pRb gets attached with the recombinant nsp15, it has been found to stimulate the endonuclease activity, along with it the immunoprecipitated of two proteins has also been noted from cellular extracts. The nsp15 expression in cellular environments has been found to alter pRb cellular distribution from the cytoplasm and thus increases pRb ubiquitination with a decreased level of pRb. It has also been found to increase the cell fraction during the S-phase of the cell cycle and thereby increases the amount of 3T3 cell proliferation site. The growth phenotype has been found to get reduced by mutation of the LXCXE/D domain. From all this information, it has been clarified that nsp15 and pRb participate in cellular proliferation and affect CoV replication.

3. **Nsp13 protein:** The nsp13 is a 66kDa protein that comprises an N-terminal zinc finger configuration that is bound to a helicase motif of the C-terminal superfamily1. The α-CoV is of 229E protein containing a histidine-labeled version which gets expressed by vectors of baculovirus or by SARS-CoV nsp13 maltose-binding protein (MBP)- fusion (Ivanov and Ziebuhr, 2004). It has been found that the processed recombinant proteins contain unwinding functions of both RNA and DNA duplex 5' to 3'. This 5' to 3' direction is directly opposite to flaviviral helicase and may be found to cause the function of reflecting the multiple sub-genomic mRNAs synthesis and their templets of negative polarity. It has been found that glutathione S-transferase labeled CoV nsp13 (GST-nsp13) gets expressed with the help of recombinant baculovirus inside the cells and also clarifies the relaxes of nucleic acids 2 to 3 times more

frequently than other helicases (His tagged or MBP-fusion nsp13 protein). Many studies claimed that nsp13 complexes act more rapid relaxation of nucleic acid when it is with nsp12 than its alone state. Some studies claimed that the addition of triphosphatase activity along with helicase helps to process out the first step of genomic capping and for mRNAs.

4. **Nsp3 protein:** The orf1a of CoV encodes the nsp3 – multifunctional protein. The 18-200kDa of nsp3 contains multiple domains, two of which are the PLP domain and an ADP-ribosome 100 phosphate or ADRP (macrodomain) which are the cause of virulence factors. The mass spectroscopic, kinase-profiling studies, and bioinformatic analysis claimed that nsp3 contains a chaperone-like motif that encodes the PLP domain downstream and a cysteine-coordinated metal ion binding domain. These two domains are the RNA binding motif. It has been found that the PLP and its analogous (PLP2) have a deubiquitinating property along with protease activity and cause activation of type-I INF antagonism (Barretto *et al*., 2005; Zheng *et al*., 2008). It has been found that the PLP causes inhibition of IRF3 and NF-kB signaling pathways (Devaraj *et al*., 2007; Frieman *et al*., 2009).

   The macrodomains are highly conserved and have been ubiquitous property. It has been found that histone-associated macro H2A which regulates the cell type sensitive regulation/ pathway for transcription (Changolkar *et al*., 2008). The CoV has ADRP which contains the activity of phosphatase and thus converts ADP-ribose 100 phosphate to inorganic phosphate and ADP-ribose (Puticset al., 2005; Putics *et al*., 2006). It has been found that the mutation of the conserved residues of the ADRP domain causes enzymatic loss and decreases the induction of inflammatory cytokines (Eriksson *et al*., 2008). It has also been found that the ADRP domain restricts the type-I IFN treatment (Kuri *et al*., 2011). It has also been found that these macrodomains having a binding activity of mono- and poly- ADP-ribose which indicates the participation of ribosylation of host protein causes mediation of apoptosis or necrosis and thus monitors various host body pathways (Egloff *et al*., 2006).

5. **Nsp1 Protein:** The nsp1, the N-terminal protein present in the orf1a polyprotein (pp1a) which has been found to get cleaned by papain-like protease (PLP) from pp1a, is also confined in orf1a (nsp3). From immunofluorescence studies, it has been found that nsp1 gets co-recruited with some proteins that facilitate replication complexes at early time infection; whereas, during late infection, this protein binds with M-protein at viral budding and assembly site (Brockway *et al*., 2004). It has been found from various studies that this nsp1 protein is RNA binding protein that may act as a regulator for viral genome translation or replication. Cell cycle gets arrested at G_0/G_1 phases due to

infection, which also found to reduce the G_1 cyclin-Cdk complexes, activates Cdk, and inadequate phosphorylation of pRb (Chen and Makino, 2004). The nsp1 has been found to get expressed from plasmid vectors in normal cells, resulting in comparable cell arrest of G_0/G_1 phases, along with inactivation of cell proliferation (Chen *et al*., 2004). During studying the cell cycle regulatory protein, it has been found that expression of p28 causes hyperphosphorylation of pRb and thereby enhances the tumor suppressor levels- p53 and Cdk inhibitor (p21Cip1). This study suggests that expression of p28 can stabilize p53 and can increase the p53 level which can activate the p21Cip1 by inhibiting Cyclin E/ Cdk2 activity and as a result of which pRb phosphorylation gets inhibited with cell cycle arrest at G0/G1 phase. The external expression of the nsp1 also has been found to decrease cell proliferation with an increased level of cell accumulation in G_0/G_1 phases (Wathelet *et al*., 2007). The pathological role of nsp1 has also been found as it causes inhibition of type-I interferon synthesis and activates the interferon-dependent antiviral protein-ISG15 and ISG56 (Frieman *et al*., 2007; Wathelet *et al*., 2007; Kamitani *et al*., 2006). It has also been found that expression of nsp1 can inhibit three translational factors- IRF3, NF-kB, and c-Jun, which are found to activate the interferon-b promoter (Wathelet *et al.*, 2007). The biochemical studies have claimed that inhibition of recombinant nsp1 during *in-vitro* translation reaction are found to bound with 40S ribosomal subunit and inhibition of 80S ribosomal synthesis but allows the synthesis of 48S complex with mRNA (Kamitani *et al*., 2009).

6. **eIF4A:** Eukaryotic translation initiation factor 4 A (eIF4A) is an associate of the DEAD-box protein helices family, consisting of two recA-like domains separated by a flexible hinge region in the center lined by conserved motifs (Fig. **4**). This preserved motif is called the DEAD-box which contains amino acid sequences like aspartic acid glutamic acid- alanine- aspartic acid. The motif of eIF4A possibly interact with nucleic acid and participates in ATP binding and ATPase activity. As a consequence, eIF4A has been demonstrated to have RNA-dependent ATPase activity, ATP-dependent duplex RNA unwinding activity, and involvement in the initiation of translation. The action of eIF4A is synchronized with other translation initiation factors, that initiate its all activities and mediate interaction with RNA for translation (Hilbert *et al*., 2011). Further, the main functions of eIF4A include removal of secondary complex structures from the 5′-untranslated region and also to displace proteins attachment to mRNA for protein synthesis (Andreou and Klostermeier, 2013; Cencic *et al*., 2011).

   The eIF4A protein is a key factor involved in translation during viral infection. A study demonstrated that viral mRNA uses eIF4A for the synthesis of its

protein (Nakagawa *et al*., 2016). Genomic mRNAs of CoV have a 5-cap structure and undergo cap-dependent translation using eIF4F. The eIF4A is a part of the eIF4F protein complex which is associated with other two translation initiation factors such as eIF4E and eIF4G, in turn, connected with eIF4A which is further connected with eIF4E. In the cap-dependent mechanism of translation, the viral mRNA is engaged with the eIF4F protein complex, which is composed of three proteins: eIF4E, eIF4A, and eIF4G. The eIF4A and eIF4F are essential for the recruitment of ribosomes in protein synthesis during CoV infection. Consequently, eIF4A is important for controlling translation and for regulating gene expression at the translational level. Inhibition of eIF4A might play an important role in the treatment of CoV (Montero *et al*., 2019).

7. **Cyclophilin:** Cyclophilins (Cyps) are a subgroup of immunophilins belong to the enzyme peptidyl-prolyl cis/trans isomerases family. Totally 80 iso-forms of dissimilar molecular masses have been illustrated in human tissues. Among these isoforms, seven are major Cyps present in humans such as CypA, CypB, CypC, CypD, CypE, Cyp40, and CypNK. Cyps are present in both extracellular and intracellular space of the cell and secreted in response to various stimuli having different nature and intensity (Rajiv and Davis, 2018). The extracellular cyclophilins CypA and CypB are concerned with cell-to-cell communication. Cyps are contemplation to be concerned in different signaling pathways such as mitochondrial apoptosis, inflammation, RNA splicing, and adaptive immunity. Cyps bind to the CD147 cell membrane receptor as well as heparins and then initiate several arrays of intracellular signaling cascades which are involved in inflammatory processes (Singh *et al*., 2018). Besides, CypA is also competent in controlling human IFN-I reactions to viral infections (von Hahn and Ciesek, 2015).

   Moreover, CypA and CypB have significant functions in the duplication of many viruses including coronavirus (CoV), human immunodeficiency virus (HIV), hepatitis C virus (HCV), measles virus, and influenza A virus (Dawar *et al*., 2017). A study demonstrated that CypA is an essential cyclophilin that acts as binding factors for CoV proteins and is required for CoV replication (Tanaka *et al*., 2017). Another study conducted using surface plasmon resonance biosensor technology reported the interaction of CypA with nucleocapsid (N) protein of SARS-CoV. This statement gets verified by another technique which observed CypA AS one of the cellular proteins integrated into purified SARS-CoV particles by using spectrometric profiling (Carbajo-Lozoya *et al*., 2012; von Brunn *et al*., 2015). Furthermore, research using nucleocapsid protein (NP) of SARS-CoV shown that segment Val235-Pro369 of SARS NP interacts with human CypA (hCypA) more accurately, and SARS NP loop Trp302-Pro310 lock into the active-site groove of hCypA through hydrogen bonding indicate

human CypA (hCypA) binds NP of SARS-CoV with high affinity, resulting in CypA play important role in CoV replication and virus growth (Luo *et al*., 2004).

8. **3CLpro :** Genomes of CoVs comprise two exposed reading frames orf1a and orf1b, programmed by host ribosomes into two specific viral polyproteins-pp1a and pp1ab. The orf1a encrypts two cysteine proteases, a protease specific to papain (PLpro) and a protease specific to 3C (3CLpro). Although PLpro cuts the polyprotein's first three cleavage sites, 3CLpro is accountable for cleavage of the subsequent 11 positions culminating in a sum of 16 non-structural proteins (nsp) being released into CoVs. The viral key proteinase (Mpro, also known as 3CLpro) regulates the coronavirus duplication complex activities. 3CLpro is a homodimeric process effective in the company of substrates. Both 3CLpros crystal structures revealed that each monomer consists of three structural domains: domains I and II construct a chymotrypsin-like framework through a catalytic cysteine and are linked by a long loop to a third C-terminal domain (Needle *et al*., 2015). 3CLpro monomer contains three domains, domain I (residues 8–101), domain II (residues 102–184), and domain III (residues 201–303), and a large coil binds domain II and III (residues 185–200). The effective zone of 3CLpro seems to have a CysHis catalytic dyad (Cys145 and His41) found in the distance between domains I and II (Yang *et al*., 2003). At the proteolytic stage, both 3CLpros choose glutamine in positions P1 and leucine, simple residues, low hydrophobic residues in positions P2, P3, and P4 respectively (Chuck *et al*., 2011). Limited residues are expected at positions P1′ and P2′; however, position P3′ shows no clear preference. Recent studies report has reported that the structure of 3CLpro from SARS-CoV2 (Liu *et al*., 2020) (PDB code 6LU7) and the available structure of 3CLpro from CoV (Yang *et al*., 2003) (PDB code 1UK4), can only be able to differentiate through their two main proteases by only 12 amino acids, along with the α carbon atoms, which all are lying at least 1 nm away from the 3CLpro active site. The substrate-binding pockets of Covid-19 main proteases display an extraordinarily high level of alignment of the prime residues involved in substrate binding, including the CYS145···HIS41 dyad, and HIS163/HIS172/GLU166. The latter residues are supposed to offer the inaugural gate for the substrate in the active state of the protomer (Yang *et al*., 2003). The PLpro and 3CLpro process ORFs and concoct 16 non-structural proteins that are vital for membrane-associated duplication complex. The PLpro was detected to be multipurpose enzymes with deISGylating (deletion of ISG15 conjugates from host cell factors) and deubiquitinating (cleavage of ubiquitin from host cell factors) properties (Clasman *et al*., 2017). This subsidized to antagonization of the host antiviral immune response and the growth of viral duplication. PLpro can hamper the behavior of the IFN-β

reporter prompted by the mitochondrial antiviral signaling protein and dwindle the event of TNF-α-induced NF-kB reporter.

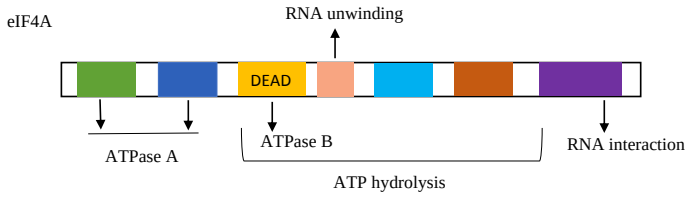


Fig. (4). Schematic representation of eIF4A.

G. CoV Associated Protein

The CoV genome is known to have encoded numerous associated proteins that have identified homologies with other host cell proteins. The CoVs associated proteins encodes within orf3a, 3b, 7a, 7b, 8a, 8b and 9b. It has been found that the encoded proteins of orf3b, 7b, and 8b are translational by the involvement of internal downstream initiation which encodes the same RNA for 3a, 7a, and 8a respectively. While investigating the viral particles, the proteins which encode in orf3a, 6, 7a, and 7b have been found (Narayanan *et al*., 2008). From *in-vitro* studies, it has been found that deletion of combined or individual orf3a, 3b, 6, 7a, or 7b in the recombinant viruses does not influence the replication. However, with the deletion of orf3a, some loss in efficiency has been found in viral replication (Yount *et al*., 2005). From various investigations, it has been found that the type-I interferon activation and signaling antagonizing capacity are influenced by orf3b and 6, whereas the orf3a and 7a also take place in apoptosis signaling pathways.

i. **Orf6:** From *in-vitro* studies and histological analysis of the patient's lungs and intestine, it has been found that orf6 contains 63-amino acid ER/Golgi membrane-associated protein (Narayanan *et al*., 2008). The protein which is encoded in orf6 has been found to cause some virulence factors (Tangudu *et al*., 2007). It has been found that this orf6 protein causes inhibition of nuclear imports and causes inactivation of interferon signaling by resisting the entry of ISGF3 (STAT1/STAT2/IRF-9). This ISGF3 is the transcriptional factor that umpires the illustration of the gene responsible for type-I interferon-stimulation or complexes of STAT1/STAT2. Near to the C-terminal end of orf6, it gets attached to karyopherin alpha2 (KPNA2) that places KPNB1, a nuclear importer complex, and thereby blocks the protein along with the help of import signals (Frieman *et al*., 2007; Hussain *et al*., 2008). It has been found that expression of this orf6 in absence of other viral proteins activates the construction of membranous structure which is very much alike to that of double-layered vesicles which takes part in viral replications and partially

placed with non-structural protein3 (nsp3), confirming the presence of orf6 role in viral replication (Zhou *et al*., 2010).

ii. **Orf3b:** During CoVs infection, it has been found that orf3b encoded proteins contain various functions (Chan *et al*., 2005). In *in-vitro* studies, it has been found that highly expressed orf3b proteins get localized initially over the nucleus of A549 cells and it causes inhibition of both interferons signaling and its activation (Vennema *et al*., 1996). From other studies, it has also been found that orf3b expression also activates cell growth arrest (Yuan *et al*., 2005) or also promotes apoptosis and necrosis (Khan *et al*., 2006).

iii. **Orf7a:** The orf7a is a 122 amino acid type-I transmembrane encoded protein, placed in the perinuclear part in SARS-infected cells (Nelson *et al*., 2005) by connecting with M and E protein (Huang *et al*., 2006). From various studies, various functions of orf7a have come into account which includes activation of apoptosis by caspase-dependent pathways (Tan *et al*., 2004), it can cause negative impulse for cellular protein synthesis, it has also been found to cause activation of p38 mitogen-activated protein kinase (Kopecky-Bromberg *et al*., 2006) and also arrests cell cycle at G_0/G_1 phases (Yuan *et al*., 2006).

Pathophysiology from a Cell Biology Perspective

CoV is the major health concern of 2020 with the everyday devastating scenario. Various studies had performed to study mortality of clinical disease but their cellular responses towards the viral disease are not known. The pathophysiology of CoV has been described in Figs. (**5A** and **B**). The basis of cell response towards infected CoV can be divided into some different clinical stages (Wu and McGoogan, 2020).

Phase I. Asymptomatic Stage (First 1-2 Days of Infection)

When the virus gets inhaled, it gets binds to epithelial cells in the respiratory tract and initiates replication. From various investigations, it has been that ACE2 is the main receptor Covid-19 (Wan *et al*., 2020; Hoffmann *et al*., 2020). During *in-vitro* studies, it has been found that ciliated cells get infected first in conducting airways (*Sims et al*., 2005). Again, it has also been found that scRNA points out the lowered expression of ACE2 in conducting airway cells (Reyfman *et al*., 2019). In this situation, there causes native diffusion of the virus but does not show any huge intrinsic immune response. In this phase, the virus is well spotted or identified by using a nasal swab. From the data generated by RT-PCR for viral RNA, it can be easily predicted that the viral load and causes infection with clinical course.

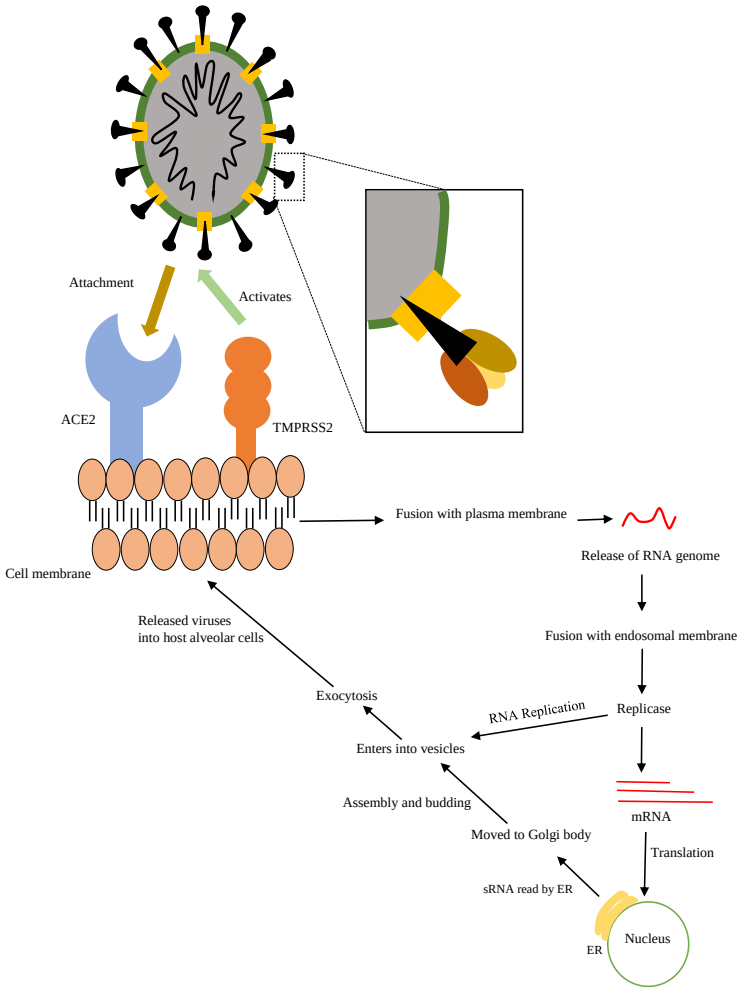


Fig. (5A). Transmission and Pathophysiology mechanism of Coronavirus inside the cell membrane.

Phase II. Upper Airway and Conducting Airway Response (Next Few Days)

In this stage, the virus grows and travels down into the respiratory tract along the conducting airways and thus triggers the initiation of immune responses. From nasal swab or sputum test can conclude the presence of the virus or by preliminary immune markers can be also used for its presence. It has been found that level of intrinsic cytokine response, CXCL-10 can cause clinical course (Tang *et al*., 2005). The epithelial cells which get infected form the main source of beta and lambda interferons (Hancock *et al*., 2018). It has also been found that the CXCL-10, an interferon responsive gene shows a strong signal towards the noise ratio that is formed in the alveolar type II cells during CoVs and influenza (Qian *et al*., 2013; Wang *et al*., 2011). It has been found that about 80% of

infected patients show mild and upper and conducting airways infection (Wu and McGoogan, 2020). These individuals need to be home quarantine.

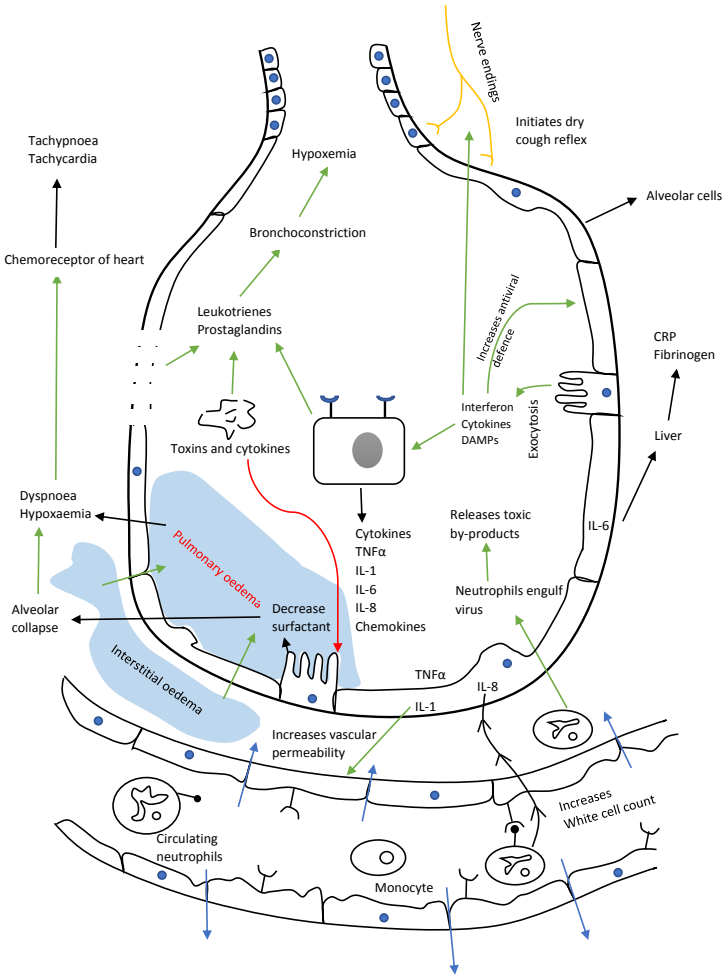


Fig. (5B). Pathophysiology of Coronavirus outside the cell membrane. The green arrow signifies activation/ enhancement and the red arrow signifies inhibition/ negative impact; the blue arrow shows permeability.

Phase III. Hypoxia, Ground-glass Infiltrates, Progression to ARDs

It has been noted that about 20% of the infected patients come to this phase and as a symptom, they show pulmonary infiltrates and sometimes develop severe illness. In this case, the virus reaches the lungs and infects the type-II alveolar cells. It has been noted that type II alveolar cells are more preferred than type I in case of CoV infection (Mossel *et al.*, 2008; Weinheimer *et al.*, 2012). The alveolar units which are infected are inclined to be peripheral and subpleural (Wu

et al., 2019; Zhang *et al*., 2020). The CoVs gradually increase into the type II alveolar cells and the release of huge viral particles causes activation of apoptosis followed by cell death (Qian *et al*., 2013). Near to the end, a self-replicating pulmonary toxin gets released as the type II cells get infected and gradually lose all these cells, initiating the secondary pathways for the regeneration of epithelial cells. It has been found that diffused alveolar damage favored by fibrin enriched hyaline membranes and the presence of few poly-nucleated giant cells are the pathological reason behind CoV (Gu and Korteweg 2007; Xu *et al*., 2020). The abnormal healing of wounds causes more severity due to the formation of scaring and fibrosis; their recovery may employ inherent or acquired immune responses for the regeneration of epithelial cells. It is harmful to administrating the epithelial growth factors, KGF, and may also enhance the ACE2 expressing cells (Nikolaidis *et al*., 2017). The lowering of immune response with an increase in age is the reason for mortality for elderly individuals and thereby decreases the capability of epithelial damage repair. As age increases the mucociliary clearance gets slowly diminished, allowing the virus to spread more vigorously into the lungs (Ho *et al*., 2011). It has been found that CD209L acts as an alternative receptor for CoV (Jeffers *et al*., 2004), and the apical cilia and microvilli on airway cells and type II cells, respectively are now found to have an important role in viral entry.

Various histopathological studies and from postmortem reports it has been found that death is mostly due to respiratory failures inpatients and at the same time it has been surprisingly found that early and progressive stages of blood clotting supply and gaseous exchange. Although the exact reason for blood clotting has not yet been excavated some mechanisms support this incidence. There are in total three debatable points for blood clotting which are as- in many patients of Covid-19 it has been found that there is an elevated level of d-dimer, a marker of thrombosis. Again, in some studies, evidence has been found that this extensive microvascular thrombosis has been observed during autopsy studies. In this study, extensive blood clotting has been observed in small lung vessels (microvascular thrombosis-MVT) which also suggests blood clotting with poor oxygenation and respiratory failure. Another point has also been found that the ACE2 receptor of the host facilitates the entry of the virus into the endothelial cells where the blood vessels lie. Autopsy studies suggest that specific contamination into cells can activate the clotting in the small blood vessels that form unresponsive respiratory failure. Various clinical reports suggest that many patients suffer from 'silent pneumonia' or 'silent hypoxia' which is due to low blood oxygen which can lead to extensive pulmonary thrombosis.

CONCLUSION

The CoVs infection is one of the major health risk factors of the current destructive outbreak worldwide. These CoVs are very well adapted human viruses in humans. The transmission of CoVs has caused a serious pandemic situation. The CoV is a single-stranded positive RNA virus that utilizes the host viral proteins and cellular components for completion of its replication cycle- viral entry, replication. Throughout the world, researchers are working day and night for the development of the vaccine. Several pathological, viral mechanisms of action, various biomarkers need to be excavated. Several series of small molecular CoVs endosomal proteins need to be discussed for a better understanding of the pathophysiology of CoV.

LIST OF ABBREVIATIONS

SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
CoV	Coronavirus
M	Membrane
S	Spike
N	Nucleocapsid
E	Envelope
PLpro	Papain-like protease
3CLpro	3C-like protease
RdRp	RNA-dependent RNA polymerase
ExoN	Exonuclease
poly U- XendoU	Specific endoribonuclease
RBD	Receptor binding domain
HR	Heptad repeat
RBM	Receptor-binding motif
ACE2	Angiotensin converting enzyme 2
TMPRSS2	Transmembrane protease/ subfamily of serine membrane 2
IL-8	interleukin-8
TRS	Transcriptional regulatory sequences
E1	Envelop-1
ERGIC	ER-Golgi intermediate compartment
VLP	Virus like particles
PBMCs	Peripheral blood mononuclear cells
I	Internal protein

pp1ab	Precursor polyprotein
MBP	Maltose binding protein
pp1a	Polyprotein
eIF4A	Eukaryotic translation initiation factor 4 A
Cyps	Cyclophilins
HIV	Human immunodeficiency virus
HCV	Hepatitis C virus
hCypA	Human CypA
nsp	Non-structural proteins
KPNA2	Karyopherin alpha2

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 5

Clinical Presentation and Comorbidities

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Abstract: Presently, the whole world is going through a historic yet a troublesome situation following COVID-19 outbreak. The clinicians have observed a wide variety of respiratory and non-respiratory clinical manifestations in COVID-19 patients. Accumulating reports revealed that the clinical features of COVID-19 may include asymptomatic/mild symptoms, neurological, cardiovascular complications, severe pneumonia and mortality. The most common features noticed are fever, dry cough, sore throat, shortness of breath, sputum production, fatigue, and myalgia. Recently, US health protection agency has also reported repeated shaking along with chills, loss of taste and smell in new case studies as additional symptoms. In addition to this, COVID-19 patients may show clinical signs like persistent pressure and pain in the chest, blue lips or face, confusion and GIT disturbances (diarrhea, nausea, vomiting and abdominal discomfort). The current ongoing pandemic has remarkably affected almost every age group of humans, starting from infants less than 3 months, adults, elder and older patients. Furthermore, the clinical presentations in these groups of COVID-19 infected patients were found to show considerable inter-individual variations. The findings also suggested that the comorbid conditions (heart injury, hyperglycemia, hypertension, neurodegenerative diseases) in elder/older patients further complicate the health of COVID-19 patients.

In the present book chapter, the clinical presentation of COVID-19 in pediatric, adults and geriatric group of population will be emphasized along with the higher susceptibility of COVID-19 in comorbid patients.

Keywords: ACE-2, Adolescents, Anosmia, Asymptomatic, Atypical symptoms, Comorbid, Comorbidity, Consolidations, Cutaneous, Dyspnea, Fatigue, Fever, Gastrointestinal, Ground glass opacities, Immunity, Incubation period, Neonates, Neurological, Pneumonia, Respiratory distress, Respiratory symptoms, SARS-CoV-2, Septic shock, Spikes, Vaccination.

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INTRODUCTION

At the beginning of the COVID-19 outbreak, the clinicians have encountered a substantial diversity in the clinical presentations, such as different incubation periods among different age groups, noticeable variations in the onset of symptoms and degree of severity (moderate, serious and critical). Besides the most common respiratory symptoms, an array of complications has been found to be associated with Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection. Moreover, asymptomatic (transmit virus but never have symptoms) and presymptomatic (transmit virus and symptoms appear later) transmissions have also raised concerns. The present chapter elaborates the typical and atypical clinical features discerned in original clinical cases of COVID-19 patients. Besides, the factors (age, Angiotensin-Converting Enzyme 2 (ACE2), gender, blood group, previous immunization, comorbidity) responsible for the high inter-individual variations of clinical outcomes have also been discussed in detail.

CLINICAL PRESENTATIONS OF COVID-19 INFECTION

In COVID-19 patients, it is found that after exposure, the virus takes an incubation period of 2-14 days. According to the findings of clinical cases, the COVID-19 infection starts showing its mild symptoms after a median incubation time period of approximately 5.1 days (McIntosh, 2020). However, Lauer and his coworker estimated that 97.5% of COVID-19 infected patients presented severe symptoms by 11.5 days (Lauer *et al.,* 2020). The diverse respiratory and non-respiratory symptoms observed to date are compiled in Table **1**.

Table 1. Respiratory and non-respiratory symptoms of COVID-19.

Respiratory Symptoms (Hassan *et al.,* 2020; Cascella *et al.,* 2020; Wang *et al.,* 2020)			
Mild Cases • Infection resides only in upper respiratory tract. • Fever, nasal congestion, dry cough, headache, sore throat and malaise.	**Moderate Cases** • Respiratory manifestations include cough, shortness of breath, and tachypnea.	**Severe Cases** • Patients are reported to have severe pneumonia. • Acute Respiratory Distress Syndrome (ARDS), PaO_2/FiO_2< 300, SpO_2 ≤93%, tachypnea, severe dyspnea. • Fever can be absent or moderate.	**Acute Respiratory Distress Syndrome** • It indicates worsening/failure of the respiratory system. • ARDS can be of different types on the basis of values of PaO_2/FiO_2. Severe (≤ 100 mmHg). Moderate (100-200 mmHg). Mild (200-300 mmHg).

(Table 1) cont.....

• Dyspnea is absent. • Radiographic manifestations are not present. • Most of the cases have been found to be mild. • Mild cases may deteriorate into severe cases if precautions will not be acquired.	• Severe symptoms are absent.	• In critical cases, cardiac injury, RNAaemia, respiratory failure or multiple organ dysfunction have also been found. • Comorbidities such as hypertension, diabetes, cancer, cardiovascular problems further increase the fatality rate in severe cases of COVID infection.	Its different values indicate different degrees of hypoxia. • Deterioration of ARDS can also be correlated with altered AST (aspartate transaminase) and ALT (alanine transaminase) levels. • Ground Glass Opacity (GGO) (86%), bilateral (76%), peripheral (33%) distribution, consolidation (29%), crazy paving (19%) are the prominent features found in computed tomography (CT) scan.
Non-Respiratory Symptoms (unusual Manifestations)			
Neurological	Altered mental state, musculoskeletal disturbance, acute necrotising encephalopathy ischaemic stroke, headache, dizziness, Guillain-Barre syndrome.		
Ocular	• In China, 32% of infected patients showed ocular manifestations, for example, chemosis and conjunctival hyperaemia. • Tears and conjunctival seepage have shown the presence of COVID-19. • In severe COVID-19 cases, pseudomembranous and hemorrhagic conjunctivitis was also found. • Development of ptechiae, tarsal pseudomembranous, mucous filaments in 63-year-old COVID-19 positive male. • External ocular infections could occur lately due to the spread of infection and physicians should take care if ocular complications exist for >2 weeks in COVID-19 patients (Navel, Chiambaretta and Dutheil, 2020).		
Taste and Smell	• Olfactory dysfunction (Anosmia) is typically found to be present only in the most severe cases. • Bilateral obstruction in olfactory clefts due to inflammation; however no abnormalities were found in olfactory bulbs and tracts (Giacomelli *et al.,* 2020; Temmel *et al.,* 2004; Eliezer *et al.,* 2020). • Anosmia may persist with or without dysgeusia and manifest itself either in the early stage of progression or in patients with mild symptoms (Xydakis *et al.,* 2020; Carrillo-Larco *et al.,* 2020).		
Cardiovascular	Heart failure, cardiac arrhythmias, pacemaker conduction defects, infections such as myocarditis and myopericarditis, chest pain (Yang *et al.,* 2020; Bonow *et al.,* 2020; Inciardi *et al.,* 2020; Driggin *et al.,* 2020; Wang *et al.,* 2020; Zhou *et al.,* 2020).		
Hematological Symptoms	Hypercoagulable state in COVID-19 patients increased the threat of thrombotic occlusion		
	Mediastinal lymphadenopathy was also found in patients with a severe form of COVID-19 (Valette *et al.,* 2020)		

(Table 1) cont.....

Cutaneous Manifestations	• Conjunctival hyperaemia and chemosis were disclosed in SARS-CoV-2 patients (Wu *et al.,* 2020). • Development of an erythematous, confluent, non-pruritic macula papular rash which started initially in the neck and trunk and spread to cheeks, palms, upper and lower extremities (Morey-Olive, 2020) • Low-grade fever accompanied by acute urticaria, rashes on face, upper extremities, trunk and lower extremities • COVID-19 infection induced chilblains (Kolivras *et al.,* 2020; Romani *et al.,* 2020).
Otolaryngological	Otitis media, tinnitus (Fidan *et al.,* 2020)

INTER-INDIVIDUAL VARIATIONS IN CLINICAL PRESENTATIONS DUE TO DIFFERENTIAL SUSCEPTIBILITY TOWARDS COVID-19

Patients with COVID-19 present a range of clinical presentations. Some are silent carriers (asymptomatic), few have only fever or regular cold, some experience reduced sense of taste and smell, while others need to get hospitalized and need the assistance of a ventilator to breathe. Such a mysterious wide range of clinical manifestations has forced the clinical researchers to find out the reasons behind the differential susceptibility in humans. Various risk factors can be discussed which may be responsible for the occurrence of a range of mild to severe symptoms. Some of these factors are described below:

Age: According to CDC reports, 14-21% and 31-59% of patients were in the age range of 20-44 and 75-84 years, respectively. It is important to discuss the clinical outcomes in individual groups such as neonates/infants, children, adults, elderly and older patients (Chen *et al.,* 2020, Liu *et al.,* 2020). Studies have revealed that children and young generation have mild symptoms with good prognosis as compared to middle-aged and elderly COVID-19 patients. The major outcomes from clinical cases in different age groups are discussed in the below sections:

Neonates or Newborns (Upto 1 Month); Infants (1 Month-2 Years), Children (2-10 Years)

Several reports have been put forth which showed that COVID-19 has not even spared the population of neonate group. During the initial stages of infection, the neonates were found to be asymptomatic, having low fever and normal chest imaging and may show only mild gastrointestinal symptoms. However, later on, these may progress to severe conditions (if having one or multiple comorbid conditions) (Yu and Chen, 2020). The innate immune system of children may be responsible for the lower susceptibility to COVID-19. The infant acquires the maternal antibodies through feeding, which the mother develops after exposure to

infection (Carsetti *et al.,* 2020). However, a significant number of findings revealed that infants under the age of one year might have a higher risk of severe illness with COVID-19. This can be mainly due to their immature immune systems and smaller airways, which make them more likely to develop breathing issues with respiratory virus infections. Newborns can become infected with COVID-19 during childbirth or by exposure to sick caregivers after delivery (Sun *et al.,* 2020; Hagmann, 2020). Thus, it is advised by the American Academy of Pediatrics to temporarily separate the newborn from the mother with special care and monitoring of both for symptoms of infection (Amatya *et al.,* 2020).

Various pathogens have already been reported to transmit contagious diseases in the fetus from the mother *via* direct contact during delivery, across the placenta or during breastfeeding. The RT-PCR test conducted on ten neonates delivered to mothers with COVID-19 suggested a tendency of coronaviruses (CoVs) to undergo vertical transmission. The pharyngeal swab samples showed negative results from 1-9 days after birth. Likewise, similar negative results were reported in the samples of neonatal throat swab, breast milk, amniotic fluid and cord blood samples from six COVID-19 infected pregnant women. However, a positive case report of a newborn (30 h after birth) born to a confirmed COVID-19 mother on Feb 5, 2020 raised the concerns of vertical transmission among obstetricians and health agencies. There are mixed reports of transmission. Hence extensive studies are required to investigate the chances of horizontal or vertical transmission *via* contact through the birth canal and breastfeeding (Peng *et al.,* 2020).

Table **2** contains major clinical cases of newborns, infants and children across the world affected by COVID-19. A collective study of these clinical cases from all over the world explains the asymptomatic picture of COVID-19 in newborns, infants and children. A deep molecular level understanding is required to strengthen these facts. The role of the immune system, acquired immunity through vaccination and level of expression of ACE2 in this group of population is warranted to develop hope for future.

Table 2. Clinical case reports of COVID-19 in new borns, infants and children.

S.no.	Patients	Clinical Symptoms/Manifestations	Salient Point of Recovery Period	References
1.	Female neonate born after 35+3weeks of the gestation period	• Baby's mother was feeling fatigued, fever and shortness of breath.	• After seven days of persisting symptoms in the mother, the baby was delivered by cesarean section.	Peng *et al.,* 2020

(Table 2) cont.....

S.no.	Patients	Clinical Symptoms/Manifestations	Salient Point of Recovery Period	References
		• Multiple patchy nodular opacities and GGO in sub-pleural spaces was revealed through the chest CT scan. • RT-PCR testing of throat swab showed the presence of SARS-CoV-2.	• After birth, the baby presented moaning and periodic breathing and tachypnea. • Baby's chest X-ray demonstrated bilateral lung volume reduction, thus she was given pulmonary surfactant. Test for COVID-19 was performed on different swabs samples such as of throat, anal, serum, and urine. • Each time the test came out to be negative upto 14 days. • As a result, the baby was discharged after 14 days of observation in the hospital. • The virus was also not spotted in amniotic fluid, breast milk, serum, anal swab, placenta, cord blood. • This case report confirmed the unlikeliness of transmission of coronavirus to fetus from mother during pregnancy.	Peng *et al.,* 2020

(Table 2) cont.....

S.no.	Patients	Clinical Symptoms/Manifestations	Salient Point of Recovery Period	References
2	Female neonate born after a gestation period of 38 + 4 weeks	• Baby's mother was reported to have high fever with severe bilateral pneumonia. • Baby's father was found to exhibit fever and gastroenteritis since the birth of his baby girl. • RT-PCR test for COVID-19 was found to be positive in both mother and father. • Initially, the baby girl was negative but later on became positive (8 days after birth).	• The baby girl was monitored carefully and was detected with intermittent hyperpnoea, oxygen desaturation during deep sleep and feeding. • GGO in the right peripheral region was visualized by a chest radiograph. • The symptoms resolved after 24 hours, and remained asymptomatic even 13 days after birth. • RT-PCR test continued to be positive • The clinicians attributed the appearance of COVID-19 in newborn to the transmission of virus by direct contact with mother.	Diaz *et al.,* 2020
3.	26 days old male infant	• The infant experienced 2 paroxysmal episodes- upward rolling of the eyes, hypertonia and facial cyanosis during sleep. • In the hospital, he did not present any abnormal movements. • However, fever, nasal discharge and vomiting was noted. • He was breastfed continuously, normal bowel activity, no history of gastroesophageal reflux.	• In the hospital, the infant was found to have fever and watery stools. • All other viral antigens tests were found to be negative, however, tested positive for COVID-19. • The male infant was isolated under strict rules in a negative pressure room and visits of outsiders were restricted. • He showed favourable outcomes and was discharged with advice to maintain isolation and a strict follow-up.	Chacon-Aguilar, 2020

(Table 2) cont.....

S.no.	Patients	Clinical Symptoms/Manifestations	Salient Point of Recovery Period	References
4.	1 month old male having Cystic fibrosis (CF)	• Male infant was detected positive for COVID-19, however, he remained asymptomatic. • Later on, it was revealed that he was in close contact with his grandfather, who was also detected with SARS-CoV-2 infection. • He was isolated from his family members and remained asymptomatic, no fever or any other signs.	First case reporting the occurrence of COVID-19 infection in a child with CF; baby did not present any respiratory illness and was asymptomatic.	Poli and Timpano, 2020
5.	7-week-old baby	• The patient was showing symptoms of upper respiratory tract infection. • Diagnosis of nasal and pharyngeal swabs was made using different assays.	• Immunofluorescence assay and nucleic acid amplification assays did not detect any virus in the nasopharyngeal aspirate. • Only cell cultures of nasal or pharyngeal samples detected SARS-CoV-2 virus. • The baby did not demonstrate any worsening of respiratory symptoms and thus discharged after 3 days from the hospital.	Calderaro *et al.,* 2020

(Table 2) cont.....

S.no.	Patients	Clinical Symptoms/Manifestations	Salient Point of Recovery Period	References
6.	3-month-old female patient	• The infant was presented to emergency with rhinorrhea and nasal congestion. • No cough, fever, diarrhea, vomiting, wheezing, or dyspnea was detected • On hospital day (HD) 3, she was reported with fever. • Nasopharyngeal swab samples revealed the presence of SARS-CoV-2.	• Chest radiographs were normal. • No dyspnea, normal breath sounds without rhonchi and crepitation • Heart rate, cardiac ultrasound was normal • Hematology reports for total white blood cell, neutrophil and lymphocyte count were also normal • The infant was put on azithromycin for 5 days • Her condition remained stable and the RT-PCR test was found to be negative after 8 and 11 days • This case represents mild upper respiratory symptoms upon COVID-19 infection and was attributed to its secondary transmission from a close family member • The infection even did not transmit to her mother despite close contacts	Le *et al.,* 2020

(Table 2) cont.....

S.no.	Patients	Clinical Symptoms/Manifestations	Salient Point of Recovery Period	References
7.	5-month-old boy (history of cardiac arrest and type I-Hurler syndrome)	• Patient was experiencing irritability, cough, low-grade fever, vomiting, runny nose for the last 24 hours • Also presented with bibasal pulmonary subcrackles, pallor and respiratory distress • Biventricular hypertrophy and cardiomegaly without consolidations were observed in chest X-ray • CT scan and echocardiography showed dilatation of left ventricle and dysfunctioning of the left ventricle • RT-PCR test was positive	• After 3 days in the hospital, the infant was presented with high fever, respiratory distress, and extensive symmetric parahilar consolidations in pericardiac region and the left side of the pulmonary base was observed • A hyperinflammation state was found 3 to 4 days after the first onset of symptoms • No lymphopenia • He was on hydroxychloroquine, ceftriaxone and and remdesivir; however, the patient had a sudden cardiac arrest • In the intensive care unit, the infant encountered a second cardiac arrest, which proved fatal. • Heart failure or cardiac myopathies in SARS-Co--2 infection may worsen the conditions	Assaad *et al.,* 2020
8.	27 week old male infant	• At 35 weeks of age, the infant was presented with sneezing, dyspnea and poor feeding for the last two days • He was assessed to have septic shock, respiratory failure, severe lactic acidosis • Bilateral air space opacification was visible in chest X-ray	• Antimicrobial treatment including cefotaxime, amoxicillin, clarithromycin, gentamicin was prescribed • Antiviral remdesivir was prescribed intravenously • This is the first report of COVID-19 infection in the premature infant which developed multiple organ injury and altered status of inflammatory markers	Cook *et al.,* 2020

(Table 2) cont.....

S.no.	Patients	Clinical Symptoms/Manifestations	Salient Point of Recovery Period	References
9.	8-month-old male patient having complex hydrocephalus (shunt malfunctioning)	• The infant was having mild temperature, dry cough, and occipital cerebrospinal fluid collection (shunt malfunctioning). • Repeated vomiting. • Shunt disconnection was also evident in the head CT scan • Interstitial pneumonia was not present in chest X-ray • However, the test of SARS-CoV-2 was positive • Later on the infant started showing upper respiratory symptoms	• After four days of surgery, vomiting worsened and it was followed by second neurosurgical surgery of the shunt • During the second surgery, no respiratory complications were reported and the baby was extubated • In this case report, it was observed that major surgeries could be performed in babies and children who are paucisymptomatic with COVID-19	Carraba *et al.,* 2020
10.	4-year-old girl; was facing unilateral nasal obstruction for one week (patient was reported to place a green sponge into the right nasal cavity during bath time)	• Swelling and mild oozing observed in the anterior nasal cavity and right nare, respectively • In the following week, an increase in sneezing, mucous drainage, foul smell and nasal obstruction was noticed • Fragments of the green sponge were removed with forceps • SARS-CoV-2 was detected through the RT-PCR test	• After one week, the infant and her mother remained asymptomatic. • First report of an asymptomatic child who had undergone surgery in the upper airway	Diercks *et al.,* 2020
11.	55-month-old girl (undergone liver transplantation and coinfection with Epstein-Barr virus (EBV)	• COVID-19 infection was confirmed 5 months after the child had undergone liver transplantation • Was having rhinitis, fever, cough, and polypnea • Liver function tests were deteriorated; Signs of anicteric cholestasis and cytolysis were also found	• The patient recovered from COVID infection despite intake of tacrolimus (immunosuppressant; 8.8 ng/mL) • Nasopharyngeal swab samples became negative after 11 days • Authors suggested that worsening of COVID-19 was not associated with liver transplantation • EBV and COVID-19 co-infections have not been found to aggravate the clinical outcome.	Morand *et al.,* 2020

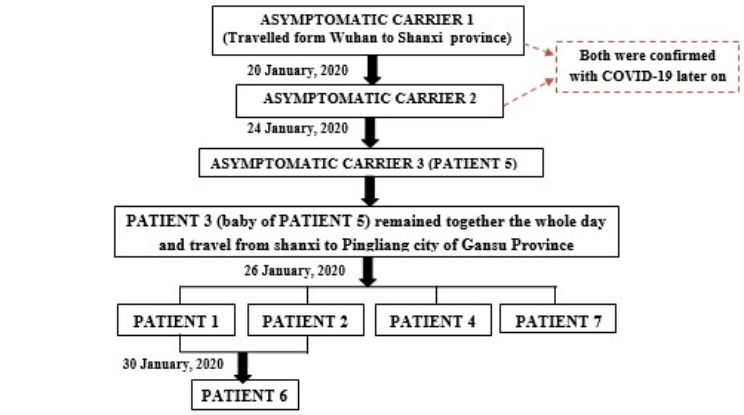
(Table 2) cont.....

S.no.	Patients	Clinical Symptoms/Manifestations	Salient Point of Recovery Period	References
12.	9-year-old male patient	• He was presented to emergency with continuous fever (38.9°C) for 3 days • There were no signs of coughing, shivering, sore throat, chest distress, abdominal pain, headaches, muscle aches and cyanosis • Chest CT scan was found to be normal, however, his SARS-CoV-2 nucleic acids test was found to be positive	• Clinical manifestations were not severe. • However, proper measures were taken for isolation to prevent further transmission of infection	Yin *et al.,* 2020

Adolescent (11-19 Years), Young (20-35 Years) and Middle Aged (36-59 Years) Patients

Liao *et al*. studied the demographic, epidemiological data from 46 COVID-19 patients in the age range of 10-35 years admitted in Chongqing Three Gorges Central Hospital. Out of 46 patients, young adults constituted a major fraction (69.56%) while adolescents comprised only 30.43% (Liao *et al.,* 2020). The data from this study suggested that no severe cases have been reported in adolescent group of patients except a few with comorbid conditions. Only 6.3% adolescent patients have been shown to be asymptomatic in comparison to 14.3% of young adults. In another study, only 7 adolescent patients showed GGO on chest CT as compared to 22 young adults. Besides, significant differences have been found in various biochemical indicators such as C-reactive protein, D-dimer, lactate dehydrogenase, alanine aminotransferase and total bilirubin with higher values in young adult patients as compared to adolescents (Liao *et al.,* 2020). The incubation period of COVID-19 has also noticed to vary in adolescents, young adults and older patients. A longer incubation period is reported to be associated with adolescents, young adults as compared to older patients. Unlike newborns and infants, adolescent and young adults tend to transmit the disease to their family relations very frequently. Huang and coworkers have recorded seven family-clustered cases of COVID-19 originating from asymptomatic patients. It was observed that transmission mainly occurred through close contact among patients without any travel history (Fig. **1**). Furthermore, the onset of symptoms varied among all the patients excluding patient 4 and 5 who remained asymptomatic during the study. On the other hand, CT findings were discovered within one week of onset of symptoms in 4 patients. However, commonly found CT findings consistent with COVID-19 pneumonia were also visible in asymptomatic patients (Fig. **1**). CT imaging manifestations include GGO with

consolidation, multilobar involvement and crazy paving signs. The study also emphasized the importance of personalized CT screening at initial stages, which may manage the further spread of infection (Huang *et al.,* 2020).



Patient number	Age (year)/ Gender (M-male;F-female)	Onset of symptoms after exposure (days)	Positive CT findings after exposure/onset (days)	Symptoms
1	29/M	4	11/7	Fever, cough, headache, limb joint pain
2	27/F	6	8/2	Fever, cough, fatigue
3	1/F	9	15/6	Diarrhoea
4	55/F	-	12/-	Asymptomatic
5	33/F	-	15/-	Asymptomatic
6	54/M	10	9/-	Shortness of breath
7	35/F	13	15/2	Cough, pharyngeal itching

Fig. (1). Family-cluster cases of 7 COVID-19 positive patients. Figure elaborates the travel history, close contact among 7 patients, clinical characteristics and correlation between onset of symptoms and initial CT imaging features.

During the initial period of pandemic, most of the clinical reports indicated that higher number of patients were from the middle aged population with a mean incubation period and a serial interval incubation of 5.2 (range 0–14 days) and 7.5 days (2–17 days), respectively. In China, the proportion of confirmed cases involving younger patients was higher. According to the reports of Chinese CDC, approximately 4,168 confirmed COVID-19 patients with an age less than 30 years were reported till February 11, 2020. Similarly, younger individuals also constitute a major proportion of COVID-19 carriers in Italy, Japan and South Korea. The overseas travelling for study, business and work has been considered as the reason behind this hike in younger generation COVID cases (Liao *et al.,* 2020). The examples of clinical cases with atypical manifestation or presentation

encountered till date have been discussed in Table **3**. The clinical cases described the development of unknown pathologies along with COVID-19 or exacerbation of patient's conditions due to concomitant co-morbid states. The studies revealed that COVID-19 may also manifest neurological, cardiovascular, cutaneous, ocular, hematological and otolaryngological symptoms in addition to common pulmonary manifestations.

Table 3. Salient clinical manifestations documented in clinical cases of COVID-19 patients (Young and middle aged patients).

S. No.	Patients	Comorbid Condition	Clinical Symptoms/ Manifestations	Outcome From Study	References
1.	21-year-old male	Severe aplastic anaemia	• Pancytopenia was observed • Multiple intravenous transfusions of stem cells were done to establish haematopoietic function • He was presented with petechiae (red or purple dots on the skin) and epistaxis (Nose bleeding) • Apheresis platelets were transfused from a healthy donor to patient • It was later on intimated that the platelet donor was detected with COVID-19 infection • However, platelet donor and recipient both remained negative for SARS-CoV-2	COVID-19 patients should avoid donating blood in their recovery period; wait for about 28 days after resolution of symptoms or completion of therapy	Cho *et al.,* 2020
2.	21-year-old male	Dependent on cocaine and methamphetamine with a smoking habit	• Fever, dyspnea, dry cough, fatigue, myalgia and diarrhea • No chills, nausea, vomiting, abdominal and chest pain • Positive test for parainfluenza virus • GGO was observed • Dyspnea worsened • SARS-CoV-2 was tested positive	Simultaneous infection of parainfluenza and COVID-19 virus can cause severe pneumonia which can further deteriorate to ARDS	Rodriguez *et al.,* 2020

(Table 3) cont.....

S. No.	Patients	Comorbid Condition	Clinical Symptoms/ Manifestations	Outcome From Study	References
3.	29-year-old male	Tricuspid atresia	• Fevers, fatigue, cough and shortness of breath • Was in close contact with family member having COVID-19 • No reports of gastrointestinal or neurological symptoms, anosmia and taste disturbances • RT-PCR test was positive for COVID-19 • Chest X-ray revealed bilateral patch opacities	Hypoxic conditions developed after COVID-19 infection can complicate the case in adults with a congenital heart defect	Ahluwalia *et al.,* 2020
4.	29-year-old	Obesity (BMI: 42 kg/m^2)	• Patient was suffering from fever, myalgia, non-productive cough, sore throat, malaise, respiratory distress, mild peripheral cyanosis from the past 8 days • Bilateral fine crackles was evidenced in lungs while consolidation was not present • SARS CoV-2 was tested positive again after 5 days • Bilateral, multifocal airspace opacities were observed in chest CT • EKG showed sinus tachycardia • Cardiac marker-creatine kinase-myocardial band initially increased and normalized after 3 days, however, cardiac troponin I, and myoglobin remained normal	COVID-19 infection increased the chances of occurrence of cardiac arrhythmias and failure	Loghin *et al*, 2020

(Table 3) cont.....

S. No.	Patients	Comorbid Condition	Clinical Symptoms/ Manifestations	Outcome From Study	References
5.	29-year-old woman	-	• Patient was experiencing fever, headaches, cough, shortness of breath and developed seizures in past few days • Neurologic exam notified decreased blink, global aphasia, facial palsy and spontaneous movement against gravity • Temporoparietal hemorrhagic venous infarct and thrombosis was observed in sinus by non-contrast head CT • Microcytic anemia was also detected (hemoglobin-5.5 g/dL) • The signals of MRI brain showed the presence of hemorrhagic infarct in different regions • Diplopia was complained • Bilateral 6th nerve palsies and papilledema was also detected	The study emphasized that young individuals are more prone to develop Venous Thrombo Embolism (VTE) along with neurologic complications	Klein *et al.,* 2020

(Table 3) cont.....

S. No.	Patients	Comorbid Condition	Clinical Symptoms/ Manifestations	Outcome From Study	References
6.	35-year-old female	--	• Patient was diagnosed with otalgia media accompanied by tinnitus • COVID-19 symptoms were absent • No comorbid diseases • Hyperemia and bulging tympanic membrane was noticed • Mild rhonchi was found in the lower region of the thorax • Hearing loss in right ear was measured by audiometry and tympanometry tests • Chest X-ray showed bilateral lung involvement • RT-PCR tests was positive for COVID-19	No classical symptoms of COVID-19 and instead presented an adult otitis media as an atypical manifestation	Fidan *et al.,* 2020
7.	49-year-old male	--	• High fever was accompanied by dry cough, shortness of breath • However, symptoms such as nausea, vomiting, skin rash, sore throat were absent • EKG reports were found to be normal • Chest X ray showed middle lobe opacity • Noticed to have bradycardia, AV block and dissociation	Case report demonstrate an unusual outcome (transient high-degree AV block) in young COVID-19 patient without any previous cardiac comorbidities or conduction disease	Kir *et al.,* 2020

(Table 3) cont.....

S. No.	Patients	Comorbid Condition	Clinical Symptoms/ Manifestations	Outcome From Study	References
			• Cardiac biomarkers including troponin I, NT-proBNP were normal. • Mild elevation in inflammatory markers, ferritin and C-reactive protein • RT-PCR test of nasopharyngeal swab indicated the presence of SARS-COV-2		
8.	33-year-old pregnant woman	Sickle cell disease, acute chest syndrome, necrosis in joints	• Presented with anemia • First nasal swab test was disclosed to be negative for COVID-19 • Patient was convinced to undergo surgical abortion at gestation period of 15 weeks (HD-11) • Placenta was evaluated for COVID-19 and the test resulted negative • On following days, patient was detected with hypotension and pneumonia • RT-PCR test was detected positive after 13 days of onset of symptoms	• RT-PCR test for COVID may show false negative results • COVID infection may transfer from healthcare workers to patients	Fang *et al.,* 2020
9.	33-year-old female; Gestational age-34 weeks	--	• Patient was diagnosed with pancytopenia • Hemoperfusion improved anemia and thrombocytopenia • RT-PCR test was positive for COVID-19 • GGO with consolidation was revealed in upper part of right lobe • Higher creatinine levels were observed (up to 6.8 mg/dl)	COVID-19 possess the tendency to develop Acute Tubular Necrosis (ATN)	Taghizadieh *et al.,* 2020

(Table 3) cont.....

S. No.	Patients	Comorbid Condition	Clinical Symptoms/ Manifestations	Outcome From Study	References
10.	34-year-old man	HIV patient	• 14 days before admission hospital, he was presented with fever, cough, and dyspnea and was attributed to influenza A infection • He was treated for influenza and again had a acute onset of fever, chills, cough and shortness of breath (HD 1) • From HD 2-HD8, symptomatic worsening was observed by chest CT • On HD 9, bronchoscopy was done • Pathology reports indicated patchy chronic inflammation in alveolar tissue, intra-alveolar fibrin, pneumocyte hyperplasia leading to acute lung injury	• Patient experienced very uncommon symptoms during hospitalization • COVID-19 rapidly progressed the pulmonary disease and was diagnosed after 9 days of illness by bronchoscopic biopsy and BAL	Harkin *et al.,* 2020
11.	35-year-old woman	--	• Drug refractory seizures were observed along with headache, nausea, dizziness • There were no motor or sensory deficits • According to MRI reports, left anterior temporal lobectomy was achieved and postoperatively, she was tested positive for COVID-19 • Encephalitis was diagnosed 5 days post surgery indicating possibility of neuroinvasion of SARS-CoV-2	• The findings confirmed the occurrence of encephalitis in COVID-19 positive patient • COVID-19 (viral RNA) can invade in brain tissues	Efe *et al.,* 2020

(Table 3) cont.....

S. No.	Patients	Comorbid Condition	Clinical Symptoms/ Manifestations	Outcome From Study	References
12.	36-year-old woman (Gestation period-33 weeks)	Diabetes mellitus, asthma, maternal obesity and preeclampsia in her previous two pregnancies	• Presented with fever, muscle pain and dry cough for last four days • Sudden onset of vomiting, nausea and epigastric pain was observed with blood sugars > 200 mg/dl • Acute pancreatitis was diagnosed on HD 2 • Chest radiograph showed diffuse bilateral infiltrates • Persistent dry cough followed by development of dyspnea • COVID-19 test remained positive even after four weeks	Human pancreatitis can develop upon COVID-19 infection	Rabice *et al.,* 2020
13.	39-year-old male	Diabetes Mellitus and hyperlipidemia	• Presented with high temperature and cough post visiting Italy and London and 8 days prior to hospital visit • Primary issues were restlessness, shivering and body aches • There were no signs of dyspnea, abdominal or chest pain, vomiting and diarrhea • Mild respiratory distress and Diaphoresis was also diagnosed • Heart was tachycardic without murmurs; Chest CT revealed GGO with consolidation • SARS-CoV-2 was found to be present in nasopharyngeal swab samples	Young COVID-19 patients with accompanying comorbid conditions presented worsening of respiratory and cardiovascular symptoms	Shah *et al.,* 2020

(Table 3) cont.....

S. No.	Patients	Comorbid Condition	Clinical Symptoms/ Manifestations	Outcome From Study	References
14.	41-year-old male	Well controlled Human Immunodeficiency Syndrome (HIV) and recurrent Herpes Simplex Virus (HSV)	• Experienced illness six days prior to start of dry cough and intermittent fever • EKG showed tachycardia • Chest CT revealed diffused and patchy nodular ground glass infiltrates • Patient presented with acute encephalopathy, lumbar puncture revealed no white blood cells • On HD 2, detection of encephalopathy, agitation, left sided ptosis and occurrence of tonic-clonic seizure, tongue laceration subsequently led to respiratory arrest • COVID-19 testing was positive	COVID-19 may increase the chances of renal injury, tachycardia, tonic-clonic seizure	Haddad *et al.,* 2020

(Table 3) cont.....

S. No.	Patients	Comorbid Condition	Clinical Symptoms/ Manifestations	Outcome From Study	References
15.	42-year-old male	--	• Diagnosed 12 days earlier with mild symptoms of COVID-19 infection • Later on dyspnea worsened; chest pain, and hemoptysis was also manifested • Mild respiratory distress along with development of bibasilar rhonchi • EKG displayed slow flattening of T waves • Computerized Tomography Angiography (CTA) reports revealed that bilateral pulmonary emboli and consolidation was present in right lower lobe • Peripheral GGO was also noted	Hemoptysis has also been detected as an uncommon symptom of COVID-19 infection	Casey *et al.,* 2020

(Table 3) cont.....

S. No.	Patients	Comorbid Condition	Clinical Symptoms/ Manifestations	Outcome From Study	References
16.	42-year-old-male	--	• Patient reported pain in abdominal, testicular, and back region for past 8 days • Was having fever two days before presentation • No signs of nausea, vomiting, rhinorrhea, sore throat, cough, and shortness of breath was observed • Chest radiographs revealed no abnormal findings, however, abdominal and pelvic region CT scan spotted pulmonary GGO and consolidation in lung bases • In addition, colitis was identified in descending and sigmoidal region of colon • Two days later, the patient was detected with COVID-19 infection	Both GGO and consolidations constitute the most common radiological findings in chest CT scan reports. It is estimated to be reported in approximately 88% of COVID-19 cases	Kim *et al.,* 2020

(Table 3) cont.....

S. No.	Patients	Comorbid Condition	Clinical Symptoms/ Manifestations	Outcome From Study	References
17.	43-year-old man	--	• Headache, fever (100.0°F) • Chest X-ray showed absence of cardiopulmonary abnormality • Patient was presenting strange actions such as talking to himself, improper eating or drinking, frequent shakings and staring at wall, sweating without having fever • RT-PCR test came positive • CT scan of head showed no acute intracranial abnormality; upon lumbar puncture, no polymorphonuclear abnormalities were disclosed in cerebrospinal fluid • The patient was described to have diaphoresis, disturbed mental status, muscle rigidity, slowed verbal responses and inability to resist gravity in all limbs	Catatonia (three or more signs- staring, stupor, rigidity, mutism, posturing, and autonomic abnormalities) has also been associated with acute COVID-19	Caan *et al.,* 2020

(Table 3) cont.....

