Review Article

Sumaira Naz, Muhammad Zahoor*, Muhammad Umar Khayam Sahibzada, Riaz Ullah*, Ali S. Alqahtani

COVID-19 and SARS-CoV-2: Everything we know so far – A comprehensive review

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Abstract: Coronavirus disease-2019 (COVID-19) emerged as a unique type of pneumonia outbreak in the Wuhan city of China in 2019 and spread to all its provinces in a matter of days and then to every continent of the world except Antarctica within 3–4 month. This paper aims to comprehensively consolidate the available information about COVID-19 and present all the possible information about this disease in form of a single paper to readers. Unparalleled research and exhaustive studies of everything about the disease and its causative virus, i.e., severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), are underway since its emergence. The genome sequence of the virus was made available within a record short time by China, making possible immediate study of its structure and characteristics. The routes of transmission of the disease, signs and symptoms, incubation period, pathogenesis, and pathophysiology have been extensively studied and presented in an organized way in this review paper. The number of confirmed cases and case fatality and mortality rates are updated regularly. The different diagnostic mechanisms have been characterized. Testing and management criteria and protocols have been adopted. Extensive efforts are underway for finding a treatment of the disease and developing a vaccine against it. A number of vaccines are available even in markets in different countries. More and more ways of personal protection, prevention, and mitigation of the disease are being explored and shared. While the outbreak has been declared as pandemic, the response of scientists was timely and enormous; thousands of publications about various aspects and impact of the diseases and its causative virus are there on the World Health Organization database and many more studies are underway. The purpose of writing this review article is to provide a comprehensive summary of the major aspects and important scientific findings so far, about COVID-19 and SARS-CoV-2, in a single article for ready reference.

Keywords: COVID-19, SARS-CoV-2 genome, epidemiology, risk factors and transmission of COVID-19, pathogenesis and clinical features of COVID-19, diagnosis, treatment, and vaccine of COVID-19, prevention and mitigation of COVID-19, reinfection with COVID-19

1 Introduction

Plagues and epidemics have been one of the greatest challenges to the survival of the human race through the course of history. Due to the factors like rapid growth in population and emerging of advanced means of communication and transportation across the globe, the humankind has become more susceptible to epidemic diseases. That's why the epidemics which took months and years to transfer from the infected area to other parts of the world now reach in every nook and corner of the globe in a matter of days or weeks. In recent history, some new epidemics appeared and took many precious lives like the Spanish Flu in 1918, Severe Acute Respiratory Syndrome (SARS) in 2002, Ebola in 2014, and Middle East Respiratory Syndrome (MERS) in 2015 [1]. The dawn of 2020 saw an outbreak of a new super spreading disease named COVID-19 (Coronavirus disease 2019) and the causative agent was identified as a novel coronavirus named initially as 2019 novel coronavirus (2019-nCoV) and later

* **Corresponding author: Muhammad Zahoor,** Department of Biochemistry, University of Malakand, Chakdara, Dir Lower, 18800, KPK, Pakistan, e-mail: mohammadzahoorus@yahoo.com
* **Corresponding author: Riaz Ullah,** Department of Pharmacognosy (MAPPRC), College of Pharmacy, King Saud University, PO Box 2457, Riyadh 11451, Saudi Arabia, e-mail: rullah@ksu.edu.sa
Sumaira Naz: Department of Biochemistry, University of Malakand, Chakdara, Dir Lower, 18800, KPK, Pakistan
Muhammad Umar Khayam Sahibzada: Department of Pharmacy, Sarhad University of Science and Information Technology, Peshawar, 25000, KPK, Pakistan
Ali S. Alqahtani: Department of Pharmacognosy (MAPPRC), College of Pharmacy, King Saud University, PO Box 2457, Riyadh 11451, Saudi Arabia

on as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The disease emerged as a novel pneumonia surfaced in Wuhan city of Hubei province, China, in December 2019 and spread not only to all the provinces of China, but to all continents of the world except Antarctica in only 3–4 months since its first reported case. The WHO declared the disease as a pandemic (a global epidemic) on March 11, 2020 [2–5]. As the new virus strengthens its hold over the globe with every emerging confirmed case of the disease, extensive research was started worldwide by the scientists to study every aspect of the virus and the disease to help design diagnostic tools, evolve strategies to combat or at least contain the disease, and finally come up with a treatment. The information about COVID-19 and the causative virus are available everywhere on the internet in form of the research articles, multiple websites, news, media, etc. Several reviews have been published, each addressing a particular aspect of COVID-19. A need was strongly felt for such an article that encompasses all the necessary elements on the topic, properly citing the scientific findings and studies that are so far carried out in each area regarding the recent pandemic. In our review article, we have tried our best to present an overview of the published data about almost all the main features of COVID-19. We have added new insights and recommendations suggested by the researchers and have tried to point out what are the next challenges to be faced by scientific community. Apart from it, there is a ready reference material for the researchers where they would find answer to most of their queries in one place. The general readers will also benefit from having a compendium of useful and authentic information presented in the simplest possible manner.

2 The virus-SARS-CoV-2

The causative virus of COVID-19, i.e., SARS-CoV-2, was identified as a novel member of a previously well-known subfamily of viruses coronavirinae of the family Coronaviruses (CoVs; corona – viridae). The family, in turn, belongs to the super family – Nidovirus (Nidovirales). The coronavirinae subfamily is further divided into three genera, viz., alpha, beta, and gamma [6]. The causative virus of COVID-19 and that of SARS both belong to beta coronaviruses, the causative virus of the latter was named as SARS-CoV and because of the striking similarity (at least 86%) between the genomes of both, routes of transmission, and symptoms of the disease between the two, the former was named as SARS-CoV-2 [7]. So far, out of all the three genera of the CoVs, the pathogenesis of only the alpha and beta CoV is known. SARS-CoV-2 is the seventh known pathogenic CoV, out of which three have caused severe epidemics, i.e., SARS, MERS, and now COVID-19 [8].

2.1 Genome and the virion

Figure 1 shows the structure of SARS-CoV-2. It is an enveloped, positive-sense RNA virus with a quite large RNA genome-a characteristic common to all corona viruses. The genome is arranged in such a manner that the replicase locus is encoded within the 5′ end (which encodes replicase gene), whereas structural proteins are encoded at the 3′ end. The structural proteins are present in the following order: (i) spike (S), (ii) a small membrane (E), (iii) membrane (M), and (iv) nucleocapsid and internal protein (I). The I is responsible for complexing with the genome RNA, making a helical capsid that is present in the viral envelope. Trimeric transmembrane peplomers (the spike) protruding from the envelope give crown-like shape to the virion and hence the name “coronaviruses” to SARS-CoV-2 and other viruses of the same family have been adapted internationally [9].