S. No.	Patients	Comorbid Condition	Clinical Symptoms/ Manifestations	Outcome From Study	References
18.	44-year-old woman	Diabetes Mellitus, hypertension, dyslipidemia, and chronic kidney disease	• Patient was noticed with fever, cough, vomiting and pain in flank region • High creatinine levels (4.0 mg/dL), presence of blood an d protein indicated acute kidney injury • Chest X-ray indicated sub-segmental atelectasis, pleural effusion on right side, and bilateral diffuse patchy opacities • COVID-19 PCR test was found to be positive • Renal functions deteriorate simultaneously • Sjogren-syndrome was evidenced *via* an autoimmune test • Kidney biopsy-bowman space showed epithelial hypertrophy and hyperplasia, collapsing glomerulopathy • APOL1 genotyping of biopsy material revealed the presence of homozygous allele (rs73885319) for the G1 risk	COVID-19 infection aggravates the kidney injury *via* direct infection or through cytokine storm occurring in sepsis	Larson *et al.,* 2020

(Table 3) cont.....

S. No.	Patients	Comorbid Condition	Clinical Symptoms/ Manifestations	Outcome From Study	References
19.	48-year-old man	Sleep-Disordered Breathing (SDB) and Obesity	• Severe COVID-19 infection presented by fever, cough, and progressive dyspnea • Bilateral patchy pulmonary infiltrates in middle and lower regions were detected through chest X-ray • SARS-CoV-2 RNA was positive • Septic shock was observed	Thrombotic complications tend to progress in COVID-19 patients accompanied with multiple comorbidities	Dumitrascu *et al.,* 2020
20.	48-year-old man	Hepatitis B infection	• Worsening of constipation, lower abdominal pain, hypoactive bowel sounds, dyspnea, periumbilical tenderness were recorded • Elevated levels of procalcitonin • GGO in right upper lung was also identified on examination • Surgery was performed to ameliorate acute sigmoid colonic obstruction • Pathological reports revealed adenocarcinoma T4aN0M0	COVID-19 RNA has been detected in fecal matter	Huang *et al.,* 2020
21.	Man in his 50s	Hypertension, kidney failure, chronic hepatitis B virus infection	• Symptoms such as non-productive cough persisted for about 7 days • Patient was admitted 2 weeks later upon progression to hypoxemia without fever and myalgia • Elevation in C-reactive protein and pro-calcitonin • Throat swab samples revealed the presence of SARS-CoV-2 infection	COVID-19 infection presented very mild symptoms despite multiple comorbid conditions	Tang *et al.,* 2020

Elderly (>60 Years) and Older (>80 Years) Patients

Geriatric population constitutes a major group of individuals. Geriatric patients have a lower immunity and the presence of comorbid conditions makes them more susceptible to bacterial/viral infections (Feng *et al.,* 2012; Low *et al.,* 2019). The prevalent medical demonstrations of COVID-19 infection in older patients are high temperature, dry cough, dyspnea and tiredness. Furthermore, some patients may develop septic shock and ARDS (Chen *et al.,* 2020; Xu *et al.,* 2020; Deng *et al*, 2020; Yang *et al.,* 2020a; Guan *et al.,* 2020). The other severe acute complications include pneumonia and acute cardiac injury. The elderly population is also highly vulnerable to COVID-19 infection and high mortality rate is reported in them (Sun *et al.,* 2020).

COVID-19 pandemic exhibited a global fatality rate of 2-5% (Ji *et al.,* 2020; Mahase Elisabeth, 2020; Wu *et al.,* 2020; Wu and McGoogan, 2020; Sun *et al.,* 2020). Nonetheless, the fatality rate in the geriatric population has been found to be 8.0% (70–79 years) and 14.8% (80 years) (Wu and McGoogan, 2020). The higher proportion of comorbidities, a weaker immune system in patients older than 80 years renders them highly susceptible to COVID-19 infection. The commonly occurring comorbidities include hypertension, diabetes mellitus, cardiac diseases, COPD, and cerebrovascular disease (6.5%) (Yang *et al.,* 2020b, Chen *et al.,* 2020). The distinct range of typical and atypical clinical manifestations found in clinical cases of COVID-19 positive elder and older patients are summarized in Table **4**.

Table 4. Respiratory and non-respiratory (typical/atypical) clinical presentations in COVID-19 positive elder and older patients.

S. No.	Covid-19 Patient	Comorbid Condition (If Any)	Major Clinical Presentation Including Typical Symptoms	References
1.	51 year old male	---	Hypertensive nephropathy, diarrhea, fever, fatigue, dyspnea, abdominal pain, lymphopenia, GGO followed by consolidation.	Wang *et al.,* 2020
2.	51 year old male	Mounier-Kuhn Syndrome (MKS)	Fever, dyspnea, deterioration of chronic coughs, GGO and bronchiectasis.	Jafari *et al.,* 2020
3.	53 year old male	High blood pressure, diabetes and chronic renal failure	Dyspnea, palpitation, diarrhea, black stool along with gastrointestinal bleeding, epigastric pain, No fever and cough.	Gulen *et al.,* 2020

(Table 4) cont.....

S. No.	Covid-19 Patient	Comorbid Condition (If Any)	Major Clinical Presentation Including Typical Symptoms	References
4.	55 year old male	---	Abdominal pain and nausea for 8 days without vomiting, diarrhea, fever and any respiratory symptoms, bilateral, multifocal areas of GGO having rounded and crazy paving morphology	Sendi *et al.,* 2020
5.	56 year old male	Active smoker	Cough, rise in temperature and respiratory distress; Chest radiograph showed pneumothorax on the left side; antracotic pigmentation on the lung surface	Aiolfi *et al.,* 2020
6.	57 year old	---	Headache and malaise, no fever, respiratory or gastrointestinal symptoms, thrombocytopenia	Sadr *et al.,* 2020
7.	61 year old male	---	Cutaneous rashes which persisted for the last 4 days; cough, dyspnea along with anxiety, headache, pain in the throat, restlessness, nasal congestion, urticarial rash and erythematous papules on thighs and forearms	Quintana-Castanedo *et al.,* 2020
8.	61 year old female	Type II diabetes mellitus	Dry cough and non-radiating, sharp periumbilical pain	Mahan *et al.,* 2020
9.	61 year old male	---	Cough, fever, acute respiratory distress, GGO, bilateral pulmonary consolidations	Zubovic *et al.,* 2020
10.	62 year old male	High blood pressure, ischemic heart disease and diabetes	Persistent hiccups for four days, GGO	Prince *et al.,* 2020
11.	62 year old female	Hypertensive, prediabetic, dyslipidemic, transient ischemic attack	2-week history of cough and cold including shortness of breath, severe dysarthia, weakness in right upper and lower extremity	Oliver *et al.,* 2020
12.	62 year old male	Gallstone	Fever (persisting for last 8 days); cough and distress in the chest (persisting for last 6 days); GGO appeared in bilateral lungs later on	Wang and Hu, 2020
13.	62 year old male	---	Fevers, malaise, and dyspnea; chest radiograph revealed airspace opacities in the lung; worsening of bilateral opacities led to refractory hypoxemic conditions	Horowitz *et al.,* 2020

(Table 4) cont.....

S. No.	Covid-19 Patient	Comorbid Condition (If Any)	Major Clinical Presentation Including Typical Symptoms	References
14.	63 year old female	Smoker, recurrent adenocarcinoma, undergoing Stereotactic ablative radiotherapy for thoracic malignancy	She remained asymptomatic upto 18 days and still she was COVID positive	Samson *et al.,* 2020
15.	64 year old female	Smoker; COPD; cT1bN0M0 Non-small cell lung cancer (NSCLC) and was undergoing radiotherapy	Cough, shortness of breath, increased fatigue, post-tussive emesis and GGO in the right lower lobe	Samson *et al.,* 2020
16.	65 year old male	Abdominal aortic aneurysm, crohn's disease, hypertension, coronary artery disease, myocardial infarction, hypothyroidism, gout, lower back and hip pain	Type III Crawford thoracoabdominal aneurysm (TAAA) was detected, diarrohea with pain, fever and respiratory distress	Resch *et al.,* 2020
17.	66 year old female	Small-cell lung cancer (T1bN2M0, Stage IIIA) and was undergoing radiation therapy, obstructive sleep apnea, emphysema	Non-productive cough, dyspnea, however, fever and fatigue were absent	Samson *et al.,* 2020
18.	67 year old female	Left ventricular ejection fraction (LVEF), Cardiomyopathy	Cough, shortness of breath, left shoulder pain and serious cardiac complications	Dabbagh *et al.,* 2020
19.	65 year old male	----	Cough, fever, dyspnea, acute progressive symmetric ascending quadriparesis indicating Guillain Barne Syndrome (GBS), neurological manifestations-acute weakness of distal lower extremities, facial paresis bilaterally	Sedaghat *et al.,* 2020
20.	68 year old female	Chronic kidney disease	High temperature, elevated inflammatory markers and Chest X-ray revealed consolidation in both lungs	Abdelnour *et al.,* 2020
21.	70 year old male	COPD, coronary heart disease, arterial hypertension, diabetes mellitus, obstructive sleep apnea syndrome and obesity (body mass index: 38 kg/m^2)	Intermittent fever (> 38.5 °C), productive cough, dyspnea which persists for one week, bilateral basal coarse reticular opacities and GGO	Prattes *et al.,* 2020

(Table 4) cont.....

S. No.	Covid-19 Patient	Comorbid Condition (If Any)	Major Clinical Presentation Including Typical Symptoms	References
22.	70 year old male	Diabetes Mellitus	Fatigue, fever, and respiratory distress, bilateral, subpleural GGO, left-side pneumothorax	Aiolfi *et al.,* 2020
23.	71 year old male	----	Fever, nonproductive cough, dyspnea and myalgia for last 10 days, coarse crackles in the basal part of lungs, multifocal infiltrates developed later on. Hypoxemia and respiratory alkalosis was also observed	Kaur *et al.,* 2020
24.	71 year old male	Hypertension, chronic kidney disease, overactive bladder and seizure	Tingling and numbness in lower extremities, no respiratory problems or fever. Later on pneumonia was diagnosed in chest X-ray	Chan *et al.,* 2020
25.	71 year old female	---	Productive cough, shortness of breath, fever, maculo-papular rashes were found on the trunk similar to Grover disease	Sachdeva *et al.,* 2020
26.	72 year old male	Diabetes, hypertension, andobesity	Cough with blood-tinged sputum. Fever, sharp pain in the head, diarrhea, myalgia, ageusia, anosmia were absent. Chest X-ray revealed peripheral pulmonary opacities, kidney injury and silent hypoxia	Wilkerson *et al.,* 72
27.	72 year old male	Diabetes Mellitus and Glucose-6-phosphate dehydrogenase deficiency	Experiencing fever, nasal congestion, dry cough and dyspnea for the last 10 days, right lung GGO, severe hypoxia and metabolic acidosis, which led to cardiopulmonary arrest and death	Chhetri *et al.,* 2020
28.	73 year old male	Had undergone surgery for the treatment of T2aN0 NSCLC in 2016	Malignancy was detected in nodules, asymptomatic for typical COVID symptoms, presence of GGO was also verified in lower lobes	Polverari *et al.,* 2020
29.	73 year old female	Breast cancer, obesity, hypertension and thrombosis	Fever, severe lung damage (50-75%); GGO having a crazy paving pattern. However, pulmonary embolism was absent	Grellier *et al.,* 2020

(Table 4) cont.....

S. No.	Covid-19 Patient	Comorbid Condition (If Any)	Major Clinical Presentation Including Typical Symptoms	References
30.	73 year old male	Hyperlipidemia	Abdominal and epigastric pain accompanied by nausea, later on, the patient developed cough, chest pain, dyspnea, became tachypneic and hypoxic, consolidations were also revealed	Alkhafaji *et al*, 2020
31.	75 year old female	Ischemic heart disease, hypertension and acid reflux disease	Loss of appetite, weakness, however, the patient denied having any respiratory symptoms, fever, myalgia, chills and rigors, the appearance of GGO, crackles and rhonchi in lungs was also observed	Chan *et al.,* 2020
32.	76 year old male	---	Altered mental status- decrease interaction, confusions and lethargy, dry cough	Jansen *et al.,* 2020
33.	77 year old female	---	Fever, cough, enlargement in lymph nodal region of the neck, maculopapular exanthem and the macular hemorrhagic rash was diagnosed on the trunk and legs, No signs of pneumonia	Sachdeva *et al.,* 2020
34.	78 year old female	Hypertension, postencephalitic, epilepsy, Herpes Simplex Virus-1 (HSV-1)	Involuntary muscular jerks on face and limbs, right central facial nerve palsy, aphasia, focal status epilepticus, pronation and drifting in arm and leg, respectively, left temporoparietal lobe was found to exhibit gliosis and atrophy, No fever and interstitial pneumonia	Vollono *et al.,* 2020
35.	78 year old male	Hypertension	Chest pain, atrial fibrillation (150 beat/min), shortness of breath, GGO with consolidation in the right lower lobe, pericardial effusion and subsegmental atelectasis	Cizgici *et al.,* 2020
36.	79 year old female	---	Fever, acute dyspnea, epigastric pain, GGO but no pulmonary embolism, arterial and venous abdominal thrombosis	Barry *et al.,* 2020
37.	79 year old female	Diabetes, hyperlipidemia and hypertension	Slurred speech, left-sided weakness, aphasia, acute thromboembolic disease, no fever, bilateral peripheral, upper lobe GGO	Gill *et al.,* 2020

(Table 4) cont.....

S. No.	Covid-19 Patient	Comorbid Condition (If Any)	Major Clinical Presentation Including Typical Symptoms	References
38.	81 year old female	---	Morning and evening fever, bilateral progression, consolidation in the left lobe, ARDS, GGO in both lobes	Zubovic *et al.,* 2020
39.	82 year old female	---	Fever, shortness of breath, persistent cough, bilateral GGO in lower lobes	Ucpinar *et al.,* 2020
40.	86 year old male	13 Chronic cases: Hypertension level III, senile heart valve disorders, lacunar infarction, unstable angina, cardiomegaly, hypoproteinemia, cerebral arteriosclerosis, cardiac function level II, hypertensive cardiopathy, chronic pancreatitis, calcification of aortic valve, diabetes mellitus, chronic kidney disease stage II, and typical GGO	Fever, cough, typical GGO in both lungs	Tu *et al.,* 2020

ACE2

The gene (21 kb) located on chromosome 17 encodes a 170 kDa protein known as ACE (Angiotensin Converting Enzyme). It is a transmembrane enzyme, abundantly present on the outer membrane of enterocytes, epithelial cells of alveoli, endothelial and smooth muscle cells of arteries and veins. These transmembrane proteins are consisted of 805-amino acid long carboxypeptidase along with an outer N-terminus (17-amino acids) and intracellular C-terminus (Donoghue *et al.,* 2000, Tipnis *et al.,* 2000). The polymorphic form ACE17 helps in conversion of inactive angiotensin I (decapeptide) into active angiotensin II (octapeptide). The angiotensin I and II play an important part in maintaining homeostasis of blood pressure *via* inactivating the bradykinin vasodilator to counterbalance the vasoconstrictive action of angiotensin II. Aldosterone release is stimulated by angiotensin II, which further prompts the kidney in absorbing sodium and water (Devaux *et al.,* 2020).

The existence of polymorphic form of ACE2 gene was initially confirmed in the population of China during a cohort study on Nicotine Dependence in teens. The wide range of genetic polymorphism in ACE2 gene has been strongly associated with occurrence of arterial hypertension, diabetes mellitus, cerebral stroke, coronary artery disease, heart septal wall thickness and ventricular hypertrophy

(Lu *et al.,* 2012, Chen *et al.,* 2016, Zhang *et al.,* 2018, Pinheiro *et al.,* 2019, Luo *et al.,* 2019). The three ACE2 variants- rs4240157, rs4646155, rs4830542 and other rs2074192, rs233575, and rs2158083 have been noticed to be correlated with hypertension (HT) and variations of blood pressure, respectively. An association of the latter mutations has also been found with HT in a study of 246 HT patients in India. In addition, Brazilian and African-American patients also revealed existence of polymorphism in ACE2 (Devaux *et al.,* 2020).

SARS-CoV and SARS-CoV-2 share phylogenetic similarities and exploit same ACE2 human cell receptor. The existence of different polymorphic forms of ACE2 worldwide may be the rationale behind the different geographical distribution of SARS-CoV infection across the globe. The polymorphism affects the perceptivity of people towards SARS-CoV-2 infection and the resulting symptomatic outcomes. During early stage infection of SARS-CoV-2, virus spread inside body tissues and impairs the ACE2 function. The binding of virus with peptide domain of ACE2 produces steric hindrance and down regulates the ACE2 mRNA expression. However, in severe cases of COVID-19, virus attacks on ACE2 other than lung resulting in multi-organ failure. Thus, it is suggested that quantification of AngII and ACE2 can be adapted as biological markers for monitoring susceptibility of COVID-19. The interaction between ACE2 and spike protein is expected to enhance or reduce depending upon the binding compatibility of spike (S)-protein of SARS-CoV-2 with ACE2 and ultimately affecting the ability of virus to infect host cell.

Gender

COVID-19 exhibits a strong gender susceptibility difference. Such difference in gender susceptibility can be correlated to the different polymorphic forms of ACE2. The gene located on X chromosome of male generally encode ACE2 protein which can be attributed to the higher ACE2 expression in lungs of male compared with women (Smith *et al.,* 2020). Similarly, in males the angiotensin II converts to angiotensin I at higher levels. About 66-75% severe clinical cases reported till date indicated higher proportion of men in comparison to women. In China, approximately 58% of the admitted patients were revealed to be male. Similar patterns were observed in Italy and USA. According to meta-analysis directed by Fu and coworkers, 29-77% of infected patients were male. The higher proportion of COVID-19 males was also spotted in Asia, South America, and New York. However, more women tested positive in Canada and European countries (Fu *et al.,* 2020).

The hospitalization rates were also higher for males than female ranging from 55 to 62% in all countries. It is recorded in Europe that approximately 73% of

patients admitted in ICU were men. The proportion of death ranged from 59-69% among men in comparison to women. In China, the death rates for men and women were found to be 2.8 and 1.7%, respectively. Similar higher death rates in men were estimated in Australia, Denmark, Austria, France, Seattle, Germany, Belgium, Italy, Europe, and New York. In Italy, the mortality rate of COVID-19 was also higher at all ages for men than women. Furthermore, men estimated for 60.3% deaths while women for 39.7% in New York (Rozenberg *et al.,* 2020).

Brener has explained the association of male predominance and androgen regulation in COVID-19 cases. The proteolytic activation of S-protein of SARS-COV-2 by Transmembrane Serine Protease 2 (TMPRSS2) assists the latter's entry inside cells. TMPRSS2 enzyme is abundantly present in high grade prostate cancers and prostate epithelium. Similar male predominance was observed in pandemic influenza A and was attributed to the presence of TMPRSS2 in host cells. This concept can be considered as a strong reason behind the scarcity of COVID-19 cases in preadolescents, as they lack androgens and cellular proteases TMPRSS2. However, factor such as smoking has been found to affect men by COVID significantly as smoking tends to raise the ratio of androgens to estrogen (Brenner, 2020).

Blood Group

Biological markers may portray a crucial part in predicting susceptibility to COVID-19. The susceptibility of various viral infections has also been associated with different ABO blood groups in past such as in hepatitis B and Norwalk. It was also noticeable during SARS-CoV attack in 2003 that individuals with blood group O were less sensitive towards infection (Lindesmith *et al.,* 2003 and Cheng *et al.,* 2005, Batool *et al.,* 2017). The blood groups of patients thus can act as biological marker for COVID-19 infection. Different blood groups have been studied as one of the responsible aspect for different medical outcomes in COVID patients. Zhao and his coworkers originally studied the correlation between COVID-19 and ABO blood group. An investigation was performed to determine the blood group of 1,775 COVID-19 infected patients admitted in Wuhan Jinyintan Hospital. The ABO distribution study revealed that 37.75% patients were spotted with blood group A as compared to 25.80% patients having blood group O. The results revealed that blood group A patients are more vulnerable to COVID-19 infection as compared to lower risk for blood group O. In addition, the proportion of blood group A (41.26%) was higher than blood group O (25.24%) in 206 dead patients. Another study performed on 113 patients in Renmin Hospital, Wuhan also revealed similar results. The blood group O was conjoined with lessened chance of infection as related to blood group A (Zhao *et al.,* 2020). In consonance with above investigations, higher proportions of blood group A

was also detected among 265 patients admitted in Central hospital of Wuhan (Li *et al.,* 2020). The research carried out by Patrice and coworkers attributed the reduced susceptibility of blood group O to the decreased adhesion of S-protein of coronavirus to ACE-2 receptor by anti-A antibodies (Guillon *et al.,* 2008). However, these speculations need further elucidation to reveal the interrelation between COVID-19 susceptibility and patient blood groups.

In addition, the lower susceptibility in blood group O patients, can also be correlated to decreased risk of cardiac complications and thrombotic disorders in comparison to other blood groups (O'Sullivan *et al.,* 2020). Otherwise, micro thrombi may disseminate through lung vasculature and contribute to emergence of ARDS. In ABO(H) blood groups, the red blood cells (RBCs) show carbohydrate structures which are usually coincided as antigens present on RBCs. The later have not only detected on RBCs but on platelets, plasma glycoproteins such as von Willebrand factor and factor VIII and endothelial cells (O'Sullivan *et al.,* 2020). These factors interact among themselves in complex pattern and play a conspicuous part in activating the pathogenesis of SARS-CoV-2 infection.

Lansiaux and his coworkers analyzed β-thalassemic heterozygote population for their susceptibility to COVID infection in three different regions (Puglia, Sardinia, Sicilia) of Italy employing multiple linear regression. They hypothesized that persons suffering from thalassemia have shown immunity against COVID-19 (Lansiaux *et al.,* 2020). However, these studies are required to be performed on large population of thalassemia patients for longer period of time. Similarly, it is requisite to establish correlation between COVID-19 and other blood pathologies.

Previous Immunization

Albert Calmette and Camille Guerin were the first to introduce Bacillus Calmette-Guerin (BCG) vaccine employing attenuated strain of *Mycobacterium bovis*. Clinically, it was administered in humans in 1921 for the treatment of tuberculosis. It is mainly indicated in neonates and infants to protect against tuberculosis, meningitis and disseminated tuberculosis (Colditz *et al.,* 1995; Redelman-Sidi *et al.,* 2014). Further, it has been reported that its immunomodulatory properties have non-specific (off-target) effects on immune system which is helpful for fighting against infections other than respiratory infections. BCG vaccine is also used as an adjuvant immunotherapeutic agent and for curing bladder cancer (Goodridge *et al.,* 2016; Pollard *et al.,* 2017).

In clinical studies, BCG-Danish has been found to reduce the mortality by 38% in neonates (Guinea-Bissau) and 73% in adolescents (South Africa) suffering from pneumonia, sepsis or infection in respiratory tract (Nemes *et al.,* 2018; Curtis *et al.,* 2020). It has also showed a reduction in cases of yellow fever, viraemia

(volunteers in the Netherlands), mengovirus (encephalomyocarditis virus) infection (Old *et al.,* 1961; Floch *et al.,* 1976). In addition, such vaccines have the tendency to induce epigenetic and metabolic alterations which can help in attaining trained immunity by upgrade the existing immune system (Netea *et al.,* 2020). On the basis of above speculations, different vaccinations have been considered to demonstrate beneficial effects against COVID-19 infection. The concept has nevertheless various limitations and is required to research at larger levels and in high risk populations.

The frequency of COVID-19 infection in neonates and children is very low. The frequent vaccinations and infections in neonates, infants and children have been considered as the major key factor in development of innate immunity system (Lyu *et al.,* 2020). The clinical cases discussed in this chapter (Table **2**) also elaborate that mild symptomatic cases have very low chances of transmission to other children or adults. Lyu and coworkers also focused that administration of vaccines for pneumonia, influenza, and tuberculosis, may act as protection shield against COVID-19 (Lyu *et al.,* 2020).

COMORBIDITY

It is a well known that COVID-19 infection have proven fatal in patient with Comorbid conditions. The investigations performed by Niu *et al*. also revealed that COVID-19 has exaggerated the severity of comorbid conditions in elder and older patients (Niu *et al.,* 2020). The common and uncommon clinical manifestations in different age groups have been documented in different clinical studies as described in Tables **2**, **3** and **4** .

CONCLUSION

COVID-19 pandemic has not only manifested itself in the form of respiratory symptoms but a myriad of other uncommon presentations also. Different age groups may present different manifestations such as asymptomatic or mild symptoms in infants, children or adult, severe and atypical presentations in elder and older group. The atypical symptoms observed in COVID-19 patients include cardiovascular, hematological, renal, cutaneous, ocular, otolaryngological alterations. Although the young population has encountered the infection in higher number, however, the severity and fatality rate is higher in older patients. Silent (asymptomatic/presymptomatic) carriers of COVID-19 also pose an additional concern regarding the silent transmission/spread of COVID-19 infection. The scientific community has continuously updated the occurrence of typical/atypical presentation of COVID-19 infection. Their knowledge may help in future clinical

cases in preventing any delay in early diagnosis and unknown transmission to non-infected individuals.

LIST OF ABBREVIATIONS

ACE2	Angiotensin Converting Enzyme 2
ALT	Alanine Transaminase
ARDS	Acute Respiratory Distress Syndrome
AST	Aspartate Transaminase
ATN	Acute Tubular Necrosis
BCG	Bacillus Calmette–Guerin
CT	Computed Tomography
CF	Cystic Fibrosis
COPD	Chronic Obstructive Pulmonary Disease
CoV	Coronavirus
CTA	Computerized Tomography Angiography
EBV	Epstein–Barr virus
ECG/EKG	Electrocardiography
FLAIR	Fluid-Attenuated Inversion Recovery
GBS	Guillain Barne syndrome
GERD	Gateroesophageal Reflux Disease
GGO	Ground Glass Opacities
HIV	Human Immunodeficiency Syndrome
HSV	Herpes Simplex Virus
HBsAg	Hepatitis B Antigen
HD	Hospital Day
HT	Hypertension
LVEF	Left Ventricular Ejection Fraction
MKS	Mounier-Kuhn Syndrome
NBS	New Born Screening
NSCLC	Non-Small Cell Lung Cancer
RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus-2
TAAA	Thoraco-Abdominal Aneurysm
TMPRSS2	Transmembrane Protease Serine 2
VTE	Venus Thromoboembolism

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 6

Diagnosis

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Abstract: Diagnosis of COVID-19 is supremely valuable in unraveling the complex dynamics involved in SARS-CoV-2 infection and in vaccine development. With an extremely high transmission rate, and initial symptoms similar to other human respiratory viruses, there has been a tremendous urge to develop and supply accurate and rapid procedures for testing the presence of SARS-CoV-2 in a plethora of patient specimens. Scientific and healthcare communities globally have been racing to develop critically needed test kits and ensure ample supply worldwide. Containing the spread of COVID-19 poses multiple challenges, including being able to correctly identify asymptomatic viral carriers that result in the silent spread of the virus, and diagnosing the infection at early stages. Current strategies employ molecular and serological testing techniques in lower and upper respiratory tract samples. The first type detects the presence of viral genetic material and can diagnose an active COVID-19 infection, whereas serological immunoassays detect viral antibodies, which can help identify individuals who have developed an adaptive immune response to the virus, as part of an active or prior infection. The newly authorized antigen tests are designed for the rapid detection of viral antigenic proteins. More elaborative diagnostic testing based on viral genomic sequencing can determine the rate and degree of mutational variability associated with SARS-CoV-2 and identifying newly emerging viral strains for more effective vaccine development. The chapter also highlights the role of rapid, easy-to use point-of-care diagnostic tests in alleviating the challenge posed by the strain on the healthcare system and mitigating the cost of care for both individuals and the government.

Keywords: Antibody, Antigen, Biomarker, Biosensor, Chest CT, COVID-19, CRISPR, Cytokine, Detection, Diagnosis, ELISA, Hematological analysis, Immunoassay, Isothermal amplification, Microarray, POC tests, RT-PCR, SARS-CoV-2, Serology, Viral RNA.

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COVID-19: A PANDEMIC DISEASE

With the current stage of the pandemic and the associated challenges presenting major hurdles, it is evident that highly sensitized and precise diagnostic measures for COVID-19 are paramount for rationalizing infection control initiatives, as well as for case identification and contact tracing (Chan *et al.*, 2020). There is an urgent necessity for rapid yet accurate SARS-CoV-2 diagnostic methods, with emphasis on what type of tests are available in the region, under what in circumstances these tests can be used, and who should be tested (Patel *et al.*, 2020).

Similar to other infectious diseases, effective COVID-19 diagnosis relies on various parameters such as patient history, respective epidemiological conditions, clinical symptoms, and, radiological and hematological analysis. However, standard guidelines state two categories of COVID-19 tests; viral RNA tests (molecular tests) to detect the presence of SARS-CoV-2 in an active infection state, and serological tests to identify whether the subject (patient) has been exposed earlier/previously to the virus and the presence of virus-specific antibodies in the serum (Li *et al.*, 2020b). Some techniques are onerous and technically taxing, hence, there is an urgent need for rapid, cost-effective, and selectively diagnostic point-of-care (POC) tests for COVID-19 capable of providing quick yet accurate, results within a duration of possibly a few minutes (Moitra *et al.*, 2020). These POC tests allow medical diagnostic testing at the time and place of patient care while offering extra benefits of speed of diagnosis and ease of use. The current POC tests available in the market utilize the aforementioned diagnosis strategies of nucleic acid detection and analysis of antigen and/or antibodies in various sample specimens (mostly nasopharyngeal swabs). POC tests are pictured to supplement laboratory testing and enable testing to be available for remote communities and populations that lack readily accessible laboratory settings. In such secluded, crisis-struck locations, small and portable mobile POC platforms are optimal for deployment, although they are lower throughput and run one sample at a time in a specified time-frame of 5-30 minutes. They can be of great value to test moderately symptomatic patients outside clinical settings (Yang *et al.*, 2020). Higher throughput, facility-based POC platforms are also available for use in hospitals and medical centers for diagnosing top-priority specimens, such as that of frontline healthcare workers and critically-ill patients. Although POC tests are a handy component of the rapid diagnostic strategy to battle the ongoing outbreak, they are strictly recommended to be used only in conjunction with PCR-based detection. Laboratory testing undoubtedly remains the foremost testing mechanism because of the presence of complex technology specifically designed to perform a sizeable number of tests at a time. Thus, parallel use of these serology-based detection techniques alongside

molecular techniques is the way forward for mitigating the global, as well as regional load of the disease (CDC-Fact-Sheet, 2019).

COVID-19: EARLY DETECTION BASED ON SYMPTOMS

Clinical Analysis

Clinical presentation of COVID-19 is vague as symptoms coincide with other seasonal respiratory infections simultaneously circulating in the respective territorial population. It is observed that infected patients present with symptoms ranging in severity. Fever (37.5° C and above) must be carefully interpreted as even in severe cases; it can be present in moderation or even absent. Upper respiratory tract viral infection, noted as the leading symptom, was reported in more than 80% infected patients which also include mild fever, dry cough, soreness of the throat with probable nasal congestion, headache, fatigue, or malaise. Patients with moderate infection present symptoms like shortness of breath or tachypnea in children in addition to mild symptoms. On the other hand, highly infected patients are diagnosed with severe pneumonia, having fever associated with severe dyspnea, respiratory distress, cyanosis in children, tachypnea, and hypoxia (Cascella *et al.*, 2020). Particularly, a chest computed tomography (CT) is the expert's recommendation for the severely infected individuals and works as an auxiliary tool alongside other standard diagnostic methods. The current pandemic status depicts that it has no age-limits as it affects the newly-born and the aged people; however, those who have crossed the age of 50 are at a relatively higher risk. Additionally, patients with a history of other comorbidities such as cardiovascular disease, diabetes, lung disease, and other types of immune suppression are also at the risk of getting an infection due to their compromised immune system. Despite measurements of the severity and other symptomatic conditions, the identification marks for the COVID-19 cases, epidemiological circumstances of the respective locations and other parameters may vary from place to place, other factors which affect the diagnosis and identification of the patients (Harapan *et al.*, 2020).

According to WHO, screening for the routine causes of respiratory illness, which delineates the season of the respective location, is the rudimentary step as and when the patient first appears with the symptoms. If a sample yields a negative result, it should be sent to a referral laboratory for SARS-CoV-2 detection. Real-time RT-PCR assay is the preferred molecular test for detecting SARS-CoV-2 infection in a clinical specimen, while serology-based techniques are used as adjunct tools. Specimen for testing must be collected from both the upper respiratory tract (*i.e.*, nasopharyngeal (NP) and oropharyngeal (OP) swabs) as well as the lower respiratory tract (*i.e.*, expectorated sputum, endotracheal

aspirate, or bronchoalveolar lavage (BAL)), depending on the symptom shown by the patient. NP and OP swabs are preferred since they are better for the patient to tolerate, safe to collect and yield higher diagnostic results. To evaluate patients suspected of having pneumonia, self-collected saliva and nasal washes may be alternative upper respiratory tract specimen (Tang *et al.*, 2020).

Hematological Analysis

To better identify the status of infection, other laboratory examinations of blood and plasma specimen are taken into consideration. These include liver and renal function tests, urine routine test, erythrocyte sedimentation rate (ESR), coagulation image, myocardial enzyme, myoglobin, C-reactive protein (CRP), procalcitonin (PCT), lactate dehydrogenase (LDH), D-dimer. Additionally, inflammatory factors such as interleukins (IL-6, IL-10, tumor necrosis factor- α (TNF-α), interferon γ (IFN-γ)), 11 items of tuberculosis subgroup, complement, anti-acid staining are also analyzed (Jin *et al.*, 2020). Blood gas analysis, in combination with analysis of lactic acid, is performed to determine the oxygenation of moderately and severely ill patients. The increased level of liver enzymes (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)), muscle enzyme, ESR and myoglobin has also been reported in certain patients (Zhongnan Hospital of Wuhan University) (Jin *et al.*, 2020). The detection of CRP and PCT is important to check for the history of bacterial infection in the lung. D-dimer was found to be significantly high, in accordance with microthrombotic formation and frequent clotting disorders in peripheral blood vessels of severely infected patients. In the early stage of the infection, a steady or decreased total white blood cell (WBC) and lymphocyte count are also observed in peripheral blood samples (Cascella *et al.*, 2020). A study of 41 infected patients admitted to the Jin Yin-tan Hospital in Wuhan, China, showed that the detection of other cytokines and chemokines, specifically IL-1β, IL-1 receptor antagonist (IL-1RA), IL-2, IL-4, IL-5, IL-7, IL-8 (or CXCL8), IL-9, IL-12 heterodimer p70 (IL-12p70), IL-13, IL-15, IL-17A, Eotaxin (or CCL11), basic fibroblastic growth factor (FGF2), granulocyte colony-stimulating factor (GCSF or CSF3), granulocyte-macrophage colony-stimulating factor (GMCSF or CSF2), IFN-γ induced protein (IP-10 or CXCL10), monocyte chemo-attractant protein (MCP1 or CCL2), macrophage inflammatory protein MIP-1α (or CCL3) and MIP-1β (or CCL4), platelet-derived growth factor subunit-B (PDGFB), regulated upon activation, normal T-Cell expressed and presumably secreted chemokine (RANTES or CCL5) and vascular endothelial growth factor-A (VEGFA) in plasma samples may help in preliminary evaluation of the patient's immune status. It was also noted that COVID-19 patients had increased amounts of IL-1β, IFN-γ, IP10, and MCP1, thus activating T-helper-1 (Th1) cell responses. Moreover, higher concentrations of GCSF, IP-10, MCP1, MIP-1α, and TNF-α

were observed in patients requiring ICU admission when compared to those that do not, indicating that the cytokine storm is related to disease severity. SARS-CoV-2 infection also initiated a surge in secretion of T-helper-2 (Th2) cytokines (IL-4 and IL-10) responsible for suppressing inflammation (Huang *et al.*, 2020).

Chest CT

The National Health Commission of the People's Republic of China published the diagnosis and treatment program (6th version), stating that the diagnosis of SARS-CoV-2 pneumonia on the basis of radiologic observations is one of the diagnostic criteria for COVID-19, with CT scan being the preferred modality. Accurate diagnosis based on chest CT examinations could indicate isolation of the virus and plays a vital role in the management of patients with suspected SARS-CoV-2 pneumonia. Singular or multiple areas of ground-glass opacities (GGO) and consolidation are two predominant signs of lesions on CT images of COVID-19 patients (Li *et al.*, 2020a).

Studies show CT imaging demonstrates five stages of COVID-19 in accordance with the time of onset and the body's response to the virus (Jin *et al.*, 2020).

1. ***Ultra-early Stage*:** The stage where patients have no significant clinical manifestations but positive reports of throat swab for SARS-CoV-2 within 1–2 weeks post-exposure. Major imaging manifestations include single, double or scattered focal GGO and patchy GGO surrounding nodules in the central lobule. Middle and lower lobes of lung show patchy consolidation and signs of intra-bronchial air bronchogram.
2. ***Early Stage*:** The period of 1–3 days post clinical manifestations (fever, cough, fatigue, dry cough, *etc.*). The alveolar septal capillary shows dilatation and congestion, along with interlobular interstitial edema and exudation of fluid in alveolar cavity. Images showed scattered (single or multiple) patchy or agglomerated GGO, separated by grid-like or honeycomb-like thickening of interlobular septa.
3. ***Rapid Progression Stage*:** The period of about 3–7 days after onset of clinical manifestations, where numerous cell-rich exudates collect in the alveolar cavity, with aggravated alveolar and interstitial edema due to vascular expansion and exudation in the interstitium. CT scan shows a large-scale, fused light consolidation containing air-bronchogram.
4. ***Consolidation Stage*:** Around 7–14 days after appearance of clinical manifestations, in which the alveolar cavity shows fibrous exudation and the alveolar wall shows no capillary congestion. CT images show lesser density and smaller range of multiple patchy consolidations in comparison to the previous stage.

5. ***Dissipation Stage*:** A period of approximately 2 and 3 weeks post onset, during which there was further reduction in lesion range and CT scans showed strip-like opacity or patchy consolidation. As time progressed, interlobular septum showed grid-like thickening, bronchial wall showed thickening and strip-like twist and some scattered patchy consolidations (Jin *et al*., 2020).

Usage of chest CT to diagnose COVID-19 is a controversial matter although detailed features of CT images of the disease have been reported. Very few are available in public forum which creates the scarcity in the appropriate understanding of the disease. With CT examination of SARS-CoV-2 pneumonia, suspected individuals can be further isolated and given the right treatment timely so that the patient management can be optimized, especially in communities or hospitals of developing and underdeveloped countries where they have the limited resources for nucleic acid testing kits. Also, it is a relevant tool for radiologists to identify that the CT findings of COVID-19 can be correlated with and compared to images of diseases caused by other viruses, such as Adenovirus, and those within the same family such as SARS-CoV and MERS-CoV. However, CT is still a limited option for the identification of newly discovered viruses like the SARS-CoV-2 (Li *et al*., 2020a).

COVID-19: MOLECULAR DETECTION OF VIRUS

Since the outbreak in December 2019, early diagnosis of COVID-19 has not only been a significant challenge, but also a critical factor in disease prevention and progression control. However, clinical characteristics alone are not sufficient to base the diagnosis of COVID-19, especially if patients present early and ultra-early stage symptoms (Tahamtan and Ardebili, 2020). In conjunction with medical diagnostic advancements, reliable laboratory detection techniques are of utmost importance to expedite public health interventions. In general cases of acute respiratory infections, nucleic acid detection-based approaches are routinely utilized to detect pathogenic agents collected from respiratory secretion specimen. The feasibility of introducing rapid and reliable nucleic acid tests at the time of global health emergencies *via* the collaboration between public and academic laboratories was previously demonstrated in cases of other human viruses like SARS, Chikungunya (Panning *et al.,* 2009) and Zika virus (Corman *et al.,* 2016). In these situations, for establishing assays and assessing their performance, virus isolates were accessible as a reference. However, in the current scenario of COVID-19, such virus isolates from diseased patients have not yet been pre-established as per the international public health community guidelines (Corman *et al.,* 2020).

Numerous SARS-CoV-2 nucleic acid detection kits have been designed and have obtained rapid approval as multiple suspected cases having typical clinical COVID-19 characteristics with related CT images went undiagnosed. Due to the overcrowded situation in many healthcare centers, more number of suspected and confirmed cases could not be efficiently identified, isolated or treated (Zeng *et al.,* 2020). Amidst such nucleic acid tests, the polymerase chain reaction (PCR) method is referred to as the 'gold standard' for the detection of specific human viruses. At present, it has been discovered that real-time reverse transcriptase-PCR (RT-PCR) is immensely valuable for detection of SARS-CoV-2 due to the advantage of being a highly specific yet simple qualitative assay with adequate sensitivity for rapidly diagnosing infections in early stages. Thereby, 'criterion-referenced' real-time RT-PCR tests can potentially be regarded as a prime technique to diagnose COVID-19 causative agent, *i.e.*, SARS-CoV-2. Besides basic PCR, several novel molecular tests that employ non-PCR-related techniques and assays also reportedly have enhanced specificity and sensitivity. Such tests include nucleic acid amplification under isothermal conditions (such as loop mediated isothermal amplification-LAMP) and nucleic acid sequence-based amplification which are intricately developed to rapidly detect coronavirus RNA (Shen *et al.,* 2020). Moreover, POC tests employing nucleic acid detection strategies have been developed to enhance the accuracy and effectiveness of current testing strategies.

RT-PCR Based Assays

The most preferred and dependable test for diagnosis of COVID-19 is RT-PCR test, carried out using upper (NP and OP swabs) and lower (expectorated sputum, endotracheal aspirate or BAL) respiratory tract specimen, blood, urine and feces. Different manufacturers use a variety of molecular targets within the SARS-Co--2 positive-sense, single-stranded RNA genome, includes structural proteins and several spike protein (S), envelope protein (E), transmembrane protein (M), helicase (Hel), and nucleocapsid protein (N) (Kang *et al.,* 2020). Additionally, some tests target species-specific accessory genes essential for viral replication, such as hemagglutinin-esterase (HE), RNA-dependent RNA polymerase (RdRp) and open reading frames *ORF1a* and *ORF1b* (open reading frame 1a/b) (Tang *et al.,* 2020).

Recently developed RT-PCR assays targeting three genes, *i.e.*, RNA-dependent RNA polymerase/helicase (*RdRp/Hel*), *S*, and nucleocapsid (*N1* and *N2*), of SARS-CoV-2. Amongst these, the COVID-19 *RdRp/Hel* assay yielded the lowest *in vitro* detection limit, but was advised to confirm positive results. However, in comparison to the *RdRp* assay, the *E* gene assay shows higher sensitivity in combination with the single-step RT-PCR system and is sufficient to detect

SARS-CoV-2 RNA (Zhai *et al.,* 2020). To identify patients in early stages, two single-step quantitative RT-PCR (qRT-PCR) assays were designed to detect two other targets of the SARS-CoV-2 genome that are highly conserved amongst *Sarbecoviruses*; the *ORF1b* and the *N* gene of expected amplicon sizes of 132bp and 110bp respectively (Chu *et al.,* 2020). Although there has been no attestation that either sequence regions are advantageous for clinical diagnostic testing, it is preferred that a minimum of two molecular targets should be included in the RT-PCR assays to prevent possible cross-reaction and probable genetic drift of SARS-CoV-2 (Tang *et al*., 2020). Researchers in various countries have devised real-time RT-PCR assays based on different combinations of these targets. In the United States, the CDC (Center for Disease Control) recommended two loci in the *N* gene, which has reportedly been performing well (Holshue *et al.*, 2020). Hong Kong used two targets for RT-PCR assays; initial screening using the *N* gene, followed by *ORF1b* for confirmation (Chan *et al.*, 2020). The combination of *E* and *RdRp* genes were selected in the territory of Germany (Corman *et al.*, 2020).

The viral load kinetics suggests the variations in different location of individual patients. Optimum type of samples and timing for maximumviral load during the course of SARS-CoV-2infection are yet to be revealed (Tahamtan and Ardebili, 2020). In a study conducted by Wang *et al.*, different clinical specimen of 205 patients with confirmed COVID-19 were tested by RT-PCR, targeting the *ORF1ab* gene. Maximum positivity was obtained in BAL specimens (93%), accompanied by sputum specimen (72%), NP swab (63%), and OP swab (32%) (Wang *et al.*, 2020). In NP swabs of most cases of symptomatic SARS-CoV-2 infection, viral RNA can be detected and measured (by cycle threshold (Ct)) on day-1 of symptoms and reaches to the peak within a week. Ct is defined as the number of replication cycles needed to yield a fluorescent signal; lower the Ct values, greater the viral RNA load in a sample. If the Ct value is lesser than 40, the patient is clinically declared as "PCR positive". By the third week, this positivity gradually starts to decline and subsequently becomes undetectable. For critically ill and hospitalized patients, Ct values are lower than that of mild to moderate cases, and positive PCR results may be persistantfor more than 21 days after infection onset, whereas majority mild cases produce negative results. However, PCR positivity indicates only the presence of viral RNA, but may not compulsorily imply viability of the virus in the sample (Sethuraman *et al.*, 2020).

Despite the higher sensitivity and specificity of RT-PCR, the usage of these assays heavily relay on the method of sample collection and their transport to the respective COVID-19 testing laboratories. Missing the time window of viral replication, incorrect sample collection timing in comparison to illness onset and lack of efficient sampling technique can confer to false-negative results. Occasionally, this could also occur due to technical errors and contamination of

reagents or samples. At this stage of the pandemic, FNRs can have serious consequences by allowing infected patients to spread the infection and hampering the measures to contain rapid spread of the virus (Guo *et al.*, 2020). All the commonly used diagnostic methods are illustrated in below given (Fig. **1**).

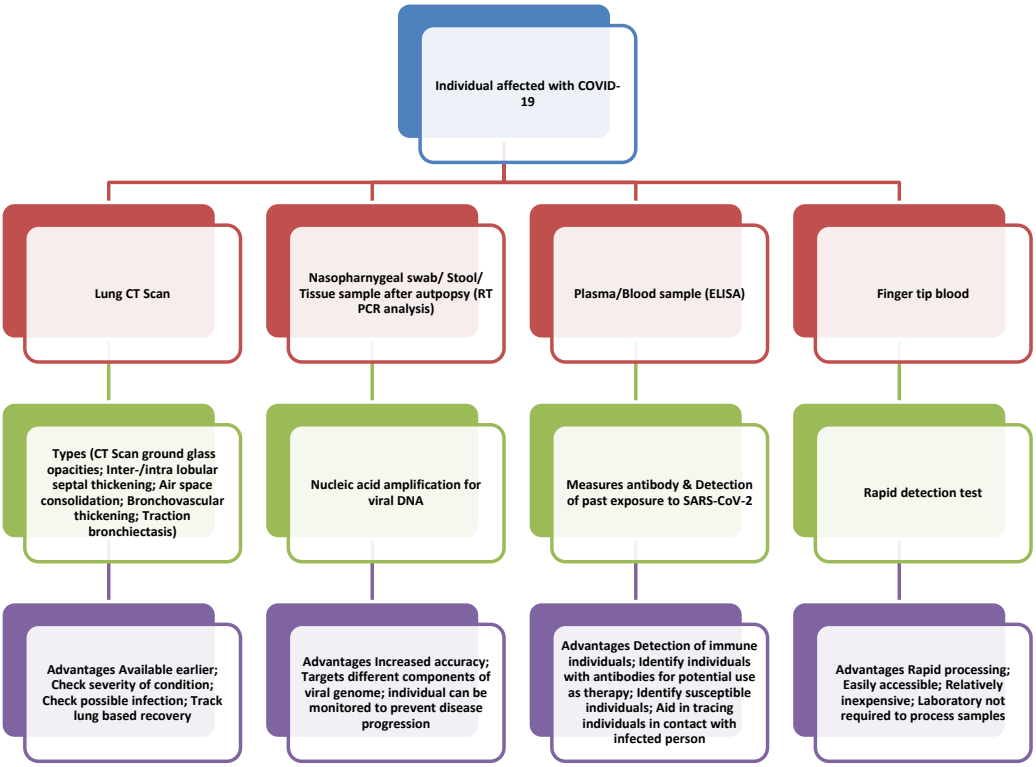


Fig. (1). Diagnostic methods and their advantages for COVID-19 (Iyer *et al.*, 2020).

Loop Mediated Isothermal Amplification (LAMP)

In PCR, double-stranded DNA is denatured using heat, and the products are used to promote the next few rounds of DNA synthesis. LAMP is a novel nucleic acid amplification technique that does not require initial heat denaturation and thus, proceeds rapidly to amplify fewer copies of nucleic acid to multiple-fold in under an hour under isothermal conditions and with increased specificity (Shen *et al.*, 2020). Isothermal conditions can be provided by a simple water bath or heating block, thus making LAMP much approachable to use, especially in the area where prompt action is required for the immediate diagnosis due to the episodes of severe illness of COVID-19 (Notomi *et al.*, 2000). Similar to the principle of RT-PCR, it uses reverse transcriptase enzyme to obtain complementary DNA (cDNA) from a RNA template, which is further amplified by DNA polymerase with a set

of two specially designed inner and two outer primers (Wong *et al.*, 2018). The initial steps require all four kinds of primers, followed by usage of only inner primers for strand displacement of DNA. Each inner primer (forward inner primer (FIP) and the backward inner primer (BIP)) comprises of a pair of distinct sequences that correspond to the sense and antisense sequences of the target DNA; one is used for priming in the initial phaseand the other for self-priming in later phases (Notomi *et al.*, 2000). The amplified product can then be identified *via* photometry or by quantifying the turbidity caused by magnesium pyrophosphate precipitate as a byproduct of DNA amplification (Carter *et al.*, 2020).

LAMP-based detection methods have previously been developed and applied in diagnosis of the SARS-CoV and MERS-CoV. In 2004, Poon *et al.* reported and demonstrated how a simple LAMP assay is a feasible technology for the immediate diagnosis of SARS-CoV. Comparative study showed that SARS-CoV detection rates and LAMP assay sensitivity is equivalent to that of conventional PCR-based methods (Poon *et al.,* 2004). Later on, Shirato *et al.* developed LAMP-based assay for the detection and epidemiologic surveillance of MERS-CoV. Here the detection of amplicons was carried out by precipitation of magnesium pyrophosphate or by naked-eye observation of fluorescence signals, eliminating the requirement for any specialized instruments. As few as 3.4 copies of MERS-CoV RNA could be detected without any cross-reaction with other respiratory viruses (Shirato *et al.*, 2014). Recently, Zhang *et al.* described a simple colorimetric LAMP-based assay for rapid detection of SARS-CoV-2 from direct tissue or cell lysate without an RNA purification step as the sample input (Zhang *et al.*, 2020b).

In addition to enhancing the accuracy in diagnosis, the RT-LAMP can also be used as an epidemiologic surveillance tool for viral infections. It is a pioneering nucleic acid amplification technique and works as a simple yet rapid diagnostic tool for detection and identification of infectious pathogens (Fig. **2**). LAMP is also an advantageous real-time assay for use in developing countries due to its easy-to-use approach in any field, under any circumstances and environment (Wong *et al.*, 2018).

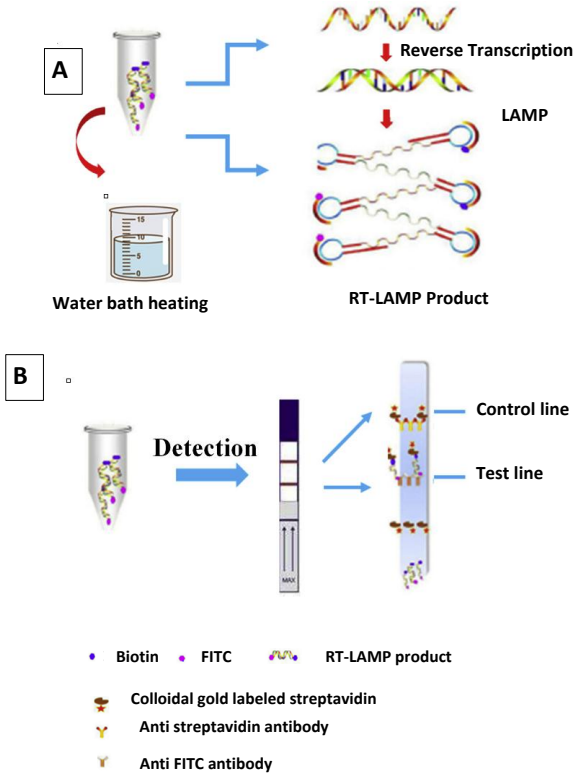


Fig. (2). Schematic illustration of the RT-LAMP-VF assay. **(A)** amplification reaction for RT-LAMP and **(B)** detection on visualization strip [Adapted with permission from (Shen *et al*., 2020)].

CRISPR-Isothermal Amplification Based Assays

Microbial clustered regularly interspaced short palindromic repeats (CRISPR) based diagnosis (CRISPR-Dx) can rapidly diagnose nucleic acids with increased sensitivity and single-base specificity(Gootenberg *et al.*, 2017). Having CRISPR array processing activity, the RNA-targeting CRISPR associated (*Cas*) enzyme '*Cas13*' targets and cleaves RNA and release individual CRISPR RNA (crRNAs). Additionally, *Cas13* has concomitant cleavage activity that has been utilized for specialized diagnostic applications, such as specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) (Gootenberg *et al.*, 2018, Freije *et al.*, 2019). SHERLOCK uses the combination of recombinase-mediated isothermal pre-amplification orrecombinase polymerase amplification (RPA), and *Cas13* for multiplexed, ultra-sensitive detection of single molecules of nucleic acids. Two guide RNAs were designed based on the SARS-CoV-2 RNA sequence, each recognizing the *S* gene and the *ORF1ab* gene respectively. To maximize the accuracy, sequences most specific for SARS-CoV-2 are selected to minimize the interference from other respiratory virus genomes (Kang *et al.*, 2020). Broughton

et al. reported the development of a quick (less than 40 minute), simple yet accurate CRISPR-Cas12-based lateral flow assay, termed as DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter), for diagnosis of SARS-CoV-2 RNA from respiratory swab specimen (Broughton *et al.*, 2020).

Microarrays

The microarray is a high-throughput detection method where the target RNA produces cDNA by reverse transcription, followed by loading of these specific probe-labeled cDNA into wells of microarray plates. The surfaces of these microarray chips contain fixed solid-phase oligonucleotides. If the cDNAs hybridize, they remain bound to the surface and the unbound DNAs get washed away. In this way, presence of virus-specific nucleic acids can be detected by target-specific probes (Carter *et al.*, 2020). The microarray assays have previously been able to identify SARS-CoV genomic mutations and up to 24 associated single nucleotide polymorphisms (SNP) in the SARS-CoVgene with an accuracy of 100% (Guo *et al.*, 2014). Due to its superior design for rapid diagnosis, microarray assays are capable of detecting a broad range of human viruses and can be accessed at or near patient POC in the future (Shen *et al.*, 2020). In a study conducted by Luna *et al.*, an inexpensive, non-fluorescent, low-density oligonucleotide array was designed for detecting the complete Coronavirus genus with a sensitivity equivalent to that of real-time RT-PCR (de Souza Luna *et al.*, 2007). Another study evaluated the mobile analysis platform (MAP), which is a compact, portable, near-POC diagnostic platform utilizing disposable assay-specific microfluidic cards. It is a promising technology for rapid, accurate automated detection and differentiation of several pathogens in clinical specimen (Hardick *et al.*, 2018).

Metagenomic Sequencing Based Methods

Metagenomic sequencing is used evaluate the background microbiome of infected patients. To identify SARS-CoV-2, this technique combines the use of amplicon-based sequencing with metagenomics sequencing(Moore *et al.*, 2020). This approach enables the rapid identification of SARS-CoV-2 as well as secondary infection causing pathogens, and study of potential contact tracing, molecular epidemiology, and viral evolution. Metagenomic approaches like sequence-independent single primer amplification (SISPA) check for sequence divergence. With relevance to the current SARS-CoV-2 outbreak, this technology is particularly useful in assessing rate of mutation and detecting probable recombination with other human Coronaviruses; both have direct impact on development and efficacy of vaccines and antivirals (Carter *et al.*, 2020). Moore *et al.* used amplicon and metagenomics 'MinION' sequencing technique to

rapidly obtain the genomic sequencing of SARS-CoV-2 and other pathogens in NP swabs of COVID-19 patients by the ISARIC 4C consortium. For the amplicon-based system, 16 primer binding sites from specific conserved regions in the SARS-CoV-2 genome were chosen to amplify about 1000bp fragments having a roughly 200bp overlapping region. These primer sets generated 30 amplicons from the cDNA, which were then sequenced using MinION (Moore *et al.*, 2020).

Shotgun metagenomics is a comprehensive sequencing technique that collectively examines all organisms present in complex samples without concerns of highly mutagenic regions that can be challenging to detect *via* other assays (Ai *et al.*, 2020). It provides an in-depth, almost complete sequence of the viral genome, highlighting valuable details such as virus function and biology that further aid immensely in implementing effective surveillance strategies to prevent transmission and infection (Carter *et al.*, 2020). Illumina developed a next-generation shotgun metagenomics sequencing platform that can detect and collectively assess multiple strains of pathogenic organisms (including Coronaviruses) present in samples of varying complexities. The workflow involves the TruSeqRibo-Zero Gold rRNA depletion kit for sample preparation, TruSeq Stranded Total RNA Library Prep for library preparation, the Illumina-benchtop sequencing system, and the LRM resequencing module or IDbyDNAExplify platform for final data analysis (Carter *et al.*, 2020).

Gold Nanoparticles-Based Colorimetric Assay

In terms of reliability, reproducibility and selectivity, many assays fail when testing viral load at its early representation or viral gene mutations during its ongoing spread. Thus, there is a need for sophisticated diagnostics to test patient samples, preferably without any access to advanced instruments (Moitra *et al.*, 2020). Colorimetric bioassays incorporating nanotechnology can be conveniently used to design biosensors with an easy-to-use platform with visual output. Gold nanoparticles (AuNPs) have been a recent trend for use in such colorimetric-based biosensing applications because of their immanent photostability, high extinction coefficient and localized surface plasmon resonance (SPR), *i.e.*, the optical property which represents a color with maximal absorbance wavelength (Kim *et al.*, 2019). AuNPs have been utilized in colorimetric assays for the detection of a broad range of chemical and biological targets like small molecules, metal ions, proteins and nucleic acids, where the change in particle color is generated by sensitive reactivity of the nano-sized particles to the external conditions (Zeng *et al.*, 2020). However, this technique implements the production of single-stranded DNA probes and gradual denaturation and annealing after PCR. Shokri*et al*., 2017 developed a simple colorimetric bioassay for the visual detection of DNA or RNA

targets on the basis of unmodified AuNP aggregation using disulfide self-assembly of terminally-modified DNA. The study showed that the resulting products exhibited a great affinity for the surface of AuNPs and also conferred protection from salt-induced aggregation (Shokri *et al.*, 2017). Based on a similar approach, Kim *et al.,* proposed a colorimetric assay to detect the presence of MERS (Kim *et al.*, 2019).

On a similar basis, Moitra*et al.*, reported a colorimetric assay using AuNPs capped with aptly designed thiol-modified antisense oligonucleotides (ASOs) exclusively for the SARS-CoV-2 *N* gene, which could be useful for detecting positive COVID-19 cases within 10 minutes from isolated RNA samples. These AuNPs selectively agglomerate in the presence of their target SARS-CoV-2 RNA sequence and demonstrate a change in the SRP. *RNaseH*-mediated cleavage of the RNA strand from the RNA−DNA hybrid forms a visually detectable precipitate. The assay can also be optimized to target other regions of the viral genome, such as the *S*, *E* and *M* genes, for pre-clinical or POC screening and to achieve utmost sensitivity with the least number of false positives (Moitra *et al.*, 2020). The currently available different molecular-based methods with their target of the gene have been summarized in Table **1** (See in the later section-including the test serology-based test).

Table 1. The list of commercially available SARS-CoV-2 Diagnostic Kits.

Manufacturer	Test Name	Test Type	Principle of Detection	Target for Detection Gene
1 drop Inc.	1copy ™ COVID-19 qPCR Multi Kit	Molecular- based	RT-PCR	*RdRp* and *E*
Abbott Diagnostics Scarborough, Inc.	ID NOW COVID-19	Molecular-based, POC	ITAP	*RdRp*
ArcDia International Oy Ltd. (Ivaska *et al*., 2013)	mariPOC® COVID-19 tests	Antigendetection, POC	-	-
AtilaBioSystems, Inc.	iAMP® COVID-19 Detection Kit	Molecular-based	ITAP	*ORF1ab* and *N*
Becton, Dickinson and Company	BD SARS-CoV-2 Reagents for BD MAX™ System	Molecular-based	RT-PCR	*N1* and *N2*
BioCore Co. Ltd.	BioCore 2019-nCoV Real Time PCR Kit	Molecular-based	RT-PCR	*N* and *RdRp*
BioMedomics Inc. (BioMedomics, 2020)	COVID-19 IgM/IgGRapid Test	Serology-based, IgG/IgM, POC	LFIA	-
Cepheid	Xpert® Xpress SARS-CoV-2	Molecular-based, POC	RT-PCR	*N2* and *E*

(Table 1) cont.....

Manufacturer	Test Name	Test Type	Principle of Detection	Target for Detection Gene
ColorGenomics	SARS-CoV-2 LAMP Diagnostic Assay	Molecular-based	ITAP	*N*, *E* and *ORF1a*
CueHealth Inc.	Cue™ COVID-19 Test	Molecular-based, POC	ITAP	*N*
DiaSorin, Inc. (DiaSorin, 2020)	LIAISON® SARS-CoV-2 S1/S2 IgG	Serology-based, IgG	CLIA	*S1/S2* proteins
EmoryMedicalLaboratories(Suthar *et al*., 2020)	SARS-CoV-2 RBD IgG	Serology-based, IgG	ELISA	*S1 RBD* proteins
GeteinBiotech, Inc. (Assaygenie, 2020)	COVID-19 Rapid POC CE-IVD Test	Serology-based, IgG/IgM, POC	Chromatographic LFIA	*S* and *N* proteins
Goldsite Diagnostics Inc. (Goldsite, 2020)	SARS-CoV-2 IgG/IgM Kit (TRFIA)	Serology-based, IgG/IgM, POC	TRFIA	*S* and *N* proteins
Hangzhou LaiheBiotech Co., Ltd.	LYHER Novel Coronavirus (2019-nCoV) IgM/IgGAntibody Combo Test Kit (Colloidal Gold)	Serology-based, IgG/IgM	LFIA	*S1*protein
Illumina Inc.	Illumina®COVIDSeq™ Test	Molecular-based	MetagenomicSequencing	-
Jiangsu Bioperfectus Technologies Co. Ltd.	COVID-19 Coronavirus Real Time PCR Kit	Molecular-based	RT-PCR	*ORF1ab* and *N*
KilpestIndia Ltd. DBA 3B BlackbioBiotech	TRUPCR® SARS-CoV-2 KIT	Molecular-based	RT-PCR	*E*, *N* and *RdRp*
Mammoth Biosciences, Inc.	SARS-CoV-2 DETECTR	Molecular-based	CRISPR-ITAP	*N2* and *E*
Mesa Biotech Inc.	Accula™ SARS-Cov-2 Test	Molecular-based, POC	RT-PCR	*N*
Ortho-Clinical Diagnostics, Inc. (OCD-Vitros, 2020)	VITROS ImmunodiagnosticProducts Anti-SARS-CoV-2 Total Reagent Pack	Serology-based, Total Antibody	Immunometric, CLIA	*S1*protein
PlexBio Co. Ltd.	IntelliPlex™ SARS-CoV-2 Detection Kit	Molecular-based	RT-PCR	*E*, *N* and *RdRp*
PreciGenome LLC.	FastPlex Triplex SARS-CoV-2 Detection Kit (RT-Digital PCR)	Molecular-based	RT-PCR	*ORF1ab* and *N*
QIAGEN	QIAstat-Dx® Respiratory SARS-CoV2 Panel	Molecular-based	RT-PCR	*RdRp* and *E*
Roche Diagnostics (Roche, 2020)	Elecsys Anti-SARS-CoV-2	Serology-based	ECLIA	*N* protein
SeasunBiomaterials	AQ-TOP™ COVID-19 RapidDetection Kit	Molecular-based	ITAP	*ORF1ab*
Shenzhen MindrayBio-MedicalElectronics Co., Ltd. (Nuccetelli *et al*., 2020)	SARS-CoV-2 IgM	Serology-based, IgM	CLIA	-

(Table 1) cont.....