2.2 The spike ‘S’-tool for the entry of the virus into the host cell

The spike is mainly responsible for the attachment of the virus with its receptor that has been reported to be angiotensin-converting enzyme 2 (ACE2) – the same receptor that is already been reported for SARS-CoV [10]. The S protein (sized 180–200 kDa) has three important components: N-terminus, a transmembrane domain, and a C-terminal segment [11]. Upon interaction of the virus

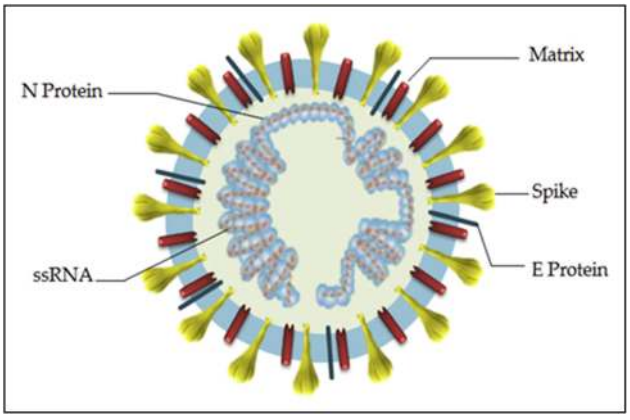


Figure 1: Structure of SARS-CoV-2 [9].

with the host cell, there occur changes in the S protein which help in fusion of the virus with the host cell membrane. Figure 2 represents structure of S and its binding to the receptor [12].

The S has 2 subunits, S1 and S2; the latter is cleaved at the border between the two subunits in such a way that both are present in a non-covalently bound form before the entry of the virion into the host cell. S1 subunit has N-terminus and a receptor-binding domain (RBD); S2 subunit which is responsible for fusion with the host cell membrane has a fusion peptide, heptapeptide repeat sequence 1 and 2 (HR 1, HR 2), a cytoplasm domain, and a transmembrane domain. Another so-called S2′ site is also present directly after S2, which is cleaved by the host proteases and results in conformational changes and the subsequent activation of the S2 for fusion with host membrane [9,13,14]. Hence, the entry of CoVs to the host cell requires both specific receptor-binding capability and subsequent proteolytic cleavage of the respective subunit (S2′) [10]. Moreover, at the junction of S1 and S2 subunits, a polybasic sequence with a leading proline is present. This sequence is actually the site of recognition for furin and other proteases. The presence of polybasic sequence is a feature that is unique to SARS-CoV-2 and is previously unknown in any of B subgroup β-CoVs (BB coronaviruses). This sequence is believed to have a role in determining the specific host for the virus and may contribute to its pathogenicity and transmissibility. Another important feature of the S is that it is lined with N-glycans that are required to neutralize antibodies (Abs) and interact with the host proteases. The cryo-EM structures of S have been reported which would be helpful in designing vaccines [9].

3 Origin of SARS-CoV-2

3.1 Is the virus lab-originated?

Though nothing could be asserted with ultimate finality at this stage as yet, most of the scientists are in the view that the virus is not lab-originated. In this connection, a strong evidence is the Anderson and his colleagues' article [15] in which they have concluded that it is very unlikely that SARS-CoV or related corona viruses would have been used for the bioengineering of SARS-CoV-2. Their conclusion is also supported by other studies as described below:

(i) The RBD sequence of the S of SARS-CoV-2 is not theoretically ideal for attachment with the human ACE2 (hACE2) as explained in the reported computational studies [15,16]. Understandably, if a virus

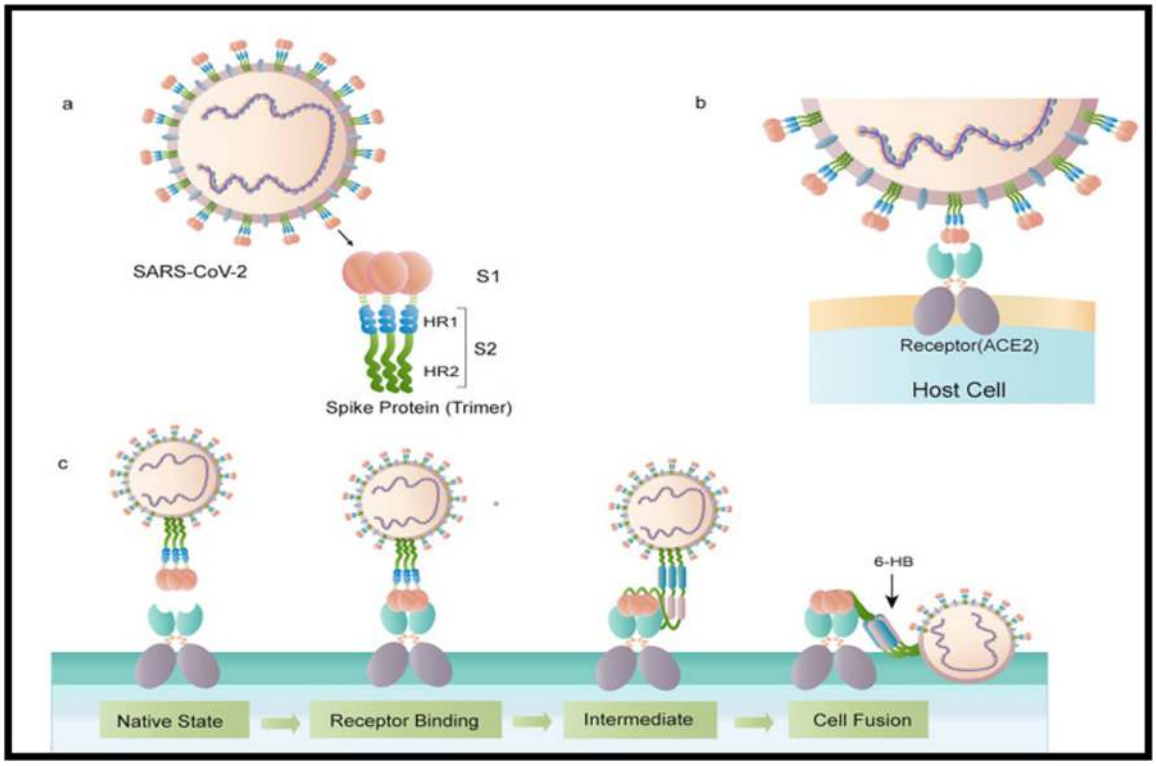


Figure 2: Structure of S and its pattern of binding to the receptor and fusion with host cell membrane. (a) The schematic structure of the S protein. (b) The S protein binds to the receptor ACE2. (c) The binding and virus–cell fusion process mediated by the S protein [9].

destined to be genetically engineered as a bioweapon, it should have optimal-binding capability with its receptor. In reality, S of the virus has been observed to have much higher affinity for its receptor than theoretically predicted. This observed high-affinity binding of SARS-CoV-2 to hACE2 can be explained hypothetically as the virus naturally select the hACE2 for attachment and the attachment further making conditions ideal for its optimal binding with the mentioned receptor [15].

(ii) To bioengineer a virus, the easy way is to borrow a genetic skeleton of a previously known/encountered pathogenic virus; however, the genome of the SARS-CoV-2 as analyzed by multiple scientists is natural that is originated wildly and has quite a similar genome to that of coronaviruses that are normally present in bats and pangolins from ages [15–24].

(iii) The sheer intelligence of the virus for acquiring the degree of natural selection to select hACE2 with more specificity than the same receptor present in other species, attaching to it, and then creating an optimal-binding environment for its entry into the cells is something that seems to be the result of mutations that have happened through the course of years and not possible through bioengineering at least with the current resources and technologies [15,17,25,26].

Another controversial theory postulated by someone is that this virus has escaped from a biosafety level-2 lab where such types of highly pathogenic microorganisms and viruses are kept for the sake of research [10]. The theory is supported by referring to the already reported cases when SARS-CoV was escaped [27]. Escaping from the lab can be ruled out by the fact that the unique and never before reported features in the virus, i.e., specific RBD sequence and the polybasic cleavage site, could not be achieved by cell culture passages that would at least require an ancestor SARS-CoV-2 and repeated prolonged passages in cell culture. Moreover, the specific predicted O-glycan could not have been produced in cell culture as its production is induced by immune response [28].

3.2 Who is the intermediate reservoir host; bat or pangolin?

The very close resemblance of the genome of SARS-CoV-2 with SARS-CoV and SARS related coronaviruses, SARSr-CoVs, suggests that bats might have played a role as reservoir host for SARS-CoV-2 [29]. However, even with the very close resemblance, the RBD sequence of the S is still different in SARS-CoV-2 [30]. Likewise, CoVs from pangolin specie *Manis javanica* have also been reported [15,25] to have analogous genome to SARS-CoV-2, and even in some of them, the RBD sequence too is exactly like that of the S in SARS-CoV-2. However, none of the BB CoVs from either bat or pangolin has so far been reported to have the same polybasic cleavage site as is present in the border of S1 and S2 subunits [31]. As the diversity in BB CoV species is very high and a majority of them are yet to be discovered/studied, it is expected that in future we may encounter with such BB CoVs that have a similar cleavage site [15]. Moreover, mutations in the S1–S2 subunit junction are quite possible and expected, so it can be assumed that this virus has acquired the unique polybasic furin cleavage site and its current form of S in either an animal host [32]. The second possibility is that its ancestor may have been transferred to humans in some other form long before assuming the current pandemic form through adaptation and mutations. In the latter case, the mutations, especially acquiring the cleavage site, may have occurred initially in human-to-human transmission stages [15]. Scientists still not have arrived to a definite conclusion whether the immediate origin of SARS-CoV-2 is pangolin or bat for which extensive animal studies are required to establish the origin and intermediate host through which transmission of the virus has taken place [33].

Owing to the extensive genetic diversity, widespread distribution, and the high rate of mutations in their genome, the family of the viruses poses a continuous serious threat to human health and even survival. SARS was the result of zoonotic transfer of the SARS-CoV from bat to civet cat and then to humans and MERS was a result of zoonotic transfer of MERS-CoV from dromedary camels to human. Keeping in view that these two epidemics were caused by CoVs, the zoonotic transfer of SARS-CoV-2 to humans is not much surprising – an event more likely to happen in the future as well [21,34].

4 Epidemiology

4.1 Geographic distribution

The outbreak started in the Wuhan city of China and spread to all its provinces affecting a total of 83,014 people with 3,343 recorded deaths. On 19th of March

2020, China announced that there is no new local case reported since last 36 h, although the imported cases are still emerging [35,36]. Next on the list was Italy with 152,271 confirmed cases and 19,468 deaths and Iran with 70,029 confirmed cases and 4,357 deaths where the epidemic is slowing down although yet continued. Next badly hit in Europe was Spain, where the number of confirmed cases was 161,852 with 16,480 deaths, surpassing Italy. The pandemic continued spreading swiftly around the world, and as on 12 April 2020, it had affected 210 countries and territories across the globe. The US has started experiencing a dramatic surge in the number of confirmed cases since late March and on 12th April 2020, it was the country with the highest number of confirmed cases (519,453) and deaths (20,283). On 22nd October, 2020, the number of total confirmed cases was 41,696,520 and the total number of recorded deaths was 1,137,204 worldwide (All the number of confirmed cases and deaths have been taken from the coronavirus resource center map at Johns Hopkins University and Medicine) [37]. A chart representing continent-wise distribution of COVID-19 cases is represented Figure 3.

These numbers were high from the mentioned cases as the number of reported confirmed cases globally as well as country-wise was affected by several factors like how many tests have been done both globally and in specific countries, the population of a country, selection bias (individuals with severe symptoms are tested preferentially), asymptomatic infected individuals, and false negatives. Globally, the cases have been reduced to minimum in late October 2020; however, with start of winter season in many countries, the number of infected people is on rise these days.

4.2 Case fatality rate (CFR)

The CFR is calculated as the number of reported deaths/numbers of reported confirmed cases. It is highly variable among various countries and quite unpredictable in the current scenario. As on 09th April 2020, the Centre for Evidence-Based Medicine estimated the CFR of each country, from the lowest 0.08% in New Zealand to the highest 13.04% in Algeria. However, the CFR of COVID-19 is affected by many factors like location, intensity of transmission, and age (older than 60 years are more at risk) of the population in the given location along with the incidence of comorbidities and poorer health care. Besides, a large number of asymptomatic undiagnosed cases are expected to be present which are therefore not reported, and hence, not included in the determination of CFR reported [37]. Although the pandemic has taken a lot of lives, still the CFR is lower than that reported for SARS (10%) and MERS (34%) [39]. The case fatality ratio and deaths per 100,000 population worldwide is graphically represented in Figures 4 and 5, respectively.