Manufacturer	Test Name	Test Type	Principle of Detection	Target for Detection Gene
Sherlock Biosciences, Inc.	Sherlock™ CRISPR SARS-CoV-2 kit	Molecular-based	CRISPR-ITAP	*ORF1ab* and *N*
Snibe Diagnostics (Isabel *et al*., 2020)	MAGLUMI IgG/IgM de 2019-nCoV	Serology-based, IgG/IgM	CLIA	*S* and *N* protein
TBG Biotechnology Corp.	ExProbe™ SARS-CoV-2 Testing Kit	Molecular-based	RT-PCR	*E*, *N* and *RdRp*
Siemens Healthcare Diagnostics Inc.	SARS-CoV-2 Total Antibodyassay (CV2T)	Serology-based, Total Antibody	CLIA	*S1* *RBD* protein
Quidel Corporation	Sofia 2 SARS Antigen FIA	Antigendetection, POC	LFIA	*N*protein

ITAP: Isothermal amplification, CRISPR-ITAP: CRISPR-Isothermal Amplification, ECLIA: Electro-chemiluminescence Immunoassay, CLIA: Chemiluminescent Immunoassay, ELISA: Enzyme-linked Immunosorbent Assay, LFIA: Lateral Flow Immunoassay, TRFIA: Time-resolved Fluorescence Immunoassay.

COVID-19: SERIOLOGICAL AND IMMUNOLOGICAL BASED DETECTION

In the current scenario for the diagnosis of COVID-19, PCR-based techniques and deep sequencing are prevalent methods for detecting the SARS-CoV-2 viral infection. Nevertheless, these methods are greatly dependent upon the presence of the viral genome in an adequate amount so as to be amplified; further, if the time window for viral replication is overlooked, false-positive results can arise. Likewise, erroneous sample collection can hinder the efficacy of qPCR-based assays, giving rise to false-negative results, which can augment the dire consequences of the current pandemic. Hence, auxiliary screening methods that enable us to detect infection in spite of low viral titers can prove to be extremely valuable (Guo *et al.,* 2020). Serological testing is generally regarded as the investigation of blood serum or plasma for the ubiety of IgM and IgG, but the term has been functionally broadened to encompass the analyses of other biological fluids such as saliva and sputum (Carter *et al.,* 2020). Immunoassays can provide information not only on the active viral infection (as in the case of qPCR-based detection), but also on past exposures to the virus. Several companies have ventured into this approach for developing SARS-CoV-2 detection kits. These assays are designed such that they mainly target those proteins in the virus that have extensively been established as suitably immunogenic – the S protein (being the most exposed protein in the virus), the N protein (for its abundant expression during an infection), and the receptor-binding domain (*RBD*) on the viral spike protein (Lee *et al*., 2020).

Serological testing can prove to be a key strategy in guiding successful public health practices to contain the pandemic. Besides rapid identification of COVID-19 positive cases, it can help us largely in the aspect of contact-tracing and in defining case-clusters, particularly in highly dense populations (Xu *et al.,* 2020). Serological testing can also enable us to go beyond these immediate uses of case identification and containment. Serological testing on the population scale can enable researchers to evaluate the total number of individuals mounting an immune response, including those infections that had a sub-clinical manifestation or those that occurred in the past (Lee *et al.,* 2020). This information can point out four cardinals pertaining to the disease epidemiology; primarily, it can help estimate major epidemiological variables (for instance, the attack rate of the disease, or its case-fatality rate), which facilitates an assessment of the burden of community transmission; next, it can direct the strategic placement of frontline workers to mitigate the viral exposure to susceptible individuals in the population; further, the information can also be used to evaluate the consequences of non-pharmaceutical interventions at the population level; lastly, it can enable identification of those individuals who set off a robust immune response to SARS-CoV-2, such that their antibody isolates can be utilized in convalescent plasma therapy (Winter and Hegde, 2020) (Fig. **3**).

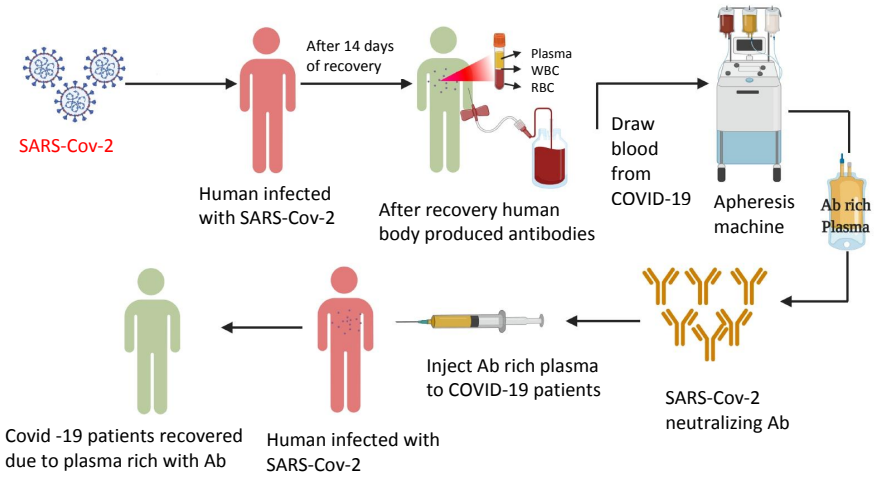


Fig. (3). Convalescent plasma (CP) therapy: The figure illustrates the process and importance of CP therapy to treat COVID-19 disease. CP therapy is immunotherapy where the humoral antibody (Ab) from the recovered patients to the severely affected diseased patients. In CP therapy, as the SARS-CoV-2 affected is infected the Ab spans out and marks the virus as an intruding agent into the human system. This, in future, will trigger the white blood cells to identify the SARS-CoV-2 virus, which deactivates the viral function in the human body. In this procedure, almost 1ltr of blood will be collected from the recovered patients and approximately 250 ml of plasma will be injected to the COVID-19 diseased patients. This might reduce the COVID-19 disease symptoms, give relief to the patients and would get recovered from this dreadful infection [Adapted with permission from (Iyer *et al*., 2020)].

Despite being fast, robust and easy to carry out, immunological/serological assays have certain limitations. The incapacity of immunoassays to detect infection during the very early stage, because immunoglobulins are generated only after a few days of exposure to antigen, is a major drawback; in view of this, a recent infection might depict false-negative outcomes with serological testing. Another setback with the usage of serological assays might arise with the possibility of cross-reactivity, a factor that crucially influences the specificity and the sensitivity of an immunoassay (Lee *et al*., 2020). Hence, serological testing is an indispensable measure that should be taken, at least in complementation with nucleic acid testing so as to account for a close window of detection between a positive PCR test and seroconversion to anti-SARS-CoV-2 IgG. This can also help address additional use cases such as to support return-to-work, immunity passport initiatives and studies aimed at the evaluation of therapeutic/vaccine candidates (F. hoffmann-la roche ltd, 2020).

BIOMARKER IDENTIFICATION: APPROACH OF SEROLOGICAL PLATFORM

Antibody Biomarkers

Numerous serological immunoassays have been developed so far for the recognition of SARS-CoV-2 proteins and for that of anti-SARS-CoV-2 antibodies in the serum or plasma. The most widely employed biomarkers in the currently available immunoassays are immunoglobulins, IgM and IgG (Vashist, 2020). IgM is the first antibody produced by the host immune system in response to the initial exposure to an antigen(Schroeder Jr and Cavacini, 2010). On the other hand, IgG, the most commonly found immunoglobulin isotype in the blood, is produced after the processes of class-switching and affinity maturation, and participates predominantly in the secondary immune responses (Vidarsson *et al.*, 2014). IgM is first detectable in serum a few days after infection, lasting for a few weeks after which class-switching to IgG takes place (Carter *et al.*, 2020). A few recent studies have pointed out that IgM can be detected as early as 3 days post-infection onset, but peaks between two to three weeks post infection onset; contrastingly, IgGis detectable 4 days post-infection onset and the levels peak no sooner than 17 days post-infection onset (Zhang *et al.*, 2020a, Long *et al.*, 2020). Thus, it follows that IgM can be an indicator of early stage of the infection whereas IgG, that of current or former infection. IgG might also be indicative of post-infection immunity (Carter *et al.*, 2020).

A study reporting acute antibody responses to SARS-CoV-2 in 285 COVID-19 patients also showed that 100 percent of patients tested positive for anti-SARS-

CoV-2 IgG within 19 days post-onset of symptoms. The study also reported plateauing of IgM and IgG titers within 6 days post seroconversion (Long *et al.*, 2020). Other investigations have shown significant rates of seropositivity for antibodies against various SARS-CoV-2 viral proteins. To *et al.* carried out serological analyses for samples available not less than 14 days post onset of symptoms; they reported 94% and 88% seropositivity for anti-nucleoprotein IgG and IgM, respectively. In the case of anti-*RBD* immunoglobulins, 100% seropositivity for IgG and 94% seropositivity for IgM was reported (Kang *et al*., 2020). Another study assessing for the seropositivity of the spike-binding immunoglobulins reported the detection of SARS-CoV-2-specific neutralizing antibodies correlating to the *S1*, *S2* and *RBD* regions on the tenth to fifteenth-day post disease onset; the findings also suggested a highly variable correlation of immunoglobulin titres with parameters such as age, blood CRP levels, lymphocyte count (Wu *et al*., 2020).

In a nutshell, three major types of antibodies, on the basis of maturity, are taken into consideration. Immature antibodies appear in the initial stage of infection, serving the purpose of bringing about an initial host response to begin grasping the virus/antigen. It is not necessary that immature antibodies will effectively recognize the antigen. IgM, immature IgA and immature IgG fall under this category. Secondly, there are neutralizing antibodies that participate in the advanced stage of the infection, as well as during convalescence. These are a subset of mature immunoglobulins and can very effectively neutralize the virus, rendering it ineffective against the host. Lastly, mature antibodies that appear only in the convalescent phase are also late immunoglobulins and these can effectively recognize the virus. These mature antibodies hold significance in hosting the viral memory for future recognition and mounting a secondary immune response. Targeting mature antibodies for sero-immunological assays may account for an increase in their specificity.

Antigen Biomarkers

In supplementation with molecular assays, rapid antigen testing that allows detection of the viral antigens can also be used (Carter *et al.*, 2020). Among the SARS-CoV-2 viral proteins, the spike proteins (S1 and S2), the receptor-binding domain (*RBD*) and the nucleocapsid are utilized as antigen biomarkers for several assays. Recombinant counterparts and biotinylated versions of these antigens have also been made commercially available. Besides these, recombinant SARS-CoV-2 methlytransferase, recombinant SARS-CoV-2 NSP9, and recombinant SARS-CoV-2 Plpro are also commercially available. The viral papain-like cysteine protease (Plpro) is involved in deubiquitinating and deISGylating activities and

plays a key role in the innate immune response to the infection. The protease is associated with the inhibition of cytokine and chemokine production in the host system. These features make the protease a significant target and a reasonable biomarker for SARS-CoV-2 infection (Rut *et al*., 2020).

Besides viral antigens, studies have reported that levels of several cancer biomarker antigens such as the carcinoembryonic antigen (CEA) and carbohydrate antigens (CA) are frequently observed to be elevated in lung inflammatory pathologies. This observation has entailed the conjecture that SARS-CoV-2-induced acute lung insult may be linked with elevated levels of these cancer biomarkers (Wei *et al*., 2020).

Procalcitonin and Interleukin-6 as Prognostic COVID-19 Biomarkers

As suggested by the name, procalcitonin (PCT) is a pro-hormone whose metabolism produces calcitonin. The hormone calcitonin plays a role in calcium homeostasis. In a health individual, procalcitonin is virtually seronegative; notwithstanding this, in sepsis condition PCT has been known to be released from hepatocytes and peripheral blood mononuclear cells (Creamer *et al*., 2019). PCT is considered to be a specific indicator of invasive bacterial infections, microbial products and pro-inflammatory mediators of host immunity. These conditions trigger a generalized induction of calcitonin mRNA and subsequent release of calcitonin precursors through a non-regulated constitutive pathway. This leads to PCT release from parenchymal tissue throughout the body resulting in a major elevation of PCT levels in circulation (Hesselink *et al*., 2009). The production and release of this prohormone is actively sustained by enhanced concentrations of IL-1B, TNF-α and IL-6 but inhibited by IFN-γ which is a hallmark in patients afflicted with viral infections. Thus, it follows that in COVID-19 patients with non-complicated infection, the PCT level would remain within the reference range; an elevation of this biomarker can be indicative of a bacterial co-infection that might steer the disease towards a more severe manifestation (Lippi and Plebani, 2020).

Cytokines are of utmost significance in the regulation of inflammation and immune responses (Zhang and An, 2007). Among these, because of its pleiotropic nature, IL-6 serves a heavy implication in disease modulation (Tanaka *et al.*, 2014). Reports have suggested an increase in IL-6 levels in patients with respiratory dysfunction, which may entail the possibility of shared contraption in cytokine-mediated lung damage associated with COVID-19 infection (Herold *et al*., 2020). Aggressive manifestation of SARS-CoV-2 infection can be linked to rapid viral replication that tends to invade the lower respiratory tract leading to elevated IL-6 mediated respiratory distress. It is known that ultimately it is the

cytokine storm that deteriorates the prognosis of COVID-19 patients. Hence, it is reasonable that assessing IL-6 as a prognostic biomarker for COVID-19 patients seeking medical aid, might recommend valuable directions in the course of therapeutic interventions (Ulhaq and Soraya, 2020).

SEROLOGICAL AND IMMUNOLOGICAL ASSAYS

The determination of SARS-CoV-2 exposure depends heavily on the finding of IgM and IgG specific to the aforementioned viral antigens. Several approaches can be adopted for this purpose and each platform carries a unique set of advantages as well as inadequacies associated with it. Assays can be tailored and optimized according to the objective of a detection kit (Carter *et al.*, 2020); diagnostic companies all around the globe are focusing on the conception of high-throughput serology kits for detection of SARS-CoV-2 infection using the platforms described in this section.

Enzyme-linked Immunosorbent Assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) is an immunological assay and is vastly applied for the quantitative and/or qualitative estimation of antibodies, antigens, and other proteins in biological samples. The assay is based on the principle of antigen capture using a specific antibody, or vice versa, coupled to an enzyme-substrate reaction for detection/quantitation of target molecule (Yolken, 1980). ELISA assays are designed such that they can be utilized in different formats – direct, indirect, sandwich, and competitive ELISA. Direct ELISA is a format wherein an antigen is immobilized on the multi-well plate surface and detected using a primary antibody (specific to the antigen) that is directly conjugated to an enzyme. The benefit of a direct ELISA assay is that it relatively less-time consuming and lesser amount of reagents are invested. The disadvantage associated with this format is that there is a high chance of background noise, besides lower flexibility arising due to necessary conjugation of the primary antibody. Similarly, in the indirect assay the antigen is immobilized on the well-plate surface. The distinction comes with a two-step process in the indirect ELISA assay: (i) a primary antibody specific to the immobilized antigen adheres to the target molecule; (ii) a secondary antibody conjugated with an enzyme adheres to the primary antibody for detection. A major advantage that the indirect ELISA format offers is that of signal amplification owing to the ability of numerous secondary antibodies to bind to the primary antibody, providing high flexibility. However, this might also account for potential cross-reactivity arising from the use of secondary antibodies (Yolken, 1980, Carter *et al*., 2020, Horlock, 2016).

Sandwich ELISA is widely utilized format, requiring two antibodies (regarded as matched antibody pairs) such that they are specific to the same antigen, but to two

distinctive regions on the antigen. One of the matched antibody pair is coated on the well-plate surface, posing as the capture antibody to mediate antigen immobilization. The other antibody is conjugated to the enzyme for detection/quantitation of the antigen. This format is expedient in terms of the high specificity, sensitivity as well as high flexibility (Gonzalez *et al*., 2008). However, the design of such an assay often proves to be demanding as finding two antibodies specific to two distinct regions on the antigen can be a challenging task (Sakamoto *et al.*, 2018). Another format, *i.e*., competitive ELISA measures the antigen titer by detecting signal interference. All of the previously described configurations can be modified to the competitive assay. By this set up, the test antigen contends with a reference antigen (pre-coated on the well plate) to bind to a limited volume of antibody conjugated with the enzyme. Prior to addition to the wells, the sample is incubated with the antibody-enzyme conjugate. Only the free unbound antibodies will be presented for binding to the reference antigen. Thus, the greater the antigen in the sample, the weaker is the signal detected (Yolken, 1980).

Lateral Flow Immunoassay

The lateral flow immunoassay (LFIA) is similar to ELISA in its layout as the base carries an immobilized target capturing entity, generally an antibody or an antigen. It is a qualitative assay based on the principle of chromatography. There are four major components in the assembly, *viz.* sample pad, conjugate pad, absorbent pad and nitrocellulose membrane. Generally a fluid sample is applied to the substrate which lets the fluid flow through a strip region of anchored antigen (Carter *et al*., 2020). Two formats of this assay are most frequently used depending on the target analyte to be tested. The competitive format is generally performed when the target to be tested has low molecular weight and/or exhibits a single antigenic determinant (Koczula and Gallotta, 2016). The analyte is complexed with a protein and coated on the test zone of a nitrocellulose membrane. The conjugate pad contains the detection antibody conjugated with a nanoparticle. If the analyte is present in the sample, it will adhere to the antibodies in the conjugate pad. Hence, as the flow advances towards the test line, the antibody-nanoparticle conjugate will not be free to bind to the previously immobilized analyte (in the test line). Another specific antibody-coated strip of area on the membrane forms the control zone, responsible for the capture of excess antibody complex; thus, the color band pertaining to this zone will always appear irrespective of the presence of analyte in the sample. This acts as a confirmation for correct development of assay. In the absence of the analyte in the sample, the antibody-nanoparticle conjugates will be free to bind to the analyte molecules on the test line, yielding a signal in the form of a second, colored band (O'Farrell, 2009).

The sandwich format of this immunoassay is employed for the detection of higher molecular weight proteins which have minimum two unique epitopes (Koczula and Gallotta, 2016). The antibody specific to one of these epitopes is conjugated with the nanoparticle, and the antibody specific to the second distinct epitope (on the analyte) is immobilized on the test line on the membrane. If the sample is positive for the analyte, binding will be observed with both, the antibody-nanoparticle conjugate, as well as with the antibody immobilized on the test line. In this format, the positive sample will yield two bands – one at the test line, another at the control line indicative of proper functioning of the assay (O'Farrell, 2009).

A group had also developed the IgM and IgG combined LFIA and tested against the blood samples of 397 PCR confirmed COVID-19 cases togetherwith 128 negative patients. It has shown the 88.66% sensitivity and around 90.63% specificity which confirm their suitabllity with compare to individual IgG and IgM test (Li *et al*., 2020b) (Fig. **4**).

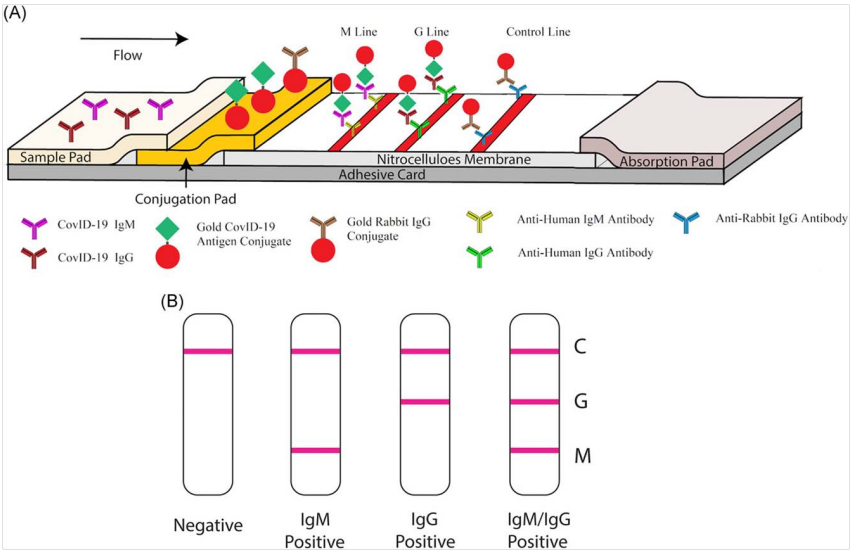


Fig. (4). Schematic illustration of rapid SARS-CoV-2 IgM-IgGcombinedantibody test. **A**, Schematicdiagram of the detectiondevice; **B**, an illustration of differenttestingresults; **C**, means control line; G, meansIgG line; M, meansIgM line. IgG, immunoglobulin G; IgM, immunoglobulin M; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2 [Adapted from (Li *et al*., 2020b)].

The result of the study confirms the functionality and suitability in different symptomatic and asymptomatic conditions which includes the various settings of laboratories. The lateral flow immunoassay provides a platform for the development of most POC tests because of ease of handling, and simple interpretation of results besides it being a rapid diagnostic test assay that provides

results within 10 to 30 minutes (Carter *et al.*, 2020, St John and Price, 2014).

Nanoparticles (NPs) have widely been used as reporter molecules in the lateral flow immunoassay. It is natural that particles larger in size, will give a stronger signal visible to the eye. However, the bulkiness of larger particles hinders the flow, thus reducing the window of opportunity for the binding event to happen. Hence, nanoparticles are most suitably employed as reporter molecules in this assay. Gold nanoparticles are among the commonly employed NPs because of two reasons – they have exceptional light absorbing property, and they provide strong surface for binding antibodies and other proteins leading to formation of robust conjugates (Ngom *et al*., 2010).

Neutralization Assays

Plaque reduction neutralization assays (PRNA) are based on the occurrence of virus-antibody interaction in a test tube or a microwellplate. Next, antibody response to viral infection is assessed by plating the mixture on cells susceptible to the virus of interest. A semi-solid medium is used to overlay the cells, restricting the spread of progeny virus. Plaques will be formed in the localized area on the plate where productive viral infection of the cells has taken place. The number of plaques is counted and compared with the initial viral concentration in order to infer the percentage reduction in total viral infectivity. In the original assay, the test serum is serially diluted and the viral load is kept constant and end-point titers are calculated for each dilution of serum at selected percentage reduction of viral activity. A modified neutralization assay based on the PRNA assay has been designed, *viz*. serum neutralization assay, wherein log serum neutralization index is used to evaluate the potency of the test serum to inhibit viral infection in susceptible cells. Contrarily to PRNA, in the serum neutralization assay, the serum concentration is held constant and it is the challenge dose of the virus that is serially increased. It is advantageous over the original assay in the fact that undiluted serum is tested which may be a better measure of the serum capacity (WHO, 2007).

The duration taken to obtain results with this test is generally 4-5 days; nevertheless, recent advances have enabled to reduce this duration to a few hours. A major drawback associated with this approach for diagnosis is that cell culture facilities and highly-skilled personnel are required. For detecting a pathogen as pernicious as SARS-CoV-2, laboratory infrastructure with biosafety level III is indeed. Nevertheless, these assays can prove to be tremendously helpful in determining the competence of neutralizing antibodies for convalescent plasma therapy and that for vaccine development (Carter *et al*., 2020).

Luminescence-based Immunoassays

In a luminescent immunoassay a fixed quantity of labeled antigen is made to compete with varying quantities of sample antigen to bind to a limited number of specific antibodies. The conjugate on the labeled antigen acts as a light-generating entity. Following incubation, the labeled antigen-antibody complex is separated from the assay mixture by either of a variety of techniques- chromatography, ion-exchange, immunoprecipitation using a secondary antibody, *etc*. The next step is to determine the luminescence emitted by by the bound fraction or that of the free fraction *via* a monitoring reaction. The result obtained is mapped on a standard curve to infer the concentration of the unlabeled antigen (Schroeder, 1982). Luminogenic conjugates that are commonly coupled to the antigen of interest include luciferase, NAD, ATP and isoluminol that are covalently coupled *via* stable amide bonds(Yeh and Ai, 2019). Luminescence detection is carried out using luminometers. Three detection systems for this assay are very widely used – (i) NADH is used with long-chain fatty acid and a bacterial luciferase, (ii) isoluminol with microperoxidase and a peroxide, and (iii) ATP with luciferin and firefly luciferase (Schroeder, 1982).

A research group has reported developing a peptide-based luminescent immunoassay to detect SARS-CoV-2 specific IgG and IgM. The peptide they have used is derived from the viral S protein. They report significant specificity exhibited by the peptide inferred from no cross-reactivity observed in 167 sera samples obtained from non-COVID-19 patients afflicted with other pathogens; this may be attributed to the low homology of the region encoding for the particular peptide among other coronaviruses (Cai *et al.,* 2020).

Biosensor Tests

Biosensors are devices that perceive a target biological analyte in the sample or in the surrounding. These are composed of three components - one that recognizes the target analyte to produce a signal, a signal transducer, and a reader (Carter *et al*., 2020). Several biosensor platforms for immunoassays have been developed over the past few decades. Electrochemical biosensors, localized surface plasmon resonance, surface enhanced Raman scattering, and piezoelectric microcantilever sensors are major types of biosensors (Mehrotra, 2016). Biosensors can potentially offer rapid, sensitive and accurate early detection of target molecules. Electrochemical biosensors are low cost, simple in design and miniaturization and one of the most widely used approaches (Cui and Zhou, 2020). Electrochemical biosensors based on electrochemical impedance spectroscopy (EIS) are commonly used in biomedical devices. Advances in technology have enabled modifications of the sensor surface using nanofabrication in order to increase the

sensitivity of EIS. This approach has been utilized to detect interactions between CLEC5A and dengue virus (Cheng *et al*., 2012). Surface enhanced Raman scattering-based biosensors have a distinct advantage in that it is an ultra-sensitive molecular spectroscopy method, devoid from interference with water. Biosensors based on this phenomenon can detect even trace amounts of analyte (Cui and Zhou, 2020). Surface plasmonic resonance is an emerging technology that evaluates interference with light incident on a solid boundary, brought about by regional disturbances like the adsorption of an antigen or that of an antibody (Carter *et al*., 2020).

Several platforms employing biosensors have also been established for the immunoassay based detection of SARS-CoV-2. A group has reported proof-o--concept development of biosensor-mediated detection of the SARS-CoV-2 S1 protein. Their design is centered on the technique of molecular identification through membrane engineering. It is a cell-based assay which enables detection of analytes on the basis of specific, selective interaction of target analytes with cellular biorecognition elements. The surface of these cellular biorecognition elements is modified to facilitate detection, *via* electroinsertion of antibodies specific to the target analyte (Mavrikou *et al*., 2020). Another group has reported a field effect transistor-based biosensor device for the detection of SARS-CoV-2 in clinical samples. It was developed by layering FET graphene sheet with anti-SARS-CoV-2 spike antibody. The study demonstrated limit of detection of their construct to be 1fg/mL in phosphate buffer saline and 100 fg/mL in clinical transport medium. Further they reported that the biosensor could differentiate SARS-CoV-2 spike protein from MERS-CoV spike protein (Seo *et al*., 2020).

Rapid Antigen Tests

Rapid antigen tests depend on specific monoclonal antibodies to facilitate the capture of viral antigens from the serum sample. Rapid antigen detection tests are not limited to any individual type of format, but rather encompass a wide range of formats such as colorimetric enzyme immunoassays, chemiluminescence assays, as well as lateral flow assays, *etc*. (Carter *et al*., 2020). One such rapid antigen test for SARS-CoV has been developed using the colorimetric enzyme immunoassay (ELISA) format. The assay employs a cocktail of three different types of monoclonal antibodies for antigen capture and yet another type of rabbit polyclonal antibodies for the purpose of detection of the antigen (Che *et al*., 2004). A rapid antigen test based on the format of lateral flow assay for the detection of SARS-CoV-2 has been reported. However, the study reports the development of only a half-strip lateral flow assay, which is a successful first step towards the development of a lateral flow platform (Grant *et al*., 2020).

Currently available rapid antigen test assays for influenza virus and syncytial respiratory virus detection are attributed with suboptimal sensitivity. It is understood that this challenge might present for the detection of SARS-CoV-2. Prototypes of these rapid antigen tests for novel coronaviruses have not been convened with regulatory approval, but attempts towards the development and enhancement of the same are ongoing (Cheng *et al*., 2020).

CONCLUDING REMARKS

The onset of COVID-19 has resulted in an ever-increasing demand for efficient and rapid SARS-CoV-2 diagnostic and screening strategies that are substantial for controlling transmission of the infection. To speed up the rate of diagnosis and simultaneously obtain accurate results, there is a strong focus on developing cost-efficient POC kits that can be produced on a large scale for mass distribution worldwide (Carter *et al.*, 2020). Current standards of COVID-19 diagnostics involve nucleic acid (viral DNA or RNA) detection strategies as well as immunological (or serological) detection of IgG and IgM antibodies. SARS-Co--2 screening also relies on the clinical symptoms observed in suspected patients, followed by confirmation of the status of the infection by molecular detection methods, specifically RT-PCR (Zhang *et al.*, 2020b). Although RT-PCR has been approved as a confirmatory test, other molecular techniques such as isothermal amplification assays, hybridization microarrays, metagenomics-based sequencing and CRISPR-associated assays are also being developed and approved for rapid and accurate SARS-CoV-2 testing. Complementary to these techniques, serological and immunological testing of not only symptomatic, but also asymptomatic patients is vital for assessing the body's immune response before, during and after the course of infection. The presence of specific antibodies against SARS-CoV-2 can be detected and studied for vaccine formulation for both, immunity against the virus and treatment of various stages of infection (Carter *et al.*, 2020).

Ever since the emergence of COVID-19, significant progress has been made in the diagnostics sector for the development of accurate, sensitive and rapid strategies to detect SARS-CoV-2 in a variety of clinical specimen (Zhang *et al.*, 2020b). The availability of test kits on a global scale, the requirement of technical expertise and access to high-throughput and elaborate laboratory facilities still remain a matter of concern. Despite the numerous challenges, constant efforts are being taken by various organizations to collaborate globally and facilitate the share of knowledge and resources for development of newer and more advanced SARS-CoV-2 diagnostic techniques.

LIST OF ABBREVIATIONS

ALT	Alanine aminotransferase
ARDS	Acute respitatorydistress syndrome
AST	Aspartateaminotransferase
AuNPs	Gold nanoparticle
BAL	Bronchoalveolar lavage
BIP	Backwardinnerprimer
CA	Carbohydrate antigen
CDC	Center for Disease Control
cDNA	complementary DNA
CEA	Carcinoembryogenicantigen
CoV	Coronaviruses
CP	Convalescent plasma
CRISPR	Clusteredregularlyinterspaced short palindromicrepeats
CRP	C-reactiveprotein
CRS	Cytokine release syndrome
CT	Computedtomography
EIS	Electrochemicalimpedancespectroscopy
ELISA	Enzyme-LinkedImmunosorbentAssay
ESR	Erythrocytesedimentation rate
FGF2	Fibroblasticgrowth factor
FIP	Forwardinnerprimer
GCSF	Granulocyte colony-stimulating factor
GMCSF	Granulocyte-macrophage colony-stimulating factor
HE	Hemagglutinin-esterase
LAMP	Loopmediatedisothermal amplification
LDH	Lactate dehdrogenase
LFIA	Lateral flow immunoassay
MAP	Mobile analysisplatform
MARS-CoV	Middle East respiratory syndrome CoV
MCP1	Monocyte chemoattractantprotein 1
MIP	Macrophage inflammatoryprotein
MOD	Multi-organdysfuntion
NP	Nasopharygeal

NPs	Nanoparticles
OP	Oropharyngeal
ORF1a/b	Open reading frame 1a/b
PCR	Polymerase chain reaction
PCT	Procalcitonin
PDGFB	Platelet-derivedgrowth factor subunit-B
POC	Point-of-care
PRNA	Plaque reductionneutralizatonassay
RBD	Receptor binding domain
RdRp	RNA-dependent RNA polymerase
RPA	Recombinasepolymerase amplification
RT-PCR	Real-time reverse transcriptase-PCR
SARS-CoV	Severe acute respiratory syndrome CoV
SHERLOCK	Specifichigh-sensitivityenzymaticreporterunlocking
SISPA	Sequence-independent single primer amplification
SNP	Single nucleotidepolymorphisms
Th1	T-helper-1
Th2	T-helper-2
VEGFA	Vascularendothelialgrowth factor-A
WBC	White bloodcells
WHO	World HealthOrganizations

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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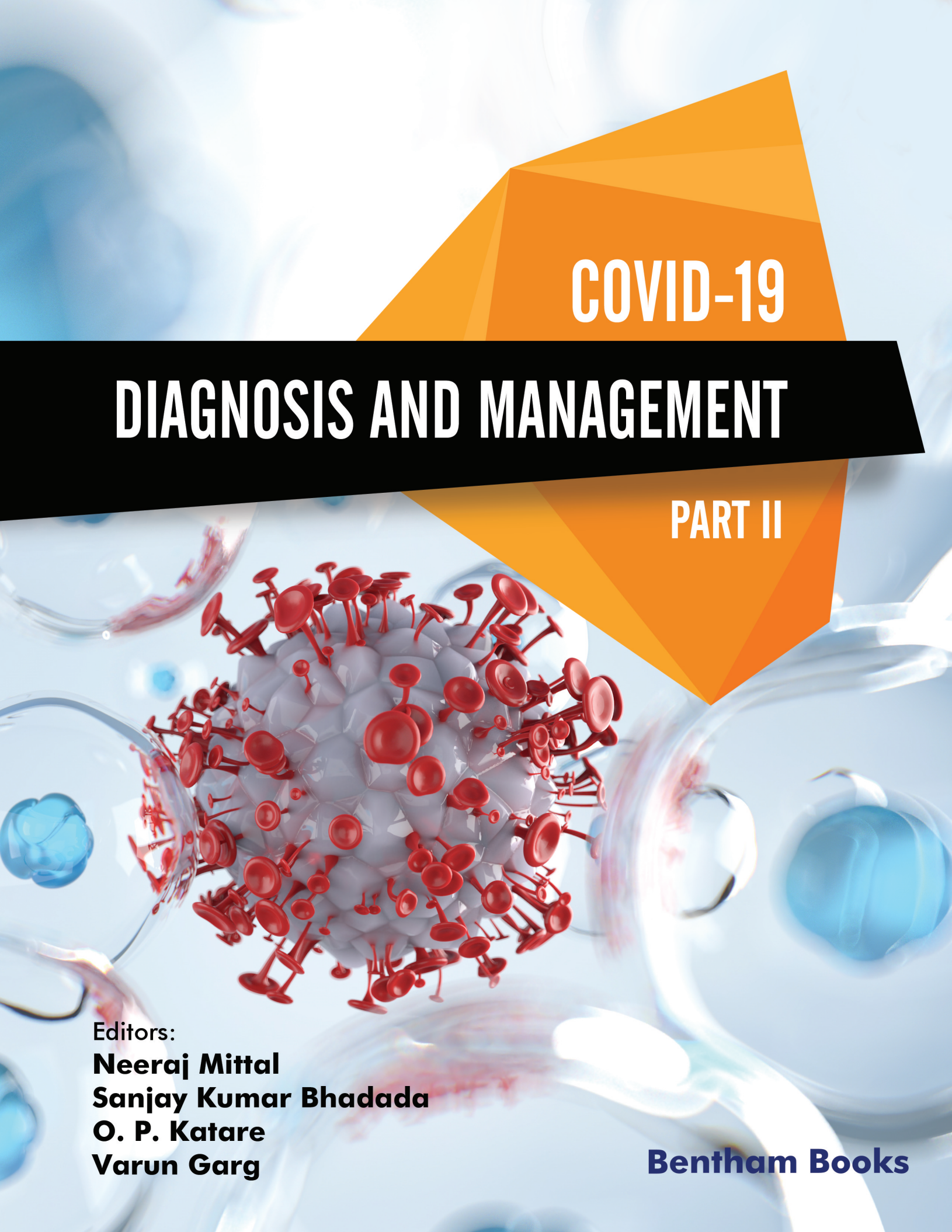
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COVID-19

DIAGNOSIS AND MANAGEMENT

PART II



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FOREWORD

It is my proud privilege to introduce the book "Covid-19: Diagnosis and Management," which is authored by a group from PGIMER, Chandigarh. The timing of this monograph is very apt as it has been about 9 months since the start of the COVID-19 pandemic, and it is now that we are starting to unravel the various mechanisms of disease pathogenesis and treatment modalities for this viral infection which has infected 29 million people, out of which about 1 million have died globally.

It has been the need of the hour to come up with a treatment for this pandemic disease. Moreover, it is of utmost importance that all the information related to COVID -19 should be compiled in one place, a goal which this book will fulfill.

Though the tests for diagnosis of the infection have been developed in the start of the pandemic, there are still some issues in diagnosis, including the sensitivity of the best test available, *i.e*., Real-Time Polymerase Chain Reaction.

The book is very well organized and has been divided into two parts; each part is comprised of 6 chapters and covers all the aspects of COVID-19 from the history to the treatment of the disease. Based on the best scientific studies available, the editors and authors have used their vast professional experience to discuss the all clinical aspects of COVID-19, including clinical presentation to diagnosis in the first part and treatment of COVID-19 in the second part, and I am very sure that this compendium will become the benchmark to refer to for any information required on COVID-19.

Whenever we write books, we must have in our minds, as clearly as possible, the affirmation of Carlyle Guerra de Macedo, who was the Director of Pan American Health Organization, relative to the responsibility of what is being published: "It must be remembered that behind each table, every report or material examined, there are lives, there are people, there is suffering, waiting for our efforts and human solidarity." Both the parts of the book are very well organized, and the readers will get a mine of information available to date on COVID-19 in one place, and it would be helpful to both the clinicians and the lab professionals for day-to-day guidance in various matters. The monograph is comprehensive but is written in a lucid manner that is easy to grasp, and even complex topics are made simple for understanding.

I am also sure that as the knowledge of the virus evolves further, the authors will certainly keep updating the work from time to time, further adding to the importance of the book. I would like to congratulate the editors/authors for this tremendous effort, and I am very sure that this book will surely be of use to readers around the world and help them in the diagnosis and management of patients with COVID-19 and will also go a long way in the efforts to help fight the pandemic, which is being faced by the humanity now.

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PREFACE

The coronavirus disease 2019 (COVID-19) outbreak has spread throughout the globe and declared as a pandemic by the World Health Organization (WHO) on 11th March, 2020. Till date on 1st September 2020, there are more than 25,327,098 confirmed cases of COVID-19 worldwide and around 848,255 deaths have been reported. The clinicians and scientists across the globe need all the information on this pandemic disease on one platform. We have already discussed history, epidemiology, and diagnosis in part I of this book. Part II of the book "COVID-19: Diagnosis and Management" is a concise and visual reference for this viral disease. It will provide comprehensive knowledge that will cover all the aspects related to the prevention and treatment methodology of this communicable disease COVID-19.

Key Features:

1. Chapter vise description and segregation of all the areas of management of COVID-19.
2. Six chapters cover prevention and treatment aspects of COVID-19.
3. Multiple tables and figures summarize and highlight important points.
4. Covering all the aspects of COVID-19 making this a perfect textbook for Virologist and medical students.
5. A summary of the current standards for the evaluation and clinical management of COVID-19.
6. A detailed list of references, abbreviations and symbols.

This book is an essential reference for practicing and training virologists, pulmologists, medical students, scientists working in various research labs, pharmaceutical and biotechnology industries on COVID-19.

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CHAPTER 1

Prevention of COVID-19: Facts to Overcome the Myths

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Abstract: The entire world has slowed down since the outbreak of a highly contagious virus, *i.e.*, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Extensive efforts are being made to reduce disease transmission, optimize the management strategy to reduce deaths and to come up with a vaccine as a preventive measure. Though several scientists across the globe are working tirelessly for developing an effective vaccine, it may still take several months to launch it successfully in the market. The behavioural interventions like maintenance of physical distancing (at least one meter), hand hygiene and cough etiquette, and use of personal protective equipment (PPE) are the currently available effective strategies to break the chain of disease transmission. All these interventions have been implemented at the population and individual level with or without linking with regulations. Based on the risk of exposure, provision and use of appropriate PPE is the need of the hour. The healthcare professionals working in COVID-19 areas have been recommended to use full PPE, which includes gloves, N-95 face masks, face shields, goggles, full-body gowns, and shoe covers. The general population has been asked to use homemade or triple-layered surgical masks in addition to the maintenance of physical distancing and hand hygiene. There are other additional strategies or measures which may or may not prevent the COVID-19 transmission. This chapter attempts to clarify the important and effective measures for the prevention of COVID-19 at the individual and community levels. It also tried to demystify the myths related to COVID-19.

Keywords: COVID-19, Diet, Disinfection, Face mask, Face shield, Filtering facepiece, Gloves, Hand hygiene, Home quarantine, Hydroxychloroquine, Institutional quarantine, N95 mask, Pandemic, Personal protective equipment, Physical distancing, Prevention, Sanitization, Screening, Transmission, Travel.

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INTRODUCTION

As it has been well-said, "Prevention is better than cure", the same applies to the pandemic situation of COVID-19 as the causative virus is novel, and there is no specific treatment available. In this situation, only the preventive measures applicable to the community and individual level will help control the spread of infection and its impact. There are several preventive measures, specifically behavioural interventions like use of face masks or personal protective equipment, maintaining hand hygiene and physical distancing, practicing cough etiquette and others to contain the spread of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which have been discussed throughout this chapter (World Health Organization, 2020c). A thorough understanding of existing evidence on the mode of transmission of the infection is important to practice the preventive measures further.

The modes of transmission of coronavirus can be broadly categorized into two types. Direct transmission is characterized by close and direct contact of a healthy person with asymptomatic (less likely) or symptomatic COVID-19 patient, especially when a healthy individual comes into close contact (up to 2 meters) and has been exposed to the respiratory droplets (>5-10μm diameter) produced by sneezing, coughing, talking and other activities (Bai *et al.*, 2020). Indirect transmission of infection happens through contact with fomites. It is generated during sneezing and coughing, which may deposit on clothes and even surfaces or objects, making it viable for transmission (Guo *et al.*, 2020). However, the evidence on airborne transmission (through droplet nuclei-diameter <5μm) and transmission through the faeco-oral route is extremely limited. Though mother-to-child transmission is highly unlikely, the newborn baby is equally at risk of acquiring the disease like others for the person-to-person transmission through respiratory droplets or through contact with fomites (Sohrabi *et al.*, 2020; World Health Organization, 2020b). Table **1** summarizes the myths and facts related to the transmission of COVID-19 (Fong, 2020).

Table 1. COVID-19 transmission associated myths and facts.

Sr. No.	Myth	Fact
1	Coronavirus cannot be transmitted in extreme weathers like hot and humid or cold/freezing temperatures	There is no evidence till date that supports these theories and has been denied by WHO as well. The virus can stay viable even at significantly high temperatures, and this fact is supported by the increasing number of cases in India.
2	The novel coronavirus can spread through mosquitoes and houseflies	No. It is not. It is spread by respiratory droplets and through contacts.

(Table 1) cont.....

Sr. No.	Myth	Fact
3	Only people with symptoms of this disease can spread it	Though transmission by an asymptomatic person is unlikely and no robust evidence available, it is possible.
4	Tobacco smoking prevents from corona infection	No. Actually, it may increase the risk of severe disease due to lowering lung function and hence increased mortality.
5	Being able to hold the breath for more than 10 seconds is a sign of the absence of virus	As more than 80% of patients present without any symptoms or with mild disease, they can hold the breath even during the acute stage.
6	Use of hand dryers can kill the virus	Hand dryers are not an effective disinfectant method against coronavirus. Alcohol-based sanitizers or hand wash using soap and water is an effective method.

MEASURES TO PREVENT THE SPREAD, ASSOCIATED MYTHS AND FACTS

The preventive measures ranged from using a simple face mask to using a full set (from head to toe) of personal protective equipment. Similarly, the preventive measures work together and effectively prevent the transmission of the disease like the use of face mask, physical distancing, maintenance of hand hygiene and others. Further, most of the measures are related to changing the behaviour and practice, some of which are linked with regulations.

Personal Protective Equipment (PPE)

The PPE can act as a physical barrier, or it is something that protects a person from any lethal biological agent when used properly (World Health Organization, 2020d).

PPE can be divided into various components, such as:

1. Head Cap/cover
2. Face Shield
3. Mask
4. Goggles
5. Gloves
6. Shoe Cover
7. Gowns or Coveralls

All the above-mentioned components together make a complete PPE, and a brief explanation on them, rationale of their use and the standards thereof are discussed below:

Head Covers

This works as a primary covering for the head and also the hair to prevent cross-contamination between the patients and healthcare providers.

Face Shield

Face shields are like the primary walls for the protection of the face, *i.e.,* nose, eyes and mouth, which are vulnerable to getting infected by the droplet transmission that is generated as a result of sneezing or coughing or talking and protects the face from direct exposure to infectious droplets as well as refrains a person from touching the face (Gupta, 2020).

The standards and specification for face shields are following:

- Must be made of a transparent plastic/polymer and provide clear visibility from either side and should be preferably fog-resistant.
- Should cover the sides and length of the face.
- An adjustable/elastic in nature band for its snug fitting to the forehead.
- May be disposable or preferably reusable after appropriate disinfection.

Mask

A face mask is the most common PPE used by all groups of population based on the risk of exposure or type of setting, *i.e.,* at the community or healthcare setting. It varies from simple homemade cloth mask or advanced masks like filtering facepiece (FFP) 3. As the cloth masks or non-medical masks are not fluid resistant and have low filtration efficiency, they are not considered to be a PPE. Further, masks can be disposable or reusable after adequate and appropriate disinfection.

Classification of Masks

Single-Use Masks

Usually with a single layer, and prevents larger dust particles. The filtration efficiency of this mask is 95% for particles above 3.0 microns.

Surgical Masks

It is usually triple-layered and the filtration efficiency up to 98% for particles

above 0.1 microns. However, effectiveness in the prevention of infection is low in case of exposure with confirmed COVID-19 patient.

Respirator or FFP Masks

These are effective masks, especially in preventing the airborne transmission of infections and smaller particles. Further, it is available with or without an exhaling valve.

Non-respirator or Dust Masks

These are worn to prevent non-toxic nuisance dust generated from various activities. However, it will not provide adequate protection from hazardous dust or infectious agents (Robertson, 2020).

FFP (Filtering Face Piece) Classification

FFP is classified into three types based on the filtering capacity and total inward leakage of a mask. The lesser the inward leakage better is the protection offered (Bode Science Center, 2020).

- FFP-1: Maximum of 22%
- FFP-2: Maximum of 8%. The N95 mask is a type of FFP2 mask.
- FFP-3: Maximum of 2%

Masks used for Prevention of COVID-19 Transmission

Masks are of several types, but specifically, two types of masks are used in the present conditions of COVID-19, which are:

N-95 Mask

It is the best suiting mask for the present situation as it has good aerosol filtration percentage, *i.e.,* 95% and it closely fits the face of one wearing it with a tighter and better seal than any other mask. These are available with and without an exhalation valve. The valve allows free movement of exhaled air and reduces the discomfort when worn. It is recommended in place of moderate to high risk of exposure to COVID-19.

The standards that a N-95 mask should meet are:

- High filtration capacity with good breathability with or without expiratory valve.
- Ensuring shape that does not collapse easily and is reliable.

It should be compliant or equivalent to the following standards:

a. National Institute of Occupational Safety and Health (NIOSH) N95, EN 149 FFP2, and
b. Fluid resistant at a minimum of 80 mmHg based on the guideline of American Society for Testing & Materials (ASTM) F1862, International Organization of Standardization (ISO) 22609 or equivalent (US Food and Drug Administration, 2020).

Triple-layered Medical Mask

As the name itself suggests, it is a triple-layered mask which is fluid-resistant and thus provides protection from the droplets and is disposable too. A triple-layered mask must be non-woven with a nose pin and ISI compliant with a filtering efficiency of 99% for 3-micron and larger particle size. It is recommended in low risk of exposure to COVID-19 or people working in non-COVID-19 areas/settings.

Goggles

They form an integral part of the protective equipment for face and should have a flexible frame making it fit tightly to the face and not allowing any particulate matter or droplets to enter the eyes (World Health Organization, 2020d). Usually, people working in high-risk area or exposure to COVID-19 need to wear goggles along with N95 masks.

The specifications and standards to be ensured for a mask include:

- Should have a good seal, transparent, made up of strong polymeric material, covering all sides with a good fitting.
- Preferably fog and scratch-resistant.
- An adjustable band that can firmly secure the position and does not lose out during the clinical activity.
- Should have an indirect vent to reduce fogging.
- It should preferably be reusable after adequate disinfection.

Gloves

The major source of getting infected after the droplet transmission is by physical contact, which is majorly through hands. Anybody who is dealing with moderate to high-risk areas (the areas where the infected or one with high risk are dealt with) has to ensure wearing gloves and also their proper disposal after use or else, they may also become a mode of transmission. However, maintenance of hand hygiene and PPE cannot be ignored even if someone is using gloves (alone) since the person may touch his face or other places after wearing the gloves.

Certain specifications and standards for gloves (non-sterile) include:

- Made up of Nitrile rubber (better resistance to chemicals than latex).
- Must be powder-free.
- Outer gloves should reach the mid-forearm (at least 280 mm total length).
- Available in different sizes.

Shoe Covers

The shoe covers are made of an impermeable material to cover the footwear and for personal protection which must be made of the same material as that of coverall and must cover the entire shoe and reach above the ankle. Shoe covers are recommended to people working in the high-risk areas or exposure to COVID-19.

Gowns or Coveralls

These are the full-body coverings generally made of polymers that ensure the safety of the body of the healthcare workers and provide a barrier to reduce or even eliminate the droplet contamination. It generally covers the whole body from head to feet and ensures complete protection if appropriately used. However, there might be some gaps at the backside of the gowns or coveralls, which may also cause contamination but ensuring a proper covering and doffing can minimize this risk. They can basically protect a person from the biologically contaminated particulate matter or solid (World Health Organization, 2020a).

The specification and standards for the coveralls and gowns include:

- Single-use and preferably light in colour to detect the contamination.
- Must be impermeable to blood and other body fluids and has an impermeability

pressure of at least 40 mmHg.
- Thumb/finger loops for anchoring the sleeves.

Must be equivalent, exceed or complaint with the following standard:

ISO 16603 Class-3 Exposure Pressure

The rationale of the use of PPEs as per the guidelines by Ministry of Health and Family Welfare, Government of India & World Health Organization, are stated below in Table **2** (EMR Division Ministry of Health and Family Welfare (Government of India), 2020b, 2020a; World Health Organization, 2020d, 2020c).

Table 2. The rationale for the use of personal protective equipment.

Sr. No.	Designated areas/Personnel	Level of Risk	PPE Recommended
Point of Entry			
1	Health desk, Immigration & Customs counters, Temperature checking, Security	Low	Triple-Layered Medical Mask Gloves.
2	Isolation, Holding Facility.	Moderate to High.	N-95 Mask Gloves Full PPE in the Isolation ward.
3	Sanitary staff	Moderate	N-95 Mask Gloves.
Hospital Setting **Out-Patient Department (OPD)**			
1	Triage, Screening area, Temperature recording station, Holding area, Doctor's chamber and Sanitary staff	Moderate	N-95 Mask Gloves.
2	Accompanying children and elderly people	Low	Triple-layered Medical Mask.
In-Patient Department (IPD)			
1	Individual isolation rooms and Sanitary staff	Moderate	N-95 Mask Gloves.
2	ICU, Treatment areas	High	Full PPE recommended.
Emergency Department			
1	Emergency cases, Attending SARI (Severe Acute Respiratory Infections) patients	Moderate to High	N-95 Mask, Gloves or Full PPE recommended.
Ambulatory and Ancillary Services			
1	Emergency transfer of SARI patients	High	Full PPE recommended.
2	Driving the ambulance	Low to Moderate	N-95 Mask Gloves.
3	Laboratory testing/radiologist	High	Full PPE recommended.

(Table 2) cont.....

Sr. No.	Designated areas/Personnel	Level of Risk	PPE Recommended
4	Sanitation, Laundry, Mortuary	Moderate	N-95 Mask Gloves.

Sr. No.	Designated areas/Personnel	Level of Risk	PPE Recommended
Community Health Workers			
1	Accredited Social Health Activists, Auxiliary Nurse Midwives, NGO workers, Supervisory doctors imparting field education	Low to Moderate	N-95 Mask Gloves
Quarantine Centre/Facility			
1	Persons being quarantined, Support staff	Low	Triple-Layered Mask
2	Healthcare staff	Moderate	N-95 Mask Gloves
Home Quarantine			
1	Person being quarantined and designated family member	Low	Triple-Layered Mask Gloves
General Public			
1	Individuals, Offices with no public dealing	Low	Triple-Layered Mask or Equivalent to Cover their Face whenever out
2	Offices with public dealing and Shops	Moderate	N-95 Mask or Triple Layered Mask or clothed mask Gloves

Note: A person wearing PPE must not ignore the basic rules of hand hygiene and public distancing and must take extra care in every aspect to avoid getting infected and transmit the disease.

SANITIZATION AND DISINFECTION

Hand Hygiene

The coronavirus pandemic is known for transmission through two major modes which include the droplet transmission or through contact. Transmission through contact occurs when a healthy person comes in contact, *i.e.,* touches an infected person, surfaces, *etc.,* with his hands and may carry the virus which may further enter in the body through nose, mouth or eyes while performing the day to day activities. To prevent this possibility of getting infected, one must always maintain the hands clean (Lotfinejad *et al.*, 2020; World Health Organization, 2020b).

Now the question is, what and how can someone achieve or maintain this hand

hygiene? Well, it's quite simple, but certain considerations have to be ensured to stay safe such as:

For Public

- One can wash his hands with soap and water for a minimum of 20-30 seconds ensuring the proper scrubbing of all the areas of palm, fingers, nails and crevices is recommended till the wrist, and then the soap is properly washed with water and ensure that the hands are dry.
- One can also consider the use of hand sanitizers, which should contain not less than 70% of isopropyl alcohol when cleaning with soap and water is not possible or hands are not visibly soiled. A person allergic to alcohol-based hand rub can maintain hand hygiene using soap and water.

Everybody must consider washing hands or sanitizing them at regular intervals, especially during the following activities, namely:

- Before preparing and eating food.
- Before and after coming in contact with any person outside the house.
- After visiting a public place like an office, hospital, sports complexes *etc.*
- Before and after treating a wound or cut.
- After using public devices, others' phones and also touching surfaces such as lift buttons, doorknobs, public transport *etc.* (Fathizadeh *et al.*, 2020).
- After coughing or sneezing or vomiting.
- Before touching the face while outdoors.

Prevention of COVID-19 Among Frontline Healthcare Workers

As the frontline working force including the healthcare professionals, sanitization workers, law enforcement departments *etc.* are more susceptible to acquire the infection being in direct contact with the infected or in contact with a lot of people every day. Hence, it is very necessary for them to take care of hand hygiene importantly (Lotfinejad *et al*., 2020). This can be done by the use of disposable hand gloves and repeatedly sanitizing the hands with a recommended sanitizer that has the alcohol content not less than 70%. Frontline workers not only have to take care of their hands but should also consider disinfecting their clothes and whole body once they relieve from their everyday duty to ensure maximum safety (World Health Organization, 2020a).

Cleaning of Vegetables-Fruits

Vegetables and fruits are an essential part of daily life and are very necessary to be consumed for maintaining sound health. But, in pandemic situations like COVID-19, there are chances of contamination of the fruits and vegetable surfaces as well, and to overcome this, certain steps have to be followed, such as:

- One should wash his hands efficiently before and after handling fruits/vegetables
- Initially, they can be classified into two types, *i.e.,* 1) Raw fruits & vegetables, and 2) Green leafy vegetables
- For the fruits and vegetables whose outer covering is removed before consumption, they can be soaked in clean water with some amount of salt or baking soda or potassium permanganate added to it and cleaned properly followed by washing them under a tap of clean running water with the application of a mild scrub or a brush for this activity, wherever applicable.
- In case of the green leafy vegetables, soaking them for 15 minutes in one part of vinegar and three parts of water or water added with a spoon of salt followed by thorough washing under running water is recommended.

Note:

- One should not use detergents or any disinfectants to clean edible substances as their consumption be harmful to our body.
- Purchase only fresh fruits and vegetables.
- Purchase prepacked fruits and vegetables, preferably which may have less contact with human hands or secretions.
- Avoid eating uncooked vegetables or improperly cleaned fruits and vegetables.
- Consumption of raw or stale meat must be avoided.

Safe Handling of Currency and Paper Documents

The paper currency notes tend to carry several microorganisms as they go hand by hand to several individuals and places, making them a potential carrier for bacteria and viruses. Hence, one must ensure proper hand hygiene as discussed above before and after handling currency, as there are possibilities of carrying the droplets over them (Oosterhoff and Palmer, 2020). Shifting towards cashless payment methods wherever possible is advised to reduce the chance of exposure.

Recently, a laboratory of Defense Research Development Organization (DRDO), India has developed a device which can sanitize items like paper currency, mobile phones, wallets, laptops *etc.* with the help of UV-C rays (predominantly 254 nm) which is an effective method to be adopted (Roza *et al.*, 1985). Few countries

which have polymer-based water-proof currency have an additional advantage of the ease of sanitizing them, making it safer for use (IANS, 2020). Further, all the routine office procedures could be shifted to digital mode to reduce the handling of paper surfaces which moves between people and risk the exposure. Besides, the movement towards digital method further increases the efficiency, timeliness and transparency.

Cough or Respiratory Etiquettes

Individual responsibility plays a major role in efficient handling of such dreadful pandemic and containing the spread of disease, especially in practicing the cough or respiratory etiquettes.

Precautions to be Taken While Coughing/Sneezing or Any Physical Contact

It is strictly advisable that a person should cover the mouth and nose with a mask always. Irrespective of use of mask, it is advisable to cover the mouth with his elbow while sneezing or coughing and to sanitize the hands after it with an alcohol-based sanitizer.

Spitting or Littering in Public

Spitting in public areas or on roads is strictly unadvisable as well as prohibited in several places or punishable under law. Spitting in places can increase the risk of infection as the virus may remain in an open area for a longer period of time (PTI, 2020).

Disinfection of Public Places

Disinfection and sanitization of public places is another measure that has to be performed effectively to check the spread of coronavirus. There are certain standard procedures and chemicals that have to be used for the disinfection of these surfaces to ensure no viable form of virus stays there to spread the infection (Oosterhoff and Palmer, 2020).

Certain guidelines for effective disinfection of surfaces are given below:

- The surfaces of offices, rooms, hospitals must be mopped using phenol or 1% sodium hypochlorite solution or a bleaching agent at least twice daily.
- The frequently touched areas such as the tables, chairs, machines *etc.*, must be

cleaned using the same solution as mentioned in Table **3** below.

- Metallic surfaces such as lifts, doors knobs, handles, locks, and keys must be cleaned using 70% alcohol because using a bleaching agent or so could result in the rusting of these items.
- All the used products and disposable wastes must also be treated with 1% sodium hypochlorite solution preferably.
- Extra care must be taken for the cleaning of restrooms and all its surfaces and must be done at regular intervals with the aid of soaps, phenolic solutions, 1% sodium hypochlorite solution or using a bleaching agent (Xiao and Torok, 2020).
- There should be arrangements for disinfection of toilet seats present in public places. Closed flushing of the toilets must be practiced avoiding the generation of aerosols.

Table 3. Preparation and chlorine content of various disinfectants.

Sr. No.	Product	Preparation	Chlorine Content
1	Sodium hypochlorite	1 part in 4 parts of water	5%
2	Sodium dichloro-isocyanurate	17 g in 1000 ml of water	60%
3	Chloramine (powder)	80 g in 1000 ml of water	25%
4	Bleaching powder	7 g in 1000 ml of water	70%

Disposal and Disinfection of PPEs

As the PPEs are used as a barrier from the virus and is used when the risk is high; thus, the chances of them getting contaminated by the virus are also high. These contaminated kits cannot be disposed of as such and are needed to be treated with a disinfectant before disposal. For the disinfection, any phenolic solution or 1% sodium hypochlorite solution can be used.

Donning of PPE kits is recommended after proper sanitization of hands followed by the stepwise wearing of all the coverings from head to toe sequentially. Doffing of PPE kits requires utmost care and attention as there is a huge risk of contracting the virus. The steps for removal of PPE kits are shown in Fig. (**1**) below (World Health Organization, 2007).

WATER TREATMENT AND WASTE MANAGEMENT

Management of Water and Faecal Waste

Till date, there has been no significant evidence that the virus can be transmitted

through sewage water, but studies have found viral fragments in the human excreta. Efficient wastewater treatment and disposal are essential to ensure that it will not pose any threat to nature or human beings. The general water treatment measures include the retention method, which is carried out for days (20 days), if sufficient and a final disinfection step may be considered to ensure better safety. Considering the safety requirements for the staff working in the respective water treatment plants, use of PPEs like heavy-duty gloves, masks, face shields, boots, goggles *etc.* must be practiced (World Health Organization, 2020e).

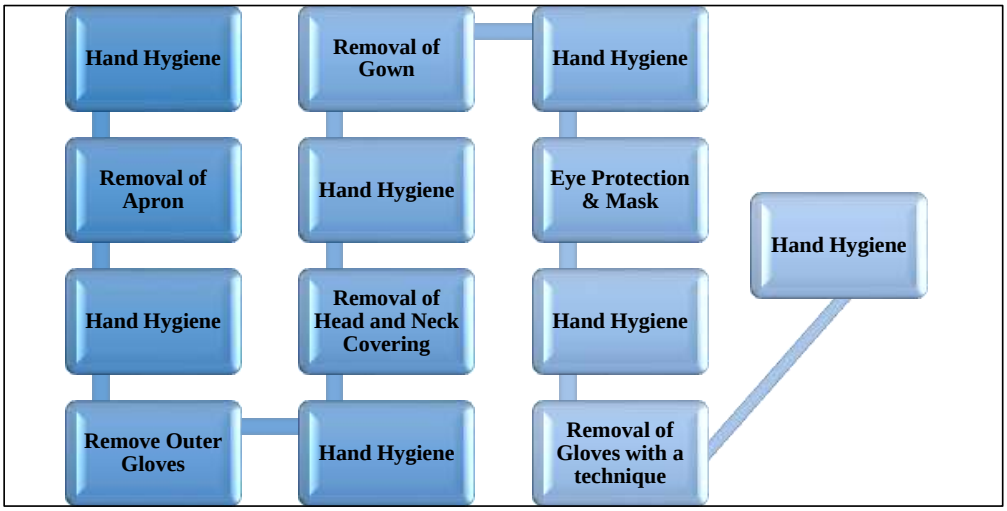


Fig. (1). Steps to doffing or removal of personal protective equipment.