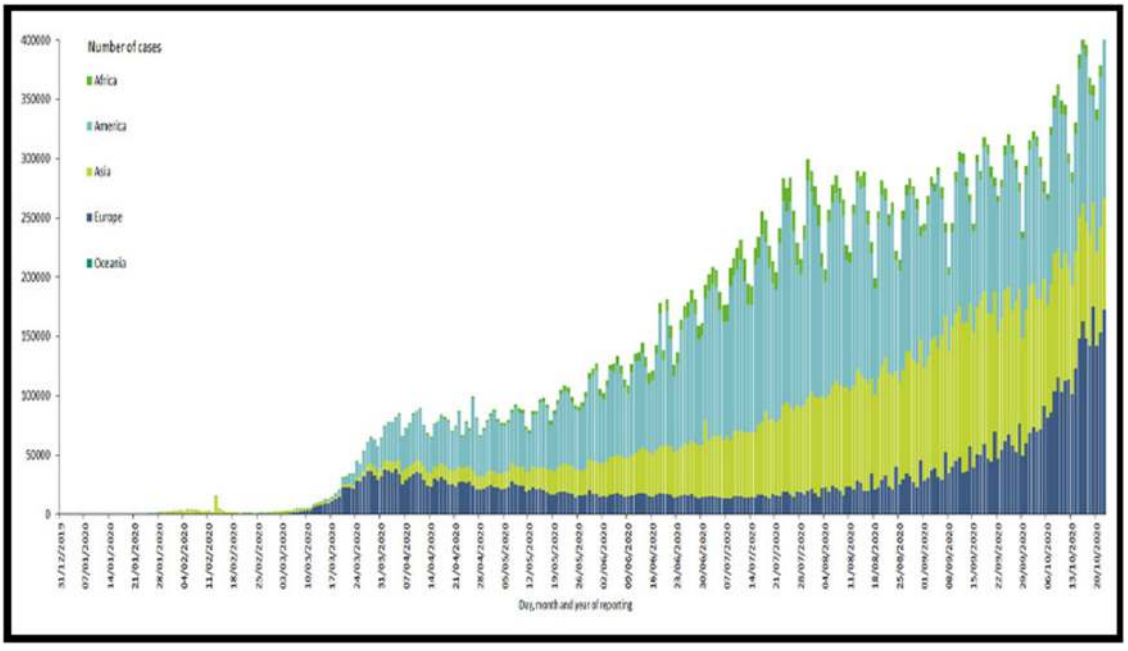


Figure 3: Continent-wise distribution of COVID-19 cases [38].

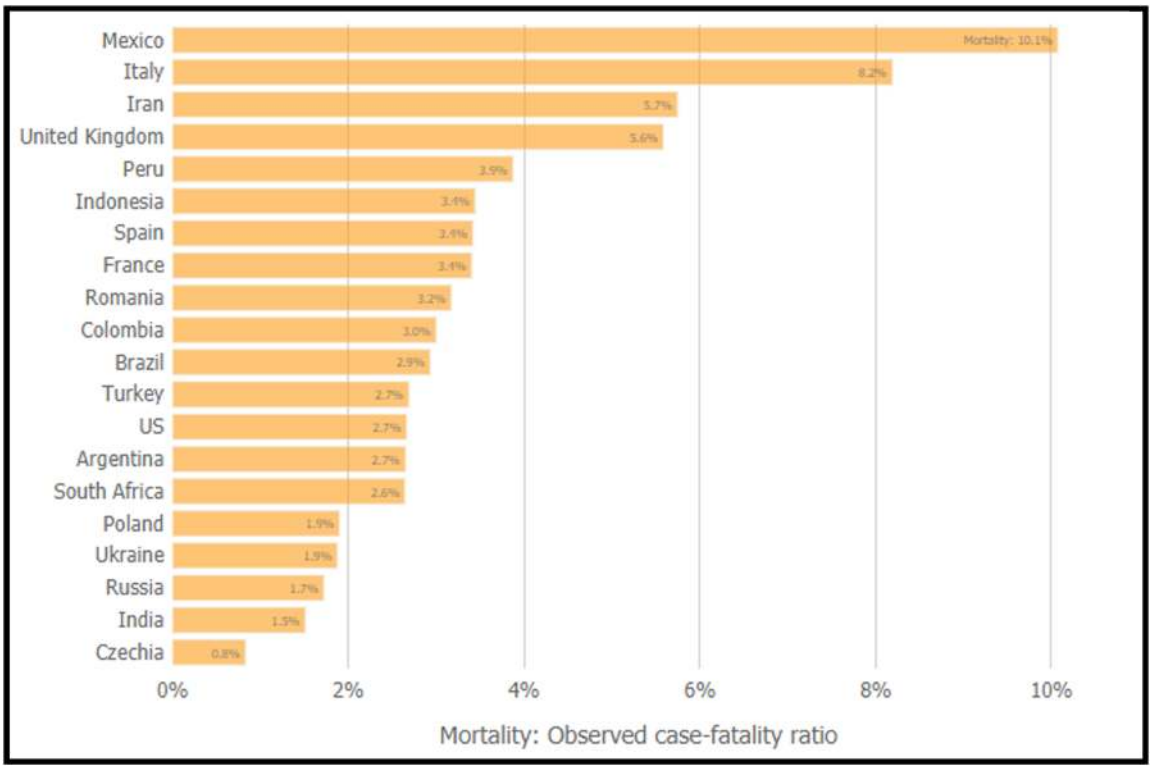


Figure 4: Global case fatality ratio, as of 22 October 2020 [36].

4.3 Mortality rate

Initially, the WHO estimated the mortality rate up to 2%, but on 3rd March 2020, the organization declared the overall approximate mortality rate is 3.4%. Mortality rate is observed to be higher in individuals with older age, from zero for children under nine and 14.8% in patients aged 80 years or above [39].

4.4 Transmission

So far, extensive research has been carried out by the scientists and they have thoroughly investigated the route of transmission of the virus, its mode, and rate of its spread so that it is easy to decide who the potential transmitter is and how it is transmitted. Human-to-human transmission can occur through any of these

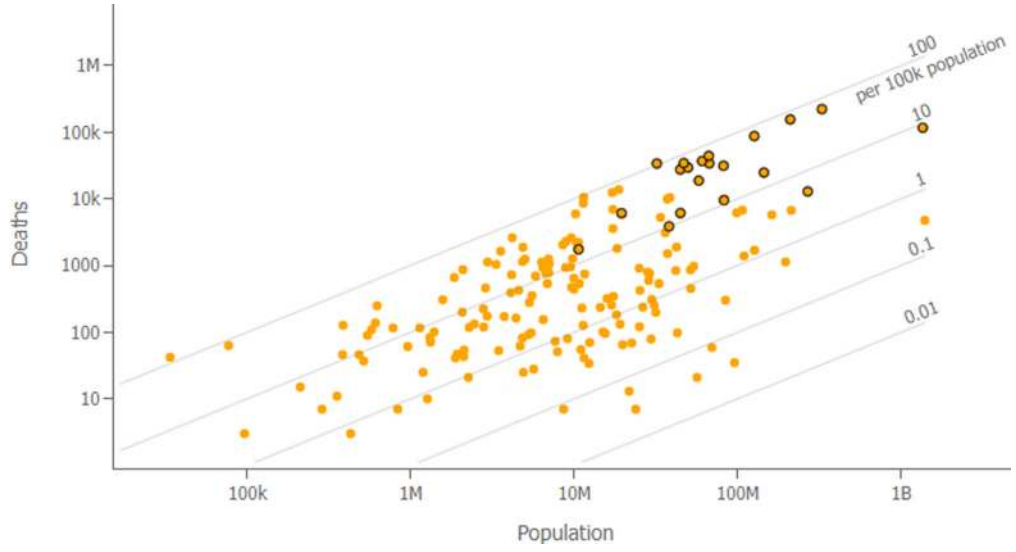


Figure 5: Global deaths per 100,000 population as of 22 October 2020 [36].