Safety of Water Supplies

The basic measures that are taken for the treatment of water are enough to destroy coronavirus as they have sensitivity towards chlorination and the amount of residual chlorine ≥ 0.5 mg/L with 30 minutes of contact time at a pH of < 8 (World Health Organization, 2020e). Ultraviolet irradiation can also add up as a feature of extra safety to eradicate the chance of any viable form of virus remaining in water to render it unfit for consumption.

SOCIAL MEASURES

Physical Distancing

Through physical distancing (or also known as social distancing), *i.e.*, maintaining a possible safe distance and avoiding the large gatherings, the contact of an infected person and susceptible person is minimized. Hence, it lowers the chances

of the spread of infection. It can also be called a non-technical infection control and preventive measure to check on the spread of the virus (Kissler *et al.*, 2020).

A thumb rule to ensure physical distancing is to maintain a distance of at least 1 meter (preferably 2 meters) from any individual. However, the more is the distance, and the lower are the chances of contracting the virus (Greenstone and Nigam, 2020). One can take advantage of technology to stay connected with their social groups, even while practicing physical distancing.

Application of door/home delivery, wherever possible, must be encouraged for the purchase of groceries, medications *etc.* If an individual is visiting any shop or a store, they must practice physical distancing.

Encouraging work from home can be another solution to avoid closed group gatherings and risk of exposures. Meetings and teaching must be conducted *via* video conferencing, or other online modes which can prevent large gatherings and yet fulfil the needs (Center for Disease Control, 2020; Ministry of Health and Family Welfare (Government of India), 2020a). The myths and facts related to social preventive measures during COVID-19 is described in Table **4**.

Table 4. Myths and facts related to social measures for preventing COVID-19.

Sr. No.	Myth	Fact
1	The practice of physical distancing can completely clear the risk of coronavirus transmission	Physical distancing can significantly reduce the risk by breaking the chain of transmission, but it cannot provide complete protection. For which, it should be augmented with proper use of mask, maintenance of hand hygiene and cough etiquette for near-complete protection.
2	Physical distancing is only for the elderly or people with high risk	Physical distancing is applicable for everyone irrespective of age, gender, or other social/demographic groups of the classification.
3	Only people being tested positive must ensure public distancing	Physical distancing should be practiced by everyone irrespective of the status of COVID-19 results as asymptomatic patients and patients in the incubation period will be in the community.
4	All human interactions need to be stopped	It is not practically possible, and the very purpose is to ensure there is no physical/close contact, but activities with a safer distance can be carried out.
5	If face is covered with a mask, there is no need for public distancing	Use of mask or maintenance of physical distancing alone will not provide complete protection from infection. All the four good practice (proper use of mask, maintenance of physical distancing, hand hygiene and cough etiquette) need to be practiced together always for reducing the risk of exposure.

Quarantine and Isolation

Quarantine is a practice of restricting the movement of an individual or a group of individuals for a particular period at a particular place who are potentially exposed to infection. This ensures early detection of infection among exposed individuals and reduces the chances of transmission through restricting the contact of a susceptible person with exposed person. Isolation can be simply termed as the separation of an infected patient from healthy/susceptible population to prevent further transmission of infection. Quarantine is traditionally employed among people who have a travel history from a place which is a hotspot for the disease or has come in contact with a person who has been diagnosed with the disease. This is basically done to break the chain of transmission (Nussbaumer-Streit *et al.*, 2020). This can be broadly classified into two types, *i.e.,* home quarantine and institutional quarantine, which is decided by the authorities dealing with the situation at the point taking several factors into consideration.

Home Quarantine

It is nothing but the restriction of movement of a person at his home itself for a period of 14 days (incubation period of the virus is 2-14 days) and taking care of necessary precautions like:

- Staying in a separate room and isolated from other family members especially the elderly, children and the ones with comorbidities.
- Avoid sharing of household items with other people at home.
- Using a mask all the time and also take proper care of its disposal like treating it with disinfectants before discarding.
- Only one person (caretaker) of the family is advised to serve the quarantined person with necessities, and that too without direct physical contact with practicing all the necessary precautions and visitors should never be allowed.
- If any symptoms of the disease are observed, then it must be immediately brought to the notice of responsible authorities.
- All the surfaces, including the objects used, must be disinfected or adequately and appropriately cleaned.
- Everyone in the house must practice hand hygiene measures and try to practice physical distancing as much as possible.
- They have to regularly update the health workers or in the given mobile application regarding their health status and presence of new symptoms.

Institutional Quarantine

Institutional quarantine is practiced especially for individuals who don't have adequate facilities like a separate room in their house and other amenities for practicing quarantine (Hu *et al.*, 2020). There are certain considerations needed to be taken care of for the efficient and purposeful functioning of a quarantine centre like:

Infrastructural Facilities

- The institute must be well maintained, has proper water supply, drainage facility and electricity.
- Well maintained beds with a distance of at least 2 meters from each other with a proper washroom facility.
- Basic amenities like fans, lights *etc.* must be ensured.

Staff Guidelines

- The entry into the institute must be restricted and strictly monitored, and also a logbook should be maintained for the movement of staff in and out of the facility
- An active medical team must evaluate all the individuals residing in the facility for any symptoms periodically (daily or twice daily)
- The staff dealing with the institutional tasks must ensure wearing proper personal protective equipment and its disposal

Utility Guidelines

- Basic needs such as food, water, housekeeping and laundry must be taken care of efficiently
- Disinfection of surfaces and washrooms must be done regularly
- Used clothes, masks or other daily use items must be washed/ disinfected/disposed efficiently

The myths and facts related to quarantine are given in Table **5**.

Table 5. Myths and facts related to Quarantine.

Sr. No	Myth	Fact
1	One can maintain close contact with other people in quarantine	A person under quarantine should not be visited by anyone except the fixed caregiver. During caregiving also, a physical distance of at least 1 meter must be practiced.

(Table 5) cont.....

Sr. No	Myth	Fact
2	One can meet people if home quarantined	Until the completion of the quarantine period, no one should be allowed to meet the quarantined person other than the fixed caregiver.
3	One can start a common lifestyle after the 14-day period	Individual tested negative at the end of the quarantine period can live a normal lifestyle. However, they have to follow all the precautions like use of face mask, practice physical distancing, hand hygiene and cough etiquette all the time post quarantine.

Lockdown and Travel Restrictions

A lockdown can be explained as a seizure of several non-essential and everyday activities in a particular area, state or country ensuring people stay at their home, thus resulting in better physical distancing, lesser gatherings and movements which will help to control the spread of the disease to an appreciable extent. As of now, to stop the spread of novel coronavirus, lockdowns and travel bans have become the frontline measures for the public and also containment of the disease.

Worldwide travel restrictions and bans have been observed to contain the spread of this deadly virus, which has proven to be an effective step towards prevention. Important myths and facts related to lockdowns and travel bans have been stated in Table **6** below.

Table 6. Myths and facts related to lockdown and travel restrictions.

Sr. No.	Myth	Fact
1	Normal life will return immediately after lockdown	The normal activities of life will resume gradually in a phased manner to check for an increasing number of cases or movement of people.
2	There is only one form of lockdown	The spectrum of lockdown can range from restriction for local or international travel to restriction of all non-essential activities to restriction of all activities. The severity of the situation decides the enforcement of a particular type of lockdown.
3	Lockdowns are the only measures to prevent COVID-19	Lockdown is not the only measure to prevent COVID-19. Measures like quarantine, early identification of cases and testing, isolation, and treatment of the patient are important measures which should go in parallel to lockdown. Further, enforcement of the use of the mask in public places, practicing physical distancing, hand hygiene and cough etiquettes are important individual level practices for prevention of COVID-19.
4	Wearing a mask and having a sanitizer is enough for safe travel	Use of mask and maintaining hand hygiene are good practices. In addition, physical distancing and avoiding unnecessary touching of objects/surfaces need to be practiced for enhanced prevention.

Zonal Classification of the Geography

Based on the number of cases reported and active cases from an area, it is classified as a red, orange or green zone based on the number of cases reported in the past 21 days, active cases and its doubling rate by the states/union territory. Further, the district authorities can demarcate it as containment zone, buffer zone and non-containment zone based on the spread of active cases and its contacts. Except for medical emergencies and essential supplies, all activities are restricted within the containment zone with a clear demarcation of entry and exit points for those movements. Importantly, active case finding through household surveys and testing will be the key strategy in this zone. Buffer zones are the areas delineated immediately around the containment zone. This is the immediate next attention area as it is the next closest area to active cases/contacts with restriction of movements to a certain extent. In non-containment zones, all normal activities are allowed as per the notification from time to time by the respective states/union territories (Thacker, 2020).

PROPHYLAXIS FOR COVID-19

COVID-19 is a disease for which an effective and safe therapy or a vaccine is yet to be discovered. In such conditions, one can rely on certain healthy practices and consume functional foods that can boost the immunity of the body and promote sound health.

Dietary Recommendations for the Prevention of COVID-19

It is always better to improve the immunity against a pathogen rather than consuming medications to treat the severity of symptoms. Certain functional foods and ingredients may help to a certain extent to improve immunity. However, an effective and proven dietary prophylaxis against COVID-19 yet to be identified.

Certain guidelines of healthy practices and foods have been issued by the Ministry of AYUSH, Government of India in accordance with the Ayurveda, Unani, Siddha and Homeopathy (AYUSH) system of medicine (traditional/ancient medicinal system of India) and are briefly discussed below (Patwardhan *et al.*, 2020).

General Measures

- Regular practice of yoga, meditation *etc.*, for at least 30 minutes a day can

relieve stress and promote physical, and psychological wellness.
- Drinking warm water.
- Use of spices like turmeric (known for the beneficial activities of curcumin), cumin, garlic, coriander, and ashwagandha which promote good health with the valuable phytoconstituents present therein, has been recommended (Golechha, 2020).

Medicinal Plant Measures/Procedures

- Consumption of herbal tea or decoction with ingredients such as basil leaves, cinnamon, black pepper, ginger, raisins *etc.*, which have also been proven to have beneficial effects with their chemical constituents and a glass of milk to which a spoon of turmeric powder is added.
- Consumption of fruits, especially citrus fruits, lemon juices *etc.*, which are rich in vitamin C are beneficial for immune functions
- Use of clove (Eugenol which is known for its anti-microbial activity), steam inhalation of the extracts of caraway seeds and mint to ease cough and sore throat.
- Simple ayurvedic procedures such as application of pure coconut oil in both nostrils or oil pulling therapy (swishing mouth with sesame/coconut oil for 2-3 minutes and spitting out followed by water rinsing) can also help ease the conditions of cough *etc.*
- All such measures have been practiced in India as part of its own medicinal system (Ayurveda) and have also proven beneficial both practically and scientifically (Ministry of AYUSH (Government of India), 2020; Tillu *et al.*, 2020). However, it needs to be proven against COVID-19 prevention, some of which are under study.

Medical Prophylaxis for Healthcare Workers and Contacts of a Positive Patient

- Being the frontline warriors (Healthcare workers), they are the ones who are in direct contact with the infected persons and are at a verge of getting infected easily, and use of personal protective equipment may not be sufficient due to underestimation of the risk of exposure and accidental exposures due to various operational issues while handling a COVID-19 positive patient.
- Governments of several countries like the USA and India have advised the healthcare workers to consume hydroxychloroquine (HCQ) tablets prophylactically on the basis of available pre-clinical and ongoing clinical data looking into the successful treatment of patients treated with it (Murthy, Gomersall and Fowler, 2020; Rathi *et al.*, 2020).

- As per Indian Council of Medical Research (ICMR), the prophylactic dose of HCQ for asymptomatic healthcare professionals is 400 mg dose twice a day on the first day and a single dose of 400 mg per week for seven weeks with meals. The duration of the regime for the asymptomatic household contacts has been restricted to three weeks (Ministry of Health and Family Welfare (Government of India), 2020b). However, use of this chemoprophylactic measure with the consumption of HCQ may be associated with certain cardiac side-effects which should be evaluated before starting the medication (Principi and Esposito, 2020).

There are a number of myths associated with various dietary recommendations or related to prophylaxis, and the common ones are tabulated below in Table **7**.

Table 7. Myths and facts related to dietary or medical prophylaxis against COVID-19 (Ministry of Health and Family Welfare (Government of India), 2020c).

Sr. No.	Myth	Fact
1	Adding black pepper to food can prevent COVID-19	Black pepper may improve general immunity. However, its effectiveness against COVID-19 needs to be proved.
2	Tobacco smoking clears the coronavirus from throat and lung due to its smoke	Not at all. Tobacco smoking reduces the lung function and causes/aggravates the inflammation which may result in severe disease
3	Tobacco chewing or smokeless tobacco use will kill the coronavirus due to the antiviral effects of the tobacco or its ingredient	Not at all. As chewing tobacco or the ingredients in the smokeless tobacco form causes or aggravates the inflammation, it may cause severe COVID-19.
4	Introducing or ingesting alcohol or disinfectant can kill the virus	Consuming such alcohol and disinfectant can cause severe damage to the gastrointestinal lining such as lesions, perforations and can be lethal in high concentration/quantity.
5	Exposing yourself to a higher temperature will prevent the infection	Exposure to sunlight or higher temperature will not prevent the infection.
6	Drinking more of hot drinks can stop COVID-19	No cool or hot drinks can stop the spread of the virus, and the practice of this may also harm the body.
7	Use of UV light on the skin can kill the virus	UV radiation should not be used to disinfect hands or other body parts as it may cause harm.
8	One should consume hydroxychloroquine to prevent COVID-19	Self-medication without a doctor's supervision is always dangerous and may cause death.

REGULAR SCREENING

Screening is an important disease containment strategy. High risk or population screening is needed based on the local epidemiological situation. Primarily,

screening includes symptomatic screening or thermal screening. Recently, rapid antigen-based screening testing kits are also added in the list. Since these are screening tools with varying sensitivity and specificity, a confirmatory test is needed for providing clinical care to the patient (Gostic *et al.*, 2020). There is a number of digital applications like Arogya Setu or others for regular assessment of risk and closeness to confirmed COVID-19 cases which can be used by anyone after installation.

The person in quarantine (home or facility) will be checked for increased body temperature or symptoms suggestive of COVID-19 on a regular interval by the healthcare worker/self/using Arogya Setu or other applications. The patient must immediately contact the concerned authorities and act based on the symptoms or screen positivity (Paules, Marston and Fauci, 2020). The person must also be advised always to cover the face with a mask and follow all the quarantine guidelines (Augusta Health, 2020).

PRECAUTIONS FOR TRAVEL

The mandatory precautionary measures during travel include the use of mask, maintenance of hand hygiene and physical distancing and practice of cough etiquettes. Following these measures can lower the risk of infection. However, it will not eliminate the chances of spread.

As per guidelines given by WHO:

- Screening of the passengers while entering and exiting is important, and if the temperature happens to be more than 38°C with or without cough, then the person needs to be evaluated for the same.
- One must always carry a hand sanitizer and should take care of hand hygiene frequently.
- Travelling is not recommended for the geriatric and pediatric population.
- The passenger must maintain a distance of at least 1 meter, *i.e.,* physical distancing.
- Persons from the containment zone and under quarantine are strictly prohibited from travelling.
- Home quarantine for 14 days is advised to ensure that there is no spread of the virus in the port of arrival with or without linked with testing.

CONCLUSION

COVID-19 outbreak was recognized in early January 2020 in Wuhan city of China and later on declared as a pandemic by WHO. Till date, it has affected

more than 200 countries. Prevention from the infection at all setting is important due to the availability of no/poor information about the exact origin of this virus, and non-availability of effective and proven treatment. Since the major route of transmission is through respiratory droplets emitted through coughing, sneezing, and through contact of fomites from surfaces, cloths or objects, use of face mask, maintenance of hand hygiene and physical distance (at least 1 metre) becomes an essential tool to prevent the spread. In light of the current scenario, authors wish to conclude with recommending the adoption of preventive measures by each member of the society which further will help to prevent other infectious diseases in the population.

LIST OF ABBREVIATIONS

ACE-2	Angiotensin-Converting Enzyme-2
ASTM	American Society for Testing & Materials
AYUSH	Ayurveda, Unani, Siddha and Homeopathy
CHW	Community Health Worker
COVID-19	Coronavirus Disease 2019
DRDO	Defense Research Development Organization
FFP	Filtering Face Piece
GoI	Government of India
HCQ	Hydroxychloroquine
ICMR	Indian Council of Medical Research
ILI	Influenza-Like Illness
IPD	Inpatient Department
ISO	International Organization of Standardization
MERS-CoV	Middle East Respiratory Syndrome Coronavirus
MoHFW	Ministry of Health and Family Welfare
nCoV	Novel Coronavirus
NGO	Non-governmental Organization
NIOSH	National Institute of Occupational Safety and Health
OPD	Outpatient Department
PPE	Personal Protective Equipment
RNA	Ribonucleic Acid
SARI	Severe Acute Respiratory Illness
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
UV-C	Ultraviolet C

WHO World Health Organization

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 2

Current Treatment Methods for Coronavirus Disease-19

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Abstract: The coronavirus disease (COVID-19) pandemic outbreak has created health havoc all over the world. Till now, no definite treatment has been found to combat the COVID-19 outbreak, probably due to a poor understanding of the molecular mechanism of this infection. As it's a health devastating situation, so due to lack of proper time for research, clinicians all over the world are exploring the already approved drug such as lopinavir, ritonavir, chloroquine (CQ), hydroxychloroquine (HCQ), azithromycin (AZ), remdesivir, favipiravir, ribavirin, nitozoxanide, interferon-α (IFN), arbidol, corticosteroids, ivermectin, teicoplanin, herbal drugs, *etc.* for anti-viral activity. Previous studies suggest that these drugs act by different mechanisms such as prevention of entry and fusion of the virus with host cell by blocking angiotensin-converting enzyme-2 (ACE-2) receptor and increasing endosomal pH respectively, inhibition of RNA polymerase and protease enzyme, inhibition of inflammatory pathway by blocking toll-like receptors (TLR's), inhibition of RNA synthesis, interference of glycosylation of cellular receptor, suppression of immune response, *etc.* Besides these drugs, few humanized monoclonal antibodies such as tocilizumab and sarilumab are also shown to be effective against COVID-19 by blocking interleukin-6 (IL-6) receptors. In addition to these drugs, convalescent plasma therapy is also being used to treat COVID-19 patients. Focus is on the development of a vaccine for COVID-19 at the earliest and indeed, many vaccines are in various stages of the development process, with some under clinical trials. This review gives an exhaustive view of current therapeutic strategies for the management of COVID-19.

Keywords: ACE-2, Arbidol, Azithromycin, COVID-19, Corticosteroids, Favipiravir, Hydroxychloroquine, IL-6, Immune response, Interferon-α, Ivermectin, Lopinavir, Monoclonal antibodies, Plasma therapy, Protease, Remdesivir, Ribavirin, Ritonavir, RNA polymerase, TLR's, Tocilizumab.

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INTRODUCTION

The coronavirus disease (COVID-19), also referred as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has been the major health outbreak nowadays. This virus was firstly detected from Wuhan city of China in December, 2019, and now it has been spread worldwide (more than 190 countries) (Wang *et al.*, 2020). Till the end of May 2020, over 6 million human beings are reported to be affected and nearly half a million deaths have been reported due to COVID-19. Health machinery all over the world is trying its very best to combat this pandemic and facing a real challenge to tide over this crisis. Till today, no definite treatment has been found to combat this COVID-19 outbreak. Besides probationary drug therapy, some preventive, supportive, and precautionary measures such as self-isolation, social distancing, avoid unnecessary travel and crowded places are being advised and exercised to prevent the spread of this devastating infection (Chen *et al.*, 2020). Due to lack of time and a health emergency, clinicians are exploring the already approved drug for anti-viral activity (Savarino *et al.*, 2006; Rolain *et al.*, 2007; Mullard, 2012; Yan *et al.*, 2013; Agostini *et al.*, 2018; Bleibtreu *et al.*, 2018; Colson *et al.*, 2020; Cao *et al.*, 2020). In the wake of this, a number of drugs such as lopinavir, ritonavir (Cao *et al.*, 2020), chloroquine (CQ), hydroxychloroquine (HCQ) (Savarino *et al.*, 2006; Yan *et al.*, 2013), remdesivir (Agostini *et al.*, 2018), favipiravir, ribavirin, interferons (IFN's), corticosteroids, oseltamivir (Bleibtreu *et al.*, 2018), *etc.* have been tested on COVID-19 infected patients and are in current practice but with variable success. Critical insight into various treatment methods currently being employed to manage COVID-19 infection is being given in this review.

OVERVIEW OF VARIOUS TREATMENT STRATEGIES FOR COVID-19

Hydroxychloroquine

Cinchona bark and its derived constituents are considered as the primary treatment for malaria (Ruiz-Irastorza and Khamashta, 2008). CQ, a 9-aminoquinoline, has been used as the most effective anti-malarial compound (Wellems and Plowe, 2001). HCQ (7-Chloro-4-[4-(N-ethyl-Nb-hydroxy-methylamino)-1-methylbutylamino]quinoline sulfate) is a hydroxyl analogue of CQ that is a more safe and efficacious molecule (Sperber *et al.*, 1995). HCQ is a widely used clinical agent not only in the treatment of malaria but also for many other disorders such as fungal infections (Byrd and Horwitz, 1991; Henriet *et al.*, 2013), rheumatoid arthritis (RA) (Rainsford *et al.*, 2015), systemic lupus erythematosus (SLE) (Jessop *et al.*, 2001; Rainsford *et al.*, 2015), sjogran's syndrome (Oxholm *et al.*, 1998) and polymorphic light eruption (PLE) (Pareek *et al.*, 2008). In addition to the above disorders, HCQ is also demonstrated to

possess anti-viral activity and has been used clinically for the treatment of several viral infections such as human immunodeficiency virus-1 (HIV-1) (Chiang *et al.*, 1996), Zika virus (ZIKV) (Kumar *et al.*, 2018), dengue virus (DENV) (Wang *et al.*, 2014) and severe acute respiratory syndrome coronavirus (SARS-Co-V) (Vincent *et al.*, 2005). HCQ has efficient absorption after oral administration with peak plasma concentration reaching after 2-3.5 hours and it has an elimination half-life around 22-45 days (Tett *et al.*, 1989; Lim *et al.*, 2009).

HCQ in Treatment of COVID-19

HCQ has become a molecule of the moment because of its utility in managing COVID-19 infection. Many *in-vitro* and controlled and uncontrolled clinical trials have substantiated the activity of HCQ against COVID-19 (Gautret *et al.*, 2020; Liu *et al.*, 2020; Yao *et al.*, 2020). These *in-vitro* reports are further supported by many clinical trial studies carried out, and being conducted in different countries such as China, U.S, Europe, *etc.* (Gao and Yang, 2020; NCT04261517; NCT04307693). Therefore, HCQ has seen variable success in these clinical trials. In a non-randomized, open-label clinical trial conducted out in France, 20 patients of COVID-19 were administered with HCQ at the dose of 200 mg three times daily (TID) and were compared with another group of patients who received supportive care. It was concluded that all patients well tolerated the HCQ and showed fast clearance of virus (Gautret *et al.*, 2020). Moreover, another study conducted in China reported that HCQ at the dose of 400 mg daily did not show any clinical benefits in mild COVID-19 patients (Chen *et al.*, 2020). Another parallel study from China conducted on more than 100 patients of COVID-19 gave evidence of less severe pneumonia, improvement in lung imaging and decreased disease duration in comparison to control group patients after receiving HCQ (Gao and Yang, 2020) and it was also documented that the treatment was safe. This study gave confidence to the experts in China to recommend HCQ at the dose of 500 mg twice daily for 10 days to mild, moderate, and severe patients of COVID-19 (Zhonghua *et al.*, 2020).

Probable Mechanism of HCQ in Treatment of COVID-19

It is being hypothesized that HCQ acts by inhibiting the replication of viral nucleic acid, glycosylation of viral proteins, assembly and transport of virus and release of virus molecule to achieve its anti-viral activity (Fox, 1993; Wang *et al.*, 2015; Kumar *et al.*, 2018) (Fig. **1**). It is suggested that HCQ has an immuno-modulatory effect, which further helps in decreasing the elevated level of cytokines, interleukin-6 (IL-6), and IL-10, which are primary contributors to multi-organ failure and death in COVID-19 patients (Chen *et al.*, 2020; Huang *et al.*, 2020). It is also being considered from the previous history of HCQ for

inhibiting SARS-CoV that it can act by increasing endosomal pH, which is a key step of virus and host cell fusion (Mauthe *et al.*, 2018). HCQ is a weak diprotic basic, lipophilic and lysosomotropic drug that can cross the cell membrane easily and accumulates in the acidic vesicles (endosomes, lysosomes, golgi apparatus) of the cytoplasm. These acidic vesicles contain a number of hydrolytic enzymes that are activated by the acidic pH of these vesicles. HCQ accumulates in the lysosomes, thereby increasing the internal pH of lysosomes from their normal pH level of 4.7-4.8 to 6 (Mindell, 2012). The increased pH of lysosomes by HCQ further leads to expansion and vacuolization of lysosomes followed by inhibition of normal lysosomal functioning such as the release of an enzyme, recycling of receptor, cellular signalling, and plasma membrane repair eventually resulting in interference of normal functioning of the immune system (Hurst *et al.*, 1988; Kaufmann and Krise, 2007).

On the other side, it is also suggested that HCQ interferes in post-translational modifications of newly synthesized proteins in host cells infected by virus molecules. In addition to this, HCQ can sometimes inhibit endoplasmic vesicular enzymes such as proteases and glycosyltransferases that further inhibit post-translational modification of a viral envelope glycoproteins and also maturation of virus molecule (Randolph *et al.*, 1990).

Moreover, it is reported that CQ/HCQ also acts by increasing the zinc ions influx into the cytoplasm of host cells by acting as an ionophoric agent. After entry of the virus into host cells, zinc ion inhibits the polymerization of the virus molecule by inhibiting RNA dependent RNA polymerase (RdRp) enzyme. Sometimes virus gets mutated inside the host cells even than zinc ions inhibit the multiplication of virus molecules into the host cells. In any case, if the virus molecule somehow escapes from the zinc ion trapping mechanism, then the virus gets released from the cytoplasm of the host cells into intercellular space, interstitial matrix and will try to infect/target the healthy cells of the host. In this case, CQ/HCQ inhibits the re-attachment of the virus molecule with host cells by inducing some conformational changes in angiotensin-converting enzyme-2 (ACE-2) receptors that further mediates the binding of virus molecule with host cells (Vincent *et al.*, 2005; Zhu *et al.*, 2020).

Although, HCQ currently is one of the main drug interventions for the clinical management of COVID-19 cases. It has some adverse effects of concern, including QT prolongation with ventricular dysrhythmia, haematological disorders and impairment of hepatic and renal function (Cortegiani *et al.*, 2020). Instead of this, the use of HCQ is contraindicated in patients with retinopathy, QT prolongation in ECG, history of allergy to HCQ, deficiency of glucose-6-phosphate, and pregnant and breastfeeding ladies (Gautret *et al.*, 2020).

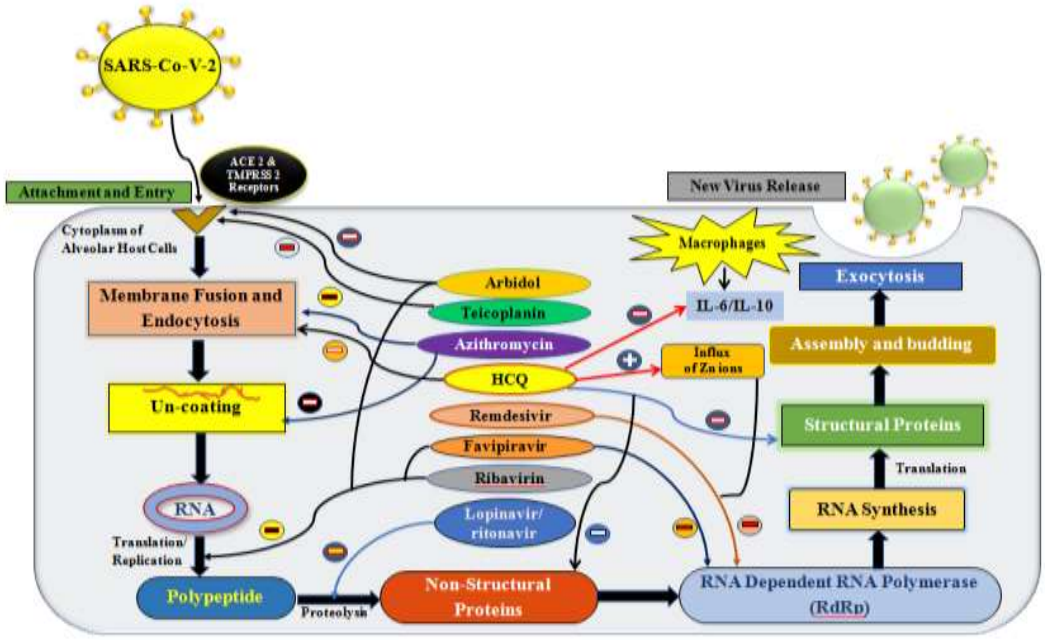


Fig. (1). Mechanism of action of various drugs at different steps of the virus life cycle into host cells. ACE-2: angiotensin-converting enzyme; HCQ: hydroxychloroquine; IL: interleukins; RNA: ribonucleic acid; RdRp: RNA dependent RNA polymerase; SARS-Co-V-2: severe acute respiratory syndrome corona virus-2; Zn: zinc.

Remdesivir

Remdesivir is another drug molecule that has gained immense popularity for its usefulness in COVID-19. It has a broad-spectrum anti-viral activity and was originally developed to treat Ebola by the Gilead Pharma Industry of France (Warren *et al.*, 2016; Mulangu *et al.*, 2019). Recently, it is also observed to possess promising efficacy in the treatment of MERS Co-V and SARS Co-V infections (Sheahan *et al.*, 2017; de Wit *et al.*, 2020). Remdesivir is a prodrug that, after activation, acts by inhibiting RdRp. Pharmacokinetic (P/K) studies on cynomolgus monkey indicated that the first-pass effect of the drug exhibits its lower distribution rate and intravenous (*i.v.*) infusion of the drug at a dose of 10 mg/kg is rapidly decomposed into triphosphates metabolites. Intravenous administration of remdesivir at the dose of 150 mg/kg for 1 hour a day showed linear P/K for a period of 14 days. Furthermore, intravenous infusion of remdesivir in a phase-1 clinical trial showed good P/K's without showing any adverse effects on vital organs (Cao *et al.*, 2020).

Remdesivir in Treatment of COVID-19

Currently, remdesivir is the second most discussed molecule after HCQ for its use in COVID-19 infection. Various pre-clinical and clinical studies have been carried and are being carried out to evaluate its efficacy in COVID-19 (Hillaker *et al.*, 2020; Holshue *et al.*, 2020). A study conducted in the USA, reported that remdesivir treatment *via i.v.* injection for 7 days produces promising results in the treatment of patients with COVID-19 (Holshue *et al.*, 2020). A clinical trial case report also depicts that remdesivir with a loading dose of 200 mg followed by 100 mg *i.v.* for the next 9 days shows promising result in 40- year- old COVID-19 patients. In addition to this, it has also been noticed that late initiation of remdesivir therapy is also effective in SARS Co-V-2 as compared to other anti-viral drugs like acyclovir and osmeltmivir (Hillaker *et al.*, 2020). One more observational cohort study on 200 patients is being conducting to evaluate the therapeutic effect of remdesivir in COVID-19 (NCT04365725).

Probable Mechanism of Action of Remdesivir in Treatment of COVID-19

Remdesivir is believed to act by interrupting viral RNA replication. When a virus infects host cells, the positive-sense RNA genome gets translated into host cells and produces viral poly-proteins which are essential for viral survival. In the next step, protein gets cleaved by viral proteases and produces 16 yield non-structural proteins called $nsp^{2,}$ which is responsible for viral replication and transcription. Furthermore, nsp^2 forms a multi-subunit complex containing enzymes RNA polymerases (a characteristic of RNA virus but not specific to host cell). Remdesivir is an RdRp inhibitor. As it is a nucleotide analogue, so it gets converted into an active form, which is nucleotide triphosphate into the host cell and competes with ATP (an active adenosine triphosphate necessary for maturation of nascent RNA) that further leads to the premature synthesis of RNA and finally halt the growth of RNA strand (Kirchdoerser, 2020). Constipation is the major side effect observed with remdesivir (Fig. **1**).

Lopinavir/Ritonavir

These are protease inhibitors comprising an important class of anti-viral drugs used to treat HIV infection (Chandwani and Shuter, 2008). A Combination of lopinavir and ritonavir offers some advantages in that ritonavir enhances plasma concentration of lopinavir *via* inhibition of *Cyt P450* to aid in a more pronounced effect (Soliman *et al.*, 2011). A Combination of both lopinavir and ritonavir has already been tested to treat severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS) infection (Chu *et al.*, 2004; Arabi *et al.*, 2018).

Lopinavir/Ritonavir in Treatment of COVID-19

Some recent clinical studies have given the evidence that these drugs may be employed as a promising therapeutic agent to curb COVID-19 (Cao *et al.*, 2020; Lim *et al.*, 2020; Ye *et al.*, 2020). A study conducted in Korea concluded that lopinavir 200 mg/ritonavir 50 mg reduced β-coronavirus load and improved clinical symptoms (Lim *et al.*, 2020). Another study was conducted by Cao and colleagues on 199 patients of COVID-19. Among these, 99 patients were administered with lopinavir/ritonavir, and 100 patients were subjected to standard care. At the end of the study, it was concluded that no benefits were observed with the treatment beyond standard care (Cao *et al.*, 2020). One more study conducted in Ruian People's Hospital, China, on 19 patients and concluded that the treatment group with adjuvant therapy shows promising results in the treatment of COVID-19 without causing any side effects (Ye *et al.*, 2020).

Probable Mechanism of Action of Lopinavir/ritonavir in COVID-19

Lopinavir is a potent viral protease inhibitor but lopinavir/ritonavir co-formulation produces its anti-viral effect by inhibiting the action of 3-chymotrypsin like proteases enzyme that further mediates the processing of COVID-19 (Zhang *et al.*, 2020) (Fig. **1**).

Favipiravir

Favipiravir is a broad-spectrum anti-viral agent that has been approved by US-FDA to treat Ebola and Influenza (Nagata *et al.*, 2015). Favipiravir is a prodrug that belongs to the pyrazine carboxamide derivative category (Bocan *et al.*, 2019). It is phosphorylated and ribosylated by the hosts enzyme and gets converted into an active form Ibofuranosyl 5'triphosphate (Furuta *et al.*, 2017). Bioavailability of favipiravir is 97.6% and the apparent volume of distribution (V_d) is estimated at 15-20 L. It has 65% plasma protein binding specially with serum albumin. Favipiravir gets metabolized and undergoes hydroxylation by an enzyme aldehyde oxidase that converts it into inactive metabolite and excretes through urine.

Favipiravir in Treatment of COVID-19

Genome sequencing of Ebola and SARS Co-V-2 exhibits similarity therefore, it is believed that favipiravir can be used a promising therapeutic agent to treat COVID-19. Although, favipiravir is not approved by US-FDA for the treatment of COVID-19. National Medical Product Administration in China approved favipiravir for the treatment of SARS Co-V-2 infection (National Medical Product Administration, China). Several clinical trials have been carried out to substantiate the safety and efficacy of favipiravir in treatment of COVID-19 (ChiCTR2000029600; Cai *et al.*, 2020). The First clinical trial was conducted in China to evaluate the safety and efficacy of favipiravir and IFN in the treatment of

COVID-19 (ChiCTR2000029600). In this trial, 80 patients were selected and reported that the drug was employed as a target agent in COVID-19 without showing any adverse effects (Chen *et al.*, 2020). Another open labelled clinical trial also documented that favipiravir (1600 mg twice a day on day 1 and 600 mg twice daily on 2-14 days) plus IFN-α by aerosol inhalation (5 million U twice daily) produce a significant improvement rate in chest imaging and also reported faster viral clearance (Cai *et al.*, 2020). Two different dosage regimen of favipiravir *i.e.*1600 mg twice a day on day 1 followed by 600 mg thrice daily on day 2-14 (NCT04333589) and 1800 mg twice a day on day 1 followed by 600 mg thrice daily from day 2-14 (NCT04336904) have been exercised in clinical practice for COVID-19. Elevation of serum uric acid level, gastrointestinal discomfort, psychiatric symptoms and teratogenicity are few common side effects noticed with favipiravir.

Probable Mechanism of Action of Favipiravir in Treatment of COVID-19

Favipiravir competes with purine nucleosides and is incorporated into RNA of the virus. After incorporation into viral RNA, it disrupts the genome sequence of the viruses. In addition to this, favipiravir also inhibits viral replication due to inhibition of the RdRp enzyme (Furuta *et al.*, 2017) (Fig. **1**).

Azithromycin (AZ)

AZ (N-Methyl-11-aza-10-deoxoy-10-dihydro-erythromycin-A) belongs to the category of macrolide antibiotics which are broadly used in the treatment of several respiratory (bronchitis and pneumonia), enteric and genitor-urinary tract bacterial infections. AZ inhibits bacterial protein synthesis after binding with the 23S rRNA subunit of the 50S ribosomal unit (Bacharier *et al.*, 2015; Pfizer, 2020). AZ has rapid absorption after oral administration and long plasma half -life *i.e.* 68 hours (Pfizer, 2020) as well as a large volume of distribution (31L/kg) (Ishaqui *et al.*, 2020). Although, AZ is not a licensed drug which can be used as an anti-viral drug, however numerous *in-vitro* studies as well as clinical trials have been carried out to check its anti-viral efficacy against several viruses including COVID-19 (Kouznetsova *et al.*, 2014; Li *et al.*, 2019; Touret *et al.*, 2020).

AZ in COVID-19 Treatment

Recent *in-vitro* studies have been carried out to explore the effect of AZ in COVID-19 treatment (Andreania *et al.*, 2020; Touret *et al.*, 2020). Besides *in-vitro* studies, clinical studies have also been carried out to check the efficacy of AZ SARS-Co-V-2 infection. A recent study, carried out on 20 patients of severe COVID-19 in which AZ was administered at the dose of 500 mg on day 1,

followed by 250 mg daily on day 2-5. It was observed that AZ has a synergistic effect if it is administered along with HCQ at a dose of 200 mg three times per day for 10 days (Gautret *et al.*, 2020).

Probable Mechanisms of AZ in COVID-19 Treatment

The exact mechanism of AZ for treating COVID-19 is still unexplored. However, from the record of previous studies of AZ for inhibiting other viruses, it is being hypothesized that it might act by increasing endosomal pH which is a key step of virus and host cell fusion (Fig. **1**). Being a weak basic drug, AZ can easily cross the cell membrane and accumulates in the acidic vesicles (endosomes, lysosomes, golgi apparatus) of the cytoplasm. When AZ accumulates in these acidic vesicles it increases the internal pH of vesicles. This increased pH by AZ further leads to expansion and vacuolization of vesicles followed by inhibition of normal functioning of these vesicles and can further inhibit endocytosis and viral replication (Tyteca *et al.*, 2002; Homolak and Kodvanj, 2020). In addition to this, it is also being predicted that AZ also inhibits the un-coating of enveloped viruses (Greber *et al.*, 1994). One another study has also proved that AZ maintains the normal functioning of epithelial cells and lungs by reducing the secretion of mucous (Cramer *et al.*, 2017). AZ shows similar effects and has similar P/K profile in elderly patients to that of young patients. However, the patients with mild to moderate renal impairment show a slight increase in mean C_{max} and area under the curve (AUC) after 1g dose administration of AZ. Moreover, patients with severe renal impairment show a significant surge in AUC and C_{max} in comparison to the patients with significant renal functioning, a point that needs to be taken in to consideration for prescribing AZ (Pfizer, 2020). Another major side effect associated with AZ is Torsades-de-Pointes.

Arbidol

Arbidol is an indole based anti-viral agent that already has been used in the treatment of influenza virus in China and Russia (Blaising *et al.*, 2014). Besides influenza, it has been used in the treatment of herpes simplex virus (HSV) (Li *et al.*, 2018), ZIKV (Haviernik *et al.*, 2018), and Ebola virus (Hulseberg *et al.*, 2019). It exerts its anti-viral effect through multiple pathways that help to inhibit the effect of the resistance of virus against the drug. Arbidol is rapidly absorbed after oral administration with an estimation of T_{max} *i.e.* 0.65-1.8 hours and C_{max} around 415-467 mg/ml (Liu *et al.*, 2009; Deng *et al.*, 2013). It gets metabolized by hepatic and intestinal microsomal enzymes through phase-1 pathways such as hydroxylation, sulfoxidation and N-demethylation followed by phase-2 conjugation (Song *et al.*, 2013; Deng *et al.*, 2013) and its plasma half -life is estimated as 25 hours (Deng *et al.*, 2013).

Arbidol in Treatment of COVID-19

Although, arbidol is not approved by US-FDA for the treatment of COVID-19, some pre-clinical and clinical studies have reported that it may be a very efficacious therapeutic molecule for COVID-19 (Deng *et al.*, 2020; Wang *et al.*, 2020). Arbidol has been included in the latest version of the guidelines issued by the National Health Commission (NHC) of the people's republic of China for the prevention, diagnosis, and treatment of novel coronavirus induced pneumonia at the recommended dose of 200 mg, 3 times/day for 10 days for adults (National Health Commission, China).

An *in-vitro* study claimed that arbidol is effective against COVID-19 at the concentration range of 10-30 μM (News: Abidol and darunavir can effectively inhibit corona virus (2020). A retrospective study conducted by Deng and colleagues reported that patients who received oral arbidol plus lopinavir/ritonavir showed improvement in clinical symptoms and reduced viral load as a compared to the patients who received lopinavir/ritonavir alone (Deng *et al.*, 2020). A randomized multi-centre controlled clinical trial of arbidol in the patients with COVID-19 is currently running in China (ChiCTR2000029573).

Besides this, one more study was conducted on 69 patients of COVID-19. In this study, it was reported that the administration of arbidol at the dose of 0.4 g, TID for 9 days improves the discharging rate from the hospital and also decreases the mortality rate of patients (Wang *et al.*, 2020). In addition, another study also reported that arbidol is more superior to the combination therapy of lopinavir/ritonavir (Zhu *et al.*, 2020).

Probable Mechanism of Action

It is hypothesized that arbidol inhibits the contact between the virus and host cell that further prevents the entry of the virus into host cells. It is also being predicted that arbidol also interferes with the late stages of virus cycle by interacting with lipids and proteins of the virus which are essential for the viral replication; probably arbidol binds with these essential components that further inhibit viral replication (Boriskin *et al.*, 2008) (Fig. **1**).

Ribavirin

Ribavirin is an analogue of guanosine and used in the treatment of several viral infections such as HCV, respiratory syncytial virus (RSV) and viral hemorrhagic fever (Martinez, 2020). It acts by interrupting the replication of DNA and RNA viruses into host cells (Chen *et al.*, 2020).

Ribavirin in COVID-19 Treatment

The anti-viral activity of ribavirin against SARS-Co-V is supported by an *in-vitro* study conducted by Chan and colleagues in 2015. They also calculated the anti-viral concentration of ribavirin against SARS-Co-V which was 50 μg/mL (Chan *et al.*, 2015).

To explore the efficacy of ribavirin in COVID-19 treatment, the government of China recommended the use of ribavirin in the diagnosis of pneumonia associated with COVID-19 based on Treatment Plan Edition-5. An oral loading dose of 4 g was administered followed by oral administration of 1.2 g after every 8 hours (Treatment Plan Edition 5, 2020). This schedule was further revised to 500 mg *i.v.* BID/TID (Treatment Plan Edition 5, revision edition, 2020). The outcome of these studies is not yet revealed (Huang *et al.*, 2020; Holshue *et al.*, 2020; Wang *et al.*, 2020). Besides this several new clinical studies have been announced to explore the efficacy of ribavirin in COVID-19 treatment (ChiCTR2000029387, 2020; NCT04276688; NCT04306497).

Probable Mechanism of Action in COVID-19 Treatment

Ribavirin shows anti-viral activity by inhibiting the polymerase activity followed by an interruption in the replication of the virus. Besides this polymerase inhibitory activity, ribavirin also acts by interfering RNA capping mechanism which further prevents RNA degradation. Also, ribavirin inhibits the generation of guanosine by inhibiting inosine monophosphate dehydrogenase enzyme naturally (Chen *et al.*, 2020) (Fig. **1**).

Ribavirin is reported to cause a reduction in haemoglobin which is very harmful to the patients of respiratory distress.

Teicoplanin

Teicoplanin is a glycopeptides antibiotic, broadly used in the treatment of gram-positive bacterial infections such as staphylococcal infections. In addition to bacterial infections, it is found to be effective in several viral infections such as Ebola, flavivirus, influenza, HIV, hepatitis C virus (HCV), and coronavirus *i.e.* MERS-Co-V and SARS-Co-V (Colson and Raoult, 2015; Pan *et al.*, 2015; Zhaou *et al.*, 2016).

Teicoplanin in COVID-19 Treatment

An *in-vitro* study, carried out by Zhang *et al.*, evident that teicoplanin can be used in the treatment of COVID-19. In the same study, it was also revealed that the concentration required to inhibit 50% viruses is 1.66 μM which is very less than

the concentration reached in human blood (8.78 μM for a daily dose of 400 mg) (Zhang *et al.*, 2020). These results of preliminary studies further demand a randomized clinical trial.

Probable Mechanism of Action

Zhang and colleagues proposed that teicoplanin acts by inhibiting the initial step of the virus cell cycle. It acts by resisting the entry of the virus molecule into host cells *via* inhibition of the low pH cleavage of the viral spike protein by cathepsin L and cathepsin B in the late endosomes of host cells thereby preventing the release of genomic viral RNA and further halt the progress of virus replication cycle (Baron *et al.*, 2020; Zhang *et al.*, 2020) (Fig. **1**).

Ivermectin

Ivermectin is an anti-parasitic drug that has been approved by FDA and employed to treat many tropical parasites (Canga *et al.*, 2008). But, originally, it was developed to treat HIV. Ivermectin is a highly lipid-soluble drug and widely is distributed in the body (Krishna and Klotz, 1993). It has strong plasma protein binding and is not detected in cerebrospinal fluid (CSF) (Klotz, 1990). It is extensively metabolized by hepatic microsomal enzymes *Cyt P450* (Zeng *et al.*, 1998). Various pre-clinical studies have indicated its efficacy in treating tropical parasitic illness (Mastrangelo *et al.*, 2012; Lundberg *et al.*, 2013; Azeem *et al.*, 2015; Gotz *et al.*, 2016). The basic mechanism for its use as an anti-parasitic drug is that it binds with a high affinity to glutamate chloride ion channel of parasite and followed by enhancement of cell permeability that further leads to hyperpolarization and lyses of parasite cells.

Ivermectin in Treatment of COVID-19

In-vivo studies have given the evidence of the efficacy of ivermectin for the treatment of COVID-19 by using Vero-h SLAM cell lines. It mitigates the rate of infection 500 folds within 48 hrs of administration but does not show any further reduction in the severity of infection after an interval of 48 hrs (Caly *et al.*, 2020). In addition to this, a hypothesis given by Angela Patrì and Gabriella Fabbrocin purposed that concomitant administration of HCQ with ivermectin may produce a synergistic effect because both drugs do not exhibit any contradiction in combination. Moreover, HCQ already has been approved as a therapeutic agent for COVID-19 by US-FDA (Patrì and Fabbrocin, 2020). By considering the above facts, ivermectin may be a promising drug target for fighting against pandemic 2019. However, no clinical study has given any evidence for its usefulness in the treatment of COVID-19.

Probable Mechanism of Action in COVID-19

The basic mechanism behind its efficacy in COVID-19 is that it inhibits importins-α/β heterodimer which is a special type of karyopherins that transports viral protein into the nucleus of host cells. As protein entry is restricted then the virus could not be able to use host machinery for its replication and growth (Jans *et al*., 2019). Ivermectin has reported rare side effects such as depression, ataxia and mydriasis.

Nitazoxanide

Nitazoxanide is a nitro-thiazole derivative which is de-acetylated *in-vivo* to tizoxanide. Nitazoxanide is used to treat protozoan infections (Rossignol, 2016). Besides, it has anti-bacterial and anti-viral properties. It has reported broad spectrum *in-vitro* anti-viral activity against influenza, parainfluenza, rotavirus, respiratory syncytial virus, and norovirus and is approved for the treatment of some human infections (Jasenosky *et al.*, 2019). Nitazoxanide is being investigated in the clinical trials (randomized controlled) for the management of influenza and other acute respiratory infections due to its broad spectrum anti-viral activity. Though, results are not encouraging or unavailable yet (Stahlmann and Lode, 2020).

Nitazoxanide in COVID-19 Treatment

It has recently been reported to demonstrate the potent *in-vitro* activity against SARS Co-V-2 (Choy *et al.*, 2020). Nitazoxanide acts by interfering the host-regulated pathways involved in the replication of virus molecule, amplifying cytoplasmic RNA sensing, and type-I IFN pathways (Rossignol, 2016). Nitazoxanide also up-regulates the innate anti-viral mechanisms by broadly amplifying cytoplasmic RNA sensing and type I IFN pathways. It also prevents the viral infection by up-regulating the precise host mechanisms that virus targets to bypass host cellular defence mechanisms (Jasenosky *et al.*, 2019). Indeed, the *in-vitro* activity of nitazoxanide against SARS-Co-V-2 is encouraging, still more data and clinical outcome are required to determine its role in the management of COVID-19 (Choy *et al.*, 2020).

Corticosteroids

Cytokine storm and inflammation are the two major hallmarks that are associated with the human coronavirus (SARS-Co-V-1, MERS-Co-V, and SARS-Co-V-2)

(Zhou *et al.*, 2014; Channappanavar and Perlman, 2017). In addition to anti-viral interventions, corticosteroids may be another supportive therapy that may suppress the aforementioned hallmarks of COVID-19. The idea behind the concept is the pharmacological nature of corticosteroids to treat lung infection and inflammation by suppressing the inflammatory genes that encode cytokines, cell adhesion molecules (Cruz-Topete *et al.*, 2015). Although, China National Health Commission released the fifth trial for prevention and diagnosis of COVID-19 that recommended the use of systemic corticosteroids at the dose of 1-2 mg/kg for 3-5 days as adjuvant therapy (http://www.nhc.gov.cn/yzygj/s7653p/202002/d4b895337e19445f8d728fcaf1e3e13a.shtml,2020).

Corticosteroids in the Treatment of COVID-19

The utility of corticosteroids in COVID-19 is like a double-edged sword which means some clinical evidence documented its promising results in the treatment of COVID-19 (Wang *et al.*, 2020; Zhao *et al.*, 2020), while other studies reported the controversial outcomes against the use of corticosteroids in COVID-19 treatment (Russell and Millar, 2020).

A retrospective study conducted in Wuhan Union Hospital depicts the early administration of corticosteroids at the dose of 1-2 mg/kg *i.v.* gives promising results by improving the severe condition of patients (returning to normal body temperature, improvement in SpO_2) (Cheng *et al.*, 2020; Fang *et al.*, 2020). In parallel to the first study's outcome, similar results were also observed when a high dose of corticosteroids with quinoline is administered for short term use (Yang *et al.*, 2020). The dosing schedule and side effects of various drugs used for the treatment of COVID-19 are summarized in Table **1**.

Table 1. Different drugs used for the treatment of COVID-19 along with their dosing schedule and side effects.

Sr. No.	Drug/Treatment	Dosing Schedule	Side Effects
1.	Hydroxychloroquine	200 mg; Three times a day for 10 days. 500 mg; Two times a day for 10 days.	QT prolongation, Ventricular dysrhythmia, Haematological disorders.
2.	Remdesivir	200 mg *i.v.* loading dose followed by 100 mg *i.v.* for next 9 days.	Constipation.
3.	Lopinavir/Ritonavir	Lopinavir- 200 mg Ritonavir- 50 mg	Diarrhoea, Stomach upset, Drowsiness, Bad taste of mouth, Troubled sleeping.

(Table 1) cont.....

Sr. No.	Drug/Treatment	Dosing Schedule	Side Effects
4.	Favipiravir	1600 mg Twice on day 1 followed by 600 mg Thrice daily from day 2-14. 1800 mg Twice on day 1 followed by 600 mg Thrice daily from day 2-14.	Elevation of serum uric acid level, GI discomfort, Teratogenicity.
5.	Azithromycin	500 mg on day 1 followed by 250 mg from day 2-5.	Torsades-de-pointes.
6.	Arbidol	200 mg Three times daily for 10 days. 400 mg Three times daily for 9 days.	No major side effects.
7.	Ribavirin	400 mg oral loading dose followed by 1200 mg after every 8 hours. 500 mg *i.v.* Twice daily.	Reduction in haemoglobin.
8.	Teicoplanin	400 mg daily.	Erythematous rashes.
9.	Ivermectin	Not defined	Depression, Ataxia, Mydriasis.
10.	Corticosteroids	1-2 mg/kg; *i.v.* for 3-5 days.	Swelling in lower legs, High blood pressure, Mood swing, Behavioural changes.

On the contrary, Russell and his colleagues summarized the outcomes of clinical studies conducted on SARS Co-V-2 patients and implied that a high dose of corticosteroids delayed viral clearance from the respiratory tract and also increase the chances of higher incidence of psychosis (Russell *et al.*, 2020).

In addition to this, another study reported that patients treated with oral corticosteroids (hydrocortisone and methyl prednisolone; *i.v.*) do not delay the viral clearance (Cheng *et al.*, 2020) but on the other side, a meta-analysis documented that corticosteroids may increase the mortality rate with severe adverse effects when administered in COVID-19 patients (Wang *et al.*, 2020). A recent study conducted by Henry Ford Hospital, USA concluded that the administration of corticosteroids worsens the condition of COVID-19 patients (NCT04374071).

As there are no fixed guidelines for the use of corticosteroid in COVID-19 and WHO also has not given any nod for its efficacy and safety (https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected). However, it is suggested that there is need to follow some precautions while using

corticosteroids in COVID-19 *i.e.* administration should be done at an early stage but not for long term use and administration should be avoided in severe ill-treated patients of COVID-19 (Liou *et al.*, 2020).

Immuno Modulating Agents-Interferons (IFN's)

When a virus invades the host cells, there is an activation of innate and adaptive immunity to protect host cells from the deleterious effects of the virus. But sometimes, due to misdirection and mutations in the virus, the immune system gets suppressed that contributes to enhanced viral replication in the host's cell. Based on this fact, non-conventional therapy which is immune modulation may be a novel approach to combat COVID-19.

IFN's are cytokines having anti-viral properties. Currently, there are three distinct types of IFN's *i.e.* INF-α, β, and γ are available (Price *et al.*, 2000; Samuel, 2001). IFN's are one of the innate immune-modulating agents that can be used in the treatment of COVID-19. Various clinical and pre-clinical trials have given the evidence and suggest that IFN's may be useful candidates to combat viral infections such as SARS-Co-V, MERS-Co-V, and SARS-Co-V-2 (Hensley *et al.*, 2004; Stockman *et al.*, 2006).

IFN's in Treatment of COVID-19

A pre-clinical study conducted on cynomolgus monkey showed that IFN-α protects primates against SARS-Co-V. Since the genome sequencing of SARS-Co-V is 80% homologous to SARS-Co-V-2 therefore, it is considered that IFN's can be used as a potential therapeutic agent for COVID-19. China also recommended the administration of 5 million IU of IFN-α *via* inhalation two times a day in combination with ribavarin (Dong *et al.*, 2020; Lu, 2020). Clinical trials have been registered to evaluate the efficacy and safety of IFN-α in combination with lopinavir/ritonavir (ChiCTR2000029387) and that of IFN-β1 with lopinavir/ritonavir and ribavarin (NCT04276688).

IFN-1 treatment has given conducible results through *in-vitro* as well as *in-vivo* studies against SARS Co-V and MERS Co-V in combination with or without lopinavir/ritonavir (Stockman *et al.*, 2006). However, IFN-1 subtype inhibits SARS Co-V (Hensley *et al.*, 2004) but IFN-β1 is more effective as compared to IFN-1 because IFN-β1 expresses more prominent activity in the lungs. It also up-regulates the CD73 in pulmonary cells that subsequently exhibits anti-inflammatory activity and inhibits acute respiratory syndrome (Chan *et al.*, 2015). Similarly, the *in-vivo* study of IFN-β with lopinavir/ritonavir against MERS Co-V and SARS Co-V reduced lung infection but did not inhibit viral replication (Sheahan *et al.*, 2020). The aforementioned pre-clinical study depicts that IFN-1

can reduce lung infection by inhibiting cytokine storm (a major hallmark of COVID-19).

Furthermore, a study also reported that human lung tissue infected with COVID-19 failed to induce expression of IFN (type 1, 2, 3) as compared to control tissue (Chu *et al.*, 2020). Similarly, it is also reported that mice lacking IFNδR1 are more prone to the infection of viruses and inflammation (Davidson *et al.*, 2016; Galani *et al.*, 2017). Hence, based on the above clinical and pre-clinical data, it is suggested that IFN's may be a promising therapeutic agent to combat COVID-19 infection.

Probable Mechanism of Action of IFN's in COVID-19

The exact mechanism of IFN's to exhibit their anti-viral activity is unclear but some of the reports suggest that IFN's promotes the activation of macrophages, that subsequently causes phagocytosis of the antigen and also block the replication of viruses (Ivashkiv and Donlin, 2014; Cao *et al.*, 2019) (Fig. **2**).

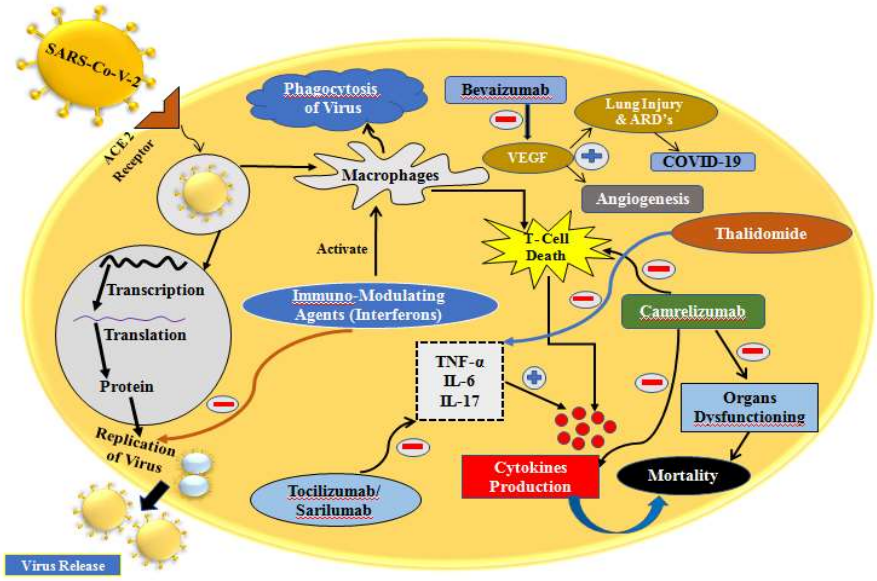


Fig. (2). Mechanism of action of different drugs at various steps of virus pathology into host cells. ACE-2: angiotensin-converting enzyme; ARD's: acute respiratory disorders; COVID-19: corona virus disease-19; IL: interleukins; SARS-Co-V-2: severe acute respiratory syndrome corona virus-2; TNF-α: tumour necrosis factor- α; VEGF: vascular endothelial growth factor.

Monoclonal Antibody Therapy

Monoclonal antibodies (mAb's) are a dynamic category of pharmaceuticals which are fortunate for the pharma industry and have a special attribute to treat a

particular diseases (Meissner and Long, 2003; Berry *et al.*, 2010; Arabi *et al.*, 2015). They represent a major class of bio-therapeutics for passive immunotherapy to fight against all type of infections. In the recent years, many mAb's have been developed to fight against viruses and some are in pipeline also (Boyapati *et al.*, 2020; Shanmugaraj *et al.*, 2020).

Monoclonal antibodies can play a crucial role to curb COVID-19 infection. Evidence of *in-vitro* and *in-vivo* studies has suggested that mAb's targeting spike protein can be potentially effective against SARS-CoV-2. These can act directly by interrupting any stage of the viral lifecycle or may bind with the receptor proteins of the host cell surface so that virus could not use host's machinery and its growth gets halted. Pre-clinical studies suggest that various neutralizing antibodies are effective against SARS-Co-V infection of humans (Arabi *et al.*, 2015). The specific neutralizing mAb's either binds against receptor-binding domain (RBD) in spike protein or binds to ACE-2 receptors and further can effectively block the virus entry into host cells. Further, evidence indicates that neutralizing mAb binds to the ACE-2 RBD of the SARS S protein (Berry *et al.*, 2010), thus block the viral entry into host cells. Moreover, mechanistically co-operative binding of mAb's leads to conformational changes in the antigen which further alters the affinity of virus to get bind with host cell (Klonisch *et al.*, 1996). The mAb's has considered a passive immunotherapy to provide a quick response against viral infection. Brink and colleagues have suggested that passive transfer of polyclonal immune serum reduces pulmonary virus load in a mouse model of SARS-Co-V infection (Van Den Brink, 2005). Therefore, immuno-prophylaxis of SARS-Co-V infection with antibodies might be an effective SARS control strategy. Another strategy includes mAb's cocktail that may exhibit more potent anti-viral activity that could increase the effectiveness of the treatment and prevent the viral escape.

80R Immunoglobulin G1 (IgG1)

80R IgG1, a human mAb, has identified as a neutralizing antibody and plays an important role in immunity. It responses to the surface glycoproteins of enveloped viruses and has anti-viral activity against SARS-Co-V spike S protein. 80R IgG1 potentially neutralizes SARS-Co-V infection by efficiently inhibiting syncytia formation as it hinders the binding of the viral agent with the receptors (Sui *et al.*, 2004). 80R has been tested *in-vitro* and found to inhibit virus entry into host cells (Sui *et al.*, 2004). The passive administration of 80R human mAb's could provide an immediate response as a prophylaxis in the treatment of SARS-Co-V-2 infection (Mair-Jenkins *et al.*, 2015).

CR3014

Study suggests that the binding site of CR3014 and CR3022 mAb's is different from that of mAb 80R (Sui *et al.*, 2004). It has been evident by a study that CR3014 protects ferrets from SARS-Co-V-2 infection at 10 mg/kg dose by completely protecting the lung pathological markers that may further aggravate the severity in patients, and also abolished the shedding of the virus (Ter Meulen *et al.*, 2004). CR3014 has been reported to reduce replication of SARS-Co-V in the lungs of infected ferrets, abolished shedding of SARS-Co-V in pharyngeal secretions, and completely prevented the development of virus-induced macroscopic lung pathology (Van Den Brink *et al.*, 2005).

Effect of SARS-Co-V-2 can be abolished by human mAb's, which target the RBD of the virus. The mixture of both human mAb's CR3014 and CR3022 by its interaction with RBD has reported producing a synergistic effect in the neutralization of SARS-Co-V. The study has suggested that a combination of CR3014 and CR3022 have potential to provide adequate protection against not only SARS-Co-V strains but also against other SARS-Co-V like viruses originate from other animals. The combination of two non-competing human mAb's CR3014 and CR3022 potentially activates the immune response and increase the aura of shielding effect against viruses (Ter Meulen *et al.*, 2006).

Monoclonal Antibody Therapy in COVID-19 Treatment

Indeed, cytokine storms had been reported in many diseases including infections and rheumatic diseases. Macrophage activation syndrome (MAS) refers to acute inflammation caused by cytokine storm (Schulert and Grom *et al.*, 2015). The fact that the biological agents neutralizing IL-6 and IL-1 are highly effective treatments for systemic juvenile idiopathic arthritis, a rheumatic disease strongly associated with MAS also arise a ray of hope that suggests that the same therapy could be beneficial for delaying cytokine storm in inflammation associated with COVID-19 (Jiang *et al.*, 2020). Two mAb's that targets IL-6 signalling *viz.* tocilizumab and sarilumab have been approved for the treatment of rheumatoid arthritis (Boyapati *et al.*, 2020).