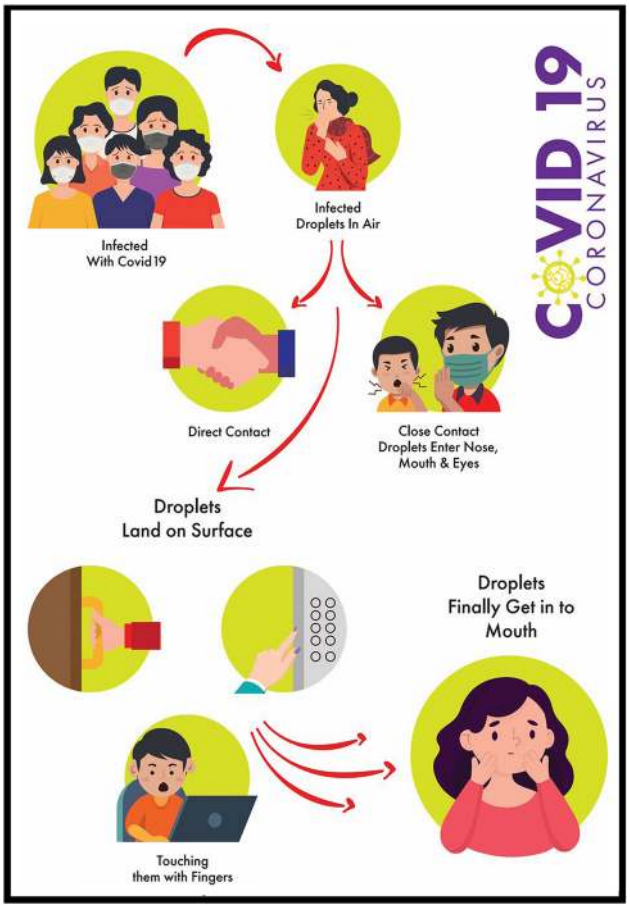


Figure 6: Modes of spread of COVID-19 [40].

routes: direct transmission, contact transmission and airborne transmissions through aerosols, and may also be through medical procedures (Figure 6). Cough, sneeze, droplet inhalation, and contact with oral, nasal, and eye mucous membranes are the common causes of its spread. Viral shedding occurs from respiratory tract, saliva, feces, and urine [40,41].

4.5 Factors affecting the transmission

The impact of the pandemic is highly dependent upon various transmission factors which are discussed in the following lines.

(i) The basic reproductive number, i.e., R_o value of SARS-CoV-2, which reflects the degree of contagiousness of a virus, is estimated to be 2–4 (median value 2.76) which means that an infected person could on average transmit the infection further to 2.76 persons [42]. The median SI (series interval) has been reported to be 4.6 which means that, on average, the infected person may transmit the virus after 4.6 days of being infected. In other words, the infected person may transfer the disease to other people before the incubation period and onset of symptoms of the disease. The R_o value is considerably affected by certain other factors, both negatively and positively. For instance, poor implementation of countermeasures like isolation, quarantine, and social distancing, the limited number of populations tested, and delayed diagnosis could add up to the value of R_o. Moreover, effective sanitization and disinfection measures, social distancing, and isolating patients and suspects could efficiently reduce the value of R_o in a given area at a given time. However, whether these measures could sustainably reduce the value of Ro is still uncertain [43,44].

(ii) According to China CDC, this is super spreading (highly contagious) disease associated with many super spreading events (SSEs). The SSEs have significantly contributed to a steady surge in the number of infected cases and sustaining the epidemic. Besides, the more symptomatic patients are carrying more oral and laryngeal load of the infection and are the superspreader of the disease [45].

(iii) The stability of the SARS-CoV-2 in transmission media is also important. A single cough by an infected person can produce thousands of respiratory droplets, of which the smaller, lighter ones remain in air in the form of aerosols and the bigger, heavier ones can land on clothes and other surfaces coming in their way like doorknobs, worktops, floors, bedsheets, etc. Different studies suggest different viability period for the virus on various surfaces, for example, according to the reported studies [46,47], the virus is viable up to 2–3 days on surfaces like stainless steel and plastic, approximately 24 h on cardboard, up to 4 h on copper, and can remain viable in aerosols for about 3 h.

(iv) Environmental and behavioral factors also decide the degree of transmission. Dense population and confined settings, e.g., health care facilities, elevators, mass gatherings, event halls, nursing homes, etc., fuel the super spreading of SARS-CoV-2. Another important transmission route is fecal-oral transmission [48]. Thus, poor hygienic habits are a big factor in transmitting the infection, while observing healthy hygienic measures is the key to prevention. The WHO and the US EPA (Environmental Protection Agency) have reported a list of disinfectants, sanitizers, and their active ingredients for public use which could be utilized safely to reduce the chances of getting infected [49].

4.6 Routes of transmission

The disease is transmitted through various respiratory and extra respiratory routes. Some of the known and more important ways of transmission are:

(i) Direct contact with the infected person, i.e., person to person transmission (main mode);
(ii) Respiratory droplets;
(iii) Oral-fecal transfer;
(iv) Infected blood and body fluids;
(v) Touching contaminated surfaces;
(vi) Viral aerosols in a given confined space;
(vii) Sewage waste, air condition systems, and contaminated water/food; and
(viii) Transfer from asymptomatic and presymptomatic infected persons as asymptomatic shedding [44,46,48–50].

Recently, a study investigating air samples of the hospital wards including Intensive Care Unit (ICU) and general ward reported that the virus can travel up to four meters (13 feet) from the source (infected patients). The investigation also reports that a high concentration of the virus can be found on floors of the ward, on bed rails, computer mouse, and other regularly touched surfaces as well as on the shoe soles of the ICU staff [51].

Continued transmission of SARS-CoV-2 suggests that tertiary and quaternary transmission routes would also be there. Now, a question arises: as compared to the primary and secondary transmission of the SARS-CoV-2, during which it was transmitted from an animal to human and then from human to human, have the rate of transmission and severity of pathogenicity of SARS-CoV-2 been affected (increased/decreased) with the continued transmission and after multiple passages in human or not? [43]. To answer this question, further investigational studies are required.

5 Clinical features

According to China CDC, the range of clinical manifestations can be broadly divided into three categories:

– First: Many of the infected individuals (81%) may remain asymptomatic with no pneumonia or mildly symptomatic having minor pneumonia. In case of mild to moderate pneumonia, the patients may experience fever (99%), fatigue (70%), dry cough (59%), sputum production (27%), and other typical symptoms like sore throat, headache, and body aches or shortness of breath that are observed in other respiratory tract viral infections as well.
– Second: About 14% patients may show severe clinical symptoms like dyspnea, hypoxia, and respiratory distress that may require mechanical ventilation.
– Third: A third class with lesser proportion of the patients (5%) who may develop severe pneumonia, respiratory failure, and from mild to severe acute respiratory distress syndrome (ARDS) followed by sepsis, septic shock, and multiorgan dysfunction syndrome [52].

Severity and duration of onset of the symptoms, however, depend upon the age of the patient, comorbidities, and the immune response of the body [4]. The overall symptoms (systemic and respiratory disorders) and complications related to the COVID-19 have been shown in the Figure 7 [53].

6 Risk factors

Although no one is at zero risk of getting COVID-19 or invulnerable to it, getting sick to different levels, chances of getting hospitalized, and mortality rate depend upon certain risk factors.

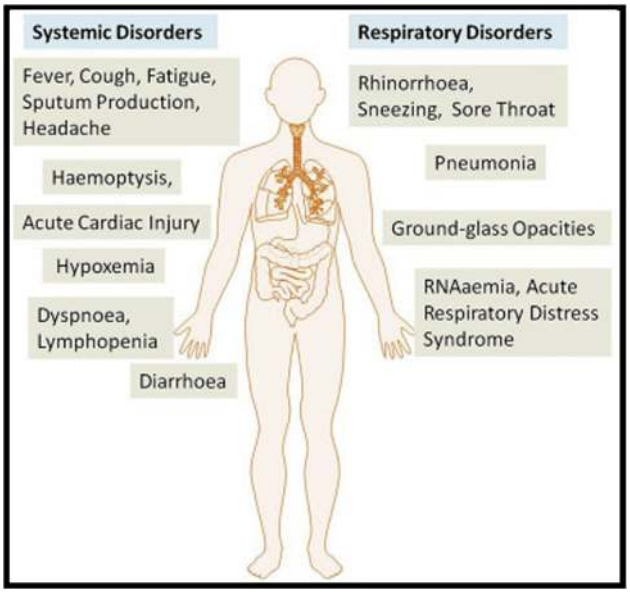


Figure 7: The systemic and respiratory disorders caused by COVID-19 infection [53].

6.1 Age

According to the reports by the WHO and China CDC, the overall rate of getting infected and developing symptoms is directly proportional to the increased age. In a study by China CDC, 87% of the cases reported were in the age group 30–87 years [52,54]. Another study has revealed that a major portion of the hospitalized patients were adults with median age in the range from 49 to 56 years [55]. US CDC has recently released its report on the survey of total hospitalized patients in the US (admitted in March 2020). The report concludes that the increase in the age had a direct effect on the rate of hospitalization; in the age group 0–4 and 5–17 years, it was recorded 0.3 and 0.1%, respectively, the rate increased to 2.5% for the age group 18–49 years, 7.4% in the age group 50–64 years, and much higher, i.e., 13.8%, in the aged individuals (65–74 years). The highest hospitalization rate (17.2%) was observed for individuals of age 85 years or above [56]. Also, CFR is observed to increase with increasing age as reported in two different studies. One was carried out in China and they reported that the CFR is 8% for patients having age from 70–79 years and 15% for patients with 80 years of age or above. The other study conducted in Italy reported that 12% CFR is for patients having age from 70 to 79 years and 20% for patients having age about 80 years and above [57]. In the same way, another study carried out in the US on 2,449 patients showed that 65% of patients were from the age group 45 years or above and 80% of all the fatalities were observed for this age group as well [58]. Besides, as shown in a number of surveys, it seems that quite a smaller percentage of children appear to be symptomatic, exhibiting only mild symptoms. In two independent studies in China and South Korea, only 2 and 6% (respectively) of all the reported confirmed cases were those having age 20 years or below [52,59]. A study conducted on much younger individuals from age 0–9 years reported that 26% of them needed hospitalization, only one of them was admitted to ICU and zero fatality was observed. It was also observed in another study that in the younger individuals, infants are at higher risk, as in more than half of the hospitalized cases of younger individuals with severe to critical symptoms were infants having age below 1 year [60,61].

At the endnote, it is also questionable whether the younger people do not get infected at faster rate like that of old age people or they do not develop severe symptoms upon infection and thus remains unreported.

6.2 Comorbidities and underlying health conditions

The older individuals are more at risk because they have other underlying health conditions normally associated with their age. A young person having an underlying health condition will be at the same risk and would be affected with the same severity as individuals of older age with the same health condition. According to the studies conducted in China, Italy, and the US, the majority of the individuals who were infected and developed severe symptoms had at least one underlying condition. In one study, carried out in China, CDC reports that 37.6% of total patients had one or more underlying health conditions, 78% out of them were admitted to ICU and had at least one preexisting comorbidity [62]. Likewise, in another report of the Italian national institute of health, out of 355 patients who reportedly died due to the COVID-19, only 0.3% had no prior underlying health conditions, whereas 99% of them had at least one, 49% had three or more, and 26% had two preexisting comorbidities [36]. A recent study reported by the US CDC estimated that out of all adult hospitalized patients admitted in March in US, 89% had one or more comorbidity [56].

The most prevalent commonly reported underlying health conditions that are associated with developing severe symptoms and mortality are discussed below.

6.2.1 Hypertension (HT) and cardiovascular diseases (CVD)

Many studies suggest that individuals with HT and CVDs have a higher risk of contracting the infection and developing severe symptoms. Although the exact mechanism is not understood, two possible reasons are proposed: (i) ACE2 – the target receptor is abundantly expressed in the cardiovascular system (CVS). The enzyme has a role in vasodilation by negatively regulating renin-angiotensin system (RAS) and thereby reducing blood pressure. The damage to CVS by the direct cytopathic effect of the virus is thus possible. (ii) Due to the flooding of CVS with cytokines produced as a result of an exaggerated response by the immune system [63].

HT is at the top of the list of comorbidities that pose risk for getting infected with the COVID-19 as reported by several studies [56,64–66]. Nevertheless, some experts disagree with listing HT as a major risk factor by questioning the sufficiency of data given to prove it. They have

compared the data in 11 different studies which have listed HT as a risk factor and conclude that the data collected so far are based on unadjusted analysis as they are collected in patients of older age group only where the prevalence of HT is just common and expected comorbidity in this age group. Whether HT is a risk factor or not can be decided after a well-adjusted study where the incidence of the COVID-19 in individuals with HT is compared with those individuals who do not have HT, taking into account the exposure history of both groups [66].

Preexisting CVDs account for 9.0% of the reported confirmed cases according to a study conducted by China CDC, 52% of which required hospitalization, out of whom, 29% were admitted to the ICU. Acute coronary syndrome in COVID-19 patients leads to cardiac insufficiency, ischemia, and critical illness [56,62]. Similarly, in another study, it was observed that 44 and 24% of all the studied patients who developed severe symptoms were those with preexisting arrhythmia and heart disease, respectively [67].