Tocilizumab

Tocilizumab is a potent immunosuppressive agent. It is a recombinant, humanized anti-human IL-6 receptor mAb's which was originally made to treat RA and has found to inhibit signal transduction pathways associated with severe inflammation (Xu *et al.*, 2020). The study has suggested the benefits of tocilizumab in controlling cytokine release syndrome in COVID-19 patients (Chen *et al.*, 2020). Tocilizumab works by inhibiting IL-6 receptor thereby reduce cytokine release

syndrome-like features in severe patients of COVID-19 (Fig. **2**). Scientists and physicians in China suspect that IL-6 may play a crucial role in the treatment of COVID-19. Furthermore, healthcare professionals from China and Italy have already started the clinical trials to access the potential of drug tocilizumab in COVID-19 patients with severe symptoms (Chen *et al.*, 2020). Recently, interleukin-6 receptor (IL-6R) mAb (tocilizumab)-directed COVID-19 therapy clinical trial has been conducting in China (NCT04356937) and it has been involved into COVID-19 management guidelines generated in China by considering the concept of cytokine storm in COVID-19 pneumonia (Zhang *et al.*, 2020). More importantly, humans have a very high tolerance for tocilizumab. Thus, tocilizumab may be an effective treatment strategy in patients with severe cases of COVID-19. A tocilizumab trial in the US and Canada in patients with severe COVID-19 pneumonia is underway (NCT04320615).

Sarilumab

It acts in similar manner as like tocilizumab (Fig. **2**). IL-6 release may be responsible for cytokine storm and also used as a prognostic marker to evaluate the progression of COVID-19 diseases and patients with high IL-6 may be likely to benefit from sarilumab. A clinical trial already has been reported that patients with high IL-6 levels are effectively responding to sarilumab (Boyapati *et al.*, 2020).

Limitations and Suggestions Regarding mAb's

Although, several mAb's showed promising results in neutralizing SARS-Co-V infection, large-scale production of mAb's requires wide ranging of labour. Moreover, it is very expensive and time consuming process which outweighs the mAb's clinical use against the emerging pathogen.

With the recent advancement production of mAb's at lower production costs could be possible. In today's scenario the sequences of mAb's that are effective against SARS-Co-V are cloned in a satisfactory expression systems such as mammalian, yeast or plant, and recombinant mAb's would be tested against SARS-Co-V-2. Plant cloned system has a great advantage that it rapidly reproduce the mAb's in a less time at the nominal cost which is considered to be a major advantage of expression system during the pandemic situations. A Low dose combination of mAb's maybe a good strategy to fight against the SARS-CoV-2 outbreak.

Triple Therapy

This is another approach that has been exercised to contain COVID-19 infection.

Triple therapy involves combined administration of IFN-β1b, lopinavir/ritonavir, and ribavarin. A multi-centred open-label randomized clinical trial has given the evidence of efficacy and safety of triple therapy to tackle COVID-19. Inclusive criteria of the study were characterized by the patient's age, minimum should be 18, and the patient should be confirmed with COVID-19 infection (Hung *et al.*, 2020). The trial comprised the administration of oral lopinavir 400 mg, ritonavir 100 mg for every 12 hours *via* a nasogastric tube; ribavarin 400 mg every 12 hours, and one to three doses of IFN-β1b on alternate days continuously for 14 days. Lopinavir/ritonavir of the same dose was administered to the control group and outcome was conquered by shortening the duration of viral load, hospital stays with nasopharyngeal swab negative (NCT04276688).

Miscellaneous Drugs

Besides the drugs discussed above for COVID-19, many other drugs are supposed to exhibit therapeutic efficacy in COVID-19 infection with disparate mechanisms. These drugs mainly act by targeting the pathogenesis of COVID-19 instead of inhibiting the replication of the virus.

A drug like PD-1 inhibitor camrelizumab, primarily used for the treatment of relapsed or refractory classical hodgkin's lymphoma (Markham and Keam, 2019). A study has reported that the drug also inhibits the apoptosis of T-cell, cytokines production, and organ dysfunctioning (Fig. **2**) (Zhu *et al.*, 2020). As it is well proven that T-cell death, cytokines production and organ dysfunctioning are the major pathological hallmarks that lead the mortality of COVID-19 patients. Based on the aforementioned information, it is believed that camrelizumab may be used as a potential agent in the treatment of COVID-19. Substantiating this mechanism, a clinical study is conducted to evaluate its efficacy with severe pneumonia patients associated with lymphocytopenia (NCT04268537).

Another drug is bevaizumab that has been approved for the treatment of metastatic colon cancer (Heinzerling and Huerta *et al.*, 2006) and exhibits its therapeutic effects by inhibiting the vascular endothelial growth factor (VEGF) (Fig. **2**) (Ferrara *et al.*, 2004) which is a key promoter of angiogenesis (US-FDA, 2008, EMA 2008). A study has revealed that VEGF plays an important role in lung injury and acute respiratory disorders (ARD'S) which are directly related to the pathology of COVID-19 (Baratt *et al.*, 2014). Based on these facts, bevaizumab is presently clinical trial in patients with COVID-19 under trial name BEST-RCT and BEST-CP (NCT04305106 and NCT04275414).

TNF-α is the primary factor that aggravates the pathogenesis of COVID-19 which is suppressed by the drug thalidomide (Fig. **2**) (Mazzoccoli *et al.*, 2012). Considering this fact, thalidomide is also under the clinical trial (NCT04273529).

Now a day, thalidomide is being used in the treatment of interstitial pulmonary fibrosis, lung damage, and myeloma (Horton *et al*., 2012).

SARS Co-V-2 vaccine which is developed by Shang and colleagues is in the clinical trial phase (NCT0428346). In addition to this, University of Pittsburgh School of Medicine also evolved the vaccine which was tested on mice. Antibodies produced by the SARS Co-V-2 vaccine were able to neutralize the virus (NCT0428346). An mRNA vaccine already has been administered to a person in the USA for treating COVID-19 (Cohen, 2020).

As we know that SARS Co-V-2 penetrates the cell membrane and gets activated by two proteins such as ACE-2 and TMPRSS2. Based on this fact, drugs like camostat mesylate and nafamosate which are synthetic candidates and can inhibit the aforementioned proteins further may prevent the emergence of COVID-19 (Hoffmann *et al.*, 2020; Zhou *et al.*, 2015). To evaluate the efficacy of these drugs, a randomized placebo clinical trial is being conducted on 180 patients (NCT04321096).

Herbal Drugs for COVID-19

In addition to various synthetic and semi-synthetic drugs discussed above, many natural compounds were also tested and tried for the management of COVID-19 infection. Most of the studies are reported with Chinese herbal drugs. Recent pre-clinical, clinical and *in-silico* data has given the evidence that Chinese herbal candidates like glycyrrhizin, scutellarin, baicalin, and hesperetin show their promising results in SARS COV-2 (Cinatl *et al.*, 2003; Pilcher, 2003; Chen *et al.*, 2020).

Glycyrrhizin

Glycyrrhizin a constituent of liquorice roots (*glycyrrhiza radix*) has earlier been reported to exert a beneficial effect in the treatment of SARS Co-V-1 (Pilcher, 2003). Besides the coronavirus, glycyrrhizin also has been used in the treatment of chronic hepatitis (Chen *et al.*, 2020). An *in-vitro* study also depicts that drug has anti-SARS activity with fewer side effects (Cinatl *et al.*, 2003). In addition to this, the clinical study indicated that glycyrrhizin produces conducible results against two strains of the virus (FFM-1, FFM2) in patients suffering from SARS who were admitted to the health care centre in Germany. It was reported that glycyrrhizin acts by inhibiting the replication of viruses and also by inhibiting adsorption and penetration of viruses into the host cells (Cinatl *et al.*, 2003). Besides these mechanisms, glycyrrhizin also up-regulates the expression of nitric acid in macrophages followed by inhibition of replication of viruses belongs to the flaviviridae family (Jeong and Kim, 2002).

Glycyrrhizin in COVID-19

This drug has a low selectivity index but it is a potent inhibitor of replication of viruses. Results of a docking study showed that glycyrrhizin binds with ACE-2 receptor with an estimated ΔG 9Kcl/mol which is an important entry point for SARS Co-V-2 into host cells (Chen and DU, 2020).

Hesperetin

Hesperetin, a bio flavonoid compound that has been found in plants *citrus aurantinum* and *citrus reticulate pericarpium*. It is a well known Chinese medicine that exhibits its dose-dependent effects and attenuates the cleavage activity of 3C-like proteases of SARS-Co-V in cell-based assays (Lin *et al.*, 2005). It also inhibits ACE-2 with an estimated value ΔG -8.3Kcal/mol, which is sufficient to evaluate its efficacy in COVID-19 (Chen *et al.*, 2020). However, there is no clinical trial evidence that delineates its efficacy in the treatment of COVID-19. Also, the *in-silico* experiment gives us hope about its efficacy in the treatment of pandemic 2019 in the future. Moreover, additional experimental studies are needed to prove its usefulness in COVID-19.

Baicalin

Baicalin is obtained from Chinese medicinal plant *Scutellaria Bericalensis Georgi* and has been reported to exert beneficial effects in oxidative stress, inflammation, and apoptosis (Chen *et al.*, 2017; Ishfaq *et al.*, 2019). Since, genomic sequencing revealed the information that almost 80% of the SARS viral genome is homologues to the genome of COVID-19. Baicalin has already proved its efficacy against SARS. Therefore, based on this fact, baicalin came under consideration as a probable therapeutic agent in the intervention of COVID-19. Moreover, the *in-silico* experiment conducted by Chen and co-workers gives the promising value of ΔG that shows its efficacy against COVID-19 (Chen *et al.*, 2020). Baicalin activity against SARS in fRhk cell lines is reported with promising results (Chen *et al.*, 2015). Also, this drug inhibits ACE-2 with an IC_{50}value of 2.24 μM and docking results also indicate that it can bind with ACE-2 with a ΔG -8.46Kcal/mol which is sufficient value for depicting its conducible results in the treatment of COVID-19 (Chen and Du, 2020).

Scutellarin

Scutellarin is another Chinese compound that is obtained from *Erigeran Brevis Capus*. Pharmacologically, the drug has a broad spectrum of effects in the treatment of inflammation and oxidative stress (Wang and Ma, 2018). An *in-vivo* study conducted by Wang and colleagues, showed that the drug inhibits the expression and activity of ACE-2 in brain tissue with an estimated IC_{50} (48.13±4.98 μM) value against SARS (Wang *et al.*, 2016). However, no *in-vivo* study has been conducted to report that this drug can inhibit ACE-2 but the *in-silico* study showed that scutellarin has the potential to treat COVID-19 with an estimated value ΔG (-14.98Kcal/mol) (Chen and Du, 2020).

Plasma Therapy

Convalescent plasma therapy is not a new concept, it's an old one, and has a similar mechanism of action based on the concept of immunization by vaccines as described by Edward Jenner. In this therapy, blood is collected from patients who have recovered from infectious illness and then transfused to patients who are currently diagnosed with COVID-19 illness. The concept behind this therapy is the production of antibodies that fights against infection. Convalescent plasma therapy has been successfully employed already, as a targeted therapy to treat influenza virus (H_1N_1), SARS-Co-V-1 epidemic, and MERS-Co-V epidemic (Hung *et al.*, 2011; Dodd, 2012). A meta-analysis done by Mair-Jenkins and his colleagues concluded that infusion of plasma attenuated the complications shown by patients suffering from a severe acute respiratory infection (Mair-Jenkins *et al.*, 2015). Moreover, the *in-vitro* trial also showed the efficacy of treatment and concluded that antibody production is responsible for blocking new infections and also for clearance of infected cells (Lu *et al.*, 2016).

Plasma Therapy in the Treatment of COVID-19

Several clinical studies have given the encouraging results for the treatment of COVID-19 (Shen *et al.*, 2020; Duan *et al.*, 2020). A recent clinical study conducted on 5 confirmed patients of COVID-19, has reported improvement in the symptoms of patients within 12 days after transfusion of convalescent plasma without showing any adverse effects (Shen *et al.*, 2020). Another study carried out on 10 patients of COVID-19, also given promising results without causing any severe adverse effects in the hospital (Duan *et al.*, 2020). To observe the efficacy of convalescent plasma therapy, the Italian National Board also gave a nod for treatment with convalescent plasma therapy but the board defined some mandatory conditions for donors which should be followed before donating plasma (Franchini *et al.*, 2020).

Besides advantages, convalescent plasma therapy also faces some challenges that are questionable regarding its efficacy (Zhao and He, 2020). Research on SARS-

Co-V confirmed that this therapy is more effective at an earlier stage and may aggravate hyper immune attacks. Convalescent plasma therapy can increase the chances of chills, fever, and anaphylactic shock. Besides this, the optimum volume of serum and optimal duration for administering convalescent plasma is required. Also, the risk of transfusion-transmitted infections can increase with this therapy (World Health Organization, 2020).

Potential of Stem Cell Therapy in COVID-19

Stem cell therapy has a special contribution to the field of regenerative medicines. This concept was given by Ernest McCulloch and James Till in 1960. Stem cells especially mesenchymal cells may also exhibit a pivotal role in the COVID-19 pandemic (Shetty, 2020). Mesenchymal cells are derived from the umbilical cord, placenta, and other tissues.

The concept behind the use of mesenchymal cells in the COVID-19 pandemic is the immune regulatory activity of cells that further inhibits ARD'S by activation of the paracrine mechanism (Fujita *et al.*, 2018). Moreover, mesenchymal stem cells also customize the attributes of both innate as well as adaptive immune cells *via* inhibiting the release of cytokines, IFN's from natural killer (NK) cells, and T-cells (Aggarwal and Pittenger, 2005). In addition to this, they also enhance the activity of phagocytes that directly activates the secretion of anti-microbial peptides and proteins (Krasnodembskaya *et al.*, 2010; Sutton *et al.*, 2016).

A clinical trial is conducted in China on four patients of COVID-19 and the study depicts that patients got recovered and discharged from hospital after administration of mesenchymal stem cell therapy. Multiple trials are under process to substantiate the efficacy of mesenchymal stem cell therapy in COVID-19 infection (NCT0425 2118, NCT04269525, NCT04273646, NCT04293692).

BCG Vaccine

Vaccination is one of the options which would be helpful to curb COVID-19 pandemic. However, the development of vaccines instantly is a tedious and time-consuming task. Therefore, researchers move towards pre-existing vaccine molecules and have started clinical trials on the molecules. BCG vaccine is one the candidate that garnered the interest of researchers to combat pandemic COVID-19. Albert Calmette and Camille Guerin had developed BCG vaccine in the 20th century and now used in the prevention of tuberculosis (Brewer and Colditz, 1995).

The idea behind the use of the BCG vaccine in COVID-19 is based upon two considerations. First, the countries which had already discontinued their universal

vaccination policies of BCG vaccine in neonates are more prone to COVID-19, for instance, United States, Italy, Netherland. After exploring the epidemiological data, Miller and colleagues found that the aforementioned countries had completely discontinued their vaccine policy and are more prone to risk for COVID-19 as compared to other countries like Japan, India. Second, the effect of the BCG vaccine is non-specific and provides heterologous immunity that further strongly activates innate and adaptive immunity. Strong innate immunity (trained immunity) on second exposure to a pathogen (in case of COVID-19) leads to some transcriptional and epigenetic reprogramming of human monocytes and may show its effect in treating COVID-19, as shown in yellow fever vaccine (Arts *et al.*, 2018).

Two clinical trials on the BCG vaccine to combat the COVID-19 pandemic are under process (NCT04327206; NCTO4328441). Until clinical trial will complete, it is necessary to adhere to WHO's recommendation because of limited supply of BCG vaccine and haphazard use may jeopardize the situation in the case where it is needed to prevent the children from infection (https://www.who.int/news-room/commentaries/detail/bacille-calmette-gu%C3%A9rin-(bcg)-vaccination-and-COVID-19).

VARIOUS REGULATORY GUIDELINES GIVEN BY NIH (NATIONAL INSTITUTE OF HEALTH) FOR TREATMENT OF COVID-19

There are some specific recommendations issued by NIH related to drugs that have been used in COVID-19:

1. If HCQ is used for treatment, then the clinician should track the patients for adverse effects especially QT interval prolongation.
2. The use of a combination of HCQ and AZ is highly prohibited because of toxicities.
3. Neither combination of lopinavir/ritonavir (Kaletra) is recommended due to unfavourable pharmacodynamics and unfavourable trial results.
4. Guidelines have also recommended the use of ACE inhibitors or angiotensin receptor blockers (ARB) for pandemic 2020, but advised to take only by the patients who are taking drugs for cardiovascular and other diseases.
5. There is no guideline in favour of or in opposition to the regular use of extracorporeal membrane oxygenation for patients of COVID-19 with refractory hypoxemia.
6. Data is also insufficient or not giving promising results for convalescent plasma therapy in COVID-19. Moreover, regulatory guidelines are neither in favour nor against for the use of IL-6 and IL-1 antagonist such as tocilizumab and anavarin.

7. The panel does not allowed the use of IFN's and Janus kinase (JAK) inhibitors such as baricitinub, because of the ineffective outcome of IFN in MERS and SARS and immunosuppressive effect reflected by drug baricitinub.
8. Panelist of NIH are against the regular use of corticosteroid in COVID-19 patients who are mechanically ventilated, but also recommends its use at a lower dose, who are experiencing refractory shock and corticosteroid or NSAID use should not be discontinued to patients of COVID-19 who are taking medication in pre-existing conditions.
9. Data are also inadequate to recommend the use of drug remdesivir in COVID-19, originally developed to treat Ebola and MERS by Gilead Sciences.

CONCLUSION

Since its outbreak, COVID-19 has become a matter of concern globally and there is a drastic elevation of mortality graph. Understanding regarding the exact cause and transmission of the virus is in the nascent stage hence, posing a difficult situation and big challenge for researchers to develop a new molecule *i.e.* vaccine in a shorter period. In the current scenario, therefore the best strategy for treating COVID-19 is to move towards existing molecules that already have been used as an intervention target in SARS, MERS (homologous to COVID-19), and other viral infections with diverse mechanisms at different steps of viral life cycle into host cells. In this chapter, we have discussed the existing molecules as a conducible target in the treatment of COVID-19. Indeed, current treatment strategies discussed above have been moderately successful to curb COVID-19 infection but off course vaccine for COVID-19 which would be the ultimate solution to eradicate the COVID-19 pandemic is still missing. Researchers across the globe are working hard to develop COVID-19 vaccine; hopefully, a successful outcome is on the anvil very soon.

LIST OF ABBREVIATIONS

ACE-2	Angiotensin converting enzyme-2
ARB	Angiotensin receptor blocker
ARD	Acute respiratory disorder
AUC	Area under curve
AZ	Azithromycin
COVID-19	Corona virus diseases-19
CQ	Chloroquine
CSF	Cerebrospinal fluid
DENV	Dengue virus
HCQ	Hydroxychloroquine

HCV	Hepatitis-C-virus
HIV-1	Human immunodeficiency virus-1
HSV	Herpes simplex virus
IFN	Interferon
IL	Interleukin
JAK	Janus kinases
mAb's	Monoclonal antibodies
MAS	Macrophage activation syndrome
MERS Co-V	Middle east respiratory syndrome corona virus
NHC	National health commission
NK	Natural killer
P/K	Pharmacokinetic
PLE	Polymorphic light eruption
RA	Rheumatoid arthritis
RBD	Receptor binding domain
RdRp	RNA dependent RNA polymerase
RSV	Respiratory syncytial virus
SARS-Co-V-2	Severe acute respiratory syndrome corona virus
SLE	Systemic lupus erythematosus
TID	Three times daily
Vd	Volume of distribution
ZIKV	Zika Virus

AUTHORS CONTRIBUTION

Kuldeep Kumar, Sonal and Pankaj Bhatia carried out the relevant literature research and prepared the primary draft; Dr. Nirmal Singh prepared the final draft of the chapter; Dr. Dhandeep Singh and Dr. Amteshwar S. Jaggi co-conceptualized and provided valuable inputs in finalizing the chapter.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 3

COVID-19 and Mortality

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Abstract: The novel coronavirus disease (COVID-19) has scourged the world ever since its outbreak in December 2019 in Wuhan, China. The disease tends to be asymptomatic or mild in nearly 80% of the patients. However, around 5% of the patients tend to have critical disease complicated by acute respiratory distress syndrome (ARDS), shock and multiple organ failure. Mortality in COVID-19, as represented by the case-fatality rate (CFR), is around 6% (as of June 4, 2020). The CFR of COVID-19 is lower as compared to other coronavirus-related diseases like the Severe Acute Respiratory Syndrome (SARS) and the Middle East Respiratory Syndrome (MERS), however, it is likely to increase as we reach the end of the pandemic. The CFR also varies widely from one nation to another with the maximum mortality being hitherto reported from the European nations and the least from Singapore, Cambodia, Vietnam and Iceland. The common causes of death in COVID-19 include respiratory failure, consequently leading to ARDS, pulmonary thromboembolism, shock and multiple organ failure. Advancing age and presence of comorbid illness are consistently associated with an increased risk of death, while certain biochemical and hematological parameters, notably C-reactive protein, IL-6, cardiac troponin, D-dimer and absolute count can also help predict mortality in patients with COVID-19.

Keywords: ACE2, ARDS, Case-fatality rate, Comorbidities, COVID-19, Cytokine storm, D-dimer, Death, Diabetes mellitus, Hypertension, IL-6, Lymphopenia, Mortality, Mortality rate, Novel coronavirus disease, Old age, SARS-CoV-2, Shock, Thromboembolism, Troponin.

INTRODUCTION

An unprecedented outbreak of 'pneumonia of unknown cause' emerged in the Wuhan City of China in December 2019 (Pneumonia of unknown cause – China, 2020). The patients presented with clinical symptoms of fever, dry cough, dyspnea, and bilateral lung infiltrates on imaging. Cases were claimed to be all

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linked to the Wuhan's Seafood Wholesale Market, which trades in fish and a wide variety of live animal species, including poultry, bats, rats, marmots, and snakes. The causative pathogen was identified from throat swab samples collected from the patients and was subsequently named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The disease was named novel coronavirus disease (COVID-19) by the World Health Organization (WHO). With the increase in the number of cases outside China, the WHO declared the outbreak of COVID-19 to be a Public Health Emergency of International Concern, posing a high risk to countries with vulnerable health systems on January 30, 2020. Subsequently, with the emergence of new epicenters in Italy and Iran, COVID-19 was declared a pandemic on March 11, 2020.

Ever since its outbreak, COVID-19 has scourged the world, affecting over 6.2 million people, inflicting more than 379000 casualties worldwide (Coronavirus disease (COVID-19) Situation Report – 135, 2020). Hitherto, the maximum number of cases has been reported from the United States of America, Brazil, Russia, the United Kingdom, Spain, Italy, India, Germany, Peru, Turkey, Iran and France (Coronavirus (COVID-19), 2020). The inherent ability of SARS-CoV-2 to withstand extremes of the ambient environment and its remarkable stability on inanimate objects and surfaces make it an ideal pathogen for human-to-human transmission. The basic reproductive number (R_0) of COVID-19, defined as the average number of secondary cases attributable to infection by an index case after that case is introduced into a susceptible population, is high, ranging from 1.5 to 6.68 with a mean of 3.28 (Liu *et al.,* 2020). The high R_0 of SARS-CoV-2 has accounted for the rapid increase in the number of COVID-19 cases worldwide. In addition, there have been instances of super-spreader events, in which an individual, who may or not be symptomatic, infects a large number of susceptible subjects (Coronavirus disease 2019 (COVID-19) Situation Report – 24, 2020.; Coronavirus disease 2019 (COVID-19) Situation Report – 36, 2020). In fact, the outbreak of COVID-19 in Wuhan might have been a super-spreader event as it had coincided with one of the world's largest mass gathering, the Chinese Spring Festival (Ebrahim and Memish, 2020).

Despite being highly contagious, more than 80% of patients with COVID-19 are either asymptomatic (no pneumonia) or have mild symptoms (mild pneumonia). Only 14% of patients develop the severe disease; the rest 5% develop critical disease manifesting as respiratory failure, septic shock, and/or multiple organ dysfunction or failure (Wu and McGoogan, 2020). Case-fatality rates in COVID-19 have been highly variable, ranging from as low as 0.5% to as high as 21.8% (Mortality Risk of COVID-19, 2020). Nevertheless, advancing age and the presence of underlying co-morbidities have been consistently associated with increased mortality in COVID-19. In addition, certain biochemical parameters

have emerged as potential predictors of mortality in COVID-19.

Herein, we have discussed in detail the multiple aspects of mortality in relation to COVID-19.

REPRESENTING MORTALITY – MORTALITY RATE *VS.* CASE-FATALITY RATE

Often the terms mortality rate and case-fatality rates are used interchangeably. Although both the parameters are used to define death rates, the two are not synonymous. According to the *Dictionary of Epidemiology*, the mortality rate is defined as "an estimate of the portion of a population that dies during a specified period". Going by the definition, the mortality rate should be ideally expressed as the number of deaths occurring per 100000 population per year, rather than representing the figure as a percentage. On the other hand, the case-fatality rate (CFR) is defined as "the proportion of cases of a specified condition that are fatal within a specified time" (Spychalski *et al.,* 2020). Accordingly, most of the estimates of death expressed as a percentage are case-fatality rates rather than mortality rates. We have referred to CFR throughout the subsequent discussion unless otherwise specified.

FALLACIES IN ESTIMATING MORTALITY USING CASE-FATALITY RATE

Case-fatality rates amid an ongoing pandemic should be interpreted with caution. The CFR is affected by the total number of cases (the denominator) as much as it is affected by the actual number of deaths (the numerator). For example, in countries where massive population screening has been undertaken, as in Switzerland and South Korea, case-fatality rates are less than 1%, as the denominator includes a large number of mild/asymptomatic cases. However, in countries where only symptomatic patients or those requiring hospital admissions are being screened, as in Italy and Spain, case-fatality rates have exceeded 5%, because the denominator is much smaller (Vincent and Taccone, 2020). Besides, the CFR might be over or underestimated based on how a 'case' is defined. During an ongoing epidemic, cases might be defined either as total cases (every confirmed case) or as closed cases (only those who have recovered or died). Hence, the denominator for calculating CFR might be either of these numbers. In the initial phase of an epidemic, the number of closed cases is relatively small, and so the CFR calculated per closed cases is an overestimate. By contrast, when the CFR is calculated per total cases, the whole calculation becomes an underestimate. As an example, early in the course of COVID-19, even before it was declared a pandemic, the WHO had opined on March 3, 2020, that the global COVID-19 CFR was 3.4% (WHO Director-General's opening remarks at the

media briefing on COVID-19 - March 3, 2020). As of June 4, 2020, the global CFR stands at 6.1% based on the WHO statistics (Coronavirus disease (COVID-19) Situation Report – 135, 2020). Nevertheless, irrespective of the method used (either total cases or closed cases), all calculations are biased in the initial part of an epidemic, and tend to merge once all the cases are closed (Spychalski *et al.*, 2020). Moreover, there exist reporting errors that become transparent towards the end of an epidemic. China had suddenly increased the death toll in Wuhan by 50% after the pandemic had subsided, stating that the newly reported 1290 COVID-19-related deaths were attributed to casualties that occurred at home at the beginning of the outbreak, as well as deaths that were wrongly reported by hospitals. Thus, once the pandemic ends and all the available statistics are reanalyzed, only then can the true CFR be estimated. Notwithstanding this fact, it seems that the CFR calculated per total cases is the least affected by reporting biases and remains the best tool to express the fatality of a disease, even though it might underestimate the figure in the initial phase of an outbreak (Spychalski *et al.*, 2020).

REPORTING COVID-19-RELATED DEATHS

A major barrier to the estimation of true CFR is the inaccurate reporting of COVID-19-related deaths ("Inaccurate Covid-19 death reporting can lower international health assistance," 2020). In addition, there have been allegations that certain nations are under-reporting or censoring COVID-19 death statistics ("Coronavirus: Europe 'wary of confronting China over deaths,'" 2020.; "Inaccurate Covid-19 death reporting can lower international health assistance," 2020; "Iran Uses Arrests, Censorship to Silence Critical COVID-19 Coverage," 2020). To bring uniformity in reporting, the WHO has introduced emergency "International Classification of Disease, Tenth Revision (ICD-10)" codes to help define and code COVID-19-related deaths. The ICD-10 codes presently recommended by the WHO for mortality coding have been summarized in Table **1** (Guidance for appropriate recording of COVID-19 related deaths in India, 2020). Of note, COVID-19 should be recorded as an underlying cause of death (UCOD) in patients with confirmed or suspected COVID-19. Often deaths in patients with COVID-19 are being attributed to underlying pre-existing comorbidities. Although underlying co-morbidities may increase the severity of COVID-19, these conditions should not replace COVID-19 as the UCOD. Such malpractice would naturally lead to under-reporting of COVID-19-related deaths and thereby skew the mortality statistics.

Table 1. Table showing ICD-10 codes presently recommended by the WHO for COVID-19-related mortality coding.

Test	Symptoms of COVID-19	Diagnosis	ICD-10 Code
Positive	None	Confirmed COVID-19	U07.1
Positive	Present	Confirmed COVID-19 documented as UCOD	U07.1
Positive	Present with co-morbid conditions	Confirmed COVID-19 documented as UCOD	U07.1
Negative	Present	Clinically-Epidemiologically diagnosed COVID-19	U07.2
Awaited	Present	Suspected COVID-19	U07.2
Inconclusive	Present	Probable COVID-19	U07.2

COMPARING CASE-FATALITY RATES OF COVID-19 WITH OTHER RELATED VIRAL DISEASES

Before the COVID-19 outbreak, the world had witnessed the outbreak of two coronavirus-related diseases, namely the Severe Acute Respiratory Syndrome (SARS) in 2003 and the Middle East Respiratory Syndrome (MERS) in 2012. A comparison of the CFR of the three coronavirus-related diseases (Coronavirus disease (COVID-19) Situation Report – 135, 2020; Middle East respiratory syndrome coronavirus (MERS-CoV); Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003) and adult seasonal influenza (Disease Burden of Influenza; Faust and del Rio, 2020) has been summarized in Table **2**.

Table 2. Table showing case-fatality rates of COVID-19, SARS, MERS and adult seasonal influenza.

Disease	Case-fatality Rate
COVID-19	6.1% (as on June 4, 2020)
SARS	9.6%
MERS	34.4%
Seasonal influenza	< 0.1%

Apparently, the CFR of COVID-19 is less than that of SARS and MERS, however, considering the magnitude of the pandemic, the absolute number of deaths are much higher in COVID-19 than either SARS or MERS. The WHO had reported only 774 SARS-related deaths and only 878 deaths due to MERS (Middle East respiratory syndrome coronavirus (MERS-CoV); Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July

2003). On the contrary, COVID-19 has already claimed more than 379000 lives globally (Coronavirus disease (COVID-19) Situation Report – 135, 2020). As the pandemic wages on and case reporting becomes more uniform and efficient, it might be expected that the CFR of COVID-19 would approach that of SARS.

COMPARING COVID-19 CASE-FATALITY RATES AMONG VARIOUS NATIONS

The COVID-19 case-fatality rates differ widely from one nation to another. As of June 4, 2020, CFR as low as 0.5% has been reported from Iceland. On the other hand, Yemen presently has the highest CFR of 21.8% (Mortality Risk of COVID-19). Overall, the European nations have high case-fatality rates, being 19.1% in France, 14.3% in Italy, 14.2% in the United Kingdom, 13.6% in Hungary, 11.6% in Spain and 11.1% in Sweden. Although the absolute number of deaths has been the highest in the United States of America (USA), the CFR (5.8%) is not exorbitantly high as in most of the European nations. This can again be attributed to the large number of COVID-19 cases diagnosed in the USA with nearly 2 million cases being reported from the continent. Mexico, on the other hand, has a high CFR of 11.6%. As far as South America is concerned, the highest case-fatality rates have been reported from Equador (8.5%) and Brazil (5.6%). Most of the Asian nations have reported a reasonable CFR, ranging from 0% in Cambodia and Vietman, 0.07% in Singapore, 2.3% in South Korea, 2.8% in India, 5.5% in China to 6.0% in Indonesia. Surprisingly, Yemen has recently witnessed a surge in the number of COVID-19-related deaths, taking its CFR to 21.8%, however, the neighboring nations of Saudi Arabia and Oman have strikingly low CFR of 0.6% and 0.5%, respectively. Russia presently has a CFR of 1.2%; similarly, Australia and New Zealand have low case-fatality rates of 1.4% and 1.9%, respectively. Case-fatality rates in different parts of Africa are variable, being highest in Liberia (8.8%) and Chad (8.0%) with no deaths yet reported from Uganda (Mortality Risk of COVID-19).

Table 3. Table showing COVID-19-related case-fatality rates in different continents/nations (as of June 4, 2020).

Nation/Continent	Case-fatality Rate
Yemen	21.8%
France	19.1%
Italy	14.3%
United Kingdom	14.2%
Hungary	13.6%
Spain	11.6%

(Table 3) cont.....

Nation/Continent	Case-fatality Rate
Mexico	11.6%
Sweden	11.1%
Canada	8.0%
Romania	6.5%
Indonesia	6.0%
United States of America	5.8%
Brazil	5.6%
China	5.5%
Japan	5.3%
Iran	4.9%
Germany	4.7%
Egypt	3.8%
India	2.8%
South Korea	2.3%
South Africa	2.1%
Australia	1.4%
Russia	1.2%
Iceland	0.5%
Singapore	0.07%
Cambodia	0.0%
Vietnam	0.0%

CAUSES OF DEATH IN COVID-19

The SARS-CoV-2 primarily attacks the lungs, resulting in viral pneumonia in a susceptible individual. In patients with mild and moderate disease, pneumonia resolves with time; however, in patients with severe and critical COVID-19, life-threatening complications ensue. Although the virus *per se* has directly been implicated in the causation of myocarditis (Inciardi *et al.*, 2020; Siripanthong *et al.*, 2020), acute renal failure (Batlle *et al.*, 2020) and pancreatitis (Wang *et al.*, 2020), complications most often result from a "cytokine storm" induced by the virus (Coperchini *et al.*, 2020). Cytokine storm, also known as cytokine storm syndrome, is a state characterized by the uncontrolled release of immune cells and excessive and disproportionate release of pro-inflammatory cytokines into circulation (Tisoncik *et al.*, 2012). Although first described in association with graft-*versus*-host disease (Ferrara *et al.*, 1993), the term has since been linked to

infectious pathogens, notably, cytomegalovirus, Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis, group A streptococcus, influenza virus (specifically H5N1 avian influenza virus), variola virus, and severe acute respiratory syndrome coronavirus (SARS-CoV) (Tisoncik *et al.*, 2012).

The concept of cytokine storm originated from the observation that patients with COVID-19 requiring admission in an intensive care unit (ICU) had higher serum levels of CXCL10, CCL2 and TNFα as compared to those with the less severe disease not requiring intensive care. In addition, several studies have shown that the serum IL-6 levels are increased in patients with COVID-19 and correlate with disease severity (McGonagle *et al.*, 2020; Zhang *et al.*, 2020). Learning from prior experience, increased circulating levels of pro-inflammatory cytokines (*e.g*, IFNγ, IL-1β, IL-6, IL-12) and chemokines (CXCL10, and CCL2) had been associated with pulmonary inflammation and extensive lung involvement in SARS patients, similarly to what had happened in MERS-CoV infection (Channappanavar and Perlman, 2017). Thus, the release of large amounts of pro-inflammatory mediators precipitates and sustains the aberrant systemic inflammatory response. The cytokine storm is readily followed by the host immune system attacking the body, which in turn causes acute respiratory distress syndrome (ARDS) and multiple organ failure, culminating in death, at least in the most severe cases of SARS-CoV-2 infection (Xu *et al.*, 2020).

Respiratory failure, secondary to ARDS, obviously remains the main cause of mortality in COVID-19 (Ruan *et al.*, 2020), as was seen in previous viral pandemics, such as the Spanish flu (1918), SARS and MERS. About one-third of the worsened patients (Chen *et al.*, 2020; Zhou *et al.*, 2020) and up to 41.8% (Wu *et al.*, 2020a) suffer from complications of respiratory failure and ARDS. Besides, COVID-19 can sometimes be complicated by shock and multiple organ failure that can also lead to the demise of the patient (Chen *et al.*, 2020). Shock is most often a result of the cytokine storm and represents a form of vasoplegic shock. Shock in COVID-19 can also result from an increased intrathoracic pressure secondary to invasive ventilation that can impede cardiac filling (Pal, 2020). Besides, cardiac involvement and secondary bacterial/fungal infections can lead to shock in patients with COVID-19 (Vincent and Taccone, 2020; Zhou *et al.*, 2020). Bacterial and fungal infections are common complications of viral pneumonia. In influenza patients, bacterial co-infection occurs in around 0.5% of young individuals and 2.5% of older individuals (Chertow and Memoli, 2013). Every one in 4 H1N1 patients during the 2009 pandemic had a coexisting bacterial or fungi infection (MacIntyre *et al.*, 2018). According to a report, 20 of 90 SARS patients had secondary lower respiratory tract infections in 2003, which accounted for 70.6% of those critical SARS patients who underwent an invasive operation. The pathogens causing secondary infections were diverse: gram-

negative bacilli were the most common but Candida was not uncommon (Zhou *et al.*, 2020). Bacterial and fungal co-infections or super-infections in COVID-19 patients have been inadequately investigated and reported thus far. Only a few articles have reported secondary infection, mostly without pathogen details. Besides, the complications resulting from bacterial or fungal infections in COVID-19 were not included in the prognosis analysis in most of the published papers. However, it should be kept in mind that most of the current infection control protocols aim to prevent the transmission and cross infection by SARS-CoV-2, thereby overlooking the prevention of bacterial or fungal secondary infections (Zhou *et al.*, 2020). As a matter of fact, in one Chinese study, secondary infections were found in 50% of the non-surviving COVID-19 patients (Zhou *et al.*, 2020).

Of late, pulmonary embolism (PE) has been implicated as a major cause of death in patients with COVID-19. Wichmann *et al.* reported the findings of 12 consecutive autopsies of patients with COVID-19. The authors reported a high incidence of PE with or without underlying deep vein thrombosis (DVT), in spite of the absence of a history of venous thromboembolism (VTE). Massive pulmonary embolism was the cause of death in one-third of the cases. Seventy-five percent were men and two-third of them were noted to have recent thrombosis in prostatic venous plexus (Wichmann *et al.*, 2020). In another autopsy study by Lax and colleagues, grossly visible pulmonary arterial thrombi were noted in all the 11 cases with associated infarcts in 9 cases (81%). During the hospital stay, most of the patients had received prophylactic anticoagulant therapy; thereby suggesting that pulmonary thrombi had formed despite anticoagulation (Lax *et al.*, 2020). Amongst non-autopsy studies, pulmonary thromboembolism has been reported on computerized tomography in patients with COVID-19 pneumonia with high as well as normal D-dimer levels (Danzi *et al.*, 2020; Scialpi *et al.*, 2020; Xie *et al.*, 2020). In a study by Klok *et al.*, computer tomography pulmonary angiogram (CTPA) and/or ultrasonography done based on clinical suspicion confirmed VTE in 27% and arterial thrombotic events in 3.7% of 184 COVID-19 patients admitted in ICU. Pulmonary embolism was the most frequent thrombotic complication (81%) (Klok *et al.*, 2020). An observational study had suggested that up to 5–10% of patients with COVID-19 who require mechanical ventilation have acute PE and/or DVT (Scialpi *et al.*, 2020). The pathophysiology of thrombotic events in COVID-19 patients involves direct endothelial cell injury by SARS-CoV-2 (Zhang *et al.*, 2020), hypercoagulability (Becker, 2020), hyperviscosity (Maier *et al.*, 2020) and traditional risk factors that include immobility inherent in a critically ill patient (Scialpi *et al.*, 2020). The common causes of death in patients with COVID-19 have been summarized in Table **4.**

Table 4. Table showing the common causes of death in patients with COVID-19.

Respiratory Failure (Acute Respiratory Distress Syndrome)
Thromboembolism
Shock (vasoplegic)
Multiple organ failure
Cardiogenic shock
Septic shock secondary to bacterial or fungal co-infection

PARAMETERS PREDICTING MORTALITY IN COVID-19

Various studies to date have pointed out at multiple factors that can predict mortality in patients with COVID-19. The factors can be broadly classified into two categories: clinical and laboratory. These have been summarized in Table **5**.

Table 5. Table summarizing the clinical and laboratory parameters predicting mortality and/or severe disease in patients with COVID-19.

Clinical Parameters	**Laboratory Parameters**
Age	C-reactive protein
Gender	Interleukin-6
Symptoms	D-dimer
Complications	Cardiac troponin
Comorbid illness	Creatinine
Smoking	Alanine transaminase
Secondary bacterial infections	Albumin
-	Blood urea nitrogen
-	Myoglobin
-	Lactate dehydrogenase
-	Ferritin
-	Total leukocyte count
-	Lymphocyte count
-	CD3+CD8+ T cell subset

Clinical Parameters

Age

Of all the clinical parameters, *advancing age* is consistently associated with

mortality in COVID-19. Multivariate logistic regression analyses have shown that the age ≥ 65 years is associated with a 3.7-fold increased risk of death (Du *et al.*, 2020). Advancing age as a predictor of mortality has been consistently replicated in multiple studies (Jin *et al.*, 2020; Martins-Filho *et al.*, 2020; Ruan *et al.*, 2020; Zhou *et al.*, 2020). The same reason has been proposed as the cause of the strikingly high CFR in Italy as compared to China. Individuals aged 70 years or older represent 37.6% of cases in Italy and only 11.9% in China. In addition, a relevant number of cases in Italy are in people aged 90 years or older. Thus, the overall older age distribution in Italy relative to that in China may explain, in part, the higher average CFR in Italy (Onder *et al.*, 2020). On the contrary, children, adolescents and young adults have strikingly low case-fatality rates (as shown in Table **6**).

Table 6. Showing age-specific case-fatality rates in Italy and China (Onder *et al.*, 2020).

Age Groups (Years)	Case-fatality Rate in Italy (As of March 17, 2020)	Case-fatality Rate in China (As of February 11, 2020)
0-9	0%	0%
10-19	0%	0.2%
20-29	0%	0.2%
30-39	0.3%	0.2%
40-49	0.4%	0.4%
50-59	1.0%	1.3%
60-69	3.5%	3.6%
70-79	12.8%	8.0%
≥ 80	20.2%	14.8%

Statistics from other countries have shown a similar trend with data from the USA showing that patients aged 0-17 years account for only 0.06% of the COVID-19-related deaths while those 75 years or above account for 48.7% of the casualties (Age, Sex, Existing Conditions of COVID-19 Cases and Deaths). The high case-fatality rate in older adults reflects their poor immune system and the high prevalence of underlying co-morbidities (Pal and Bhadada, 2020a). Elderly patients have higher Pneumonia Severity Index score (PSI score) and involvement of multiple pulmonary lobes compared to young and middle-aged patients (Liu *et al.*, 2020).

Gender

Gender disparity in COVID-19-related mortality has been reported in a few studies (Jin *et al.*, 2020; Martins-Filho *et al.*, 2020). In a meta-analysis involving

852 patients (489 male and 363 female) with confirmed SARS-CoV-2 infection by real time-polymerase chain reaction, it was found that male gender was associated an increased risk for in-hospital death (relative risk 1.3, 95%CI 1.1 to 1.4) (Martins-Filho *et al.*, 2020). In another study by Jin *et al.*, it was found that while men and women had the same prevalence, men with COVID-19 were more at risk for worse outcomes and death, independent of age (Jin *et al.*, 2020). The reasons underlying high mortality in men are obscure; high circulating angiotensin-converting enzyme 2 (ACE2) in men have been proposed as a plausible reason (Jin *et al.*, 2020).

Symptoms

Presenting symptoms in patients with COVID-19 have been evaluated as potential predictors of subsequent mortality. Ruan *et al.* had compared initial symptoms in 150 patients with COVID-19 who had either died or had been discharged. Out of fever, cough, sputum production, dyspnea, fatigue, myalgia and hemoptysis, only dyspnea was found to be statistically more prevalent in patients who had died as compared to those who were discharged (87% *vs.* 62%, *P* value=0.001) (Ruan *et al.*, 2020). Similarly, the presence of dyspnea was found to increase the risk of in-hospital death by 1.8-folds to 2.6-folds (Martins-Filho *et al.*, 2020; J. Xie *et al.*, 2020). The presence of dyspnea suggests impending ARDS and hence portends a poor prognosis in patients with COVID-19. The early recognition of respiratory distress can make a difference in the final prognosis of the patients (Martins-Filho *et al.*, 2020).

Complications

Complications of SARS-CoV-2 infection that are commonly seen in critically ill patients, notably, ARDS (relative risk 7.4), acute cardiac injury (relative risk 6.9), acute kidney injury (relative risk 22.6), disseminated intravascular coagulation (DIC) (relative risk 27.1), and sepsis (relative risk 2.4) have been found to be risk factors for the COVID-19-related death. Xie *et al.* found that hypoxemia was independently associated with in-hospital mortality. An oxygen saturation (SpO_2) cut-off of 90.5% had 84.6% sensitivity and 97.2% specificity for prediction of survival. Higher SpO_2 levels after oxygen supplementation were associated with reduced mortality independent of age and sex (Xie *et al.*, 2020). Another Chinese study from Wuhan showed that a higher Sequential Organ Failure Assessment (SOFA) score at admission was an independent predictor of in-hospital death with an odds ratio of 5.65 (Zhou *et al.*, 2020). In another report from Wuhan, mortality was 62% among critically ill patients with COVID-19 and 81% among those requiring mechanical ventilation (Yang *et al.*, 2020). Thus, mortality is high

among patients with COVID-19 who have severe disease and suffer from complications.

Comorbid Illness

Patients with underlying comorbidities are invariably at a high risk of mortality with COVID-19. The pre-existing comorbidities that have been consistently associated with poor outcomes in COVID-19 include hypertension, coronary heart disease, cerebrovascular disease, diabetes mellitus, obesity, chronic lung disease, chronic kidney disease and malignancy (Martins-Filho *et al.*, 2020; Pal and Bhadada, 2020a; Ruan *et al.*, 2020; Tian *et al.*, 2020). The effect size of individual comorbidities varies from one study to another. The presence of hypertension has been associated with a 1.5-fold to 2.5-fold increased risk of mortality in COVID-19. Underlying concurrent coronary heart disease (cardiovascular disease) and cerebrovascular disease have been shown to increase the chances of mortality by 3-fold to 3.8 fold and 3.3-fold, respectively. Likewise, the presence of diabetes mellitus has been associated with a 1.6-fold to 2-fold increased probability of dying with COVID-19 (Martins-Filho *et al.*, 2020; Tian *et al.*, 2020). Similarly, chronic lung disease (especially chronic obstructive pulmonary disease), chronic kidney disease and malignancy have been reported to be associated with mortality in COVID-19 (Guan *et al.*, 2020a; Martins-Filho *et al.*, 2020).

The association between hypertension, cardiovascular and cerebrovascular diseases and COVID-19-related mortality is probably mediated by the confounding effect of angiotensin-converting enzyme inhibitors (ACEi) and angiotensin-receptor blockers (ARBs), drugs that are widely used in this subset of patients (Pal and Bhadada, 2020a; Schiffrin *et al.*, 2020). The use of ACEi/ARBs is associated with the upregulation of angiotensin-converting enzyme 2 (ACE2), a monocarboxypeptidase that cleaves angiotensin-II and to a smaller extent angiotensin-I to angiotensin (1-7) and angiotensin (1-9), respectively (Pal and Bhansali, 2020). Unfortunately, the SARS-CoV-2 uses ACE2 as a receptor to gain entrance into the host pneumocytes. Thus, the upregulation of ACE2 with the use of ACEi/ARBs would prove to be counterproductive (Pal and Bhadada, 2020a). On the other hand, ACE2/angiotensin (1-7) system is known to exert a profound anti-inflammatory and anti-oxidant effect and thereby protect against inflammation-related lung injury. In this regard, upregulation of ACE2 by ACEi/ARBs would be expected to protect the lungs against ARDS (Pal and Bhansali, 2020; Schiffrin *et al.*, 2020). Whether ACEi/ARBs are actually beneficial or potentially nefarious in patients with COVID-19 is yet to be explored in large-scale studies.

The association between diabetes mellitus (DM) and increased mortality in COVID-19 is multipronged. Diabetes mellitus, especially uncontrolled DM is associated with a dysfunctional immune system. The innate immune system, the first line of defense against SARS-CoV-2 is compromised in people with DM (Geerlings and Hoepelman, 1999). Even short-term hyperglycemia has been shown to transiently stun the innate immune system (Jafar *et al.*, 2016). Moreover, DM is characterized by an exaggerated pro-inflammatory cytokine response, notably IL-1, IL-6 and TNFα, in the absence of an appropriate immunostimulation (Geerlings and Hoepelman, 1999); this is further exaggerated in response to a stimulus. This has been depicted in COVID-19 patients wherein serum levels of IL-6, C-reactive protein and ferritin were significantly higher in patients with DM than those without DM (Guo *et al.*, 2020). This suggests that people with diabetes are more susceptible to an inflammatory cytokine storm eventually leading to ARDS, shock and death in COVID-19 (Pal and Bhadada, 2020b). In addition, COVID-19 patients with DM also had higher D-dimer levels than those without DM (Guo *et al.*, 2020), perhaps signifying an over-activation of the hemostatic system. Amid an already underlying pro-thrombotic hypercoagulable state predisposed by the mere presence of DM, over-activation of the coagulation cascade in COVID-19 can lead to thromboembolic complications and eventual mortality (Pal and Bhadada, 2020b). Besides, DM is associated with reduced expression of ACE2 and low ACE2 expression might partly explain the increased incidence of severe lung injury and ARDS with COVID-19 (Pal and Bhansali, 2020; Tikellis and Thomas, 2012). Herein, also lies the confounding role of ACEi/ARBs that are also commonly prescribed because of their additional renoprotective effects in people with DM (Pal and Bhadada, 2020b; Pal and Bhansali, 2020). In addition, certain drugs used in the management of type 2 diabetes mellitus (T2DM), notably, liraglutide and pioglitazone have also been shown to upregulate ACE2 (Pal and Bhadada, 2020c). Although there exists little literature on people with type 1 diabetes mellitus (T1DM), the recently published CORONADO study has shown that people with T1DM and T2DM are equally at a high risk of endotracheal intubation for mechanical ventilation and/or death with 7 days of admission (primary outcome). The study also highlights the fact that death in people with DM is independently predicted by BMI, age, treated obstructive sleep apnoea, micro- and macrovascular complications. Surprisingly, long-term glucose control as reflected by HbA1c neither admission plasma glucose as not associated with the primary outcome on multivariate analysis (Cariou *et al.*, 2020).

Obesity has also been found to be associated with severe disease and mortality in COVID-19 (Dietz and Santos-Burgoa, 2020; Kassir, 2020; Pal and Banerjee, 2020). Adipose tissue express ACE2; with higher adipose tissue, more would be the overall ACE2 expression that would act as receptors for SARS-CoV-2

(Kassir, 2020). As in diabetes mellitus, even in basal state, obese patients have a higher concentration of several pro-inflammatory cytokines such as TNFα, IL-6 and MCP-1, produced by visceral and subcutaneous adipose tissue (Richard *et al.*, 2017). This could again predispose an obese individual to an exaggerated cytokine response in the presence of SARS-CoV-2, manifesting as severe disease, ARDS and finally, death (Pal and Banerjee, 2020).

In patients with chronic obstructive pulmonary disease (COPD), there is inactivation of the innate immune system and underexpression of pulmonary interferon-β, a cytokine involved in the defense against coronavirus (García-Valero *et al.*, 2019), thereby explaining the increased severity of COVID-19 in this subset of patients. Patients with underlying chronic kidney disease and malignancy are also immunocompromised, thereby explaining an increased propensity for severe disease and mortality in COVID-19 (Pal and Bhadada, 2020a).

Smoking

The association between smoking and COVID-19 is controversial. A systematic review and meta-analysis involving 11 case series with a total of 2002 cases had concluded that the pooled odds ratio of ongoing smoking and the development of severe COVID-19 was 1.98 (Zhao *et al.*, 2020). Another systematic review incorporating five studies had reported that the smokers were 1.4 times more likely (relative risk 1.4) to have severe symptoms of COVID-19 and approximately 2.4 times more likely to be admitted to an ICU, need mechanical ventilation or die compared to non-smokers (relative risk 2.4) (Vardavas and Nikitara, 2020). The plausible reason for this apparent adverse association between smoking and COVID-19 lies in the fact that smoking has been shown to upregulate ACE2 (Brake *et al.*, 2020). However, another meta-analysis involving 5 studies, totaling 1399 COVID-19 patients, 288 of whom (20.6%) had severe disease, did not find any significant association between active smoking and disease severity (Lippi and Henry, 2020).

Secondary Infections

Ruan *et al.* had shown that the prevalence of secondary infections was significantly higher in COVID-19 patients who had died as against those who had been discharged from the hospital (16% *vs.* 1%, *P* value=0.002). Secondary infections (bacterial or fungal) would increase the chances of septic shock and subsequent mortality.

Laboratory Parameters

An array of biochemical and hematological parameters has been associated with increased mortality in COVID-19. The biochemical markers are either indirect indicators of the severity of the underlying inflammation (as an example, C-reactive protein, erythrocyte sedimentation rate, IL-6, ferritin) or pointers towards underling end-organ damage (as an example, cardiac troponin, lactate dehydrogenase, creatinine, blood urea nitrogen, myoglobin, alanine transaminase) (Martins-Filho *et al.*, 2020; Ruan *et al.*, 2020; Tian *et al.*, 2020; Zhou *et al.*, 2020). Interleukin-6 needs a special mention. The cytokine has been implicated as one of the primary mediators of cytokine storm and hence, is elevated in patients with severe COVID-19. A meta-analysis of five studies reporting data on overall mortality and serum IL-6 in COVID-19 patients demonstrated that increasing mean IL-6 on admission was associated with an increased likelihood of mortality (Aziz *et al.*, 2020). A serum IL-6 cut-off of 55 pg/ml and 80 pg/ml has been proposed as predictors of severe disease and mortality in COVID-19, respectively (Aziz *et al.*, 2020; Chen *et al.*, 2020). Regarding C-reactive protein, a cut-off of 26.9 mg/l has been proposed as being predictive of aggravation of non-severe COVID-19 (Wang *et al.*, 2020).

As per as ferritin is concerned, Wu *et al.* found that higher serum levels of ferritin was associated with ARDS (hazard ratio 3.53); the association with survival however did not reach the level of significance (hazard ratio 5.28; *P* value=0.10) (Wu *et al.*, 2020a). At their univariate analysis, Zhou *et al.* pointed out at an association between higher serum ferritin levels and mortality, but subsequently, multivariate analysis was not presented (Zhou *et al.*, 2020). Similarly, non-survivors, as compared to survivors, presented more often with high LDH and high procalcitonin. Increased LDH has also been shown to be associated with a higher risk of ARDS (Wu *et al.*, 2020a), ICU support (Fan *et al.*, 2020) and death (Martins-Filho *et al.*, 2020; Wu *et al.*, 2020a) across multiple published studies. A meta-analysis of four published studies showed that increased procalcitonin values were associated with a nearly 5-fold higher risk of severe infection in COVID-19 (Lippi and Plebani, 2020).

Between 7% and 27.8% of COVID-19 patients may have elevated troponin levels (Guo *et al.*, 2020; Shi *et al.*, 2020; Wang *et al.*, 2020; Yang *et al.*, 2020). The plausible reasons for troponin elevation in patients with COVID-19 are multiple. First, SARS-CoV-2 induced cytokine storm can lead to myocardial inflammatory damage (Yang *et al.*, 2020). Second, SARS-CoV-2 can cause cardiac damage through direct viral invasion. Third, it is also possible that the elevated troponin levels are due to coronary microvascular ischemia mediated by direct binding of SARS-CoV-2 to the endothelial ACE2 receptor (Gupta *et al.*, 2020). Lastly, the

hypercoagulable state seen in patients with COVID-19 can lead to microvascular thrombi and secondary myocardial infarction with subsequent elevation in serum troponins (Tang *et al.*, 2020). Several studies have shown that those with elevated troponin levels at baseline are predictive of severe disease, increased intensive care unit admissions and higher mortality (Guo *et al.*, 2020; Martins-Filho *et al.*, 2020; Shi *et al.*, 2020; Tang *et al.*, 2020; Tian *et al.*, 2020). In a cohort study, the presence of elevated troponin levels was associated with mortality with a hazard ratio of 4.26 (Shi *et al.*, 2020). In another single-center retrospective analysis of 187 COVID-19 patients, Guo *et al.* studied the relationship of baseline troponin levels and other comorbidities with mortality. They reported that the risk of death could be stratified according to the presence of elevated troponin and/or previous history of cardiovascular disease. Notably, elevated troponin levels carried a strong prognostic value even in the absence of cardiovascular disease history. In addition, the authors reported that in survivors, during the hospitalization period, the troponin levels remained stable and within normal limits. On the other hand, non-survivors showed a trend of gradual and progressive increase in troponin levels (Guo *et al.*, 2020). Du *et al.* reported that cardiac troponin I $\geq$ 0.05 ng/mL (odds ratio 4.077) was associated with an increased risk of mortality in COVID-19 independent of age, sex or presence of comorbid illness (Du *et al.*, 2020).

Amongst hematological parameters, elevated D-dimer has consistently been found to be associated with severe disease and mortality in COVID-19 (Martins-Filho *et al.*, 2020; Tang *et al.*, 2020; Tian *et al.*, 2020; Wu *et al.*, 2020a; Zhou *et al.*, 2020). Among 201 patients with COVID-19, increased levels of D-dimer were significantly associated with increased risk of both ARDS and death (Wu *et al.*, 2020a). In a multicenter retrospective cohort study from China, increased D-dimer levels (> 1μg/mL) were significantly associated with in-hospital death in the multivariable analysis. D-dimer levels also showed a sequential increase with time among non-survivors as against those who survived (Zhou *et al.*, 2020). In another retrospective study by Tang *et al.*, encompassing data from 183 consecutive patients with COVID-19, non-survivors had significantly higher D-dimer levels compared with survivors at initial evaluation (Tang *et al.*, 2020). Elevated D-dimer points towards the possibility of hyper-activation of the fibrinolytic system as an adaptive measure to counteract the hypercoagulable state induced by COVID-19. It could also imply an underlying state of compensated DIC (Terpos *et al.*, 2020).

Lastly, circulating blood cell counts can also help predict mortality. Significant lymphopenia in patients with COVID-19 often coincides with cytokine storm (Terpos *et al.*, 2020). Several factors may contribute to COVID-19 associated lymphopenia. Lymphocytes express ACE2 receptor on their membrane (Xu *et al.*, 2020); SARS-CoV-2 may directly infect them and lead to their lysis. Besides, the

cytokine storm is characterized by markedly increased levels of interleukins and TNFα that may promote lymphocyte apoptosis (Terpos *et al.*, 2020). Cytokines may also lead to atrophy of lymphoid organs, like the spleen, and further impair lymphocyte turnover (Chan *et al.*, 2020). In addition, coexisting lactic acid acidosis may also impair lymphocyte proliferation (Fischer *et al.*, 2007). Whatever may be the underlying cause, lymphopenia is associated with severe disease (Guan *et al.*, 2020b; Huang *et al.*, 2020; Wang *et al.*, 2020), ARDS (Wu *et al.*, 2020b) and mortality in COVID-19 (Martins-Filho *et al.*, 2020; Ruan *et al.*, 2020). In addition, lymphocyte subtyping has shown that CD3+ CD8+ T-cell count ≤ 75 cell/μl is an independent predictor of mortality in COVID-19 (Du *et al.*, 2020). Apart from lymphocyte count, total leukocyte count and absolute neutrophil count have also been shown to predict mortality. Leukocytosis and neutrophilia have been reported to be associated with mortality in a few studies (Martins-Filho *et al.*, 2020; Ruan *et al.*, 2020; Wu *et al.*, 2020b).

CONCLUSION

The novel coronavirus disease (COVID-19) tends to be asymptomatic or mild in most of the patients. However, around 5% of the patients tend to have critical disease complicated by ARDS, shock and multiple organ failure. Mortality in COVID-19, as represented by the case-fatality rate, is around 6% (as of June 4, 2020) with maximum mortality being reported from the European nations. Respiratory failure ensuing from ARDS and pulmonary thromboembolism seem to be the most common cause of death in COVID-19. Advancing age and presence of comorbid illness are consistently associated with an increased risk of death, while certain biochemical and hematological parameters, notably C-reactive protein, IL-6, cardiac troponin, D-dimer and absolute count can also help predict mortality in patients with COVID-19.

ABBREVIATIONS

ACE2	Angiotensin converting enzyme 2
ARDS	Acute respiratory distress syndrome
CFR	Case-fatality rate
COVID-19	Novel coronavirus disease 2019
CRP	C-reactive protein
DM	Diabetes mellitus
DIC	Disseminated intravascular coagulation
DVT	Deep vein thrombosis
ICU	Intensive care unit

IL-6	Interleukin-6
MERS	Middle East Respiratory Syndrome
SARS	Sever Acute Respiratory Syndrome
WHO	World Health Organization

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 4

Long Term Complications of COVID-19

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Abstract: Coronavirus disease 2019 (COVID-19) is an acute respiratory illness. It is caused by a novel coronavirus (SARS-CoV-2). It has gained widespread recognition after it originated from China. The World Health Organisation (WHO) declared the outbreak of COVID-19 as an international public health emergency. Patients of COVID-19 develop long-time complications along with severe health problems as it majorly affects the respiratory system, utilizing angiotensin-converting enzyme 2. Characteristic symptoms include fever, cough, and dyspnea, although some patients may be asymptomatic. People need extensive care to be protected from anxiety and depression. This outbreak harmed various organ systems and led to the development of long-term complications such as respiratory failure, pneumonia, pancreatic complications, cardiac injury, secondary infections, renal disorders, disseminated intravascular coagulation, and rhabdomyolysis. Individuals with comorbidities are at higher risk of illness and mortality. The risk is also increasing in older people, especially people the age of 60-80 years or more. The most prevalent comorbidities are asthma, neurologic disorders, diabetes, obesity, cardiovascular disease, and malignancy/hematologic conditions. As there is no specific treatment available so far, therefore complications of this disease are also increased by the use of non-specific drugs. The recovery of these patients is another major challenge for health care professionals. So in this chapter, we will discuss long-term complications associated with COVID-19.

Keywords: Cardiovascular diseases, Coronavirus, COVID-19, Diabetes, Mortality, Neurological effect, Pandemic, Renal effect, Respiratory effect, Rheumatoid arthritis.

INTRODUCTION

The latest coronavirus 2019 (2019-nCoV) or SARS-COV-2, a respiratory synd-

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rome, has rapidly spread from the Wuhan Town of Hubei, China, to the rest of the world. Coronavirus 2019 (COVID-19) is a consequence of severe acute respiratory syndrome that is now a significant concern for global public safety and local communities (Li *et al.*, 2020b). More importantly, following considerable initiatives and a rising number of remedies in China, the disease has now started to spread from China to Europe, North America, and other Asian countries (Wu and McGoogan, 2020). Coronavirus is a positive-sense RNA virus with spike-like projections having a diameter of about 60-140 nm. Under the electron microscope, it shows its crown-like appearance; thus, the term coronavirus has been used for it. There are four different types of coronaviruses (HKU1, NL63, 229E, and OC43). They are responsible for causing mild respiratory diseases in humans (Singhal, 2020). If an individual is suffering from COVID-19, their clinical symptoms range from asymptomatic disease to respiratory failure and finally leading to multiple organ dysfunction. Such kind of developed complication leads to the death of patients. The commonest health symptoms in COVID-19 include fever, cough, headache, sore throat, myalgia, fatigue, and respiratory illness. Conjunctivitis is also reported in some of the patients of COVID-19. Therefore it is difficult to distinguish this from other respiratory infections (Lima, 2020).

By the completion of the first week after the reports of the spread of COVID-19, cases of pneumonia, respiratory failure, and death were reported. This breakthrough is related to the significant increase in inflammatory cytokines like IL2, IL7, IL10, and TNFα. A person of any age group is susceptible to this disease. Symptomatic patients of COVID-19 release their large droplets through cough and sneezing. These incidences cause the spreading of infection of COVID-19. This spread of infection may also arise from asymptomatic individuals even before the occurrence of symptoms. In contrast with the mouth, higher viral loads have been observed in the nasal cavity. Moreover, patients can remain contagious as long as the virus survives in them, and also after they are fully recovered (Wu *et al.*, 2020a, Singhal, 2020). The development of such a condition of infection is due to the passing of the virus through the mucous membranes. It enters the human body through nasal and larynx mucosa. After that, it enters into the lungs through the respiratory tract. As a result of this, fever and cough are the most common symptoms of infection to appear. Further, it causes viremia when it invades into peripheral blood through the lungs. Then the virus attacks the targeting organs that express Angiotensin-converting enzyme 2 (ACE2), such as the lungs, heart, renal, gastrointestinal tract. The fecal samples have also been found to have evidence of SARS-CoV-2. The reason behind this detection is due to the entry of this virus takes place through the lung to blood and then it propagates from the blood to the intestines. During the process of infection, the count of white blood cells is normally or slightly less in peripheral blood at an

early stage only. But as time progresses the lymphopenia is observed in patients (Cheng *et al.,* 2020a). The critical stage is appearing in this disease as a reduction of B lymphocyte occurs in the early stage of this disease only, which may affect the production of antibodies. It is also noticed that the patients having a severe stage of the COVID-19 reduction of lymphocytes significantly takes place (Cheng *et al.,* 2020a). The increased level of neutrophils, D-Dimer, blood urea nitrogen, and creatinine was reported in non-survivors than the survivors. Based on these recently reported facts and assumptions, the clinical stages can be divided as follows:

a. The viremia stage.
b. The acute stage (pneumonia phase) and
c. The recovery stage (Guan *et al.*, 2020, Wang *et al.*, 2020a, Letko *et al.*, 2020).

The coronavirus disease pandemic is causing significant morbidity and mortality in patients having a long history of any diseases associated with primary disease. Older age, diabetes mellitus, cancer, chronic kidney disease, rheumatoid arthritis, hypertension, and obesity greatly raise the likelihood of hospitalization and mortality in patients with COVID-19 (Guo *et al.*, 2020a). In this context, we explore the long-term complications of COVID-19 in patients suffering from various diseases.

LONG TERM COMPLICATIONS OF COVID-19

The meaning of the long history of diseases is chronic complications and it can be defined as the clinical state of suffering that tends to arise over the years and persists for decades. In this, patients used to suffer from not only the various complications of primary diseases but also secondary or multiple diseased states. Even though a large number of people are impacted by the COVID-19 pandemic, the most vulnerable are the aged, under-represented minorities, and underlying diseases. The increasing consumption of an unhealthy diet with excessive amounts of saturated fats, carbohydrates, and sugar persuade the pervasiveness of obesity and type 2 kind of diabetes and may raise the likelihood of seriousness of COVID-19 disease and its mortality (Butler and Barrientos, 2020). A healthy diet is very important for the development of immunity. Immunity and lifestyle (daily habit of food intake) are related to each other. So if the person taking an unhealthy diet or careless about the daily nutritious diet intake invites stimulation of the innate immune response and impairment of adaptive immunity which is inducing persistent inflammation, and finally brings disruption of the host defense system. Besides, the long-standing impact of peripheral inflammation induced by COVID-19 contributes to chronic medical disorders such as neurodegenerative disorder and dementia. These conditions are developed possibly through

neuroinflammatory pathways, which can be accelerated by an unhealthy diet (Connaughton *et al.*, 2016).

All these diseases are responsible for long-term complications of COVID-19 (Table **1**). Moreover, there is no specific treatment of COVID-19 available so far therefore various nonspecific drugs that are used nowadays also lead to serious complications. Pre-exposure prophylaxis or post-exposure vaccination is not yet authorized. At this moment, there is no approved specific antiviral therapy for is available for COVID-19. The medication is given to the patients is primarily symptomatic and in special circumstances respiratory medication and ventilator assistance being the primary recovery methods for severely infected patients. Supported mechanical ventilation is essential for the prevention of respiratory collapse in serious patients. Hemodynamic assistance is provided to treat circulatory dysfunction and a septic diseased state (Wu and McGoogan, 2020). In the absence of any FDA-approved COVID-19 therapies, several physicians and scientists are shifting to established medicines for the prevention or treatment of the disease (Harrison, 2020). Many medicines that have been studied based on hypothesis or insufficient data include antiviral (Lopinavir, Ritonavir, and Remdesivir), monoclonal antibodies (Tocilizumab), chloroquine/Hydroxychloroquine, arbidol, and azithromycin, *etc*., which have multiple side effects and toxicities even they are failed to provide relief to the patients. Hence, other studies have pointed out that repurposing of some drugs, rationalized identification and strict clinical testing may provide a step forward for the treatment of COVID-19. These drugs could be chloroquine, hydroxychloroquine, and Azithromycin, as well as anti-diabetics such as metformin, angiotensin receptor inhibitors such as sartans, or statins such as simvastatin (Nabirotchkin *et al.*, 2020), Human immunoglobulin, Darunavir-cobicistat combination, Recombinant human interferon α2β, Carrimycin or lopinavir-ritonavir or arbidol or chloroquine phosphate, Danoprevir-ritonavir and interferon inhalation or lopinavir-ritonavir or TCM plus interferon inhalation, Xiyanping or lopinavir-ritonavir-interferon inhalation, Thalidomide, Vitamin C, Pirfenidone and Methylprednisolone, *etc*. (Rosa and Santos, 2020, Mercorelli *et al.*, 2018).

Table 1. Complications in patients with COVID-19.

No. of Patients	Diabetes (%)	HTN (%)	CVD (%)	COPD (%)	CKD (%)	Reference
1099	7.4	15.0	3.8	1.1	0.7	(Guan *et al.*, 2019)
7162	10.9	-	9.0	9.2	3	(Gangopadhyay, 2020)
138	10.1	31.2	19.6	2.9	2.9	(Wang *et al.*, 2020a)
191	19.0	30	8.0^{+}	3.0	1.0	(Zhou *et al.*, 2020a)

(Table 1) cont.....

No. of Patients	Diabetes (%)	HTN (%)	CVD (%)	COPD (%)	CKD (%)	Reference
201	10.9	19.4	4.0	2.5	1.0	(Wu *et al.*, 2020a)
140	12.1	30	8.6	1.4	1.4	(Zhang *et al.*, 2020a)

[α] Dead COVID-19 Patients, [+] coronary heart disease only, CVD-cardiovascular disease, HTN-Hypertension, CKD-chronic kidney disease, COPD-chronic obstructive pulmonary disease.

COMPLICATIONS OF COVID-19 IN VARIOUS DISEASES

Diabetes Mellitus (DM)

Older age groups and the presence of diabetes mellitus (DM), extreme obesity, and hypertension (HTN) have now been well-known to raise morbidity and mortality in COVID-19 patients. Despite the increased incidence of cardiovascular diseases (CVD), HTN, and obesity, in DM patients, it is unclear whether DM individually leads to an elevated risk. However, plasma glucose and DM are acting as a predictor for mortality and morbidity separately for Severe Acute Respiratory Syndrome (SARS) patients. Potential pathways are represented in Fig. (**1**). May cause elevation of COVID-19 responsiveness in DM patients. It involves viruses that have:

a. High cellular entry (in terms of its affinity and efficiency)
b. Reduction of its clearance and reduction in the function of T cells
c. Increased sensitivity to hyper-inflammation as well as cytokine storm syndrome; and
d. Presence of CVD (Muniyappa and Gubbi, 2020).

The binding of SARS-CoV-2 at a cellular level is supported by an increase in expression of ACE2 in alveolary AT2 cells, kidney, myocardium, and pancreas. Its excessive expression was investigated and reported in the liver, intestine, heart, and pancreas when the preclinical evaluation was conducted in diabetic animals. Further, it was also reported that insulin therapy causes suppression inactivation of ACE2. On the other hand, glucagon-like peptide 1 (GLP-1) agonists, ACE inhibitors, and statins are known to use as hypoglycemic medication increase the expression of ACE2 (Guo *et al.*, 2020b). Till now, it was not clear that DM was causally related to ACE2 expression in humans in the lung. But recently Rao *et al.* studied that diseases or their characteristics could induce a rise in ACE2 expression in the lungs and found that DM. It was also indicated that it is correlated with increased expression of ACE2 in the lungs. It is also found that Furin, a cell protease promotes viral entry through the breakdown of the S1 and S2 spike protein and the level of this is also increased in the patient of DM (Rao *et*

al., 2020). ACE is responsible for the conversion of angiotensin (Ang) I to octapeptide, Ang II. ACE2 causes the transformation of Ang II to Ang1–7. Ang II causes Vasoconstriction and proliferation kind of activity performed by Ang II *via* stimulation of Ang II type 1a. On the other hand, it is very important to note that Ang1–7causes vasodilation with the reduction of proliferation of cells. The increased pulmonary ratio of ACE/ACE2, favors the development of Ang II in patients of respiratory distress syndrome (ARDS) (Wösten-van Asperen *et al.*, 2013, Kuba *et al.*, 2005). SARS-CoV causes decreases in the expression of ACE2 but the non-opposed activity of Ang II leads to lung damage. The ACE2 attachment alone will not do significant harm to the heart, as can be seen with other CoVs (NL63) (Case *et al.*, 2010). The evidence about SARS-CoV-2 triggers the pulmonary downregulation of ACE2 is still uncertain and imprecise. In the absence of more risk or benefit data, the United States Heart Association as well as the American Society of Hypertension advised the patients to start their regular antihypertensive treatment (Danser *et al.*, 2020).

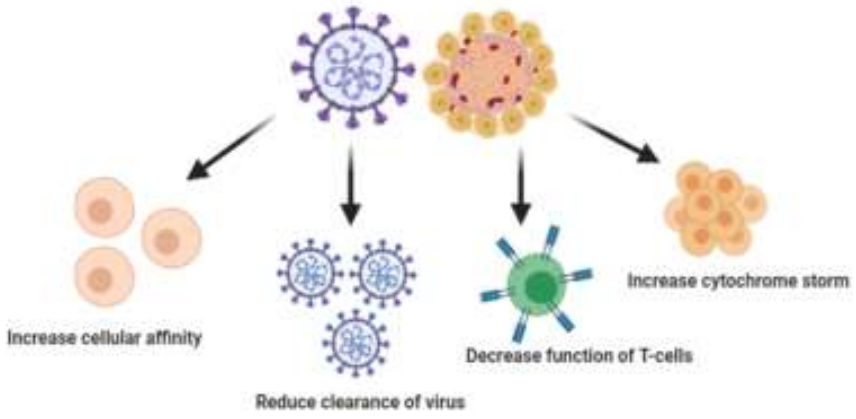


Fig. (1). Influence of COVID-19 in patients of diabetes.

Moreover, diabetes is a metabolic disorder and its complications are so vast that it can not only cause vascular diseases but also responsible to develop the chronic inflammatory disease. In case of infection with DM can impair pathogenic responses of individuals. The chronic inflammatory responses in the case of DM are because of the appearance of glycosylation (AGE), pro-inflammatory cytokines lead to evoke oxidative stress, as well as activation of adhesive molecules. This condition can be more aggravated with an increase in hyperglycemia and insulin resistance (Knapp, 2013). The inflammatory cycle contributing to higher infection susceptibility, this is worse in diabetes patients.

Several immune deficiencies have been attributed to hyperglycemia, but the therapeutic significance of these *in vitro* abnormalities remains uncertain (Petrie *et al.*, 2018). Poorly regulated diabetes has been associated with the suppressed proliferative lymphocyte response to various forms of stimuli, as well as the disrupted activities of macrophages and neutrophils. In patients having diabetes, irregular hypersensitivity reaction, and disruption in complement activation were also identified (Geerlings and Hoepelman, 1999). An increase in influenza virus infection, as well as its replication, was observed in *in vitro* experiments in which lung epithelial cells have also shown access to elevated glucose cells. This experiment suggesting that hyperglycemia can increase *in vivo* viral replication(Kohio and Adamson, 2013). Structural pulmonary modifications in animal models have been connected to diabetes, such as increased permeability of the vasculature and also the disintegrated alveolar epithelium. Forced vital capacity and forced expiratory volumes are related to the increased glucose level in the blood. Patients with DM typically have a significant decrease in their forced vital capacity and forced expiratory volumes (Popov and Simionescu, 1997, Lange *et al.*, 1989). There are no data available so far for the effective management of SARS-CoV-2 in diabetes patients and no treatment approach also available for COVID-19 patients who develop glycaemic decompensation. Rigorous control of glucose and careful consideration of drug reactions may reduce the severity of symptoms and adverse effects. While hyperglycemia is typically the major concern, in this case, the risk for hypoglycemic episodes should not be ignored due to the relationship between medication usage, viral pathogenesis, and normal diabetes metabolic disturbances. Patient treatment approaches and optimal control thresholds of glucose should be developed based on disease seriousness, co-existence and risks, age, and other aspects correlated with diabetes (Zhang *et al.*, 2020a). In Wuhan, China, 258 confirmed COVID-19 patients who suffered from diabetes had a significant increase level of leukocyte, neutrophil with higher levels of fasting blood glucose, serum creatinine, urea nitrogen, and creatine kinase isoenzyme MB in comparison with non-diabetic patients (Zhang *et al.*, 2020b). In Hong Kong also patients who suffered from diabetes had an increased mortality rate from pneumonia. The age of these patients was observed as $\geq$ 75 and reported to have CVD and cancer causes increases in the risk of mortality (Bloomgarden, 2020). Hence, during the prolonged hospitalization and recovery periods, a collaborative team strategy, involving infectologists, endocrinologists, lung biologists, psychiatrists, nutritionists, and fitness therapy specialists, can be essential. Special attention should be paid to diabetes nephropathic patients or heart disorders related to diabetes (Hussain *et al.*, 2020) as it can be fatal.

Hematological Complications

In the case of viremia, SARS-CoV-2 prominently damages the tissues responsible to express elevated ACE2. It generally takes place in the lungs, heart, and gastrointestinal tract. As the day progresses initial symptoms become worst and it is observed from day 7 to 14. Further, an unexpected rise in other clinical manifestations also occurs. It is characterized by the systemic surge of inflammatory mediators and cytokines storm. This is the time when significant lymphopenia becomes evident in patients. Here the meaning of cytokine storm can be stated as an abnormal increase in the levels of interleukins. The components that take part in this storm are helpful to promote the apoptosis of lymphocytes. It includes IL-2, 6, and 7, granulocyte colony-stimulating factor, interferon-γ inducible protein 10, MCP-1, MIP1-a, and TNF-α. (Li *et al.*, 2020d, Xu *et al.*, 2020a, Singh *et al.*, 2014, Liao *et al.*, 2002). COVID-19 is a systemic infection that affects the hematopoietic and hemostasis systems significantly. Lymphopenia is considered as a cardinal laboratory observation having prognostic value. The ratio and amount of neutrophil/lymphocytes can also be used to determine severe cases (Table **2**). Coagulation disorders are fairly common among patients with COVID-19, especially those with severe disease. During the first 2 months of an outbreak in China, the longitudinal multi-center analysis showed that 260 out of 560 patients having COVID-19 was confirmed with an increased level of D-dimer and the elevation was more prominent in severe patients (Guan *et al.*, 2020). Sepsis is another factor to be considered in patients with a severe case of COVID-19.

Table 2. Impact of COVID-19 on lymphocyte count.

Location	Duration of Study	No. of Patients	Identifying Features	Remarks	References
General Hospital of Central Theater Command, Wuhan, China	-	90	Lymphocytes at time point 1. day 10-12 from symptom onset (>20% or < 20%) and on day 17-19 (>20%, 5-20%, and < 5%).	Pre-severe disease state was observed at time point 1 while on time point 2 it turns to critical illness.	(Tan *et al.*, 2020)

(Table 2) cont.....

Location	Duration of Study	No. of Patients	Identifying Features	Remarks	References
Tongji Hospital, Hankou branch of Central Hospital Wuhan, China	1 Jan 2020 to 21 February 2020	-	Lower lymphocyte count (median: 0.63) at admission . Non-survivors have a lower lymphocyte/WBC ratio (median: 7.10)	A significantly lower level of lymphocytes was observed in the non-survivor group of patients The lymphocyte/WBC ratio decreased continuously from the time of admission to death during hospitalization.	(Deng *et al.*, 2020)
Seattle region, Washington State, USA	24 February 2020, to 9 March 2020	24 ICU patients	Lymphocyte counts were a continuous variable	The exact causes of lymphocytopenia were not provided.	(Bhatraju *et al.*, 2020)
Evergreen Hospital, Washington State, USA	20 February 2020, to 5 March 2020	21 ICU patients	Less than 1000 cells/μL	Low lymphocyte count was observed in 67% of critically ill patients.	(Arentz *et al.*, 2020)
Jinyintan Hospital and Wuhan Pulmonary Hospital, Wuhan, China	25 December 2019, to 31 January 2020	191	Lymphocyte counts treated as a continuous variable ($\times 10^9$/L)	Lower lymphocyte count was associated with higher odds of death at the univariate analysis.	(Zhou *et al.*, 2020a)
Jinyintan Hospital, Wuhan, China	24 December 2019, to 9 February 2020	52 critically ill patients		85% of lymphocytopenia in critically ill patients	(Yang *et al.*, 2020)
National Centre for Infectious Diseases, Singapore	23 January to 28 February 2020	69	Lymphocyte count =<0.5×10^9/L.	Lymphopenia at admission (4/9 of ICU patients vs 1/58 non-ICU patients, $P < .001$) and nadir lymphopenia during hospital stay (7/9 of ICU patients vs 1/58 non-ICU patients, $P < .001$) were associated with the need for ICU.	(Fan *et al.*, 2020)

It leads to increased activation of neutrophils, delaying of apoptosis, depletion with the exhaustion of CD4 and CD8 T cells in patients having the severe condition of COVID-19(van der Poll *et al.*, 2017, Zhou *et al.*, 2020c). The level of disseminated intravascular coagulation (DIC) can be measured to check the

condition of sepsis in patients. It was observed that the DIC level increases in nonsurvivors of COVID-19. Similarly, sepsis-induced coagulopathy (SIC) is a newly proposed category of global coagulation tests, which has been established as an early warning signal of DIC. Hence, hematological parameters might provide a method for classifying the severity of COVID-19 and the SIC scoring system might be a useful tool to manage critically ill patients (Iba *et al.*, 2020, Liao *et al.*, 2020).

The dynamics of the D-dimer can represent severity and are associated with increased rates of adverse effects for community-acquired patients. Hypercoagulation of the blood is normal in COVID-19 patients. High rates of D-Dimers and its gradual rise during disease are regularly recorded particularly linked with the worsening of disease (Snijders *et al.*, 2012). SARS-CoV-2 is likely to boost a massive increase in fibrin formation and its deposition. It causes a very high level of D-dimer in these patients. Hence, deposition of fibrin in the alveolar sac and interstitial lung spaces may contribute to worsening of respiratory condition which turns to its failure and advocates prolonged mechanical ventilation, poor prognosis, and death (Tang *et al.*, 2020, Spiezia *et al.*, 2020). Some abnormalities of coagulation contribute to life-threatening conditions. It includes an increase in fibrin degradation products with severe intravascular thrombocytopenia which disseminated intravascular coagulation. This shows that hospital-based and ambulatory COVID-19 infected patients are at elevated risk for venous thromboembolism. Apart from this, venous thromboembolism was also recorded in 20% to 69% of patients with extreme COVID-19 and may be linked to poor prognosis. Warm or cold autoimmune hemolytic anemia has been documented after the beginning of COVID-19 symptoms that may be due to cytokine release syndrome (Fig. **2**). It is still not clear whether hemolytic anemia is correlated with COVID-19 infection or not (Lazarian *et al.*, 2020). On the other hand, venous thromboembolism was recorded in 20% to 69% of patients with extreme COVID-19 and may be linked to poor prognosis. Pulmonary embolism in 23% of patients at one US center and 20% to 30% of patients in France were also recorded (Llitjos *et al.*, 2020). Such patients were more likely to require intensive treatment and artificial ventilation than those having no pulmonary embolism (Fig. **3**). The level of D-dimer changes from mild to a significant level as per the occurrence and development of dyspnoea as well as chest imaging changes from mild to severe. It is also very important to note that these fluctuations also affect the prolongation of prothrombin time and a gradual decrease of fibrinogen with platelet.

The identification of patients at high risk with the COVID-19 is essential for the implementation of venous thromboembolism prophylaxis measures (van Nieuwkoop, 2020). Ischemic changes comprise ecchymosis of the fingers and

toes. In many non-survivors of ischemic changes, it has been clinically observed that they were also suffered from the worse condition of kidney and heart function. Hence, it is indicated that early anticoagulation may be the reason for the blocking of clot formation and causes the reduction of microthrombus. It causes a decrease in the risk of major organ damages (Li, 2020, Lin *et al.*, 2020).

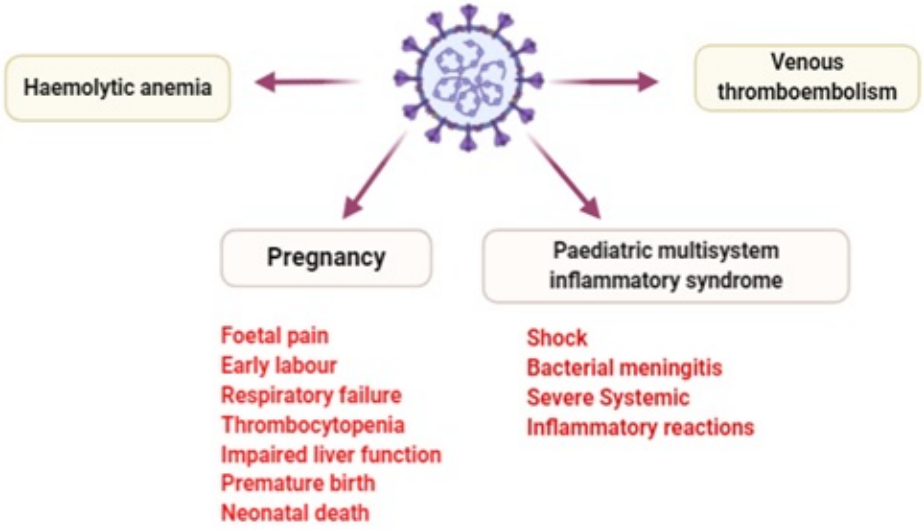


Fig. (2). Effects of COVID-19 in hemolytic anemia and venous thromboembolism.

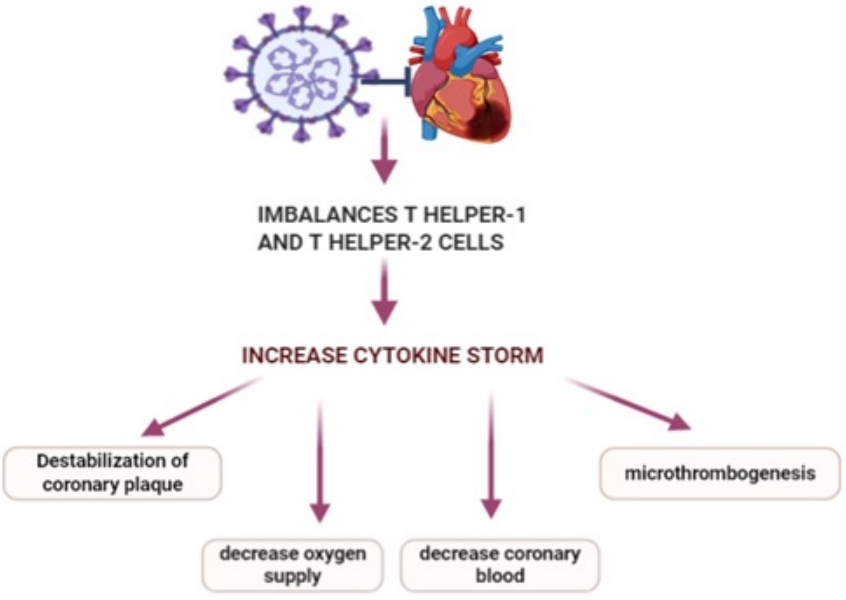


Fig. (3). Impact of COVID-19 in cardiovascular diseases.

Cardiovascular Diseases

A meta-analysis found that patients having CVD were more prone to MERS-CoV. 50% of MERS-CoV disease patients were suffering from hypertension and diabetes and up to 30% of people were suffering from other heart diseases. Likewise, for the Latest Coronavirus Infection, the people with comorbidities are more susceptible to infection caused by SARS-CoV-2, especially to the individual having a history of hypertension, cardiac disease, or diabetes. Besides, these infections a patient having CVD are liable for the development of complications of COVID-19. Moreover, such patients account for a significant proportion of deaths due to COVID-19. This can be understood properly by one of the case studies in which, 58%, 25%, and 44% cases have hypertension, heart disease, and have arrhythmias respectively among patients with severe COVID-19 conditions. As per the report released by NHC, mortality occurs in SARS-CoV-2 patients who had a history of high blood pressure (35% cases) and had a background of cardiac disease (17% cases). Moreover, these results suggested that more chronic and serious pneumonia occurred in patients having on or above 60 years of age diagnosed with SARS-CoV-2 as compare to the patients aged lesser than 60 years (Chan *et al.*, 2020). Therefore, it suggests that if the individual suffering from an infection of SARS-CoV-2 associated with clinical evidence of CVD will be also suffered from pneumonia, and such condition may raise the severity state to the patients.

Moreover, patients infected with SARS-CoV-2 with the coronary syndrome (CS) often have a poor prognosis. Heart disease is more likely to appear in a patient infected with SARS-CoV-2 and the situation of these patients suddenly deteriorates. In Wuhan, it was also observed that the patient of COVID-19 had prior ACS resulted in serious illness and a high mortality rate. The infection of SARS-CoV-2 may act as an exacerbating indicator in patients having cardiac insufficiency that leads to death. The present research indicates that patients with existing CVD and other co-morbid disorders are more likely to develop myocardial damage during COVID-19. The viral disease may destroy more myocardial cells *via* a variety of pathways, including direct exposure to the virus, systemic inflammation reaction, destabilized coronary plaque, as well as exacerbated hypoxia, in patients having CVD, including coronary heart disease, hypertension (HTN), and cardiomyopathy (Fig. **3**). Therefore in CVD patients, there is a greater risk of myocardial injury after COVID-19 and a higher risk of mortality (Zheng *et al.*, 2020). While the actual pathophysiological process of myocardial damage triggered by COVID-19 is not well known, an earlier study has revealed that 35% of patients with severe acute corona viral syndrome (SARS-CoV) infection had severe cardiovascular disease and virus genome was identified in the heart of patients. This increases the possibility of direct

cardiomyocyte disruption by the virus. SARS-CoV-2 could have the same mechanism as SARS-COV because the genome of the two viruses is highly homologous (Li *et al.*, 2020b).

Current scientific reports providing a shred of evidence for the progression of the disease, myocardial damage could directly join the inflammatory pathogenesis. Invading of viral particles takes place through respiratory mucosa and as time progresses it simultaneously infects other cells. Such kind events further lead to cause a cytokine storm, as well as a series of immune reactions, occur. Huang *et al*., also illustrated that in the patient of COVID-19 inequality of T-Helper 1 and 2 develops, which leads to an increase in cytokine storms and acts as a contributing factor to cause myocardial injury (Li *et al.*, 2020b). The inflammatory cytokines release following infection can lead to a reduction in coronary blood flow, reduced oxygen supply, microthrombogenesis, and destabilization of coronary plaque (Guo *et al.*, 2020a).

Central Nervous System Complications

Viral Encephalitis

Encephalitis leads to inflammatory disorders, including neural disruption and inflammation, in the brain's parenchyma triggered by pathogens. It leads to fatigue, fever (primarily elevated temperature), diarrhea, convulsions, and disruption of awareness (Ellul and Solomon, 2018). The Beijing Ditan Hospital research team reported that SARS-CoV-2 was present in the cerebrospinal fluid of patients having COVID-19, which culminated that is responsible for viral encephalitis(Xiang *et al.*, 2020). It gives strong evidence that encephalitis is induced by CoV (Fig. **4**). Viral infections such as Herpes simplex virus (HSV), Varicella zoster (VZV), cytomegalovirus (CMV), influenza virus, and various other respiratory-influenza viruses, such as Severe SARS-CoV and Middle East Airborne Virus (MERS-CoV) are part of a more severe underlying etiology of encephalitis or inflammation. SARS-CoV-2 may also have neurotrophic effects as well as typical respiratory symptoms. Poyiadji *et al.*, recorded another case of a female who had 3 days of experience of fever, coughing, and impaired mental state during their fifties, and diagnosed with COVID-19 through the identification of SARS-Cov-2 nucleic acid. Her CSF analysis was negative for bacteria, type 1 and 2 HSV, and varicella-zoster virus. Throughout, the longitudinal thalami, medial temporal lobes, and subinsular areas, hemorrhagic ring enhanced lesions associated with necrotizing encephalitis are seen. The above case report could support the possible idea of encephalitis by SARS-CoV-2. Poyiadji *et al.,* reported that the SARS COV-2 virus will not penetrate the blood-brain barrier explicitly, and cause necrosis encephalitis (Ahmed *et al.*, 2020).

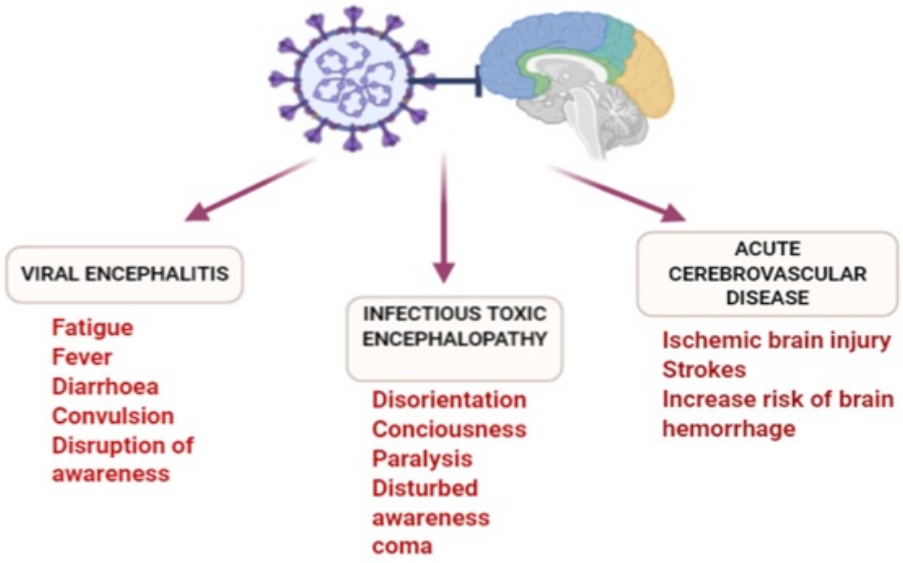


Fig. (4). Impact of COVID-19 in CNS.

Infectious Toxic Encephalopathy and Cerebrovascular Disease

A type of brain impairment syndrome is induced by various reasons which include systemic toxemia, abnormalities linked with metabolism, and hypoxia. At the time of the development of infection, it is referred to as infectious toxic encephalopathy. Cerebral edema with little proof of inflammation on the cerebrospinal fluid analysis is the fundamental pathological change to this disease. It has diverse and varied health manifestations (Tauber *et al.*, 2017). Headache, mental disorder, delirium, and dysphoria may develop in patients with a minor course of the disease. Patients who are seriously impaired will suffer from disorientation, consciousness loss, paralysis, and coma. Acute virus infection is also a significant source of the illness, as in the case of COVID-19 infection. COVID-19 patients also suffer extreme hypoxia and viremia possibly contributing to toxic encephalopathy (Guo *et al.*, 2020c). Additionally, about 40 percent of COVID-19 patients experience pain, disturbed awareness, and other signs of brain impairment, and an autopsy analysis recorded edema in brain tissue (Mao *et al.*, 2020, Xu *et al.*, 2020b). It collectively explored that COVID-19 can induce infectious toxic encephalopathy, although comprehensive studies are still required. Substantial data suggest that a respiratory infection, in particular, is a distinct risk factor for severe cerebrovascular disorders. Data obtained from experimental animal models indicate that influenza will exacerbate ischemia brain injury by causing a cytokine cascade as well as increasing the risk of brain hemorrhage COVID virus infection (Fig. **4**).

Specifically, SARS-CoV-2 is widely reported for cytokine storm syndrome, which may cause CBD (Cerebrovascular disease) (Warren-Gash *et al.*, 2018). Seriously ill patients with serious SARS-CoV-2 infections also generally have high concentrations of D-dimer and drastic platelet reduction, which can make them more likely to develop strokes. Thereby, people at risk of developing cerebrovascular disease during CoV infections (Wang *et al.*, 2020b, Wu *et al.*, 2020b).

Chronic Kidney Disease (CKD)

The elevated incidence of inpatient, as well as outpatient pneumonia, is correlated with chronic kidney disease (CKD). The death rate associated with pneumonia further appears to be 14–16 times greater in CKD patients than in the general population (Henry and Lippi, 2020). The pathogenesis of the occurrence of kidney failure in COVID-19 patients is probably more common. First, the current coronavirus may have immediate kidney tissue cytotoxic consequences. Current coronavirus using ACE 2 receptor is the same as SARS-CoV which was recorded in 2003. Also, it is confirmed by a polymerase chain reaction. In this reaction, it was observed that the chain reaction fragments of coronavirus are available in both the blood and the urine (Peiris *et al.*, 2003, Zhou *et al.*, 2020b). Second, the accumulation of immune complex of the viral antigen or unique immunological effector processes triggered by the virus (primary T cell lymphocyte) may be devasting for the renal system which severely affects the kidney.

However, kidney microscopy samples also provided evidence of normal architecture of glomerular components as well as lack of electron-dense deposits in patients who already had SARS (Chu *et al.*, 2005). Potential pathological shifts in the kidney of COVID-19 patients need further analysis. Thirdly, cytokines induced by viruses or mediators can have indirect consequences, like hypoxia, shock, and even rhabdomyolysis (Fig. **5**). In some patients with the 2009 H1N1 virus relatively had an elevated level of serum creatinine (Kumar *et al.*, 2009). According to a recent report, patients admitted to an intensive care facility having COVID-19 appeared to have elevated levels of creatinine and the patients with renal involvement may even have increased levels of creatine kinase (Wang *et al.*, 2020a, Cheng *et al.*, 2020b). The increased incidence of serious COVID-19 infection tends to be correlated with CKD. Therefore, extra care should be taken to reduce the risk of the virus in patients with CKD. Doctors should also be closely scrutinized the potential COVID-19 CKD cases, to detect the signals of disease development. Finally, in forthcoming COVID-19 risk stratification models the existence of CKD is deemed to be a significant factor (Henry and Lippi, 2020). Cheng and his colleagues examined a large cohort of 710 patients (more than 18 years of age) having COVID-19 infection for kidney disease. Those

with a history of renal transplantation and maintenance dialysis were omitted from the study. In 110 hospitalized cases, base-line serum creatinine was increased. In the elevated serum creatinine community, most of the cases were older with a greater proportion of males and shorter days from the initiation of the illness to hospital entry. Those individuals were also seriously sick and having co-morbidities contrary to those having normal baseline creatinine levels. Also in these patients, the kidney injury was more severe, rates of mechanical ventilation and mortality were also high (Adapa *et al.*, 2020).

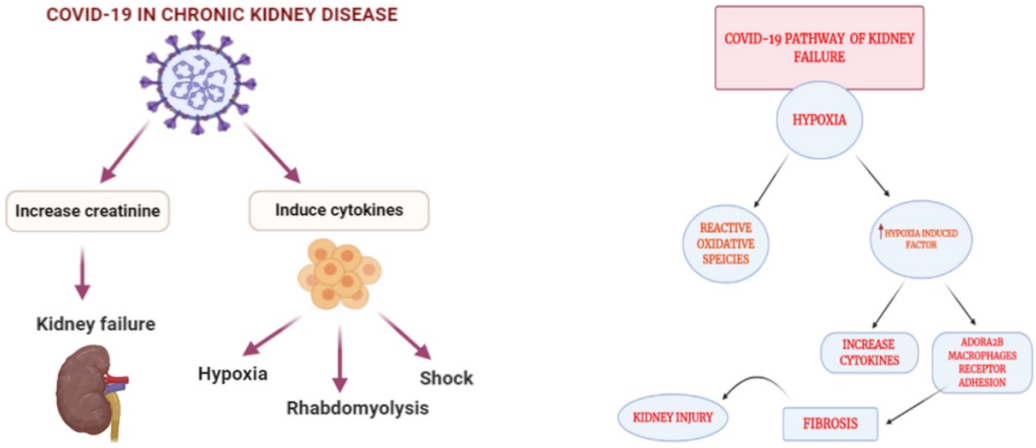


Fig. (5). Effect of COVID-19 in CKD.

Asthma and Respiratory Disorders

There is already considerable evidence that asthma patients are highly represented among the adult patients admitted to Hospital having (COVID-19). This overrepresentation could be triggered by asthma exacerbations, like other infections, which render asthma a contributing factor for the morbidity of COVID-19. The most severe symptoms of COVID-19 are dry cough and air scarcity. Fever is correlated most frequently with COVID-19 but can express any infection-induced asthma exacerbation. COVID-19 screening protocols will apply to someone who has intensified respiratory signs, like asthma. The incidence of asthma did not seem to raise previous novel coronaviruses, SARS-CoV, and MERS-CoV, unlike COVID-19 that increases the risk of asthma (Zheng *et al.*, 2018). The most severe symptoms related to COVID-19 cover fever, exhaustion, dry cough, myalgia anorexia, and dyspnea. Most complications of asthma do not escalate to fever, exhaustion, and anorexia. Typical asthma patients can exacerbate even the signs of COVID-19, indicative should be viewed with good treatment.

Oral steroids would be confined to the lowest dosage possible for the shortest

duration. Patients with COVID-19 infection also have asthma should keep on their current regime of asthma (Fig. **6**). Oral corticosteroids should only be used when COVID-19 causes exacerbation of asthma, again restricting treatment dosage and duration as much as practicable (Pennington, 2020). The risk factors and worsening of Acute Respiratory distress syndrome (ARDS) to death were older age, neutrophilia, and dysfunction of an organ as well as coagulation. It has been found that some ARDS growth factors (*e.g.* comorbidity, CD3 and CD4 numbers, T-cell numbers, AST, pre-albumins, creatinine, low-density lipoprotein, serum ferritin, *etc.*) have not been linked with death. The gap between mortality and recovery classes in median D-dimer was greater than the one between ARDS and non-ARDS, suggesting that certain patients were on their path to mortality with disseminated intravascular coagulation. Also, the production of ARDS was positively linked to high fever and was negative to death which indicates the findings of a Schell-Chaple *et al.,* 2015.

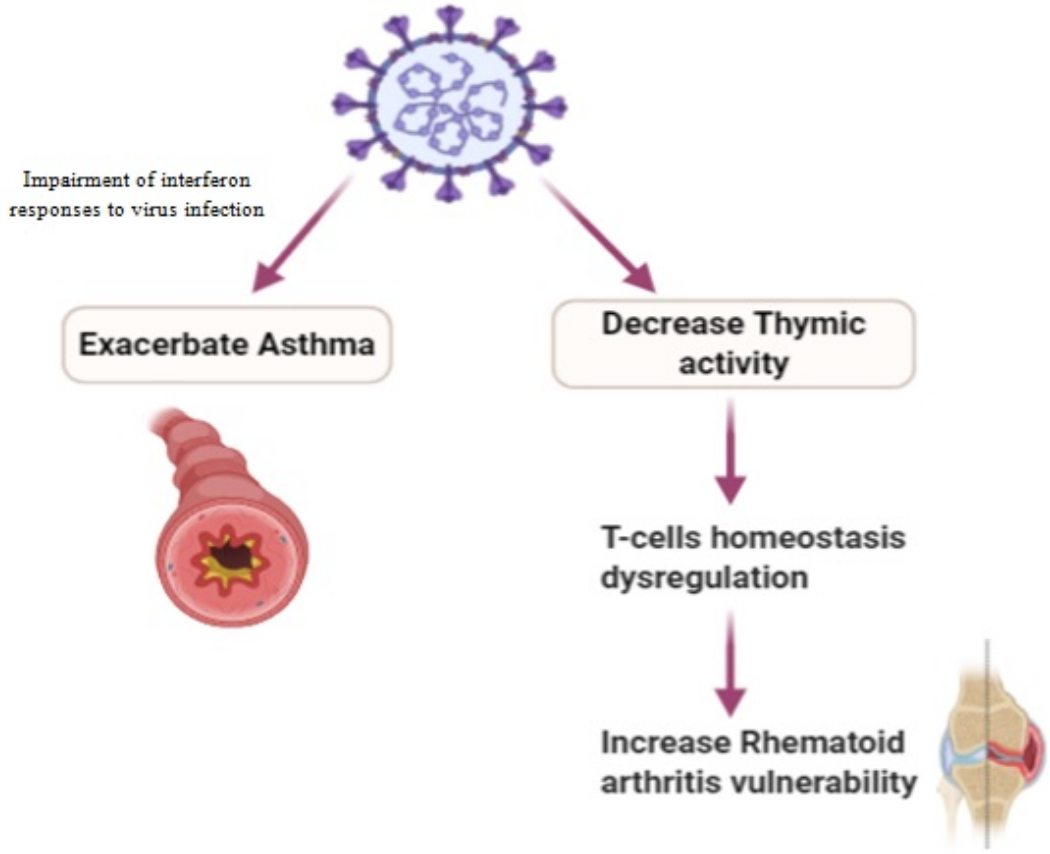


Fig. (6). Role of COVID-19 in asthma and rheumatoid arthritis.

The variations in patient temperature among groups were quite low and self-reported before hospital admission, so evidence on high fever should be viewed cautiously. Extremely pathogenic human coronavirus exact pathophysiology is still not completely known. Cytokine storms, as well as cellular immune responses, are believed to play a significant role in the seriousness of the disease (Fig. **7**). Neutrophilia has been recognized in patients suffering from both peripheral as well as lungs infected with SARS-CoV. In MERS patients, lung infections were linked with the substantial pulmonary accumulation of neutrophils and macrophages and higher levels of these cells in their peripheral blood. The primary causes of chemokines and cytokines storm are neutrophils that are responsible for the extreme acute respiratory syndrome that is a major cause of death (Cui *et al.*, 2003). In recent research, it has been found that patients with pneumonia COVID-19 who developed ARDS had slightly greater numbers of neutrophils than those without ARDS, which could contribute to the stimulation of neutrophils to carry out an immune response against the virus but also cause cytokine storm. This clarifies the favorable correlation between high fever and ARDS observed at the early stages of COVID-19. However, the findings of recent research revealed that older age is linked with a reduced immune capacity which's why they are more prone to ARDS and mortality. Older age correlated with mortality may also be related to less effective immune responses. From studies, it is also revealed that higher T-cell counts of CD3 and CD4 could protect patients against ARDS. CD8 numbers in those who were alive were slightly higher. These findings show the important functions of CD4 and CD8 T cells in the pneumonia of COVID-19. Previous studies have shown that SARS-CoV which has the same receptor as SARS-CoV-2, could invade immune cells, including T lymphocytes, monocytes, and macrophages. T-cell counts for CD3, CD4, and CD8 were reduced at the start of illness; this decline lasted until SARS-CoV pneumonia was restored. CD4 and CD8 numbers of T cells have reduced in the peripheral blood serum of patients with lethal SARS-CoV pneumonia. Studies have shown that T-cell reactions can prevent the over-activation of innate immunity. T-cells were recorded to help remove SARS-CoV, and a suboptimal T-cell response was observed in the mice with SARS-CoV to induce pathological changes (Li *et al.*, 2004, Wu *et al.*, 2020a). We presumed the need for persistent and gradual changes in lymphocyte response for successful immunity to SARS-CoV-2 infection.

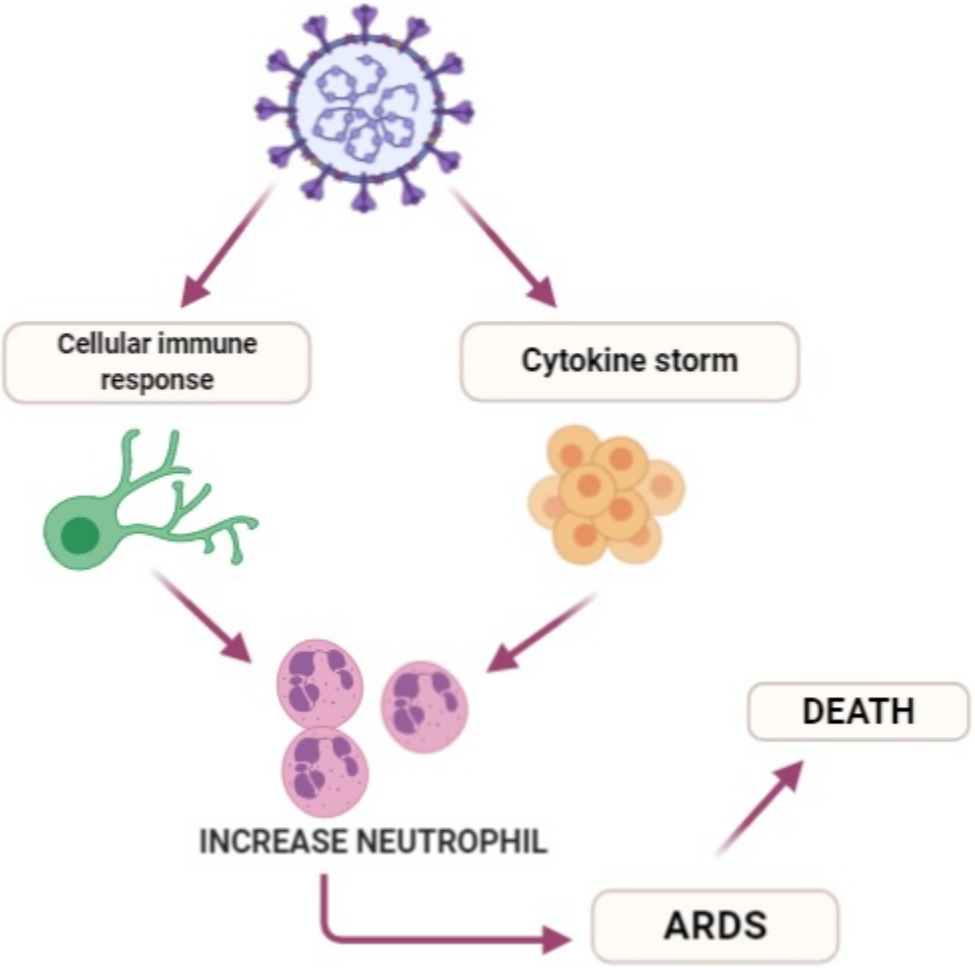


Fig. (7). Impact of COVID-19 in acute respiratory syndrome.

Apart from that Pulmonary fibrosis is also an important factor to consider as a challenging issue in the patients of COVID-19. It was reported in about 62% of the survivors. Its manifestation includes fibrous stripes, subpleural lines, and traction bronchiectasis. It may develop in the early stage in patients with SARS and diagnostic reports after recovery also suggest it as long-term complications (Antonio *et al.*, 2003) (Chan *et al.*, 2003). The reason behind the clinical state is deregulated release of metalloproteinases during the inflammatory phase of ARDS. It causes injury of epithelial as well as endothelial cells with unchecked fibroproliferation (Davey *et al.*, 2011, Zemans *et al.*, 2009).

The most fundamental aspect of all respiratory conditions is increased ACE2 expression after infection. 48h after all the pathogenesis of COVID-19 begins to

start along with its transcription. Inflammatory responses associated with the ACE2 expression indicated by the availability of RPS3, RPS8, and PRS9 kind of ribosomal proteins. Along with this VCP, LARP1, UBA52, PRKN, EIF3A, and EIF3L are also important for protein-protein interaction. Finally, SRC, CASP1, and RIPK were Hub proteins responsible for cytokine secretion. Hence, patients with such conditions are at higher risk of death (Li *et al.*, 2020a).

Rheumatoid Arthritis (RA)

Previous reports provided evidence that rheumatoid arthritis (RA) is associated with an increased incidence and complications of respiratory infections (Vinogradova *et al.*, 2009, Blumentals *et al.*, 2012). These findings may be attributed to the immunological impairment of circulating T cells in patients suffering from RA (Fig. **6**). Consequently, it stops patients from responding to the infections caused by an infectious agent. The decreased thymic activity and enhanced peripheral T-cell turnover, which may lead to peripheral T-cell homeostasis dysregulation, may also increase the RA vulnerability to infection (Koetz *et al.*, 2000). Such fundamental aspects of scientific knowledge suggested a complex relationship between RA and infection as external microorganism can develop the chronic state of RA either by:

a. Direct colonization by the pathogen at joints or
b. It may be due to an aberrant autoimmune response

Several scientific works of literature are also available indicated the severity of RA due to respiratory viral infections (Arleevskaya *et al.*, 2017, Mathew and Ravindran, 2014, Bogdanos *et al.*, 2013). Besides, RA patients are often diagnosed with drugs including steroids or DMARDs, which are immunosuppressive. Giving these immunosuppressive agents further enhances the prevalence of infections in RA patients (Bernatsky *et al.*, 2007). COVID-19 therapy in RA patients is scientifically problematic as COVID-19 infection could worsen by immunosuppressive agents. Temporary cessation of most DMARDs is advised for patients hospitalized with severe infections to facilitate RA patients to acquire defensive immunity and remove pathogens. Besides, stopping the administration of DMARDs may lead to an increased incidence of RA, which is normally controlled by increased doses of immunosuppressives (Ledingham *et al.*, 2017). The impact of steroids on COVID-19 infection is still uncertain. Steroids may exacerbate infections by decreasing the immune response, but systemic steroid treatment will prevent the inflammatory cascade of pneumonia (Ni *et al.*, 2019). Earlier studies have indicated that steroid therapy was linked to increased mortality in patients having a viral infection like influenza and pneumonia. In these instances, to decreases, the incidences of COVID-19 leflunomide and

steroids were stopped and hydroxychloroquine administration persisted owing to its potential antiviral activity, as well as its anti-rheumatic impact (Yao *et al.*, 2020, Song *et al.*, 2020).

CONCLUSION

This review provides an insight into the COVID-19 present situations and represents a picture of long-term complications associated with it. Future epidemiological studies and case records should elucidate the exact incidence of complications, pathogenesis with therapeutic options. This report of long-term complications of COVID-19 also provided an alarming signal to the patients of diabetes, central nervous system, respiratory disorders, hematological conditions, kidney disorder, and rheumatoid arthritis. These all diseased conditions are associated with an increased incidence of infections in case, if the symptoms persist to these patients they seek medical care in early stages only and follow the advice of healthcare professionals strictly.

ABBREVIATIONS

ACE2	Angiotensin-converting enzyme 2
ARDS	Acute respiratory distress syndrome
CBD	Cerebrovascular disease
CKD	Chronic kidney injury
CMV	Cytomegalovirus
COVID-19	Coronavirus epidemic 2019
CS	Coronary syndrome
CVD	Cardiovascular diseases
DIC	Disseminated intravascular coagulation
DM	Diabetes mellitus
FBG	Fibrinogen
GLP-1	Glucagon-like peptide 1
HSV	Herpes simplex virus
HTN	Hypertension
IL	Interleukin
MERS-CoV	Middle East Airborne Virus
PT	Prolonged prothrombin time
RA	Rheumatoid arthritis
SARS	Severe Acute Respiratory Syndrome
SIC	Sepsis-induced coagulopathy

TNF	Tumor necrosis factor
VZV	Varicella zoster

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 5

Vaccine Development

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Abstract: The current COVID-19 pandemic is a wake-up call pointing towards the vulnerability of humankind as it has outstretched its arms to almost all the continents, sparing few socially isolated ones. The highly contagious nature, ultra-stable genetic makeup, novel modifications in open reading frame (ORF) region, air-born route of transmission, and ability to cross the species barrier prove the potential of COVID-19 to elicit the global pandemic situation. In current times, when even known antibiotics for combating several diseases are being rendered inefficacious owing to the rising multidrug resistance among pathogenic strains, the panacea to a wide array of diseases can be vaccination. A prominent characteristic for COVID-19 vaccine development is that numerous technologies from lipid nanoparticle (LNP)-encapsulation, dendritic cells (DCs)-based vaccines, antigen-presenting cells (artificial-APCs)-based vaccines, and DNA plasmid-based platforms to viral vector approaches are being evaluated for the cause. Certain vaccine development technologies may be better suited for some parts of the global population, while others may prove to be more efficacious for the other population subtypes. This may not only be arising due to geographical or ethnic distinctions, but also physiological differences such as the presence of comorbidities, immune profile of subjects, *etc.* In this chapter, we attempt to bring forth the various approaches or molecular platforms that have been taken up or proposed for the development of a vaccine against coronavirus disease. We also attempt to elaborate on the pros and cons associated with each of the approaches that may be feasible due to the distinctions in the various population subtypes.

Keywords: Air-borne transmission, Animal model, APCs-based vaccines, Artificial-APCs, Comorbidities, COVID-19, DCs-based vaccines, DNA plasmid-based platforms, Genetic makeup multidrug resistance, Global population, Immune profile, Lipid nanoparticle (LNP), Molecular platforms, Novel modifications, Pathogenic strains, Physiological differences, Population subtypes, Vaccine development, Viral vector approaches.

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COVID-19: VACCINE DEVELOPMENT

Over the past decade, vaccine technology has undergone immense evolution, and different approaches for the development of vaccines against viruses and other pathogens have emerged. The advancement has enabled researchers to develop a wide range of vaccine candidates such as nucleic acid-based (DNA/RNA) vaccines, recombinant protein-based vaccines, and viral-vectored vaccines, *etc*. The rapid revelation of the genetic map of the SARS-CoV-2 viral genome has opened avenues facilitating several vaccine development programs against COVID-19 (Liu *et al.*, 2017). As of the current scenario, there is no antiviral drug available as a frontline treatment for the disease. To conquer the threat of COVID-19, urgent therapeutic intervention is the need of the hour, and therefore several strategies for vaccine development, besides drug development, have been initiated (Taghizadeh-Hesary and Akbari, 2020). As a result of these, there are currently 115 potential vaccine candidates across the globe, 78 of which are established as active projects; 5 among those candidates have already successfully crossed the pre-clinical stages (Grifoni *et al.*, 2020).

It is a remarkable feature of research & development for the COVID-19 vaccine that a wide assortment of platforms is being tried out. As mentioned earlier, platforms including nucleic acid-based vaccines, recombinant proteins, live attenuated virus, as well as inactivated virus-based approaches, alongside viral peptides, virus-like particles (VLPs), and replicating/non-replicating viral vectors are being explored for the development of SARS-CoV-2 vaccine. It is advantageous that several approaches have been taken up, especially concerning the fact that certain immunization technologies may be efficient for a specific group of population. This specific effectiveness relates not only in terms of age, ethnic, or epigenetic differences but also correlates to the physiological variances such as the presence of certain comorbidities (Grifoni *et al.*, 2020). The extensive study on the SARS-CoV-2 virus and its genome has revealed potential targets for vaccines and drug interventions. Bioinformatics and computational tools have a significant role to play in recognizing the most competent targets. A large number of databases have been created for easy and correct identification of potential vaccine targets (Taghizadeh-Hesary and Akbari, 2020). The characterization of the SARS-CoV-2 spike glycoprotein has enabled the researchers to identify precise immunogenic epitopes that can be taken into account for vaccine development. Their extensive analysis pointed out 13 and 3 epitopes for MHC-I & II respectively on the spike glycoprotein of the virus that can be incorporated in the formulation of a multi-epitopic peptide vaccine. Further, these candidate epitopes have been assessed for their effective immunogenicity through molecular docking with TLR-5; However, *in vitro* and *in vivo* validations are required to confirm the potential of this vaccine candidate. The fact that the SARS-CoV-2

virus is proximally related to MERS and SARS viruses which has allowed researchers to utilize structure-function comparisons to hasten the process of recognizing potential vaccine candidates (Taghizadeh-Hesary and Akbari, 2020).

Nevertheless, there are certain challenges associated with vaccine development. After the identification and *in silico* validation of a vaccine candidate, it is taken forward for a formulation, which itself is a long process. In the first phase, it passes through the rigorous tests for the efficacy and safety in animal models. It is safe to consider the fact that developing suitable animal models for SARS-CoV-2 may be a challenge in itself, as the virus is known to not propagate in wild-type mice; Further, this virus only induces a mild form of the disease in transgenic animals that are engineered to express human ACE2 (Liu *et al.*, 2017). However, even in the absence of animal models, it is not entirely impossible to assess the vaccine candidate. Serum obtained from the immunized animals can be further validated through *in vitro* testing *via* neutralization assays. The remaining part of this tier is to gauge the vaccine candidate for its safety in animals in a manner acquiescent to the GLP (Good Laboratory Practices) guidelines and usually lasts for a minimum period of 6 months. This is followed by the production of vaccine compliant to current good manufacturing practice (cGMP) that assures the quality and safety of the vaccine. The second tier that includes the clinical phase I (small trials to evaluate safety on humans), phase II (aimed at establishing dosages and formulations to substantiate efficacy in humans), and phase III trials (both efficacy and safety of the vaccine candidate should be validated in a larger population). However, in certain unexpected conditions, such as during the pandemic of COVID-19, the guidelines may be flexible, and the process for approval may be accelerated with necessary precautions taken.

COVID-19 VACCINE DEVELOPMENT: MODULATING THE HOST IMMUNE SYSTEM

To understand the mechanisms of immunization that can facilitate the modulation of the host immune system in response to SARS-CoV-2, it is substantial that we apprehend the natural manifestations that the host system gradually adapts to modulate the response to the viral infection. The virus can successfully invade the cells which express the ACE2 and TMPRSS2 (transmembrane protease serine 2) surface receptors. Once the cells get infected, the viral genome undergoes active replication followed by cascade steps and releases the copies of the packaged virus from the infected cell. It is followed by pyroptosis, a mechanism through which cells release the damage-associated molecular patterns (DAMPs). These DAMPs are recognized by surrounding cells such as epithelial cells, endothelial cells, and alveolar macrophages, which in turn trigger a pro-inflammatory condition in the milieu by inducing the generation of cytokines and chemokines,

primarily IL-6, macrophage inflammatory protein (MIP)1α, MIP1β, interferon gamma-induced protein-10 (IP-10), and monocytes chemoattractant protein-1 (MCP-1). This conglomeration of pro-inflammatory cytokines stimulates the important players of the host-immune system to drive monocytes, macrophages, and T cells towards the site of infection. This results in the augmentation of inflammatory conditions, hence establishing a pro-inflammatory feedback loop (Tay *et al.*, 2020).

Both the branches of the host immune system, the innate and the adaptive immunity, are involved in response towards SARS. Due to the genomic resemblance of SARS-CoV-2 to SARS, a similar immune response is expected in the case of SARS-COV-2 (Taghizadeh-Hesary and Akbari, 2020). The innate wing of the immune response against a viral infection profoundly depends upon the type I-IFN and the subsequent cascade which induces appropriate immune responses. Nevertheless, the direct targeting of macrophages and T cells in the case of SARS and SARS-CoV-2 is yet to be determined. The innate immune sentinels should essentially recognize the viral invasion usually facilitated by PAMPs, in the form of genomic RNA or virus replication dsRNA intermediates. These virus-associated PAMPs are recognized by any of the receptors such as TLR 3 (toll-like receptor 3), TLR 7, RIG-I (retinoic acid-inducible gene I) & MDA 5 (melanoma differentiation-associated protein 5), and endosomal RNA receptors/cytosolic RNA sensors (Prompetchara *et al.*, 2020). This elicits a cascade of downstream signaling events resulting in the suppression of viral replication and propagation at an initial stage. In addition to this, the cascade which involves signaling-mediated activation and translocation of NF-κB (nuclear factor- kappa-B) and IRF 3 (Interferon regulatory transcription factor 3) towards the nucleus, facilitates the expression of pro-inflammatory cytokines, especially type I-IFN, which further conjunct with IFNAR (interferon α/β receptor), initiates the JAK-STAT signaling pathway, and activates the STAT1/2 proteins which form a complex with interferon regulatory factor-IRF 9. The cascade of this complex-formation signals the transcription of IFN-stimulated genes in the nucleus that enables the innate immune system to mount an efficacious response against the virus (Prompetchara *et al.*, 2020).

Adaptive immunity is specific in its nature of the action and is mediated majorly by T and B cells (Tay *et al.*, 2020). Typically, the central role in mediating adaptive immunity against virus infections is played by the Th1 cells (Prompetchara *et al.*, 2020). Th1 (T helper type 1 cells) cells mainly secrete IFN-γ, IL-2, and TNF-β (tumor necrosis factor β) cytokines, which further engage macrophages, hence involving the CMI (cell-mediated immunity) as well as inducing the response mechanisms dependent on phagocytic activity (Romagnani, 1999). Th1 subset is generally produced as a measure to combat intracellular

bacterial and certain viral infections. Complementarily, another cell type, Th2 cells, are involved in the production of IL-4, IL-5, IL-10, and IL-13, which trigger a cognate-antibody production and stimulate the eosinophils, thus facilitating a phagocyte-independent immune response. It suggests that the Th2 subset is principally involved in generating immune responses against nematodes invading the gastrointestinal system (Romagnani, 1999). Alongside the Th cells that orchestrate the adaptive immunity, the cytotoxic T (Tc) cells majorly take part in identifying and eliminating the virus-infected host cells. Besides the role in mediating adaptive immunity, humoral mediated immunity (HMI) further comes into the picture at the later stage of the infection which is administered by B cells through the virus-specific antibody production. This results in the immediate neutralization of invading antigen and also generates the immunogenic memory which can eliminate the same antigen much rapidly during the relapse of infection.

In this regard, several studies based on structural proteome of various coronaviruses were conducted which also includes having the close resemblance to the SARS. The results of these studied have discovered several immunogenic epitopes which can further use as a potential vaccine candidates/components, given the feasibility of cross-reactivity with SARS-CoV-2 epitopes. As mentioned in the prior section, the approach of immunoinformatics and related computational tools can play a central role in facilitating the identification of such epitopes. For this purpose experts have utilized the database of IEDB (Immune epitope database and analysis resource), which is based upon the mathematical algorithm to fulfill the criteria in finding an appropriate epitopes for a given sequence. Another widely used repository in this regard is ViPR (virus pathogen resource) that offers data pertaining to viruses exhibiting human pathogenicity and encompasses genome/protein sequences, immunogenic epitopes and reveals the mechanism of host responses which might play a role for particular viral infections (Grifoni *et al.*, 2020). This study was the first attempted to define immunodominant regions within the SARS genome, wherein B cell epitopes derived from the genome were mapped back to a reference sequence using IEDB; following this, sequences of the SARS B cells epitope region were aligned against SARS-CoV-2 sequence to determine the similarities among the two regions. As a result, out of the 10 identified regions, six regions have showed an identity of more than 90% with SARS-CoV-2 sequences and another two have been matched with 80% to 89% identity (Grifoni *et al.*, 2020). Interestingly, their analysis depicted that the T cell epitopes hence recognized, show predominant association with the spike glycoprotein and the nucleoprotein. This study also sought to address identification of potential SARS-CoV-2 T cell-based epitopes *via* predictive algorithms that were used to map hundreds of human epitopes so as to account for HLA (human leukocyte antigen) polymorphism.