6.2.2 Poor immunity

When a person is infected with SARS-CoV-2, the innate immune system is supposed to be the first responder. It identifies the viral RNA through pattern-recognition receptors (PRRs), and in the first step, produces an array of cytokines (the type-1 interferon is produced specifically in viral infection) to wall off the virus immediately and to prevent the spread of infection and viral replication. The presence of cytokines in the blood activates the second component of innate immunity, i.e., macrophages and dendritic cells which respond by phagocytizing infected cells and producing even more cytokines and signal the acquired immune system to switch on, if the viral infection is still not eliminated. The innate immune system continues its action until the acquired immune system gets active, which by the production of specific Abs against the virus finally clears the viral load completely [68]. This is at least the expected scenario in recovered patients and in those who get vaccinated for the infection, although no evidence-based study is available yet.

In older patients or those with low immunity resulting from underlying health condition/s, the immune system is compromised and thus the immune response is delayed [69]. This gives a better coping and replicating time to the virus and spreading of the infection. Innate immune response is best if given in the very start of the infection, but once the infection spreads, the immune response given by the innate system in the form of more and more cytokines production causes inflammation, which, if becomes uncontrolled, can cause much harm than good by damaging the body's uninfected cells and tissues – a phenomenon called "cytokines storm" [70].

It is assumed by the experts that in 80% of individuals with milder or no symptoms of the infection, the innate response is quick and effective enough to control the infection at the very first step and also quick in activating the acquired immune response. But in the individuals with immunodeficiencies, both the responses are delayed and the innate immune system continues to produce its components (which are amplified steadily) and overstated inflammation that result in widespread damage to the cells and lead to the damage of healthy tissue and organs, making the patient severe or critically ill [55,68].

6.2.3 Other underlying health conditions

According to a report of China CDC, concluded from a study conducted on quite a large number of patients (122,653), 10.9% had diabetes, 9.2% had chronic lung diseases, 2.1% were former smokers, and the other 1.3% were found to be current smokers [62].

6.3 Gender – has it any role?

Multiple studies from Italy, China, and the US showed that men seem to be more affected, hospitalized, and became critically ill as compared to women. CFR was also high for men as reported in these studies. In South Korea, however, more women were found affected than men but still, the CFR was high for men. Likewise, in Spain, men and women were equally affected according to the report of the health ministry, but still, men were likely to end up in ICU and develop severe symptoms. It is yet to be confirmed if male sex is a risk factor or not; however, various behavioral differences, for example, more men are smokers as compared to women in a given population, may be most probable reasons. However, men are considered to be more at risk due to development of underlying health conditions like CVD and type-2 diabetes, which are established risk factors [37,56,71–73].

7 Diagnosis

7.1 Testing criteria

The WHO has urged for testing more and more people around the globe to restrict the pandemic. In a given location, who should be tested is decided by the government, local health authorities, and the doctors. There are standard criteria and guidelines given by the WHO, ECDPC (European Centre for Disease Prevention and Control), and the US CDC which need to be followed in deciding who should to be tested first. According to their guidelines, hospitalized patients should be the first priority, followed by patients with a higher risk of developing complications and having comorbidities and then those who work in health care and infrastructure or any suspected case from the community [74–76].

7.2 Sampling for testing

The US CDC has provided a proper guideline for the collection of samples and its handling. Commonly, nasopharyngeal and oropharyngeal swabs, expectorated sputum of a productive cough (not induced), bronchoalveolar lavage, and feces can be tested with accuracy rate of 32, 72, 95, and 44%, respectively. A small percentage (3%) of blood samples also exhibited positive results in a study. To avoid false negatives and to improve the sensitivity of the test, studies have recommend that samples from multiple sites should be taken and tested [76]. Furthermore, if a suspected person tested negative, the test should be repeated with resampling from multiple sites according to the WHO guidelines [77].

7.3 Types of diagnostic tests

The symptoms expressed by COVID-19 patients are nonspecific and cannot be used for an accurate diagnosis. Molecular techniques are more suitable than syndromic testing and CT scans for accurate diagnosis because they can target and identify specific pathogens.

7.3.1 Molecular test

The test extracts the genetic material of the virus (if any) and uses real-time polymerase chain reaction (rRT-PCR) for its detection after amplifying its number of copies.

7.3.2 Immunoassay test

The test could be either antibody (Ab) or antigen (Ag)-based; it uses ELISA, i.e., enzyme-linked immunosorbent assay, where specific Ab or Ag are quantified after their attachment with the Ab or Ag used, depending upon the type of the assay [62].

7.3.3 Nonspecific tests

(i) Observing signs and symptoms of the disease like fever, cough, and gastrointestinal symptoms.
(ii) Computed tomography (CT) scan (of chest) has been shown to be a very important tool for diagnosis and suggesting potential infection. According to a study, most (86%) of the observed patients had ground-glass opacities (GGOs) and 64% had consolidation plus GGOs in the CT scans. These characteristics were observed in critical patients and patients with mild symptoms. Although critically ill patients were older than those with mild symptoms, there were no significant differences between the CT scans of both groups. The specific findings of the CT scan may be present even before the onset of symptoms and detection of viral genetic material in the samples from the upper respiratory tract [62,72]. Table 1 presents details about the available diagnostic methods.

8 Incubation period

According to the WHO, the incubation period (the time taken in the appearance of symptoms after infection) is between 1–14 days, and from a study carried out on 181 confirmed cases of the disease, it was concluded that mean incubation period was 5.1 days with the possibility that some (1%) may still develop the symptoms after 14 days of monitoring [78]. Moreover, according to a study, the duration of incubation is also dependent upon age

Table 1: Available major diagnostic methods for COVID-19

Test	What is detected	Sample type
RT-PCR	Viral genome	Nasopharyngeal swabs, saliva, and sputum
ELISA	Antibody	Blood
CT scan	Nonspecific characteristics like GGOs and consolidations are observed	NA

and immunity; more the age and weaker the immune system, the shorter will be the incubation period [79].

9 Pathogenesis

9.1 Entry of SARS-CoV-2 in the target receptor

The target receptor as discussed earlier for SARS-CoV-2 is ACE2 enzyme, whichever cell in any organ throughout the body that possesses it will expectedly be the target cell for the virus. Studies [17,80] show that this virus has much more affinity for its target as compared to that of SARS-CoV. A recent study through the release of 3D electron micrograph of the virus has proposed that once SARS-CoV-2 gets access to human's upper respiratory tract through the mouth or nose, it can stay and replicate there for a while before going to the lungs because ACE-2 is expressed in the mucous membrane of mouth and larynx. Although the expression of ACE-2 there is quite small than that in the lungs, because of the very high affinity for its receptor, SARS-CoV-2 can still bind and enter the ACE-2 present in the mucous membrane of nose and larynx and multiply there before going to the lungs contrary to SARS-CoV which was observed to replicate only in lungs [81]. After the primary replication, the virus goes to the lungs where it enters its target receptor-expressing cells. The virus can enter the peripheral blood theoretically from the lungs and also into the gastrointestinal tract (GIT) and start approaching to the cells that have ACE2 expressed like CVS, kidney, intestine, and liver. The presence of the viral infection in areas other than the lungs is evident from studies where blood and fecal samples tested were found positive for the presence of the virus. In some cases, quite a large proportion of individuals' fecal test was positive repeatedly after negative results of the respiratory samples. Moreover, gastrointestinal problems as reported in some studies and the observed acute damage to liver, kidney, and heart also suggest the presence of infection in these areas [69,82,83]. As ACE2 is also expressed in seminiferous tubules' lining, damage to testicular tissues is pointed out in a reported study [84].