TYPES OF VACCINES AND THEIR CHARACTERISTICS

With the development and advances in the field of therapeutic interventions in last 200 years, vaccine development has emerged as one of the promising tools of medical technology to boost up the host immunity and/or to fight against a wide range of diseases (Rauch *et al.*, 2018). This section sheds light on the various major vaccine development approaches and their key features.

Live-attenuated Virus and Inactivated Whole Virus Vaccines

Live-attenuated vaccines are based upon direct mimicry of natural immune response that is elicited by the pathogens. This is the most primitive and the familiar approaches dating back to Edward Jenner's landmark work. These approaches, by and large, are centered on employing the disease-causative pathogen in an exterminated form or in a live, mitigated form (mitigation of the pathogenicity is achieved either through artificial means or through *in vitro* cell culture propagation). Such causative pathogen does not lead to clinical infection, but induces immunity at the same time (Loomis and Johnson, 2015). It has been reported that live-attenuated virus vaccines elicit strong HMI and CMI response by generating cognate B and T cells, respectively (Tse *et al.*, 2020). Overwhelming benefits of this approach that have been observed globally; for instance, MMR (mumps, measles and rubella) vaccine developed using this approach has helped in dampening the global load of mumps, measles and rubella (Loomis and Johnson, 2015). Nevertheless the design of this approach also has a few limitations that have urged researchers to venture into more advanced approaches. It is not necessarily feasible for all pathogens to be cultivated *in vitro;* it might not be possible to replicate certain conditions that support the culture of particular types of pathogen *in vitro.* Further, there is always a risk of inadequate practices that might lead to insufficient attenuation/inactivation, rendering the vaccine as lethal or detrimental as the pathogen itself. The possibility that an attenuated vaccine can elicit unwanted immune responses cannot be ruled out, indicating that these approaches might be extremely unsuitable for certain pathogens, especially those who demonstrate antigenic hypervariability; this might also hold true for pathogens that have a distinct intracellular phase in their pathophysiology (Rappuoli, 2004, Loomis and Johnson, 2015). In Beijing, Sinovac Biotech has developed two inactivated vaccines, one with alum as an adjuvant which is currently under Pre-clinical, Phase 1/2 stage trials. Similarly, Beijing Institute of Biological Products, China National Biotec Group, China has a candidate inactivate viral vaccine under phase 1/2 stage trial.

Subunit Vaccines

To tackle the diseases that cannot be challenged by the first generation vaccines,

other technologies need to be ventured into. Subunit vaccines, as the name suggests, utilize only a part of the pathogen that is generally a conserved region and easily accessible/legible (in contrast to whole pathogen in the aforementioned section) which can elicit an appropriate immune response (Loomis and Johnson, 2015). Subunit vaccines are constructed on the basis of synthetic peptides or recombinant proteins which are specific in targeting well-defined neutralizing epitopes with enhanced efficacy or immunogenicity. Several vaccines for MERS and SARS have been developed with this approach. For both, the S protein has been regarded as a major candidate; not only the full-length S protein, but also its antigenic fragments such as the N-terminal domain (NTD), the C-terminal domain (CTD), S1 and S2 subunits can assume the role of potential targets for the subunit vaccine (Wang *et al.*, 2020). Nevertheless, though full-length S protein-based subunit vaccine could invoke a strong immune response, there are reports about certain antibodies triggered by these vaccines elevated the viral infection in *in vitro*, raising a red flag in the use of this approach. Further, as subunit vaccine candidates, the NTD fragment and the S2 fragment generate prompt immune responses. However less immunogenicity and generation of lower antibody titers are the major concerns associated with these vaccine candidates. Contrarily, a subunit vaccine constructed using the receptor-binding domain (RBD) of the virus consists of only the main neutralizing domain, and also elicits an apt immune response; besides other counterparts like S1 fragment, RBD can be considered as critical target for the development of a subunit vaccine, especially when the criteria of safety and efficacy are taken into consideration (Wang *et al.*, 2020). Besides the S protein, other candidates have also been considered as potential subunit vaccine targets for coronavirus diseases. The N protein of SARS and MERS viruses is unable to generate neutralizing antibodies which can hinder the virus-receptor interactions or nullify the infection. Although, the N protein might be able to induce cellular immune response as well as specific antibody response; several immunodominant epitopes for B and T cells, that are conserved among mice, non-human primates and humans, have been found in the N-proteins of both these viruses (Veit *et al.*, 2018, Wang *et al.*, 2020). Considering this, N-proteins might be used as an additional target, if not as the solitary target in a subunit vaccine.

There are several factors that affect the overall quality and the expression of the proteins that are used in the formation of subunit vaccines, especially in the case of the recombinant protein-based ones. A study carried out by Chang *et al.*, reported that incorporation of an intron splicing enhancer to S protein fragments resulted into the higher protein expression in mammalian cells with compare to the same using exon splicing enhancers in SARS subunit vaccine candidate (Chang *et al.*, 2006, Wang *et al.*, 2020). Besides molecular factors, other factors that affect the immunogenicity and efficacy of subunit vaccines might also come

into play. For instance, the route of administration of the vaccine might affect the immunogenicity differently. Administration of adjuvants to stimulate higher Ig (immunoglobulin) titers and IFN-γ secretion is also a suitable approach to enhance the efficacy of subunit vaccines (Wang *et al.*, 2020).

Few groups of scientists designed subunit vaccine which failed since they either only used single protein or only CTL (cytotoxic T-lymphocytes) epitopes for vaccine design or neglected the importance of B-cell or helper T (Th) cell epitopes. Kalita *et al.,* (2020) constructed a subunit vaccine comprising of adjuvant, cytotoxic T cells (Tc), Th cells, and B cell epitopes connected with specific linkers. They retrieved the amino acid sequences of three SARS-CoV-2 proteins like nucleocapsid protein (N), membrane glycoprotein (M), and surface spike glycoprotein (S) from NCBI database, to ensure an optimal immune response since they are known to have an eminent role in host receptor recognition, pathogenicity and entry of the virus. 6 Th cells, 18 Tc cells, and 9 B-cell epitopes obtained from three proteins and human β-defensin as linker were used to construct the vaccine. Evaluation of the binding energy of vaccine with its TLR3 (Toll-like receptor) receptor was carried out with vaccine-receptor docking. The complex with lowest energy and −1491 kJ/mol binding energy was selected for MD simulation. Furthermore, the epitopes were checked for their potential toxicity. The subunit vaccine turned out thermostable, antigenic, and non-allergenic. Optimization of production process will lead to rapid testing and commercialization. Computational studies demonstrated good safety and protective efficacy of this multi-epitope vaccine against SARS-CoV-2 (Kalita *et al.*, 2020).

Vaccines Based on Virus-like Particles (VLPs)

The structural proteins of several viruses are capacitated such that they can direct the assembly of repetitive clusters or VLPs falling in the range of 22 to 150 nm (Grgacic and Anderson, 2006). Strikingly, these VLPs closely resemble to the viruses but are devoid of the ability to cause an infection as they lack the viral genetic material. These are attributed with greatly repetitive and high-density display of epitopes, which enables the vaccine design to prompt resilient host immune response. These virus-resembling particles induce not only the humoral arm, but also the cellular arm of the adaptive immunity. Moreover, the size range in which VLPs fall is optimal, as is the case with liposomes, for uptake by APCs such as dendritic cells (DCs) (Grgacic and Anderson, 2006). Because of this unique property, they do not require any additional adjuvants to stimulate the immune response. Their self-adjuvant attribute facilitates their uptake by DCs followed by the cascades of processing and MHC-II presentation. Further, exogenously designed and delivered VLPs can also be processed and presented

via MHC-I pathway, leading to cross-presentation based activation of $CD8^+$ T cells (Tc cells). These properties give VLPs and edge over the more superseded approaches in the prospect of vaccine development. In fact, successful VLP vaccines against viral diseases like hepatitis B and human papilloma virus (HPV) have already been devised and licensed for human use (Grgacic and Anderson, 2006). However, viruses those pose a greater challenge to the immune system have certainly put this technology to test. Adaptations in their construct, in terms of factors like particle size and route of administration can be made in order to obtain optimal immune responses using this approach (Grgacic and Anderson, 2006). Assembling chimeric VLPs is a good tactic for incorporating those antigens in the construct, which cannot self-replicate in a particulate system; VLPs can be generated in various expression systems inclusive of mammalian, yeast and specific virus expression systems (Masavuli *et al.*, 2017). Utilizing adenoviral and lentivial vectors following BSL-2 practices, the pseudo-viral system was formed and used as experimental models to study the entry of enveloped viruses, including SARS-CoV-2 (Nie *et al.*, 2020, Ou *et al.*, 2020). A study on VLP-based vaccine construct reported that a chimeric construct incorporating SARS S protein plus MHV-CoV (mouse hepatitis coronavirus) E, M and N proteins stimulated higher production of antibodies specific to SARS (Zhang *et al.*, 2014, Lokugamage *et al.*, 2008). It is also regarded that vaccines designed with this approach tend to have abridged immunogenicity as compared to live attenuated vaccines or inactivated whole virus vaccines. To overcome this shortcoming, a handful of strategies can be utilized; for instance, molecules instigating APCs can be integrated to the VLP construct *via* genetic engineering protocols (Zhang *et al.*, 2014).

As an attainable relevant and safe substitute of naturally occurring viruses, there is a high demand of construction of SARS-CoV-2 VLPs for current fight against COVID-19. In a study carried out by Xu *et al.,* 2020, SARS-CoV-2 VLPs are efficiently constructed using mammalian expression system that helps the maintenance of protein glycosylation patterns. It revealed that out of four SARS-CoV-2 structural proteins, small envelope protein (E) and membrane protein (M) expression is critical for formation and release of SARS-CoV-2 VLPs (Xu *et al.*, 2020). M protein is found in abundance responsible for packaging of other structural proteins, just like in other members of *coronaviridae* family. E protein has also been found helpful for viral morphogenesis. The evaluation of SARS-CoV-2 VLPs morphology in Vero E6 cells showed increased incorporation of S glycoproteins on the surface. Thus the approach adopted by Xu *et al.,* 2020 confirmed the molecular and morphological resemblance between native virion particles and SARS-CoV-2 VLPs.

Viral Vector-based Vaccines

The viral vector-based vaccine is very distinct from the subunit or inactivated whole virus vaccines, the latter working as exogenous immunogens. On the contrary, a viral vector-construct utilizes the DNA carried by it that encodes immunogenic components in the host cell followed by their encoding for the antigenic peptide/protein, after which the antigen is expressed intracellularly. As a result it instigates the broad-spectrum of immunity that comprises of both, the HMI and CMI of the immune system. It is a lucrative approach because of the ease of development and the direct production of virus stock (Loomis and Johnson, 2015). In general, these vaccines are regarded as live vaccines, but due to their replication being diminished, they can be considered safe and used even for immunocompromised individuals (de Vries and Rimmelzwaan, 2016). The virus from which it is derived lays the platform for specific properties possessed by the vector and hence different viral vector-based vaccines have unique and distinctive set of advantages and limitations. However a major issue associated in general with this approach is the utmost requirement of assessment of safety and efficiency that covers several parameters of genotoxicity, replication insufficiency or the capacity of the vector to elude-pre-established immunity in the host (Ura *et al.*, 2014). Nevertheless, this approach is among the most popular ones in developing MERS vaccine; in fact two in three candidates for MERS vaccine making it to clinical trials are viral vector-based vaccines (Yong *et al.*, 2019). Among several viruses that are used to develop vaccines with this approach, vaccinia virus and MVA (modified vaccinia virus Ankara) are among the most extensively exploited ones. Particularly, these have been indicated in the development of influenza vaccines; though vaccinia virus vectors have exhibited great immunogenicity in animal models, frequent reports of substantial reactogenicity have also emerged. This makes it necessary to address the issue of reactogenicity by developing modified/engineered forms of this vaccinia virus vectors (de Vries and Rimmelzwaan, 2016). MVA is an attenuated form of the vaccinia virus that was developed by serial passaging of chorioallantoisvaccinia virus ankara in chicken embryo fibroblasts. This resulted into the deletions and alterations in the viral genome, helped in developing a viral vector that exhibited certain advantages over the original viral vector. It accounted for easy insertion of antigens in the vector genome, transient expression of heterologous antigens in *in-vivo* settings and subsequent stimulation of HIM and CMI in animal models and humans. To augment for favorable features offered by MVA viral vector, it also exhibited incapacity to elude the host immunity, and its safety was confirmed in immunodeficient mammals (de Vries and Rimmelzwaan, 2016). A study has also reported that MAIT cells (mucosal immunity-associated invariant T cells) might be playing a key role in the immunogenicity rendered by adenovirus vectors that are replication-deficient in nature. The activation of MAIT cells by

this virus vector depicts requirement of transduction of monocytes for the production of IL-8, and pDCs (plasmacytoid dendritic cells) resulting in the generation and secretion of IFN-α (Provine *et al.*, 2019).

ChAdOx1 nCoV-19 is an adenovirus-vectored vaccine that encodes the spike protein of SARS-CoV-2. This candidate vaccine elicited a robust cell-mediated and humoral response and was found immunogenic in mice. The immunization with ChAdOx1 nCoV-19 (prime-only and prime-boost regimen) showed a balanced Th1/Th2 humoral and cellular response in rhesus macaques. Significant reduction in viral load of alveolar lavage was observed. Although, there was no difference in nasal shedding of vaccinated and control animals (Van Doremalen *et al.*, 2020).

DNA Vaccines

The approach of DNA vaccines gained popularity in the 1990s when the reports of intramuscular or intradermal delivery of plasmid DNA can induce antibody responses against viral/non-viral antigens. These vaccines are constructed using DNA coding sequences (that encode the candidate antigen protein) inserted in a suitable plasmid consist of a eukaryotic regulatory promoter. Other than the promoter, the plasmid should essentially possess a convenient cloning site for incorporating the gene of interest, a polyadenylation termination sequence, a prokaryotic origin of replication, and a selectable marker. The plus side of this approach is that DNA vaccines can be administered through various routes and a wide array of delivery mechanisms can be used; moreover, these can induce all-encompassing immune response inclusive of $CD8^+$ and $CD4^+$ T cells, and antibody production (Nichols and W. LeDuc, 2009). Additionally, the unmethylated DNA motifs in the vaccine can potentially prompt TLR-9 mediated innate immune responses (Kwissa *et al.*, 2007).

The conventional way of DNA vaccine delivery is mediated through intramuscular, intradermal or subcutaneous injection, majorly addressing the myocytes and keratinocytes, and the APCs in the surrounding milieu (Hobernik and Bros, 2018). Prior to internalization of DNA vaccine into the cell, it is translocated into the nucleus for transcription and subsequent translation into the cytoplasm. Henceforth, in the case of intradermal/subcutaneous route of administration, keratinocytes generate the specific antigen which is released by exosomes and internalized by APCs; the APCs present the antigen to $CD4^+$ T cells through MHC-II presentation. This leads to priming of B cells that mount an antibody response against the antigen. $CD4^+$ T cells also mediate complete activation of $CD8^+$ T cells (Hobernik and Bros, 2018, den Haan *et al.*, 2014). In the case of intramuscular delivery, the myocytes in which transfection has

occurred successfully, undergo apoptosis and the apoptotic bodies are engulfed by APCs. The APCs cross-present the antigen (in the exogenous form) to $CD8^+$ T cells *via* MHC-I pathway (den Haan *et al.*, 2014, Lazzaro *et al.*, 2015).

Despite the positive outcomes of this approach, it still holds a question for its effectiveness, majorly because the success these exhibit at pre-clinical stages does not translate to the clinical stage as of yet. The major hindrance is the generation of mitigated immunogenicity in bigger animals and humans. This, in most probability, is due to the fact that upscale of the DNA amount used in smaller animals, for human system is a difficult process. To overcome this, certain measures can be taken up. These include optimization protocols such as the use of a hybrid promotor or of one exhibiting APC-specific activity; incorporation of immunostimulatory sequences, A/T rich sequences, nuclear localization sequences and so on. Other optimization protocols include adopting antigen specific modifications such as codon optimization, or combination of different Ags. Another approach that can be considered to address this challenge is to co-administer adjuvants such as the immunostimulatory CpG oligonucleotides (Hobernik and Bros, 2018).

Other means of DNA vaccine delivery such as gene gun-mediated delivery (Chen *et al.*, 1999), electroporation (Lee *et al.*, 2015) and nanoparticles mediated delivery, have been also assessed by several studies. Among these approaches, nanoparticle-based delivery holds great potential in enhancing immune response generated by DNA vaccines. The optimal size of the nanoparticles (<200 nm) facilitates their uptake by the host cells. Encapsulation of the DNA vaccine candidate in nanoparticles also prevents degradation of the nucleic acid which resulted in stabilizing the respective formulation (Shah *et al.*, 2014). Elastic liposomes entrapping a DNA vaccine candidate can easily undergo extravasation owing to their ultradeformability and enable us to overcome the challenges faced by conventional methods of transdermal immunization (Tyagi *et al.*, 2015).

Despite the promising approach that a DNA-based vaccine is, it cannot be neglected that its administration is not immunogenically inert. Genotoxicity arising from the integration of the DNA vaccine candidate into the genome, causing unsolicited mutations and subsequent adverse effects has always been in question. Another concern is that the administration of such formulations can generate the possible risk of producing anti-DNA antibodies by the host itself, a classic hallmark of several autoimmune disorders. In this regard, several studies are under pipeline over the years to assess the concerns of autoimmunity associated with DNA vaccines and a few studies have shown that DNA vaccines neither induce, nor worsen autoimmunity. However, their suitability and efficacy needs to be checked before taking them to the next stage.

NOVEL APPROACHES: USAGE OF MULTIDISCIPLINARY FIELDS

SARS-CoV-2 has turned out to be challenging which needs efforts of several disciplines to rejoin at the biggest platform to eliminate or to overcome from the dire condition. The pathophysiology of this infection has kept physicians and scientists under bleak condition where they have to search for every possible option to fight against the SARS-CoV-2. Therefore, several new options are explored and some of them are discussed below:

Serum Albumin Strategy

In addition to the existing approaches, adoption of a strategy which can target intracellular and extracellular viral particles is one of the prime priorities for the research. The drugs that can block the entry of viral particles or curb the intracellular viral component hold enormous potential (Kruse, 2020). Serum albumin is a multifunctional protein that interacts with a wide range of endogenous and exogenous compounds. Hence it can act as an efficient vehicle for wide range of targets such as viral protease, polymerase and RNA. Several clinical and experimental data suggests the correlation between the levels of albumin and inflammation where larger amount of albumin is taken by the cells, which are under the stress and inflamed condition (Don and Kaysen, 2004). Similarly, recent studies support the idea of albumin therapy in COVID-19 patients as there has been established a relationship between albumin and ACE-2 receptors which are responsible for virus entry in to human cells. Considering all above facts, a combinational therapy will be most beneficial where one component blocks the entry of virus and other targets internalized viral components. Furthermore, usage of serum albumin will aid the efficient internalization of drug and enhance pharmacological effects. A group of researchers proved that the use of epigallocatechingallate (EGCG) and curcumin is successful in neutralizing the range of viruses like hepatitis B, porcine circovirus type 2, influenza virus, zika and chikungunya (Chen *et al.*, 2010, Lai *et al.*, 2018, Li *et al.*, 2020). It was reported that EGCG and curcumin helps by inhibiting viral replication of SARS CoV-2 (Yang *et al.*, 2017, Siu *et al.*, 2019). If such drugs are internalized with the help of albumin in combination with molecules that inhibit the entry of virus in human cells, it will really help in improvement of patients' health.

Natural Product (Plant) Based Technology

Plant based vaccines have demonstrated great potential which even resulted in a few candidates being tested in clinical trials against swine flu, influenza, rabies, and hepatitis B (Rosales-Mendoza *et al.*, 2012). Hence plant based vaccines holds potential but the development process is very slow as of the novel approach in the

field of therapeutic vaccines. History is suggestive of the fact that this technology can prove helpful in our quest for fighting COVID-19 pandemic. These vaccines are considered comparatively safe and their immunogenic characteristics were revealed through various *in vitro* assays which demonstrated positive outcome with no adverse effects. Plant based vaccine can serve a greater purpose of delivering cost effective alternative since they don't require any purification for antigen isolation. This technology can be used to trigger gut associated lymphoid tissue (GALT) with the help of plants that do not produce toxic metabolites (for example rice or corn transgenic lines or transplastomic lines) (Rosales-Mendoza and Salazar-Gonzalez, 2014). Additionally, it can overcome the usage of injection and risks associated with their invasive routes of administration. Few shortcomings like poor antigen stability and bioavailability pose concerns in development of plant based technology against COVID-19.

At present, accessible foreign protein expression methodologies in plants is dependent on nature of selected antigen. The use of VLPs is one of the most distinguished ways to develop plant based vaccine which can avoid shortcomings of attenuated or live vaccines however it attains the enough immunity to fight the infections. It has been observed that mice models injected with SARS CoV-2 VLPs subcutaneously show elevated titers of antibodies against SARS. Furthermore, cellular immunity was shown to be elicited with higher levels of cytokines IFN-γ and IL-4 (Lu *et al.*, 2007).

Another prominent approach is the development of multi-epitope based vaccines which will help us obtain rational vaccine design that can elicit robust immune response involving Th, B, and T cell epitopes. One noteworthy advantage of this approach is it can provide protection against the genetic variability (of virus) observed in SARS CoV-2. Given that, multi-epitope vaccine is successful translated in a plant system and the production of these vaccines will be highly feasible (Govea-Alonso *et al.*, 2013a). One of the several approach, is production of immune complexes (IC) which is comprise of antigens that are recognized by antibodies to form a complex and exposed to macromolecular entities which ultimately leads to antigen processing and presentation by APCs. This induces effective HMI and CMI based response. Plant cell machinery can be maneuvered as IC producing factories. Nonetheless, the major shortcoming faced here is requirement of defined antibodies targeting antigens which is not available for SARS CoV-2 yet.

Purification of antigens for injectable vaccines is a cumbersome and expensive process which can be overcome with the help of elastin-like polypeptide (ELP) fusions. ELPs possess a unique characteristic which precipitates the protein of interest by simply altering the temperature. For example, tobacco was used to

express *M. tuberculosis* antigens Ag85B and ESAT-6 by fusing it with ELPs (Govea-Alonso *et al.*, 2013b, Phan *et al.*, 2013). Perhaps, the main challenge as faced by other candidate vaccines would be assessing their efficacy at larger stage of clinical trials and fulfilling all regulatory demands to assure safety. Although the motivating fact is a plant-made biopharmaceutical against influenza is under clinical trials with promising safety and efficacy.

Nanotechnology and Material Science

Spread of virus can be significantly attributed to air and surface contamination therefore recently the focus towards the development of anti-viral surfaces has opened a new area. It has been hypothesized that developing efficient air filtering system would highly contribute to dampen the viral load in environment and tackle the spreading of infections in health care workers due to the defective personal protective equipment (PPE) kits (Elias and Bar-Yam, 2020). This purpose can be served by using the approach of nanotechnology to design contamination-safe equipment. Antimicrobial properties of nanostructures copper, silver, and zinc species can be exploited. Release of copper ions and reactive oxygen species (ROS) leads to inactivation of SARS CoV-2 virus on copper and copper alloy surfaces (Van Doremalen *et al.*, 2020). Development of PPE kits can be accomplished using copper salt nanoparticles and/or solutions (chloride, iodide, sulfide, *etc.*) that render the virus ineffective. Sustained and controlled release can be obtained from metal nanoparticles acting as ion reservoirs. Noteworthy antimicrobial property has been observed on the metal-grafted graphene oxide (GO) used for non-woven tissues which is investigated for the treatment of PPE (Orłowski *et al.*, 2018). The anti-viral properties of nanoparticles are not fully explored, although silver nanoparticles (AgNPs) are well recognized for their anti-viral properties. It was reported that AgNPs interact with host receptors and blocks the entry of HIV-I (Kerry *et al.*, 2019). It has been established that metal NPs (<10 nm) can pierce through the cell membranes and curb viral replication. A nanotechnology-based solution containing titanium dioxide and silver ions was reportedly used to disinfect streets in Milan, Italy (Sportelli *et al.*, 2020). Nanomaterials based antiviral, packaging solutions, antiviral coatings, non-woven disposable products, synergistic/multifunctional surfaces, air-conditioning filters demand our prompt attention. Nanomaterials community has been encouraged to use their profound knowledge of Nanoscience that can be used to fight this pandemic (Sportelli *et al.*, 2020).

mRNA Based Approaches

One of the frontrunners in COVID-19 vaccine development program is mRNA

based vaccines accounting to 18 potential candidate vaccines. Conventional vaccines developed with live or attenuated viruses or its antigens, require lengthy period for production and has limited manufacturing capacity. Meanwhile, mRNA based vaccines are a novel approach where antigen coding mRNA is introduced in an individual to trigger immune response as it gets expressed. This results in better immunogenicity, safety and efficacy as compared to traditional vaccines. mRNA vaccine candidate mRNA-1273 which encodes spike protein (S protein) of SARS-CoV-2, developed by Moderna is about to enter a Phase II study in Q2 2020.

There are two types of RNA vaccines exist; one is non-replicating mRNA vaccine and another is self-amplifying or replicon RNA vaccine (Iavarone *et al.*, 2017). The first step for exogenous mRNA to reach cytoplasm is to cross the lipid membrane barrier prior to getting translated in to protein (Midoux and Pichon, 2015). There are several factors that affect the mRNA delivery and organ distribution. Moreover, cellular selectivity is also observed. Irrespective of all the hurdles, mRNA vaccine is still holds enormous potential since it can be scaled up swiftly and does not pose any danger to the recipient. The hypothetical advantages of mRNA vaccines seem strong, although the factors affecting its stability and delivery cause concerns. The outcome of the phase I trial of the mRNA-1273 vaccine is still awaited.

ANIMAL MODELS TO TEST THE IMMUNOGENIC POTENTIAL OF CANDIDATE VACCINE ANTIGENS

Animal models are crucial in all facets of vaccine research and development. They are considered significant for predicting vaccine effectiveness and factors affecting their role as such include the robustness of the animal challenge model, the pathogen in question, and other known correlates of protection (Golding *et al.*, 2018). There are proof-of-concept (POC) studies that are carried out in small animal species, permitting the use of a large number of animals as well as multiple iterations of vaccine products. POC studies thus enable the identification of the optimal vaccine candidate, as well as the primary recognition of immunological end points (Golding *et al.*, 2018). Animal models can be developed to meet two resolutions: characterization of viral pathogenesis, and assessment of vaccines/anti-viral agents. Moreover, they play a significant role in comprehending the aforementioned in those infectious diseases for which carrying out clinical studies is not feasible. Animal models susceptible towards viral infection and exhibiting similarity with the clinical course are considered ideal. The straight-forwardness, ease of handling availability, generating statistically amenable data, and cost effectiveness are advantages with small laboratory animal models over larger ones (Sutton and Subbarao, 2015).

Animal Models Currently in Practice

Small animals such as mice are extensively used as a first-line screening of vaccine or a therapeutic candidate. Murine model is advantageous due to low genetic variability in inbred strains, and availability of a range of molecular biology and immunological reagents compatible for studies. The cost & effectiveness of murine models are also an added benefit for their use in R&D studies. However, one of the major shortcomings associated with these murine models lies in the fact that the viral pathogenesis observed in these animals, and the immunogenicity rendered by a vaccine in these models may not be extrapolated to humans in all cases. Another crucial drawback in using mice models is the limited blood volume sampling, and the need of several passages of viral load in the animal for an expedient infection (Ruiz *et al.*, 2017).

Several mouse models have been assessed for the study of infection by coronaviruses including SARS and MERS (Sutton and Subbarao, 2015). Balb/c murine models have been widely used for this purpose. A study reported that inoculation of Balb/c murine models with SARS, through the intranasal and oral routes simultaneously, resulted in replication of the virus in not only lung tissue, but also in the intestinal tissue (Wentworth *et al.*, 2004). The robust mouse models reiterating the pathogenesis of SARS-CoV-2 in humans have yet to be developed owing to incompatibility between the SARS-CoV-2 spike protein and the ACE2 receptor in mice (Wan *et al.*, 2020). However, transgenic mouse model (humanACE2-hACE2) has been developed for the study of SARS-CoV-2 pathogenicity. A study reported that transgenic hACE2 mice that were infected with the virus exhibited clinical infection symptoms like weight loss, besides viral replication in the lung tissue. Further, histopathological studies depicted interstitial pneumonia alongside classic immunological markers like infiltration of alveolar interstitium lymphocytes and monocytes, amassing of macrophages in alveolar cavities; viral antigens were also found in bronchial and alveolar epithilia. Moreover, these hallmarks were not exhibited in wild type mice with SARS-CoV-2 infection (Bao *et al.*, 2020). In another successful attempt ACE2 knockout mouse model (Imai *et al.*, 2005) and TMPRSS2 knockout (KO) mouse model (Iwata-Yoshikawa *et al.*, 2019) have been engineered, and these models were instrumental in unraveling the role of spike protein in SARS viral infection and development of pulmonary symptoms. Thus KO mice models may be utilized for evaluating the efficacy of proteins as vaccine/therapeutic targets. The knockout approach has also been extrapolated for various immunological proteins to facilitate various SARS studies. For instance, Stat1 KO models have been found to support SARS replication and allow progression of the disease in lungs; such a model was employed by a study to determine the effectiveness of a vaccine

candidate which reports that the live, attenuated RNA virus vaccine provided protection to aged, immunocompromised mouse models (Graham *et al.*, 2012).

Small animals other than mice have also been commonly used as animal models for preclinical studies. The respiratory tract of ferrets is regarded as akin to that of humans, both, anatomically and histologically. Their larger size (when compared with mice) facilitates more frequent and larger blood samples. While these points pose ferrets as favorable animal models, there are drawbacks associated with their usage. They are outbred that might lead to inconsistent responses for a given parameter and to overcome this drawback greater numbers of animals should be incorporated in the study to obtain statistical significance. Additionally, transgenic ferrets are not accessible and molecular biology reagents compatible for their study are inadequate; admonitions arising with requirement of greater space and the tendency to exhibit aggressive behavior when procedures are repeated and other limitations associated with handling ferrets. Nevertheless, a ferret model of SARS-CoV-2 infection has been reported. Ferret models infected with the virus showed clinical signs of the disease such as elevated body temperature, alongside viral replication. Moreover, the study reported the infected ferret shed SARS-CoV-2 in nasal washes, saliva, and urine as well as in excreta for up-to 8 day post-infection. The study utilized the ferret model to address the transmission aspect of the disease; transmission in all naïve direct-contact ferrets at two days post-contract and in a few naïve indirect contact ferrets was reported. This suggests considerable recapitulation of the human disease in the model which advocated their use in R&D of SARS-CoV-2 therapeutics and vaccines (Kim *et al.*, 2020). Golden Syrian hamsters have also been recounted as highly permissive to SARS infection. A productive infection in these animals has been widely reported with the highest viral replication on 2-3 days post-infection in the nasal turbinates and the lungs. The pulmonary histology exhibited inflamed interstitial foci but the animals did not demonstrate explicit clinical disease or mortality (Sutton and Subbarao, 2015).

The next in line as animal models are non-human primates. These animals are genetically the most proximal species to humans; hence progression of disease and responses of the host immunity to the pathogen are closely in resemblance to those in humans. Though, ethical concerns and norms related to experimentation on non-human primates are the most stringent, dictating the study to be designed with utmost care and in a meticulous manner such that the smallest number of these animals is incorporated in the study (Ruiz *et al.*, 2017). Six non-human primates (african green monkeys, rhesus macaques, cynomolgus macaques, squirrel monkeys, the common marmoset, and mustached tamarins) have been assessed as models for SARS infection. All models but squirrel monkeys and mustached tamarins, were found to support SARS replication. Further, all species

exhibited substantiations of interstitial pneumonia, though none among those were found to consistently reproduce clinical disease and none of the species exhibited mortality (Sutton and Subbarao, 2015). Studies using cynomolgus macaques have also been carried out to address comparisons of the three coronaviruses- SARS, MERS and SARS-CoV-2. The study reported absence of severe symptoms (that are observed in humans) in the models, but that the lung pathology that was observed in the models was similar to that in humans (Rockx *et al.*, 2020).

Humanized Mice Models

Small animal models have been the most effectual method for studying human afflictions, especially for that of certain human retroviruses. Currently available simian models are undoubtedly robust, but high expense and difficulty in maintaining those models is a major setback (Van Duyne *et al.*, 2009). There is a rising stipulation for effective models to facilitate experimental studies on human immune system and human hematopoietic system without putting individuals in danger. To overcome this challenge major breakthroughs have been made over the past few decades, beginning with the discovery of CB17-*Prkdc* (protein kinase, DNA-activated catalytic subunit) severe combined immunodeficiency (SCID) mice in 1983. These mice were successfully engrafted with human fetal tissue (regarded as SCID-Hu model) and peripheral blood mononuclear cells (Hu-PB--SCID model) for the first time in the year 1988. Subsequent breakthroughs in this field came with the development of NOD (Non-Obese Diabetic)/SCID mice and engrafting those with human hematolymphoid tissue in 1995. The NOD/SCID model has enhanced grafting capacity than the initial SCID-Hu mouse model. The next upgrade was brought about by developing an immunodeficient model *via* targeted mutation in IL-2 receptor common gamma chain in the early 2000s. This IL2rγ ^{null} mouse model reported significant engraftment increase and enhanced human hematolymphoid function even in comparison with NOD/SCID model (Pearson *et al.*, 2008).

For the purpose of vaccine development studies, the IL2rγ knockout model on NOD/SCID or Balb/c Rag2$^{-/-}$ background can be a remarkably useful platform. Findings have pointed out adequate purport for the suitability of engrafting human PBMCs (peripheral blood mononuclear cells), CBMCs (cord blood mononuclear cells) or CD34^{+} cells along with target tissue (respective to the vaccine candidate) in the aforesaid model construct, so as to obtain a unique human-like entity to test for the efficacy of vaccines. Despite being the most permissive host for human tissue engraftment, the SCID-IL2Rγ$^{-/-}$ knockout model holds substantial scope for improvisation in order to further promote its application in research and development. Transgenic mice can be engineered to carry human HLA I and II. This can confer the model with the capability of human-like MHC presentation in

their tissues, and can prove an indispensible asset for evaluating the effects of various therapies/formulations with refined and clinically relevant human resemblance (Koo *et al.*, 2009).

A study reported that K18 mice engineered for transgenic expression of hACE2 in the respiratory and other epithelial tissue developed a lethal infection post intranasal inoculation of SARS strain infecting humans. Their findings show macrophage and lymphocyte infiltration lung tissue post infection, as well as upregulation of proinflammatory cytokines in the lungs and brain, suggesting the usefulness of this model in pathophysiology studies and for antiviral R&D (Soo *et al.*, 2009). Recently, a Russian study group has reported a proposal to create a humanized mouse model for the application in SARS-CoV-2 research. They have designed the model to comprise *hACE2* and *hTMPRSS2* by introducing the genes in the mouse genome *via* CRISPR/cas9 technology. This will enable the model to be successfully infected by SARS-CoV-2 virus, as well as render the pathophysiology in close resemblance to that in humans (Soldatov *et al.*, 2020). In a major advancement, a recent study has reported a humanized lung-only mouse model. Bone marrow liver thymic (BLT) mice were implanted with human lung tissue, comprising of up-to 40 distinct cell types. The model has been demonstrated to facilitate replication of clinically relevant human viruses, and exhibits robust antigen-specific HMI & CMI responses (Wahl *et al.*, 2019).

With the robust advancement in the development of humanized models, several hurdles in the fight against the SARS-CoV-2 can be overcome and direct the research towards an efficient and safe vaccine/anti-viral intervention.

Novel Approach of Using Zebrafish (Their Advantages)

Animal models mimicking various human diseases have proven revolutionary in identifying and then screening of leading drug candidates for developing specific therapeutic targets. Among the choice list of researcher's, murine model stands on first however, a more convenient vertebrate model, is surfacing as a better substitute (Callaway, 2020). Scientifically named as *Daniorerio,* the zebrafish is a prominent vertebrate model organism in research for more than a century (Sullivan and Kim, 2008). Notably, zebrafish exist as an exceptionally important tool for research in genomics, genetics, embryology and immunology (Santoriello and Zon, 2012). What makes zebrafish distinct from presently available testing models is; it's easy maintenance, reproduction and morphological attributes. Owing to the simplicity of their natural habitat and versatile nature, these organisms are robust and easy to uphold and provides scope for maintaining them in laboratory at ease than other existing mammalian models. Small size and petite generation time of 3–5 months shortens the overall experiment duration making

their culturing cost-effective (Saleem and Kannan, 2018) and ideal for mutant and transgenic cell line production. Additionally, these fish undergo external fertilization, making it simpler for studying early embryonic development which is difficult in rodent models due to the internal mode of fertilization (Dooley and Zon, 2000). Reports suggest that embryos are pretty malleable to genetic manipulation by morpholino antisense oligonucleotide, transgenes, mRNAs and genome editing techniques like CRISPR-Cas9, TALENS (Hwang *et al.*, 2013, Schmid and Haass, 2013, Hisano *et al.*, 2014). One noteworthy advantage of working with zebrafish is its embryos with unrivalled optical clarity, permitting visualization of body physiology and genes individually throughout the course of development, using low power microscope, fluorescent imaging or by using non-invasive imaging techniques which can then prove boon in studying mutations and genetic manipulations (Lieschke and Currie, 2007). As a matter of fact, despite the physiological differences, humans share 70% of genes and 84% disease-related genetic factor with zebrafish (McKie, 2013, Howe *et al.*, 2013). Having high genome homology with humans, it's not strange that zebrafish shows same major tissue and organs alike human counterparts. Their blood, muscle, eyes and kidney share many lineaments with human systems. In recent years, zebrafish animal model is gaining lot of popularity in the scientific community, in reference to assets it has, especially as a model for human disease. It has been used extensively in research for alzheimer's disease, different types of cancer, cardiac disease, kidney disorder, hematopoiesis and obesity (Dooley and Zon, 2000). Accompanying to this, zebrafish is also used widely as a means for study of host-pathogen interactions and infectious diseases of fungal, viral or bacterial origin (Varela *et al.*, 2017). The pathogensis of tuberculosis is a sensible example to discuss, were *Mycobacterium marinum* (a close relative of *Mycobacterium tuberculosis* which infects human) was used to establish infection in zebrafish larvae, mimicking model of mycobacteria infecting humans and were later screened (Takaki *et al.*, 2013). Statistic data revels, countries with more the inhabitants tested for SARS-CoV-2 tend to have least number of deaths from COVID-19 and thus WHO also recommends for mass scale testing of native population but dearth of rapid testing kits posse a big hurdle in this task achievement. In direction of identifying new alternatives for rapid testing, Charlie-silva and his group proposed a novel strategy to develop a quick COVID-19 infected patient detection and tracking kit using zebrafish as animal model. According to the idea, viral peptides will be dosed into female zebrafish at an interval of a week as per two immunization sections with a target of generating plasma antibodies. Transfer of antibodies passively to eggs occurs naturally as stated by Wang and their colleagues (Wang *et al.*, 2012). After successful immunization, female fishes will be motivated to mate and generate the eggs with desired antibodies, which can then be isolated, filtered and introduced in a test

strip (Charlie-Silva, 2020). Regardless of how serious the effect of COVID-19 might be, promising and progressive ventures between research organizations with advance zebrafish facilities under the leadership of top scientists globally, the application of the above-discussed zebrafish techniques could offer novel results soon.

CURRENT STATUS OF VACCINE DEVELOPMENT

SARS-CoV-2 genetic sequence was published on January 11, 2020 by researchers (Wan *et al.*, 2020) creating a ripple in global R&D activity for vaccine development against the disease. New generation vaccine technology through novel paradigms is driven by economic and humanitarian impact of COVID-19 pandemic in order to speed up the vaccine development. The first candidate vaccine entered clinical trials with striking swiftness on 16 March 2020. This chapter will include vaccine development programs reported by WHO's authoritative as well as other ongoing projects retrieved from proprietary sources.

A commendable feature of the vaccine development program for COVID-19 is the variety of technology platforms being explored. At present under the Global Vaccine Development Program (GVDP) there are 115 candidate vaccines are there and out of which 78 are active while 37 are unconfirmed (WHO, 2020). Currently, 73 vaccine candidates from active ones are at preclinical stage meanwhile the most advanced candidate vaccines are under clinical development which involves Ad5-nCoV from CanSino Biologics, mRNA-1273 from Moderna, INO-4800 from Inovio and LV-SMENP-DC as well as pathogen specific aAPC from Shenzhen Geno-Immune Medical Institute.

Candidate vaccines mentioned in Table **1** include novel platforms based on DNA or mRNA that will offer great liberty to scientists for antigen manipulation which will aid the speed of development. Indeed after 2 months of publishing COVID-19 gene sequence, Moderna started clinical trials of its mRNA-based vaccine mRNA-1273 (ClinicalTrials.gov: NCT04283461). Viral vector based vaccines express high levels of proteins, offer stability for long duration along with strong immune response following vaccination. These candidates can also advantage from pre-existing scale-up process.

Table 1. The list of clinical-phase vaccine candidates for COVID-19 (Source: Regulatory Affairs Professional Society-last updated on August 27, 2020).

Sr. No.	Candidate Vaccine	Phase Status	Outcome	Other Information	Location
1.	Inactivated vaccine	III	• Results of phase I/II have shown the promising results in terms of appropriate neutralizing Ab responses • Phase III study is ongoing in Morocco, United Arab Emirates and Peru	Randomized, double-blind (ChiCTR2000031809)	Funded by Ministry of Science & Technology, China
2.	CoronaVac (PiCoVacc)	III	Results of phase I/II has depicted the safety & immunogenicity Phase III study is underway in collaboration with InstitutoButantan in Brazil	Enrolled 743 healthy volunteers & received two dosage of vaccines/placebo Phase I-143 Patients (NCT04352608) Phase II-600 Patients (NCT04383574)	Sinovac Research & Development Co.Ltd, China
3.	mRNA-1273	III	Results of phase I study shown the production of neutralizing Abs (dose of 25/100 µg) Phase III study involves almost 30,000 patients who are at the higher risk of COVID-19 infection and receive 100 µg of dose/ placebo followed up to 2 yrs (NCT04470427)	Phase I-120 Patients (NCT04283461) Phase II-600 Patients (NCT04405076) (Evaluated 50 & 100 µg dose level) All are healthy individuals	Moderna, Inc. USA

(Table 1) cont.....

Sr. No.	Candidate Vaccine	Phase Status	Outcome	Other Information	Location
4.	Ad5-nCoV	III	Results of phase I study have confirmed its humoral and immunogenic response and in phase II neutralizing Ab and IFN-γ based immune responses was observed The Central Military Commission of China has approved the use of Ad5-nCoV for the initial period of 1 year and phase III study is ongoing in Saudi Arabia	A recombinant novel coronavirus vaccine includes adenovirus types 5 vector (Ad5) Phase I have 108 participants and received low, medium and high dose (ChiCTR2000030906; NCT04313127) and followed by Phase II (ChiCTR2000031781)	CanSino Biologics, China
5.	AZD1222/Covishield	II/III	Results of the I/II study have confirmed its safety at acceptable level and noticed the Ab production after 1st dose in almost all patients and after two doses all the participants have shown Ab generation Phase II/III study will shortly begin in India	A chimpanzee adenovirus vaccine vector AZD1222 (ChAdOx1) is a candidate Phase I/II (NCT04324606), a single-blinded, multi-centric study enrolled 1090 healthy volunteers having four regimen of treatment	The University of Oxford, Ministry of Health, UK & BARDA
6.	BNT162	II/III	Results of phase I/II have confirmed their usage for phase II/III (on the basis of their available data of immune response and tolerability) The modRNA candidate (BNT162b2) was finally selected for advanced phase II/III study	Initially there were four vaccine candidates; two consist of nucleoside modified mRNA-based (modRNA), one is uridine containing mRNA (uRNA) and the last one is self-amplifying mRNA-based (saRNA)	Pfizer and BioNTech

(Table 1) cont.....

Sr. No.	Candidate Vaccine	Phase Status	Outcome	Other Information	Location
7.	NVX-CoV2373	IIb	Results of phase I have shown the development of Abs after multiple dosages and also cross the safety level Phase IIb trial is ongoing in South Africa	It is a perfusion protein nanoparticle vaccine candidate used in randomized, observed-blinded trial included 130 healthy volunteers (NCT04368988)	Novavax, Department of Defence, USA
8.	Adjuvant recombinant vaccine	II	Phase II is underway	Phase I results will be expected in September	Anhui ZhifeiLongcom Biopharma. & Institute of Microbiology of the Chinese Academy of Science
9.	ZyCoV-D	II	The enrolment for the phase II trial was begun	It is a plasmid DNA vaccine candidate which targets the entry of viral membrane protein of the virus Phase I was accomplished in the month of August	ZydusCadila, Gujarat, India
10.	Covaxin	II	The results of the candidate vaccines was found to be encouraging and as per Indian Council of Medical Research (ICMR) it has entered in phase II	It is inactivated vaccine candidate	Bharat Biotech, National Institute of Virology, India
11.	BBIBP-CorV	I/II	The results of the phase I have confirmed its higher efficiency and its potential in rhesus macques It is currently in phase II (ChiCTR2000032459)	It is inactivated vaccine candidate which is formulated with Beijing Institute of Biological Products	Ministry of Science & Technology, China
12.	GRAd-COV2	I/II	They have expected the completion of phase I in 3 months before their phase II at multinational level	A DNA vaccine candidate used in randomized 190 healthy participants	Genexine, South Korea

(Table 1) cont.....

Sr. No.	Candidate Vaccine	Phase Status	Outcome	Other Information	Location
13.	Sputnik V	I/II	The Ministry have a target of covering more than 40,000 people from 45 different medical centers which would be consider equivalent to phase III	It is a non-replicating viral vector candidate & it has been declared as the first vaccine for COVID-19. In phase I/II 38 participants received the vaccines (NCT04436471)	Gamaleya Research Institute of Epidemiology and Microbiology, Health Ministry of Russian Federation
14.	Self-amplifying RNA vaccine	I/II	Animal testing is ongoing Phase I/II will enroll around 300 individuals followed by efficacy trial in October	The vaccine has been developed within 14 days after the sequenced received from the China	UK Secretary of State for Health (Imperial College of London)
15.	LUNAR-COV19	I/II	Pre-clinical study have shown the adaptive ($CD8^+$) and balanced (Th1/Th2) immune response. Phase I/II will be initiated in Singapore	It is the combination of self-replicating RNA and nanoparticle non-viral delivery system.	Arcturus, Duke-NUS Medical School, Singapore
16.	INO-4800	I/II	Results of early phase I study have confirmed the immune responses in all the patients and it also inhibits the replication of SARS-CoV-2 in mice The phase I/II will be conducted in coordination with Korea National Institute of Health (KNIH)	It is a DNA based vaccine and it is given by intradermal route through device In phase I (NCT04336410) 40 healthy participants were enrolled in a non-randomized trial In pre-clinical study (in mice and guinea pigs) neutralizing Abs and humoral and T cell responses were observed	Inovio-Pharmaceutical

(Table 1) cont.....

Sr. No.	Candidate Vaccine	Phase Status	Outcome	Other Information	Location
17.	Ad26.COV2-S	I/II	Earlier study have confirmed the neutralizing Ab responses as well as the complete or mostly-complete protection in challenge study The phase III will be enrolled up-to 60,000 participants from international level and USA (NCT04505722)	It consists of AdVac and PER.C6 system which were earlier used to develop Ebola vaccine. It is a double-blind, randomized, placebo-controlled study of phase I include 1045 healthy individuals of US and Belgium (NCT04436276)	Johnson & Johnson
18.	mRNA based Vaccine	I	Results of pre-clinical studies have shown the titer of neutralizing Abs and T-cell responses.	It is mRNA based vaccine which will work by using the nucleotides which are modified non-chemically for appropriate immune responses	CureVac, German federal government
19.	SCB-2019	I	The results of the phase I study will be expected in the August.	It uses the combination of Trimer platform of Clover's, AS03 adjuvant from GSK's and CpG 1018 adjuvant of Dynavax's The phase I study (NCT04405908) has around 108 participants	CEPI
20.	COVAX-19	I	The expected results includes the formation of neutralizing Ab formation and T cell responses against the spike protein of COVID-19.	It is monovalent recombinant protein vaccine developed by Vaxin Pty Ltd. and phase I (NCT04453852) is a randomized placebo controlled trails having 40 healthy adults. Two doses will be given at the interval of three weeks.	NIAID

(Table 1) cont.....

Sr. No.	Candidate Vaccine	Phase Status	Outcome	Other Information	Location
21.	Plant-based adjuvant COVID-19 vaccine candidate.	I	The animal study confirms the positive Ab responses within the 10 days Phase I study is ongoing and phase II will be start in the month of October 2020.	It has been developed by Medicago who has developed the VLP-influenza vaccine and the candidate vaccine has been tested alongside the two additional adjuvants from GSK and Dynavax.	Medicago
22.	Molecular clamp vaccine	I	The earlier results have confirmed the formation of neutralizing Abs Phase I is ongoing and if the clinical trial have the successful results than it can be available by the end of 2021.	This vaccine candidate helps in identification of protein on virus Phase I has 120 individuals and the university has collaborated with CSL and CEPI (Coalition for Epidemic Preparedness Innovations).	The university of Queensland
23.	V590	I	The phase I is currently recruiting participants at both the centers (NCT04497298).	It is based on the Merck's rVSV (recombinant vesicular stomatitis virus) technology used for Ebola vaccine.	Merck and BARD (Biomedical Advanced Research and Development Authority).
24.	GRAd-CoV2	I	Phase I is underway in Italy	It is adenovirus based vaccine.	ReiThera (Italy), Leukocare (Germany) and Univercells (Belgium)
25.	AdimrSC-2f	I	Earlier animal studies have shown the positive results and now they are planning randomized phase I study (NCT04522089).	This vaccine candidate targets spike protein of SARS-CoV-2.	Adimmune (Taiwanese)
26.	bacTRL-spike	Pre-clinical	Currently it is being tested in phase I (NCT04334980) having 84 healthy individuals with various dosages.	It is a bifidobacteria monovalent DNA based oral vaccine.	Symvivo Corporation, Canada

(Table 1) cont.....

Sr. No.	Candidate Vaccine	Phase Status	Outcome	Other Information	Location
27.	PittCoVacc	Pre-clinical	Animal studies in mice have shown an effective results delivered *via* a fingertrip patch.	UPMC has recently received the fund from CEPI to develop this vaccine candidate.	University of Pittsburgh School of Medicine (UPMC).
28.	V591	Pre-clinical	Animal studies in mice have shown the promising results.	UPMC, Themis Biosciences and Institute Pasteur alongside Merck will develop the vaccine candidate.	Merck
29.	Ii-Key peptide COVID-19 vaccine	Pre-clinical	The vaccine development program is underway which can develop and tested in humans in 90 days.	-	Generex
30.	Recombinant vaccine	Pre-clinical	Earlier animal studies have shown the SARS-CoV-2 Abs after the 1st and 2nd dose The phase I study will initiate in the second half of 2020.	It is a oral recombinant vaccine candidate.	Vaxart
31.	LineaDNA	Pre-clinical	Earlier pre-clinical studies have shown the strong Ab and T-cell responses The human trials will be begin in fall.	It is a DNA based vaccine mainly produced by Takis Biotech and Applied DNA science.	Takis Biotech
32.	AdCOVID	Pre-clinical	Animal studies have shown the neutralizing activity, strong Ab response and potent mucosal immunity The clinical trials will be expected in the fourth quarter of 2020.	The technology used for COVID-19 vaccine is similar to used for influenza vaccine (NasoVAX). It can be injected through intranasal route to activate the entire immune response (humoral, cellular and mucosal).	Altimmune

(Table 1) cont.....

Sr. No.	Candidate Vaccine	Phase Status	Outcome	Other Information	Location
33.	T-COVIDTM	Pre-clinical	Despite the same mechanism for vaccine development, it works through different mechanism.	The technology used for COVID-19 vaccine is similar to used for influenza vaccine (NasoVAX). It can be injected through intranasal route to activate the entire immune response (humoral, cellular and mucosal).	Altimmune
34.	Protein subunit vaccine	Pre-clinical	Early studies have shown the generation of immune response. Animal studies is ongoing.	The international vaccine center and the university of Saskatchewan's Vaccine is developing this vaccine based candidate.	-
35.	Adenovirus-based vaccine	Pre-clinical	Animal studies have shown the generation of $CD4^+$and $CD8^+$T-cell which are Ag specific.	It is adenovirus based vaccine which can taerget spike and nucleocaspid DNA in SARS-CoV-2.	ImmunityBio&NantKwest
36.	AAVCOVID	Pre-clinical	-	It is a gene based candidate vaccine.	Wyc-Grousbeck
37.	Recombinat vaccine	Pre-clinical	Early non-human studies have confirmed the generation of immune response The phase I/II will begin in the month of September.	It is designed on the basis of rDNA technology which was earlier used for the SARS vaccine.	Sanofi and Translate Bio
38.	Halovax	Pre-clinical	Two different sets of this candidate is currently under animal studies	The candidate vaccine will be designed using the Self-Assembling Vaccine (SAV) platform.	Voltron Therapeutics and Hoth Therapeutics
39.	mRNA-based vaccine	Pre-clinical	Animal studies in monkeys and mice have shown the successful Ab response The clinical trial will begin in the month of September.	This particular vaccine candidate is similar to the mRNA based vaccine of Moderna.	University's Center of Excellence in Vaccine Research & Development, Thailand

(Table 1) cont.....

Sr. No.	Candidate Vaccine	Phase Status	Outcome	Other Information	Location
40.	HDT-301	Pre-clinical	The animal studies have demonstrated the robust Ab responses in mice and primates. At the earliest, the phase I trial will begin.	It is a replicon RNA vaccine candidate.	University of Washington, National Institute of Health Rocky Mountain Laboratories & HDT Bio Corp.
41.	gp96-based vaccine	Pre-clinical	The animal studies have confirmed the immune response Especially the latest report suggest that it can generate human-HLA-restricted T-cells against the immunodominant epitope of SARS-CoV-2 spike protein.	It uses the gp96 heat shock protein in the designing of the vaccine.	Heat Biologics
42.	ChAd-SARS-CoV-2	Pre-clinical	Pre-clinical studies in animals have shown the strong immune response in terms of formation of neutralizing Abs specifically in upper and lower respiratory track. At the earliest non-human primate and human clinical study will be initiated.	It is based on the chimpanzee adenovirus vectored vaccine. The route will be intranasal and targets the spike protein of the virus.	Washington University School of Medicine in St.Louis.
43.	mRNA lipid nanoparticle vaccine.	Early-phase	The time line for this candidate vaccine has not been disclosed.	It uses the Precision Nanosystem's RNA vaccine platform.	CanSino Biologics & Precision Nanosystem.

For some vaccine candidates, adjuvants would strengthen the immunogenicity and enhance the viability of lower doses (Le *et al.*, 2020), thereby avoiding the risk of compromised protection of individuals. Developers like GlaxoSmithKline, Seqirus and Dynavax have indicated plans of delivering adjuvants AS03, MF59 and CpG 1018, respectively which will be used for manufacturing COVID-19 vaccine supplemented with adjuvants. A huge number multinational pharmaceuticals like Janssen, Sanofi, Pfizer and GlaxoSmithKline have engaged in COVID-19 vaccine development, still lead developers involve small or inexperienced vaccine manufacturers in large-scale production hence, co-ordination will be critical to meet the supply demand.

The worldwide vaccine R&D in response to the COVID-19 pandemic is unmatched in terms of scale and speed. It has been indicated that by early 2021, vaccine for emergency use or under likewise protocols will be made available. This would speak to an essential advance change from the customary immunization improvement pathway which takes on an average 10 years, even if we compare the first fastest delivery of Ebola vaccine which took 5 years. Hence, COVID-19 vaccine would demand extraordinary scale-up manufacturing and innovative regulatory process to ensure safety and eliminate involved risks.

BENCH TO BEDSIDE: OBSTACLES AND OUTCOMES

Immunization has extraordinarily diminished the weight of irresistible infections. Absurdly, an intense demand for vaccine development thrives regardless of undeniable achievement of immunization programs against in the past fearsome diseases that are now rare in evolved countries. Studies on contagious disease and its pattern of infectivity, especially in industrialized countries gave evidences for a successive decline in pre-reproductive mortality since 19th century's end, and this lowering in cases of death from previous thought 'incurable diseases' was due to development of 'concept of immunization'. Apart from enhancement in sanitation, housing and nutrition facilities, vaccination has also played an indisputable role in laying strong foundation stone on which mankind stands today. Death rate prior to vaccination phase during previously encountered pandemics of smallpox and measles were devastating, were half of the population was cleared and a bit less lethal for population affected from measles (Greenwood, 2014).

The problem of vaccine acceptance has been dwelling in the society. The only possible route to answer this issue is by providing data that is scientifically validated but unfortunately, it's far easy to say than to execute on real ground because the major demerit of the game humans are playing is that, it is not governed by the rules that generally exist in field of science and justifiably, public attention is more inclined towards vaccine safety then its effectiveness, but clinical observations and data has verified that vaccines designed are far safer and secure than restorative medicines till date (Vaccines *et al.*, 2012, Andre *et al.*, 2008).

Vaccine, does not aim to prevent the disease but instead goal to safeguard the vaccinated population from the risk of future re-encounter. The level we target the vaccination program tells us the expected output, like at individual scale it may help to modify or prevent the infection while at population scale, the intension is to cease the spread of disease in mass. As thought, knowledge of personal protection and grip on epidemicity will definitely eradicate the causative

pathogen. Yet, any vaccine effectiveness is governed by some crucial factors enlisted (Graham, 2013).

- Understanding and identifying the challenge at biological front and developing strategies to overcome.
- Technological advancements that can aid to solve small and large scale operational hurdles.
- Political and social consent of local institutions, communities and governing bodies.
- Other environmental parameters like resources, genetic buildup, demographic localizations *etc.*

Microbiological evidences stand as another determinant to narrate about the load or incidences of pathogenicity. To explain the point, nothing can be better than HPV vaccine licensing, which was developed not to prevent the infection but on contrary to save the patient from cervical neoplasia (Garland *et al.*, 2007). Another example, HIV and HSV vaccines were formulated to aim for complete viral clearance, but these viruses posed some major obstructions for the vaccinologists in achieving abortive infection or sterilizing immunity after the infection rather than treatment for any physiological symptoms attributed to their ability to establishing latency, evading host's natural immune-surveillance and clearance with high risk of relapse and recurrence in time-independent manner.

Looking at their genetic framework designed for establishing latency, evading body's natural immune-surveillance and clearance, altogether with high risk of relapse and recurrence were the issues vaccinologists were answerable for. The role assigned to any therapeutic vaccine candidate, is to aid in rapid clearing of both virus and virus infected cells so as to avoid the chance of infection persistence rather than actually inducing sterilizing kind of immunity. VZV (varicella Zoster Virus) vaccine works on the similar principle of avoiding persistent infection from natural wild strain in children and cases of HSZ and postherpetic neuralgia in adults, then to avert the pathology (Oxman *et al.*, 2005). A new concept of charitable vaccine proposed, designed to protect from transmission then benefiting on individual level, though none vaccine formulation fits in the definition, yet few have been developed for diseases prevention *via* passive immunization, RSV (respiratory syncytial virus), congenital rubella syndrome and CMV (cytomegalovirus) are on the list.

Lessons from Previous Vaccine Development

Complete eradication of virus from a population stands as sign of success for the vaccine. CMI and HMI are key-role players during adaptive immunological

response and have a characteristic marker of memory formation. Antibodies are equipped with the ability to catch a virus before it can enter the cell, in association with peculiarity for target specificity and high avidity, thus making them an ideal candidate for vaccine development (Graham, 2013). Antibody works on any of the three major mechanism in general; immobilization or aggregation of viral particle, blocking of viral attachment facilitating proteins and receptors on host cell, and lastly, post-attaching neutralizing reaction tendency. Cytotoxic T cell mediated response is primly concerned with recognizing and clearing cells infected with virus.

In recent times, both vaccine industry and scientific community have been asked to quickly reciprocate to global pandemics of H1N1 influenza (2009), Ebola (2013-2016), Zika (2015-2016), and currently ongoing SARS-CoV-2. Vaccine for H1N1 influenza was commercialized in very less time owing to previously gathered knowledge and strategies on the paradigm of cell-based platforms, as getting licensed was easy and profitable. Although, northern hemispheric countries had to wait for the monovalent H1N1 vaccine, but soon the remedy was commercially available in addition to the seasonal flu-vaccines. Antagonistic to this route, vaccines for Zika and SARS were not into existence by the time epidemic got under border and thus central funding agencies were forced to reallocate funds that were committed for respective vaccine research and development, leaving a void in financial gain and setting back vaccine programs operating in parallel.

Ebola vaccine production was kept on hold between 2013-2016 by the Public Health Agency of Canada, which was then funded by the USA and transferred to private sector company, Merck. The company kept the production on even after the virus was almost uprooted from the infected demographic area generating huge stockpiles. CEPI (coalition for epidemic preparedness innovation) additionally favors development of new platforms for “Disease-X” -like some novel epidemic, one being COVID-19. Of all those concepts under trial for vaccine design, the utmost promises are of RNA and DNA- based programs and then the leading candidates are recombinant-subunit vaccines. One prime advantage of RNA and DNA-based vaccines is, synthesis independent of fermentation or cell culture procedures making it ideal choice for oncology vaccines as personalized medicine. No licensed RNA-vaccine exists till now but many of them are already under clinical trials and seem to have promising results in near future. Recent advancements like, next generation sequencing (NGS) and reverse genetics using the ground of genomics and proteomics and link with phylogenic relationship may facilitate to cut down the development time, which was a major challenge for conventional vaccines.

Even with such innovative platform, designing a correct vaccine for SARS-CoV-2 is still a challenging task, attributed to elements such as;

- Area for targeting the viral spike protein, receptor domain or whole length protein using adjuvants or other subunit type.
- Absence of reliable animal model for testing and assurance
- Lessons from SARS and MERS epidemics where intensified cases of lung severity, as a cause of direct of antibody medicated implication was witnessed
- Th2 medicated CMI response which can be more damaging then benefiting
- Lack of experimentally validated data from immunopathological front
- Insufficient data on dosing pattern, mode and duration of immunity furnished as correlated with SARS and MERS epidemics scenarios

To introduce a vaccine for mankind, it necessitates many years of intensive research with N number of candidates having high conflicting opinions on its efficacy and long lasting impact on wider scale with high financial input and uncertainty of failures (Gouglas *et al.*, 2018). These risks force the manufactures to follow a linear sequence of development with ample of halts and step-to-step data evaluation which in longer run slows down the development process considerably. As a solution, carrying out phase I clinical trial simultaneously with animal studies can benefit to certain extend but again this will heighten the risk of financial losses to a next level. However, looking at current pandemic crisis, this huge time investment seems likely to be unaffordable and thus a novel paradigm with multi-dimensional planning is the need of the hour.

LIST OF ABBREVIATIONS

AgNps	Sliver nanoparticles
CMI	Cell-mediated immunity
CTD	C-terminal domain
CTL	Cytotoxic T-lymphocytes
DAMPs	Damage-associated molecular patterns
DCs	Dendritic cells
EGCG	Epigallocatechingallate
ELP	Elastin-like polypeptide
GALT	Gut associated lymphoid tissue
GO	Graphene oxide
HLA	Human leukocyte antigen
HMI	Humoral mediated immunity

HPV	Human papilloma virus
IC	Immune complex
IEDB	Immune epitope database and analysis resource
IRF 3	Interferon regulatory transcription factor 3
KO	Knockout
LNPs	Lipid nanoparticles
MDA 5	Melanoma differentiation-associated protein 5
MHC	Major histocompatibility complex
MMR	Mumps, measles and rubella
NF-Kb	Nuclear factor- kappa-B
NOD	Non-Obese Diabetic
NTD	N-terminal domain
ORF	Open reading frame
PBMCs	Peripheral blood mononuclear cells
pDCs	plasmacytoid dendritic cells
PPE	Personal protective equipment
RBD	Receptor-binding domain
RIG-I	Retinoic acid-inducible gene I
SCID	Severe combined immunodeficiency
TLR	Toll like receptor
ViPR	Virus pathogen resource
VLPs	Virus like particles

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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CHAPTER 6

The Future of COVID-19 Treatment

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Abstract: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) or coronavirus disease or COVID-19 is a disease that has led to colossal mortality worldwide. The fast spread of this disease has caused havoc and panic among individuals, which has further worsened with the unavailability of vaccines or some proven drug regime. To date, only 12 new antiviral drugs have been approved by the FDA (8 against hepatitis C virus (2 in combinations for HIV). Thus, it becomes of utmost importance to identify drugs for new and re-emerging viruses, including the coronavirus. Considering the quest, we have put forth this book chapter to update readers about current repurposed and experimental drugs for this novel coronavirus. The viral lifecycle assisted in providing vital potential targets for drug therapy. The present chapter also deals with the existing mechanism of action of the drugs, their category, and clinical data reported.

Keywords: ACE2 inhibitors, Antiarrhythmic, Antibacterial, Anticancer, Anticoagulants, Antidiabetic, Antifungal, Anti-inflammatory, Antimalarial, Antioxidant, Antiparasitic, Antiprotozoal, Antiviral, Chelating agents, COVID-19, Drug repurposing, Natural products, Psychotropics, RAAS-ACE-ARBs inhibitors, SARS-CoV-2.

INTRODUCTION

Considering the COVID-19 pandemic, it becomes of utmost importance to identify drugs that possess potential and efficacy in treating COVID-19 disease (Mercorelli *et al.*, 2018, Cupertino *et al.*, 2020). The COVID-19 disease is notably marked with attacks at the lower respiratory system that leads to viral pneumonia along with affecting kidney, liver, heart, central nervous system, and gastrointestinal system leading to multiple organ failure responsible for high morbidity (Su *et al.*, 2016, Zhu *et al.*, 2020). Recently, drug target validation in

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SAR-CoV-2 has led to the identification of newer targets for therapeutic development (Mani *et al.*, 2019). To date, (June 4, 2020) 53 targets have been identified.

The important one includes non-structural proteins, spike proteins, membrane protein, nucleocapsid proteins, *etc.* The identified targets/proteins possess enzymatic activities and catalyze the virus-cell cycle by hijacking the host cells.

The virus division cycle (Fig. **1**) consists of six phases, which include attachment of virus with the host cell receptors, allowing its entry, followed by translation, replication, and release, and the subsequent damage resulting from the compromised immune system.

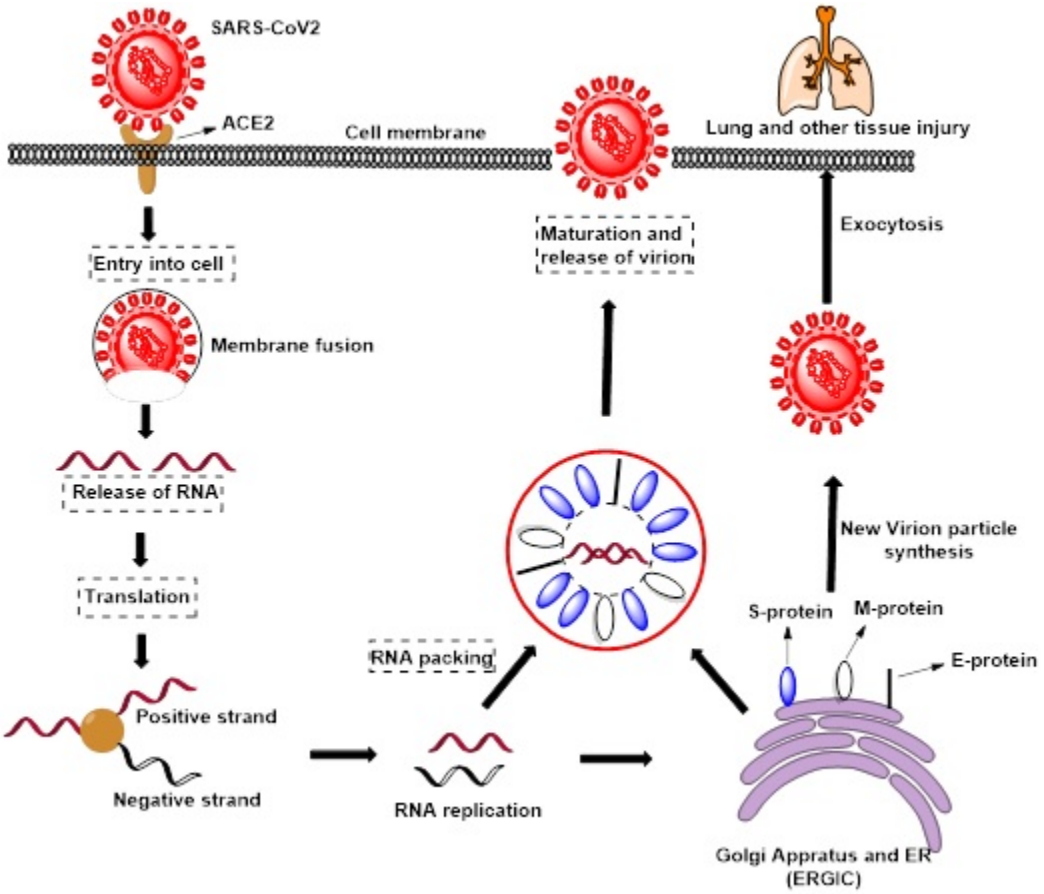


Fig. (1). SARS-CoV-2 life cycle in the host cell. The cycle consists of critical phases starting from the attachment of the virus with host cell receptors, endocytosis, proteolysis, translation, replication, maturation, and release of mature virions that ultimately are shredded, leading to subsequent damage of the organ system resulting from the compromised immune system.

Taking into account the reported interaction pattern of the virus with the host cell, many drugs have been repurposed so far. The drugs are currently under various clinical trials, and their efficacy and potential are still awaited. Drug repurposing characterizes a practical drug discovery approach from already known drugs (for different therapeutic use). It could minimize the time and decrease the cost associated with the *de novo* drug discovery (Zhou *et al.*, 2020b). This practice has been gaining popularity in recent years (Aubé, 2012). The approved drug requires regulatory approval for novel therapeutic applications which were already optimized for efficacy and safety in a particular disease (Chong *et al.*, 2007). The strategy used for drug repurposing involves identification of ligand for an indication of interest, drug assessment in preclinical models by mechanistic interpretation, efficacy evaluation in phase II clinical trials. There are various approaches used for drug repurposing, such as computational and experimental approaches. Computational approaches include molecular docking (Kitchen *et al.*, 2004), signature matching (Hieronymus *et al.*, 2006), pathway mapping(Smith *et al.*, 2012), genetic association (Sanseau *et al.*, 2012), novel data sources (Althouse *et al.*, 2015), and retrospective clinical analysis (Hurle *et al.*, 2013). Experimental approaches, such as phenotypic screening (Moffat *et al.*, 2017) and binding assays, are involved in the identification of relevant target interactions (Molina *et al.*, 2013). Some of the success stories of drug repurposing in other diseases include the use of paromomycin (from antibiotic in acute and chronic intestinal amebiasis to visceral and cutaneous leishmaniasis) (Ben Salah *et al.*, 2013), doxycycline (from bacteriostatic to antimalarial) (Tan *et al.*, 2011), miltefosine (from cancer to leishmaniasis) (Dorlo *et al.*, 2012), allopurinol (from cancer to gout) (Yasuda *et al.*, 2008), amantadine (from antiviral to tremors) (Gironell *et al.*, 2006), atomoxetine (from antidepressant to attention deficit hyperactivity disorder) (Turgay, 2005), colchicine (from gout to post-pericardiotomy syndrome) (Imazio *et al.*, 2010), and bromocriptine (from Parkinson's disease to type 2 diabetes) (Shaughnessy, 2011). Some examples of repurposed drugs used for the previous counterpart of SARS-CoV-2, *i.e.*, SARS and Middle East Respiratory Syndrome (MERS), include nitazoxanide (Rossignol, 2016), hydroxychloroquine (Zhou *et al.*, 2020a), emetine (Sharif-Yakan *et al.*, 2014), terconazole (Omrani *et al.*, 2015), lopinavir (Chan *et al.*, 2015), ritonavir (Chan *et al.*, 2015), ribavirin (Khalili *et al.*, 2020), homoharringtonine (Mustafa *et al.*, 2018), *etc.* The drugs for repurposing for the treatment of COVID-19 disease fall into multiple categories possessing various essential mechanisms. The essential categories include antivirals, anticancer, Angiotensin Converting Enzyme-Renin–Angiotensin–Aldosterone system (ACE-RAAS) inhibitors, anti-inflammatory, immune modulators, antibiotics, antirheumatics, *etc.* Besides this, some of the herbal drugs and nutraceuticals have also exhibited significant inhibitory potential against SARS-CoV-2 (Khan *et al.*, 2020, Liu *et al.*, 2020b).

The fast spread of this disease has caused havoc and panic among individuals, which has further worsened with the unavailability of vaccines or some proven drug regime. Considering the quest, we have put forth this book chapter to update readers about current repurposed and experimental drugs for this novel coronavirus. The viral lifecycle has assisted in providing vital potential targets for drug therapy. In the present chapter, we have focused primarily on small molecules (< 900 Daltons molecular weight) as drug candidates for COVID-19, along with their classification, mechanism of action, categories, preclinical and clinical evidence. It is to be noted that detailed discussion on vaccine development and current treatment is out of the scope of the present chapter as it is dealt with separately.

DRUGS AFFECTING VIRAL REPLICATION AND TRANSLATION IN COVID-19 DISEASE

Many antivirals, such as ribavirin, lopinavir/ritonavir, and arbidol are incorporated for the prevention and treatment of COVID-19 disease (Dong *et al.*, 2020). They inhibit the $3CL^{pro}$ protease of SARS-CoV-2, which plays a crucial role in the viral replication cycle. Another drug, umifenovir, blocks the fusion of the viral membrane with the endosome after endocytosis. NS3/4A protease is having an essential function in viral replication. Danoprevir plays a crucial role in inhibiting NS3/4A protease and decreases the host response against viral infection. It also reduces the hyperactivation of the host immune system.

For the replication and transcription, SARS-CoV-2 relies chiefly on the RNA-dependent RNA polymerase (RdRp). Drugs, including remdesivir, and favipiravir (nucleoside analog), inhibit RNA polymerase, and terminate the RNA transcription (Agostini *et al.*, 2018, Chen *et al.*, 2020). Remdesivir is a nucleoside analog prodrug, for which USFDA issued emergency use authorization for the treatment of COVID-19 on May 1, 2020, based on two Phase III clinical trials (Zheng *et al.*, 2020). Another trial conducted by Gilead's global SIMPLE trial (NCT04292899) found that remdesivir-treated patients were discharged in less than a week overcoming COVID-19 symptoms. According to the USFDA, remdesivir is administered intravenously to treat suspected or confirmed severe COVID-19 cases requiring intensive breathing support. However, some trials failed to show any benefit of remdesivir in patients with severe COVID-19 (Wang *et al.*, 2020b). Still, 19 clinical trials are in several phases for remdesivir that are investigating its efficacy and safety. Remdesivir is the part of WHO's SOLIDARITY trial for COVID-19.

Another drug, galidesivir attaches to a viral RNA polymerase in place of the natural nucleotide. As a result, there is a structural alteration in the viral enzyme

because of restructured electrostatic interactions. Interruption of viral RNA polymerase function leads to premature termination of the elongating RNA strand (Westover *et al.*, 2018). Emtricitabine inhibits the reverse transcriptase, which results in the reduction of viral load, thereby preventing the entry of virus RNA into human DNA (Orkin *et al.*, 2018).

Interestingly, non-steroidal anti-inflammatory agents (NSAIDs) such as indomethacin a potential cyclooxygenase (COX) inhibitor, are found to affect viral replication and translation by blocking viral RNA synthesis and anticipated to be useful in the management of COVID-19 disease (Amici *et al.*, 2006). Further, antirheumatic drugs such as leflunomide inhibit virus replication and thus inhibit the development of activated autoimmune lymphocytes through blocking the cell cycle progression (Zhong *et al.*, 2020).

In the category of antidiabetic drugs, dapagliflozin, which is a sodium-glucose cotransporter-2 (SGLT2) inhibitor, is recently found to inhibit sodium–hydrogen exchanger-1(NHE) directly (Ye *et al.*, 2018). Dapagliflozin results in the lowering of increased lactate levels, which occurs due to increased LDH (Lactic Acid Dehydrogenase) levels by cell destruction in COVID-19 infection (Cure *et al.*, 2020). Thus, the drug helps lower cytosolic pH and reduces the viral load in the COVID-19 disease. Considering antiarrhythmic drugs such as amiodarone, block voltage-gated calcium and potassium channels, and thereby block viral entry. Amiodarone has also been reported to interfere with the endocytic pathway and inhibits virus replication (Yang *et al.*, 2020). A report by Zhang *et al.* further disclosed that calcium channel blockers might extensively reduce the post-entry replication of SARS-CoV-2 (Zhang *et al.*, 2020b). All the drugs which affect viral replication and translation with their NCT (National Clinical Trial Number) (if any) are collected in Table **1**, and their chemical structures are given in Fig. (**2**).

Table 1. List of repurposed drugs affecting viral replication and translation in COVID-19 disease.

Repurposed Drug (Trade Name) /NCT No.[a]	**Clinical Trial Phase**	**Class**	**Target**	**Mechanism of Action**
Umifenovir (Arbidol) NCT04260594	Phase 4	Antiviral	Viral glycoprotein	Block the entry of viral membrane by inhibiting endocytosis.
Darunavir (Prezcobix) NCT04252274	Phase 3	Antiviral	Gag-Pol	Inhibits cleavage of Gag-Pol, an encoded viral protein.
TMC-310911	Preclinical	Antiviral		

(Table 1) cont.....

Repurposed Drug (Trade Name) /NCT No.[a]	Clinical Trial Phase	Class	Target	Mechanism of Action
Ritonavir (Norvir) + Lopinavir (Kaletra) NCT04295551	Unknown	Antiviral	$3CL1^{pro}$	Inhibit the $3CL1^{pro}$ protease activity thus blocking the formation of polyproteins.
Ribavirin (Rebetol) + Ritonavir + Lopinavir (Kaletra) NCT04276688	Phase 2	Antiviral	-	-
Favipiravir (Avigan) NCT04336904	Phase 3	Antiviral	RNA-dependent RNA polymerase (RdRp)	Inhibit viral replication by blocking RdRp
Remdesivir (Captisol) NCT04365725 NCT04292899	Phase 3			
Levovir (Clevudine) NCT04347915	Phase 2			
Galidesivir (BCX4430) NCT03891420	Phase 1	Antiviral	Nucleoside RNA polymerase (antimetabolite)	Structural alteration in the viral RNA polymerase enzyme
Emtricitabine NCT04334928	Phase 3	Antiviral	Non-nucleotide reverse transcriptase	Inhibition of reverse transcriptase and blocks the viral transcription
Ribavirin (Rebetol) NCT04276688	Phase 2	Antiviral	RNA-dependent RNA polymerase	Inhibit viral replication by blocking RdRp
Naproxen (aleve) NCT04325633	Phase 3	NSAID	blocks viral RNA replication	Interferes with the RNA replication process
Indomethacin (indocid) NCT04344457	Phase 2	NSAID		
Leflunomide (Arava) NCT04361214	Phase 1	Antirheumatic	mitochondrial enzyme dihydroorotate dehydrogenase	inhibits the development of activated autoimmune lymphocytes through blocking the cell cycle progression.
Dapagliflozin (farxiga) NCT04350593	Phase 3	Antidiabetic	SGLT2	Lowers cytosolic pH and reducing the viral load
Amiodarone (Nexterone) NCT04351763	Phase 2	Antiarrhythmic	endosome	Blocks virus replication

(Table 1) cont.....

Repurposed Drug (Trade Name) /NCT No.[a]	Clinical Trial Phase	Class	Target	Mechanism of Action
Nicardipine (cardene) NCT04330300	Phase 4	Calcium channel blockers	Calcium channel	Activator of autophagy
Nimodipine (Nimotop)	Phase 4			
Nifedipine (Adalat)	Phase 4			
Felodipine (Plendil)	Phase 4			
Diltiazem (Cardizem)	Phase 3			

[a] National Clinical trial (NCT) number retrieved from https://clinicaltrials.gov

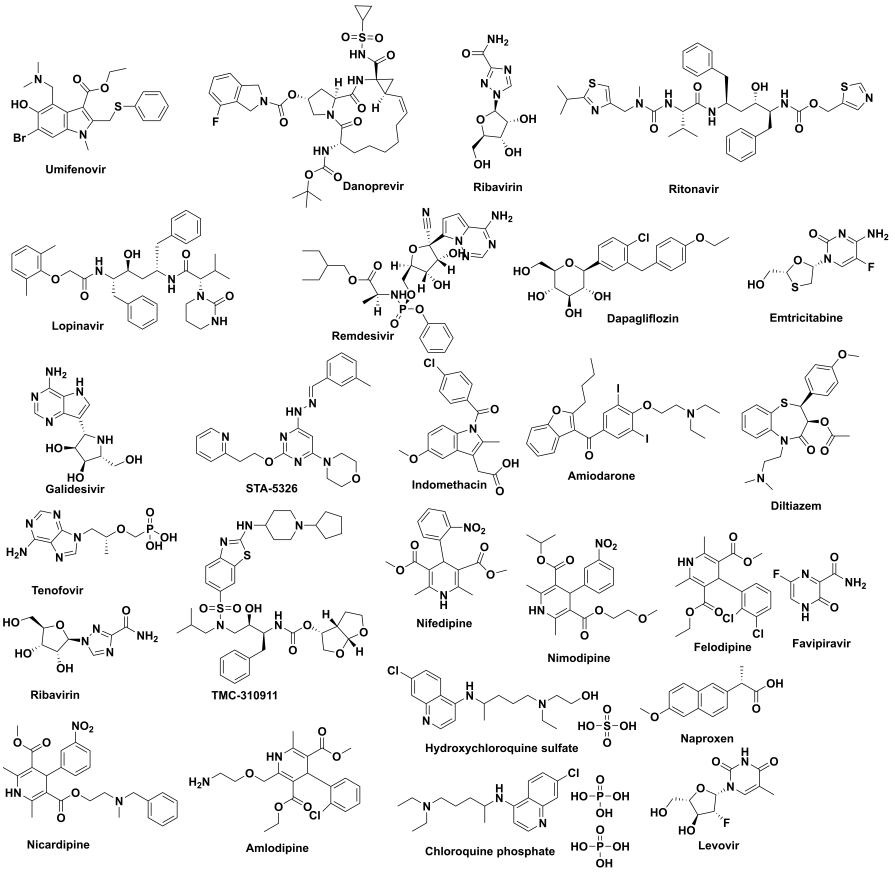


Fig. (2). Chemical structures of drugs affecting viral replication and translation.

DRUGS INTERFERING WITH INFLAMMATION AND OVERCOMING CYTOKINE STORM IN COVID-19 DISEASE

Due to the host inflammatory response, there is lung damage and subsequent

mortality during the treatment of severe cases of COVID-19. As visualized in COVID-19 disease, Calcitonin gene-related peptide (CGRP) is recognized for the increased cytokine-dependent Il-6 production; (Sakuta *et al.*, 1995). Therefore, drugs used in migraine treatments that block the CGRP pathway, *e.g.*, vazegepant, a nasal spray, is found to show its mark in hyperinflammatory response in COVID-19 patients (Robertson, 2020). Further, it is also evidenced that during the progression of COVID-19 disease, substance P interacts with neurokinin-1 (NK-1) receptor resulting in severe lung parenchymal injury and a viral infection where tradipitant has found to be a suitable candidate in overcoming NK-1 mediated interaction (Sanders *et al.*, 2020, Jean *et al.*, 2020). Further, inhalational anaesthetics are also found useful in reducing lung inflammation and dilate airways *via* γ-aminobutyric acid (GABA) type A receptors (Forkuo *et al.*, 2018, Fortis *et al.*, 2012). Another class of drugs such as antioxidants like vitamin C quenches free reactive oxygen species and free radicals. It may show beneficial effects in viral infected patients by increasing α/β interferon production and downregulating pro-inflammatory cytokines production (Frei, 1994, Carr *et al.*, 2017, Kim *et al.*, 2013). Recently, vitamin C is shown to assist in the growth and maturation of T lymphocytes and NK (natural killer) cells against viral agents by enhancing immune response. Also, it aids in the modulation of the cytokine network typical of systemic inflammatory syndrome (Klein *et al.*, 2018). Many psychotropics agents such as valproic acid and haloperidol are newly added to a list of repurposed medications with the possibility for action against COVID-19 after they exhibited anti-inflammatory and mild intrinsic antiviral effect *in vitro* (O'Meara *et al.*). Drugs like fluvoxamine have shown their potential to overcome the cytokine storm by reducing the development of inflammatory response during sepsis (Rosen *et al.*, 2019). Melatonin is also known to exhibit antioxidant and anti-inflammatory properties (Li *et al.*, 2020a). Another category of drugs belonging to anticoagulants is anticipated to play a vital role in COVID-19 disease; for example, camostat, a serine protease inhibitors (Yamaya *et al.*, 2015), blocks the mechanism of SARS-CoV-2, which uses TMPRSS2 (serine protease) for docking to the ACE2(Bittmann *et al.*, 2020, Hoffmann *et al.*, 2020). This hampers the binding of the spike protein of the virus to the host cell. The drug is also found to affect inflammatory pathways mediating *via* NF-κB on the host cell membrane by inhibiting Toll-like receptors and significantly increasing the expression of the **MYD88** gene (Totura *et al.*, 2015). This alternatively could facilitate a decrease in lung infection and improve the survival of COVID-19 patients. Also, antihyperlipidemic agents have demonstrated that statins stabilize MYD88 levels after a pro-inflammatory trigger, such as hypoxia (Yuan *et al.*, 2014). Considering antipsychotic drugs, chlorpromazine (CPZ) has shown anticholinergic, antihistaminic effects that may assist in overcoming inflammation in COVID-19 disease(Baller *et al.*, 2020). Another drug, fingolimod, which is a

Sphingosine 1-phosphate receptor (S1P) analogue, and develops lung endothelial cell integrity, has been found to manage the vascular permeability. As a high level of cytokines was observed in COVID-19 patients, so the immunomodulatory effect of fingolimod is a better therapeutic option. Another example includes tacrolimus, which upon binding with host immunophilin FKBP-12, decreases peptidyl-prolyl isomerase and forms a new FKBP12-FK506 complex that inhibits the T-lymphocyte signal transduction and IL-2 transcription. Table **2** summarizes the list of drugs interfering with inflammation and overcoming cytokine storm in COVID-19 disease, whereas their chemical structures are compiled in Fig. (**3**).

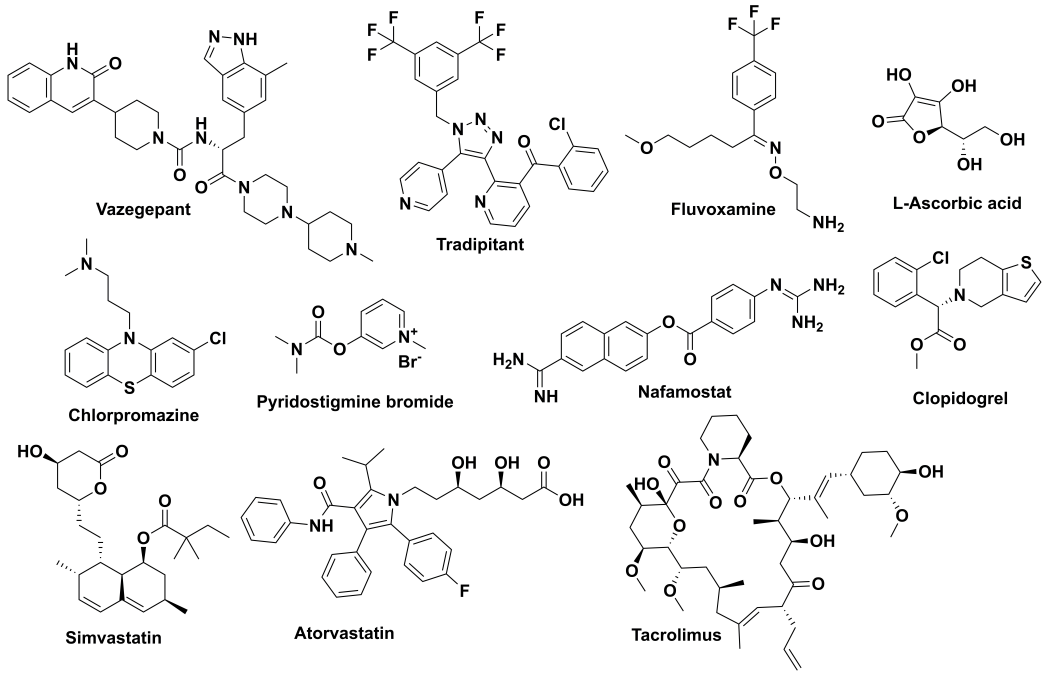


Fig. (3). Chemical structures of drugs interfering with inflammation and overcoming cytokine storm in COVID-19 disease.

Table 2. Drugs interfering with inflammation and overcoming cytokine storm in COVID-19 disease.

Drug (Trade Name)/NCT No. [a]	Clinical Trial Phase	Class	Target	Mechanism of Action
Vazegepant (BHV-3500) NCT04346615	Phase 2	Antimigraine	Calcitonin gene-related peptide receptor	Blocks CGRP receptor-mediated effects.
Tradipitant NCT04326426	Phase 3	neurokinin 1 antagonist (experimental)	neurokinin-1 receptor (NK-1R)	Blocks signaling of substance P.
Sevoflurane (sojourn) NCT04359862	Phase 4	Anesthetic agent	GABA A receptor; NMDA receptor	Overcome acute respiratory distress syndrome (ARDS) by vascular dilation, improves oxygenation in ventilator patients.
L-ascorbic acid (vitamin C) NCT04363216	Phase 2	Antioxidant	NK (natural killer) cells	able to scavenge damaging reactive oxygen species and increases the immune function.
Fluvoxamine (Luvox) NCT04342663	Phase 2	antidepressant	sigma-1 receptor	Prevents cytokine storms
Enoxaparin (Lovenox) NCT04366960	Phase 3	Anticoagulant	Antithrombin-III, Coagulation factor X	Inhibits viral attachment
Camostat NCT04353284	Phase 2	Anticoagulant	TMPRSS2	Inhibits serine protease TMPRSS2, preventing the priming of the viral spike protein
Chlorpromazine (Thorazine) NCT04366739	Phase 3	Antipsychotic	postsynaptic dopamine receptors	Inhibits viral replication
Pyridostigmine Bromide (Mestinon) NCT04343963	Phase 2	Cholinesterase inhibitor	AChE receptor	Diminishes T cell activation and proliferation, as well as increase the anti-inflammatory cytokine IL-10 and decrease the pro-inflammatory cytokine IFN-gamma
Simvastatin (Zocor) NCT04343001	Phase 3	Antihyperlipidemic agents	HMG-CoA reductase	Decreases IL levels by inhibiting MYD88 pathway
Atorvastatin (Lipitor) NCT04343001	Phase 3			

(Table 2) cont.....

Drug (Trade Name)/NCT No. [a]	Clinical Trial Phase	Class	Target	Mechanism of Action
Fingolimod (gilenya) NCT04280588	Phase 2	Immunomodulators	Sphingosine 1-phosphate receptor 5	Reduce lymphocyte circulation thereby overcoming inflammation
Tacrolimus (Prograf) NCT04341038	Phase 3	Immunosuppressant	Calcineurin	Inhibits viral replication and maturation by inhibiting T lymphocyte activation and proliferation in response to antigenic and cytokine.

[a] National Clinical trial (NCT) number retrieved from https://clinicaltrials.gov

ANTICANCER DRUGS IN THE TREATMENT OF COVID-19 DISEASE

The entry of the virus results in the activation of the innate and adaptive immune system to release numerous cytokines and chemokines, including IL-6. Activation of adaptive immunity results in the release of T- and B-cells for antiviral action and promote the activation of inflammatory cytokines. The release of these pro-inflammatory factors leads to an increase in vascular permeability and ultimately causes dyspnoea and even respiratory failure (Zhang *et al.*, 2020a). These activations of cytokines, including IL-6, are through the Janus kinase (JAK) signaling. The JAKs were encoded as the crucial facilitator of cytokines. From the uncountable research, it is clear that JAK-STAT signaling is one of the major causes of inflammatory responses (Liu *et al.*, 2020a). The potential use of JAK inhibitors for suppressing the hyperactivation of the immune system by targeting IL-6 and cytokines through JAK dependent signaling pathways is one of the many approaches to control the extreme level of cytokine signaling (Spinelli *et al.*, 2020). For the inflammation chiefly mediated *via* JAK pathways, JAK inhibitors such as siltuximab, ruxolitinib, baricitinib are found to possess a critical role in preventing elevated levels of cytokines (including interferon-γ), which significantly mediate signaling *via* JAK-STAT pathways(Stebbing *et al.*, 2020, Huang *et al.*, 2020). Ruxolitinib, apart from inhibition of JAK-STAT also inhibits interleukins, IFN-(α, β, γ), and growth factors (GM-CSF, TGF-β, erythropoietin, and thrombopoietin) (Virtanen *et al.*, 2019). Cardama *et al.* assessed JAK1 and JAK2 potential of ruxolitinib in cell and enzyme-based assays and testified their IC_{50} values 3.3 ± 1.2 and 2.8 ± 1.2 μM, respectively (Quintás-Cardama *et al.*, 2010). The drug has also shown good results in inhibition of phosphorylated STAT3 following stimulation with IL-6 (JAK1 prototype cytokine signal) or TPO (JAK2 cytokine signal) in whole blood assays with IC_{50} in nanomolar range (300 nM) (Quintás-Cardama *et al.*, 2010).

Further, Bruton's tyrosine kinase (BTK) inhibitors are analyzed for their capability to suppress the hyperactivation of cytokine release positively seen in COVID-19 patients. Treon and associates reported that the BTK inhibitors impart in the protection of lungs from various injuries in COVID-19 patients (Treon *et al.*, 2020). SARS-CoV-2 is coded with viroporins. These viroporins promote the immune signaling receptor NLRP3 through lysosomal disruption, which results in the release of cytokines (IL-1β and IL-6), tumor necrosis factor (TNF), triggering inflammation. Thus, the NLRP3 inhibitors were found to be effective and promising approaches in the treatment of COVID-19 (Shah, 2020). Table **3** summarizes the list of anticancer agents that are under investigation as COVID-19 therapeutics, whereas their chemical structures are compiled in Fig. (**4**).

Table 3. Anticancer agents in the treatment of COVID-19 disease.

Drug (Trade Name)/NCT No. [a]	Clinical Trial Phase	Class	Target	Mechanism of Action
Ruxolitinib (Jakafi) NCT04354714	Phase 2 (Withdrawn)	Anticancer	JAK-STAT pathway	Inhibits Janus associated kinases 1 and 2 and modulates their signaling pathways *via* reducing phosphorylation and activating STATs resulting in inhibition of cytokine and chemokine transcription Suppress the immune-hyperactivation.
Baricitinib (Olumiant) NCT04358614	Phase 2			
Selinexor (Xpovio) NCT04349098	Phase 2			
Acalabrutinib (Calquence) NCT04346199	Phase 2	BTK inhibitor Anticancer	BTK pathway	
Ibrutinib (Imbruvica) NCT04356690	Phase 2			
Nintedanib (Vargatef) NCT04338802	Phase 2	TNF-α and VEGR	EGFR	
Colchicine (Colcrys) NCT04322565	Phase 2	Anticancer	NALP3	Decreases IL-1beta activation and it blocks the inflammasome complex in both neutrophils and monocytes.
Thalidomide (Thalomid) NCT04273581	Phase 2	Anticancer	NLRP3 inflammasome	Decreases the synthesis of TNF-alpha.
Lenalidomide (Revlimid) NCT04361643	Phase 4	Anticancer	ubiquitin E3 ligase	Stimulates or regulate host cell immune.

[a] National Clinical trial (NCT) number retrieved from https://clinicaltrials.gov

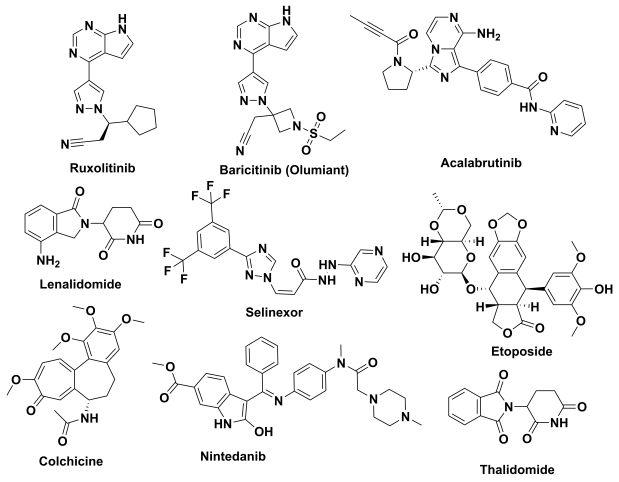


Fig. (4). Chemical structures for anticancer drugs repurposed for COVID-19 disease.

ANTIPARASITIC, ANTIPROTOZOAL, AND ANTIFUNGAL DRUGS IN THE TREATMENT OF COVID-19 DISEASE

Antiparasitic drugs such as nitazoxanide and niclosamide are among FDA-approved drugs that inhibit the expression of the viral N protein and consequently block viral replication. Nitazoxanide blocks TMEM16A ion channels, causing bronchodilation (Wang *et al.*, 2020a). It exhibits an antiviral mechanism by amplifying cytoplasmic RNA sensing and type IFN pathways and also upregulates host cellular defense mechanisms (Jasenosky *et al.*, 2019). Antimalarial drugs, including hydroxychloroquine (HCQ) and chloroquine, alter the pH of endosomes and inhibits entry of the virus into the host cell, thereby inhibiting endocytosis of the virus within the host cell (Devaux *et al.*, 2020, Olofsson *et al.*, 2005). Also, these drugs inhibit the addition of sialic acid moiety and the intracellular glycosylation of the ACE2 (trafficking), resulting in inhibition of virus fusion and its subsequent entry into the host cell (Savarino *et al.*, 2006). HCQ has also been explored in autoimmune diseases, including lupus erythematosus and rheumatoid arthritis, immunomodulator and viral diseases such as H1N1, Zika, hepatitis B, and HIV (D'Alessandro *et al.*, 2020, Browning, 2014). Recently, HCQ made its way back to the headlines in the middle of the COVID-19 disease (Downes *et al.*, 2020). USFDA issued guidelines for the emergency use of CQ/HCQ on March 28, 2020 (Food *et al.*, 2020). Findings from studies revealed HCQ to be more efficacious than CQ with *in vitro* IC_{50} of 5.47 μM. Further, there have been limited *in vivo* studies of HCQ to elucidate the mechanism. *In vivo,* clinical results were first disclosed in February 2020, by the Chinese government, where it was revealed significant improvement in pneumonia and lung imaging along with the reduction in the span of illness in

COVID-19 patients (Gao *et al.*, 2020). Another Chinese study involving 62 patients disclosed that HCQ could reduce the time of clinical recovery (Chen *et al.*). National Institute for the Infectious Diseases "L. Spallanzani" IRCCS in Italy published its recommendations for the use of HCQ (400 mg/day) for treatment of COVID-19 in combination with other antiviral drugs (Nicastri *et al.*, 2020). These encouraging results further assisted the Indian Council of Medical Research (ICMR) to recommend the use of HCQ for chemoprophylaxis on March 22, 2020. The guidelines recommended the use of HCQ by asymptomatic health workers (400 mg twice on day one, followed by 400 mg once every week for seven weeks) or household contacts of positive patients (400 mg twice on day 1, followed by 400 mg once every week for three weeks). However, few reports did not support the use of HCQ for treatment of COVID-19 disease. One of the reports by Ferner and Aronson disclosed disparity between laboratory and clinical experiments due to complex pharmacokinetics that would makes it difficult to extrapolate drug concentrations in culture media to human doses (Ferner *et al.*, 2020). Further, Molina and co-researchers emphasized on lack of evidence in support of the efficacy of a combination of HCQ and azithromycin in patients with severe COVID-19 infection (Molina *et al.*, 2020). The study by Lane and group on the use of HCQ alone or in combination evidenced an increase in QT interval prolongation leading to cardiovascular adverse events and death (Lane *et al.*, 2020). Another study by Mehra *et al.* (retracted) denied the beneficial effect of CQ/HCQ when used alone or in combination with a macrolide (Mehra *et al.*, 2020). However, the study was retracted owing to the shortcomings and expression of concern raised interrogating the accuracy of data and demanding a third-party review (Lancet, 2020). Considering these flaws, USFDA revoked authorized use of HCQ/CQ recently on June 15, 2020. Nevertheless, there is much debate on whether to use these drugs for COVID-19 or not.

Further, antifungal agents such as sirolimus act as an effective inhibitor of mTOR and have displayed significant outcome H1N1 pneumonia patients with acute respiratory failure (Wang *et al.*, 2014). Details of drugs are compiled in Table **4** and Fig. (**5**).

Table 4. Antiparasitic, antiprotozoal, and antifungal drugs in the treatment of COVID-19 disease.

Drug (Trade Name)/NCT No. [a]	Clinical Trial Phase	Class	Target	Mechanism of Action
Nitazoxanide (Alina) NCT04348409	Not Known	Antiprotozoal	viral transcription factor immediate-early 2 (IE2) and eukaryotic translation initiation factor 2α	Inhibit expression of the viral, N protein, and viral replication. Also blocks TMEM16A ion channels, causing bronchodilation.

(Table 4) cont.....

Drug (Trade Name)/NCT No. [a]	Clinical Trial Phase	Class	Target	Mechanism of Action
Ivermectin (stromectol) NCT04373824	Phase 2	Antiparasitic	IMPα/β1 heterodimer	It dissociates the IMPα/β1 heterodimer, and it is accountable for nuclear transport of viral protein.
Levamisole (ergamisol) NCT04360122	Phase 3	Antiprotozoal	Inhibits PL_{pro} of SARS-CoV-2	Decrease level of TNF α and IL-6 and virus shedding.
Sirolimus (Rapamune) NCT04371640	Phase 1	Antifungal	mTOR	Efficiently restrict the expression of viral protein and virion release.
Carrimycin (Bite) NCT04286503	Phase 4	Antibacterial	Ribosomal protein subunit of the host cell and Furin protein	Involved in the inhibition of viral fusion with the targeted membrane.
Azithromycin (Zithromax) NCT04359316	Phase 4			
Chloroquine phosphate (Aralen phosphate) NCT04344951	Phase 2	Antimalarial	Endosomes of host cells cellular receptors of SARS-CoV	Decreases the viral copy amount of the cell supernatant and viral infection.
Hydroxychloroquine sulfate (Plaquenil sulfate) NCT04303507	Withdrawn			Increasing the pH of endosome which inhibits the viral entry in the host cell.

[a] National Clinical trial (NCT) number retrieved from https://clinicaltrials.gov

DRUGS AFFECTING THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM IN COVID-19

The Renin-Angiotensin-Aldosterone System (RAAS) exhibits a crucial role in maintaining blood volume that affects the cardiac output. SARS-CoV-2 interacts with the RAAS due to ACE2, a protein that physiologically promotes the activation of RAAS. It is identified as a functional receptor for SARS-CoV-1 and 2 (Vaduganathan *et al.*, 2020). It is also responsible for viral entry for SARS-CoV-2. With the last shreds of evidence, it is clear that ACE2 has a prolonged function in the pathogenesis of SARS-CoV-2 (Patel *et al.*, 2020). The SARS-CoV-2 codes spike S protein-containing receptor-binding domain (RBD), which is attached with the host ACE2, and leads to membrane fusion and acceptances of the SARS-CoV-2 into target cells (lung) through endocytosis, which commands the host cells for the activation of protein synthesis mechanism for the synthesis of viral proteins and viral replication. As RAAS emerged as a central part of COVID-19, so hitting the essential component(s) of RAAS might be suitable for the treatment. In RAAS, renin (kidney) helps in cleaving angiotensinogen (liver)

to produce angiotensin I. Angiotensin I is further cleaved by ACE to give angiotensin II. The feasible pathway for COVID-19 therapy would be to hit the RAAS (Table **5**). Conventional pulmonary vasodilators such as an inhaled nitric oxide (iNO) (Alessandri *et al.*, 2018, Vickers, 2017, Khan *et al.*, 2009) reduce mean pulmonary artery pressure and enhance oxygenation in COVID-19 patients with acute respiratory distress syndrome (ARDS) (Åkerström *et al.*, 2005). Another category, such as vitamins, including β-carotene, retinol, and retinoic acid, is a group of fat-soluble compounds useful in the immune function system and lowers infection susceptibility (Huang *et al.*, 2018). Vitamin A and isotretinoin help in the down-regulation of ACE 2 (Sinha *et al.*, 2020).

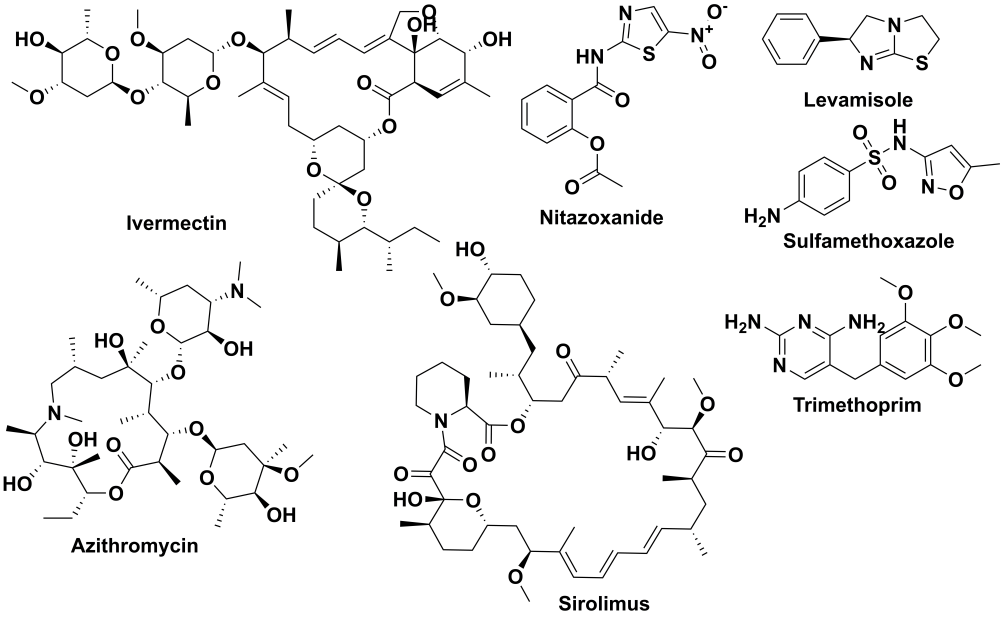


Fig. (5). Antiparasitic, antiprotozoal, antifungal, and antibacterial drugs in the treatment of COVID-19 disease.

Table 5. Drugs affecting renin-angiotensin-aldosterone system in COVID-19.

Drug (Trade Name)/NCT No. [a]	Clinical Trial Phase	Class	Target	Mechanism of Action
NORS (Nitric Oxide Releasing Solution) NCT04337918	Phase 2	Gasotransmitters	Endothelium-derived relaxing factor (EDRF)	Inhibits *S*-nitrosylation of viral proteins thus inhibiting viral protein and RNA synthesis.
Nitric oxide Gas (Inomax) NCT04338828	Phase 2			

[a] National Clinical trial (NCT) number retrieved from https://clinicaltrials.gov

MISCELLANEOUS

Endogenous protease plasmin of the host cell acts on the SARS-CoV-2 virus, thus cleaving a furin site in the S protein of the virus that results in viral infection (Mittal *et al.*, 2020). Patients with various diseases such as hypertension, diabetes, coronary artery disease, cerebrovascular illness, lung disease, and kidney dysfunction commonly have higher amounts of plasmin/plasminogen. Therefore, drugs that reduce the conversion of plasminogen to plasmin, such as tranexamic acid, might be useful (Paumgartten *et al.*, 2020). Chelating agents such as deferoxamine can be administered as an antidote for the management of chronic iron overload and acute iron toxicity. In systemic toxicity inversion, deferoxamine could remove extra iron from tissues and plasma (Yuen *et al.*, 2019) and act as a useful supportive agent in the management of the viral infection (Ghasemiyeh *et al.*).

Further, methylprednisolone an immunosuppressive drug that helps in the delay of pneumonia progression, has been found sufficient to overcome acute respiratory distress syndrome (ARDS) associated with COVID-19 disease. In the recent study, Wu *et al.* demonstrated a reduced risk of death (up to 62%) by administrating methylprednisolone in 201 participants with COVID-19 infection having severe ARDS conditions (Wu *et al.*, 2020, Veronese *et al.*, 2020, Huang *et al.*, 2020).

Further, memantine is used for the treatment of cognitive dysfunction, Parkinson, and alleviates fatigue and might be protective against COVID-19 by targeting the NMDA receptor (Rejdak *et al.*, 2020). Natural thyroid hormones block viral infection and affect the activation of p38 mitogen-activated protein kinase (MAPK). MAPK plays a crucial role in the replication of SARS-CoV-2. Also, it protects tissue from injury and shows antiapoptotic action with promising effects on the immune system and viral load in infected tissue (Velavan *et al.*, 2020, Paumgartten *et al.*, 2020). The phosphodiesterase-5 (PDE-5) inhibitor, such as sildenafil citrate, is a vasodilator used for treating erectile dysfunction (Scaglione *et al.*, 2017) has recently shown indication for pulmonary arterial hypertension (PAH) (Galiè *et al.*, 2005). Sildenafil may be useful in idiopathic pulmonary fibrosis (Rogosnitzky *et al.*, 2020) and renoprotective in interstitial lung disease (Webb *et al.*, 2015). It is currently under phase 3 clinical trial to treat patients with COVID-19 (NCT04304313). Another category, such as bronchodilators, including formoterol, is used in the management of chronic obstructive pulmonary disease (COPD) and asthma and also relaxes muscles in the airways to improve breathing (Arya *et al.*, 2020). A detailed list of various drugs with their mechanism is compiled in Table **6**, however, their chemical structures are collected in Fig. (**6**).

DISCUSSION AND CONCLUSION

There has been tremendous research going on to find a putative lead to treat COVID-19. To date, a total of 315 small molecules falling in the categories of antiviral, antimalarial, anticancer, anti-infective, anti-inflammatory, and many more are under consideration for the treatment of COVID-19 disease. Further, there have been many advancements in the development of robust *in vitro* and *in vivo* models to assess anti-SARS-CoV-2 drug candidates. The important *in vitro* models include human airway epithelial cells, vero E6 cells (both wild type and TMPRSS2 overexpressing), caco-2 cells, calu-3 cells, HEK293T cells, and Huh7 cells. Considering the *in vivo* studies, various animal models comprising mice, Syrian hamsters, ferrets, cats, cynomolgus macaques, rhesus macaques are being used for testing drug candidates (Li *et al.*, 2020b).

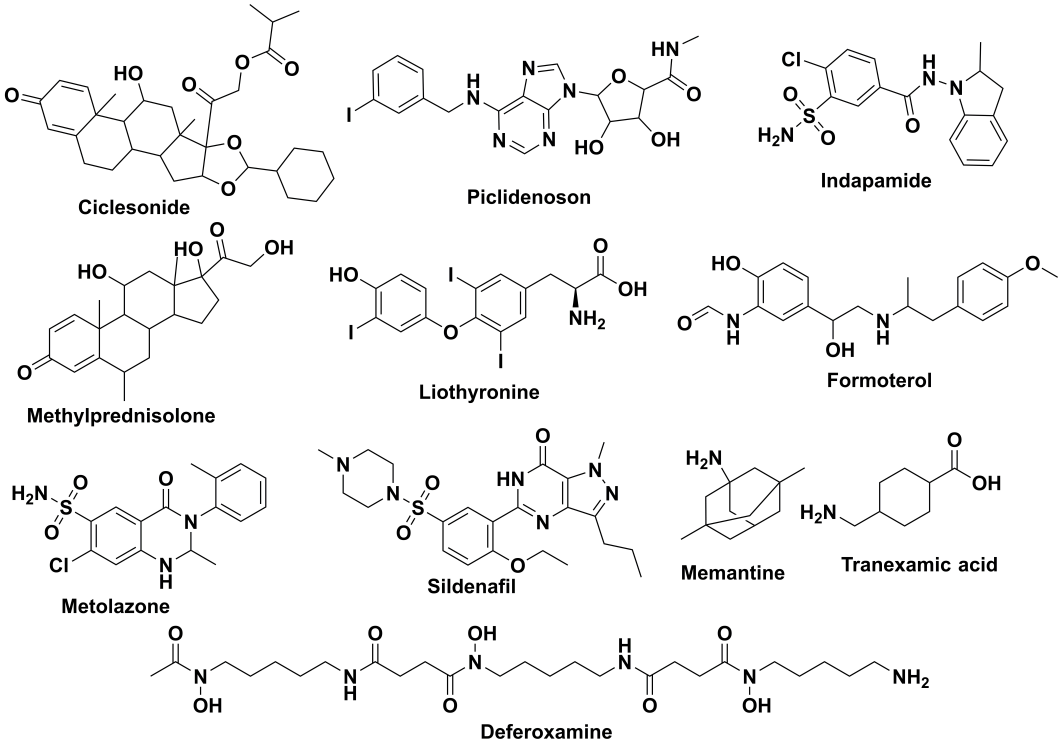


Fig. (6). Miscellaneous drugs used for the treatment of COVID-19.

Table 6. Miscellaneous drugs are being used for the management of COVID-19 disease.

Drug (Trade Name)/NCT no.[a]	Clinical Trial Phase	Class	Target	Mechanism of Action
Ciclesonide (Alvesco) NCT04381364	Phase 2	Anti-inflammatory	Nsp-15	Inhibit Nsp-15 thereby affecting viral replication.
Methylprednisolone (Medrol) NCT04374071	Study Completed	Anti-inflammatory, immunomodulator	NF-κB and TNF	Decreases circulating lymphocytes thereby inhibiting TNF-α expression and NF-κB by activating reformed gene expression *via* activating specific nuclear receptors
Sildenafil (Viagra) NCT04304313	Phase 3	Phosphodiesterase (PDE) inhibitors	PDE5	Blocks PDE5, thus restoring cGMP levels *via* the release of nitric oxide (NO), causing dilation of the blood vessels in the lungs
Formoterol (Atock) NCT04331470	Phase 2	Bronchodilators	β2-adrenergic agonist	reduces the activation of hypersensitivity mediators (*e.g.*, histamine, leukotrienes)
Liothyronine (Cytomel) NCT04348513	Phase 2	A synthetic derivative of a natural thyroid hormone	p38 MAPK activation	Improves the immune system and decrease viral load in infected tissue.
Tranexamic acid (Cyklokapron) NCT04338126	Phase 2	Antifibrinolytic	Plasminogen inhibitor	Inhibits plasmin, which acts on coronavirus and cleaves the newly inserted furin site at the S protein portion.
Memantine (Axura)	Not Known	Anti-Alzheimer	Cathepsin L	Inhibits cathepsin L, a lysosomal protease involved in SARS-CoV-2 entry to cells

[a] National Clinical trial (NCT) number retrieved from https://clinicaltrials.gov

Further, to overcome the ethical issues in *in vivo* drug monitoring, various 3D models are being developed. The important one includes organoid technology, which comprises human bronchial organoids, human lung organoids, human kidney organoids, human liver ductal organoids, human intestinal organoids, and human blood vessel organoids (Takayama, 2020, Jeon *et al.*, 2020, Touret *et al.*, 2020). Besides this, technologies such as bioprinting, single-cell analysis, and bio-fabrication are coming up to test drug candidates for COVID-19 disease.

As per data assessed for the clinical trial for COVID-19 (https://clinicaltrials.gov/) on July 7, 2020, at 14:00 Hrs IST), around 2427 clinical trials are reported. The data suggested (Fig. **7**) that among all the studies 25 are undergoing at preclinical phase, 141 in Phase 1, 539 in Phase 2, 327 in Phase 3, and 71 in Phase 4. Further, data also indicated that among all the studies, 182 had been completed, 1421 are either ongoing/recruiting/active, not recruiting/enrolling by invitation, whereas 41 have been either suspended/terminated or withdrawn, while remaining have unknown status.

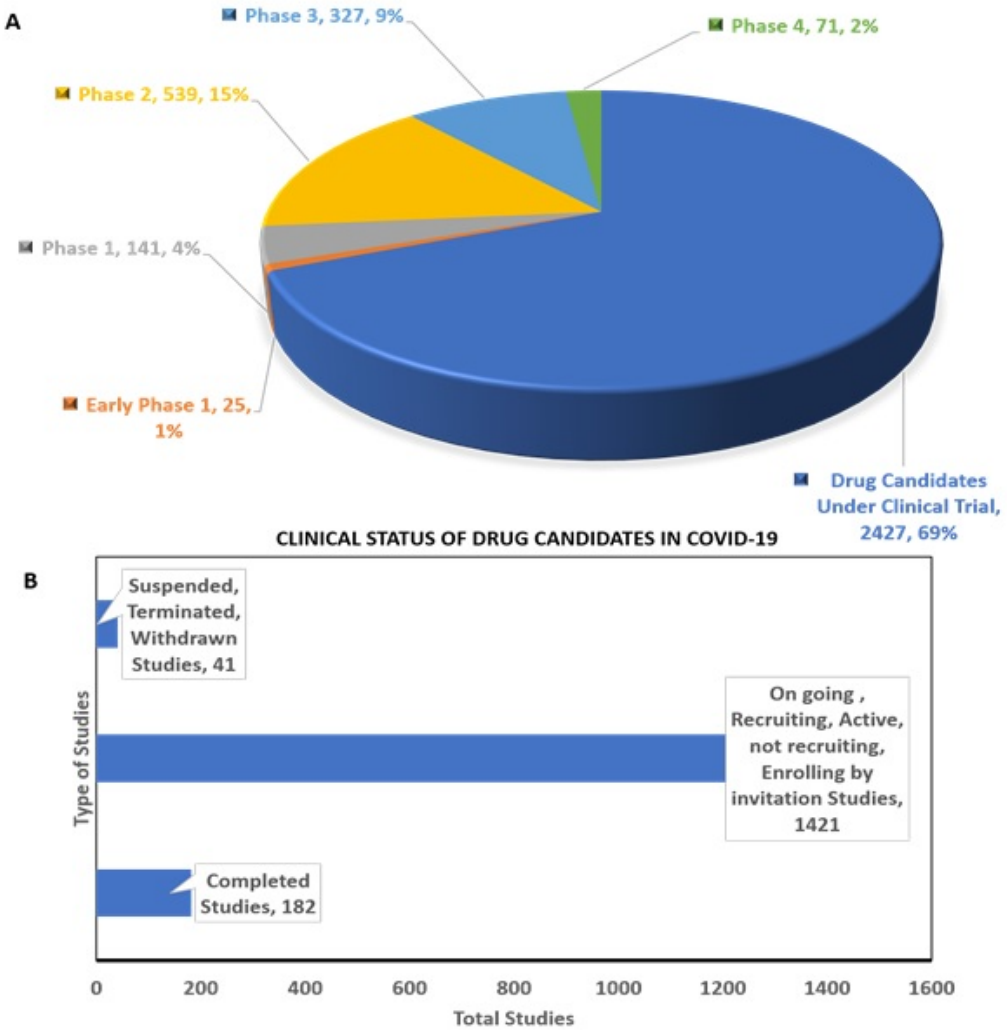


Fig. (7). (A) Pie-chart illustrates drug candidates in various stages of clinical trials for COVID-19 disease; **(B)** Bar graph represents drug candidates for COVID-19 signifying their clinical status.

Not only the small molecules but also vaccines or antibodies, natural products, and natural product derived chemicals have been actively tested for their potential in COVID-19 disease. The primary focus on the current scenario includes the use of these products/drugs to boost the immune system and provides preventative care in COVID-19 disease. Among various natural drug sources, ashwagandha has been found to boost immunity through modulation of host Th-1/Th-2. It may also be useful in inducing downregulation of TNFα, IL-1, IL-6, and other inflammatory mediators, which play a vital role in mediating cytokinin storm (Patwardhan *et al.*, 2020). Further, 17 organosulfur compounds from garlic have been reported to possess the inhibitory potential against host ACE2 protein and the main protease of SARS-CoV-2 (Thuy *et al.*, 2020). Other critical natural constituents like epicatechin gallate, quercetin, kaempferol, dimethoxy-curcumin, apigenin-7-glucoside, luteolin-7-glucoside, naringenin, catechin, allicin, oleuropein, curcumin, zingerone, and gingerol have been investigated as potent inhibitors of the Mpro, an essential protease possessing a role in SARS-CoV-2 translation (Khaerunnisa *et al.*, 2020). Additionally, Ayurveda Rasayana, such as *Asparagus racemosus* (Shatavari), *Phyllanthus emblica* (Amalaki), *Tinospora cordifolia* (Guduchi), *etc.* have immunomodulatory properties that may be beneficial against SARS-CoV-2 infection (Patwardhan *et al.*, 2020). Also, researchers and scientists involved in research on COVID-19 are exploring viral proteins to find some putative new leads or repurposed drugs through computer-aided drug design. Some of the important targets with their 3D structures are illustrated in Fig. (**8**). The thorough exploration of the active site of these proteins, including others involved in the SARS-CoV-2 life cycle, could be beneficial, leading to efficient drug design, and may speed up the quest to identify potent inhibitors shortly.

To summarize, SARS-CoV-2 was declared a pandemic by the WHO in 2020, leading to COVID-19 disease. Nevertheless, no drug or vaccine has been approved for the COVID-19 disease. Numerous clinical trials are undergoing in the quest to search for the best lead drug among the repurposed drugs. These drugs belong to diverse classes, which broadly include antiviral, anti-inflammatory, anticancer, antiparasitic, antiprotozoal, antifungal, antibacterial, RAAS-ACE-ARBs inhibitors, and natural products, along with other vital classes. These drugs importantly target the virus life cycle at different stages, and some are used as immunomodulators for developed cytokine storm with the progression of COVID-19 disease. As of now, most of the drugs in trials have exhibited good potential in a limited observational or non-randomized clinical study. The potential benefits are still awaited to come in the form of the first approved drug marking its presence for COVID-19 disease; the only remdesivir is gaining emergency authorization use to date. The authors believe that a multi-targeted approach to inhibit multiple targets involved in the virus life cycle using a

combination of repurposed/new drugs explored through rational drug design would lead to significant results than monotherapy alone.

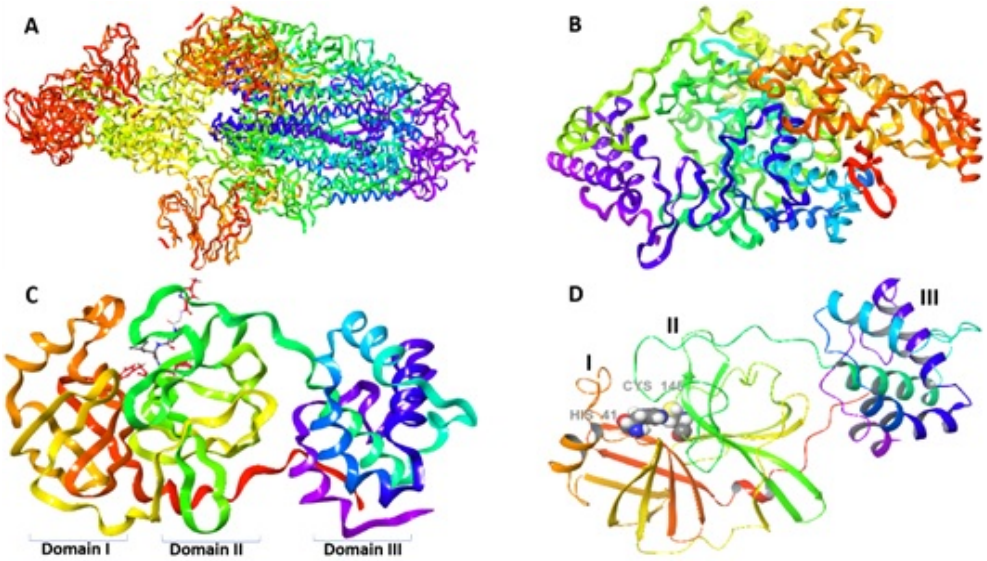


Fig. (8). Three-dimensional representation of SARS-CoV-2 molecular machines. **(A)** Ribbon diagram of the S protein (PDB ID: 6NB6). **(B)** Structural diagram of RdRp (Nsp12) (PDB ID: 6M71). **(C)** Illustration of 3Clpro in complex with its inhibitor N3 (PDB ID: 6LU7). **(D)** Three-dimensional structure of 3Cl pro showing amino acid residues CYS 145 and HIS 41 of the catalytic site and the domains I, II & III (PDB ID: 2BX4).

ABBREVIATIONS

ACE-RAAS –	Angiotension Converting Enzyme-Renin-angiotensin-aldosterone
ACE2-	Angiotensin-converting enzyme 2
CGRP –	Calcitonin gene-related peptide
COPD –	Chronic obstructive pulmonary disease
CPZ –	Chlorpromazine
HCQ –	Hydroxychloroquine
IL-6 –	Interleukin 6
$_i$NO	inhalational Nitric oxide
MAPK-	Mitogen-activated protein kinase
JAK-STAT	Janus Kinase/Signal Transducer and Activator of Transcription
MERS-CoV –	The Middle East respiratory syndrome coronavirus
mTOR -	Mammalian target of rapamycin
NK-1 -	Neurokinin-1
NK-	Natural killer

PAH-	Pulmonary arterial hypertension
PDE-5-	Phosphodiesterase-5
RAAS-	Renin-angiotensin-aldosterone system
RBD-	Receptor-binding domain
RdRp -	RNA-dependent RNA polymerase
S1P -	Sphingosine 1-phosphate receptor
SARS-CoV-2-	Severe Acute Respiratory Syndrome Coronavirus 2
SGLT2 -	Sodium-glucose cotransporter-2
TNF-	Tumor necrosis factor

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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