9.2 Entry to the target receptor in the lungs

Reportedly, type-2 pneumocytes and enterocytes are the ACE2 expressing cells, and hence, are entry sites of the virus in the lungs. The virus as discussed earlier enters its target through its spike S; S1 subunit of the S has RBD that recognizes and attaches to ACE2 membrane, while S2 anchors in the membrane; S2′ site (present directly after S2) is cleaved by the target cell's protease, i.e., TMPRSS2 (transmembrane protease, serine-2), which changes the conformation of S2 and let it ready for fusion with the target cell membrane [10,85].

9.3 Replication in the target cells

Once the virus enters into the cell, its capsid is removed and it releases its RNA in the host cell's cytoplasm. The RNA, on one hand, uses the host cell ribosome and translation machinery to produce its two polyproteins that are used for the synthesis of its component proteins and enzymes. Structural proteins are also synthesized for making glycoproteins, spike, and nucleocapsid; to do so, it uses host proteinases and nucleocapsid proteins. On the other hand, the RNA uses the host cell's RNA-dependent-RNA polymerase to make its copies. The viral cell components and RNA so formed are finally assembled making many numbers of virus particles. They are released outside of the cell through apoptosis/pyroptosis of the cell [86].

9.4 Immune response to the viral entry and replication

When the virus particles are released, they attack other cells and are recognized by PRR present on the antigen-presenting cells to provoke an immune response. The immune response can be divided into two stages.

9.4.1 Primary immune response

The engagement of the viral PRR leads to the release of antiviral cytokines which are reported to be interleukins (IL) of several types like IL-1β, IL-4, and IL-10; interferon (IFN)-γ inducible protein-10 (IP-10) and monocyte chemoattractant protein (MCP)-1 are also released in the very early response of innate immunity [87]. Based on the knowledge of what happened in the case of SARS and MERS [70,86], our basic knowledge of immunity against viruses, the expected series of events appear to be like in the following lines.

The rapid viral replication and subsequent massive cell apoptosis lead to vascular leakage and increased release of pro-inflammatory cytokines which increase capillary permeability and vasodilation causing interstitial space and alveolar edema. Surface tension is increased because of decreased surfactant levels in the alveoli and the leakage and damage of type-2 pneumocytes that are responsible for surfactant production. This leads to alveolar collapse, causing difficulty in gas exchange, hypoxemia, and increase work of breathing. On the other hand, the inflammatory mediators lead to the release of neutrophils which release ROS, hydrolases, and proteases to destroy the affected cells and when the immune response goes uncontrolled as occurs in SARS-CoV which happens here too, they also start damaging the unaffected pneumocytes (both type 1 and type 2). This leads to impaired gaseous exchange and decreased surfactant production. According to a very comprehensive review about the immune response to SARS-CoV-2 [88], the infection causes pyroptosis in the macrophages and lymphocytes; their subsequent pulmonary infiltration along with the debris of the destroyed cells, fluids, and other destroyed immune products results in alveolar consolidation, and hence, altered gaseous exchange. Side by side, downregulation and shedding of ACE2 are expected due to the action of the virus and due to the release of pro-inflammatory cytokine, especially tumor necrosis factor (TNF)-α. The ACE2 thus released in the blood is called soluble ACE2 (sACE2) that is suggested by some authors to have a role in the pathogenicity of the virus. The loss of ACE2 function leads to dysfunction of the RAS, vasodilation, and alveolar edema, thus worsening the inflammation. More likely, most patients with good innate immune responses can cope with the infection at the first stage, decrease or clear the viral load, and subsequently recover from the inflammation [60,65,70,88].

9.4.2 Secondary immune response

So far, very limited data are available on the humoral response to SARS-CoV-2, but based on the humoral immune response (adaptive immunity) given by the body in viral infection which has been observed previously in case of SARS and MERS, the expected response is the production of neutralizing Abs (NAb)-IgM and IgG. A specialized IgG is also formed specifically against the spike S called anti-S-IgG. In positive outcomes, that happen mostly; the virus is neutralized by the NAb, viral load is decreased, and the infection subsides gradually along with the production of memory Abs. But in worst case scenario, the anti-S-IgG may trigger Fc receptors-mediated inflammatory response – a phenomenon known as antibody-dependent enhanced immunity (ADE) where a NAb is not able to efficiently bind to the virus attached to it and hence only partially coat it. When these partially coated virions are taken up by macrophages/monocytes, they replicate there, infecting them, and cause an abnormal immune response, leading to severe inflammation and acute lung injury. The anti-S-IgG ADE was first observed in SARS, where the patients in whom the levels of anti-S-IgG rapidly reached to the peak had more severe lung injury, persistent infection, and higher chances of death. A proposed potential therapeutic approach to prevent the anti-S-IgG ADE phenomenon is to block the FcR and prevent the binding of anti-S-IgG to them, hence preventing the inflammatory response [70,72,89].

In a nutshell, if the infected person provides a better immune system's primary response and control the resultant inflammation at the earlier phase, the better will be the outcome because most of the times, secondary inflammatory response can go wrong and lead to critical illness and fatality. However, the uncontrolled cascade of events either in the primary immune response, i.e., the enhanced viral replication, cell apoptosis and pyroptosis, and ACE2 shedding, or in secondary immune response, i.e., FcR-mediated ADE, can result in an overstated immune response resulting in cytokines storm. The presence of cytokines and chemokines in high concentrations has been reported in a study where the critical ICU patients had higher levels and more types of cytokines in their blood as compared to non-ICU patients [60]. The cytokine storm activates an exaggerated immune reaction resulting in more harm than good, damaging the body's healthy tissues, organs, and systems. This leads to ARDS and multiorgan failure and fatality [90–92]. Figure 8 shows the schematic representation of various stages of pathogenesis caused by SARS-CoV-2 [93].

9.5 Sepsis and septic shock

The cytokines leaked into bloodstream reach to multiple organs and cause vasodilation in the blood vessels, which in turn decrease total peripheral resistance leading to hypotension, low perfusion to different organs, and subsequently cause systemic inflammatory response (SIR) followed by sepsis leading to multiple organ failure and septic shock [62,68].

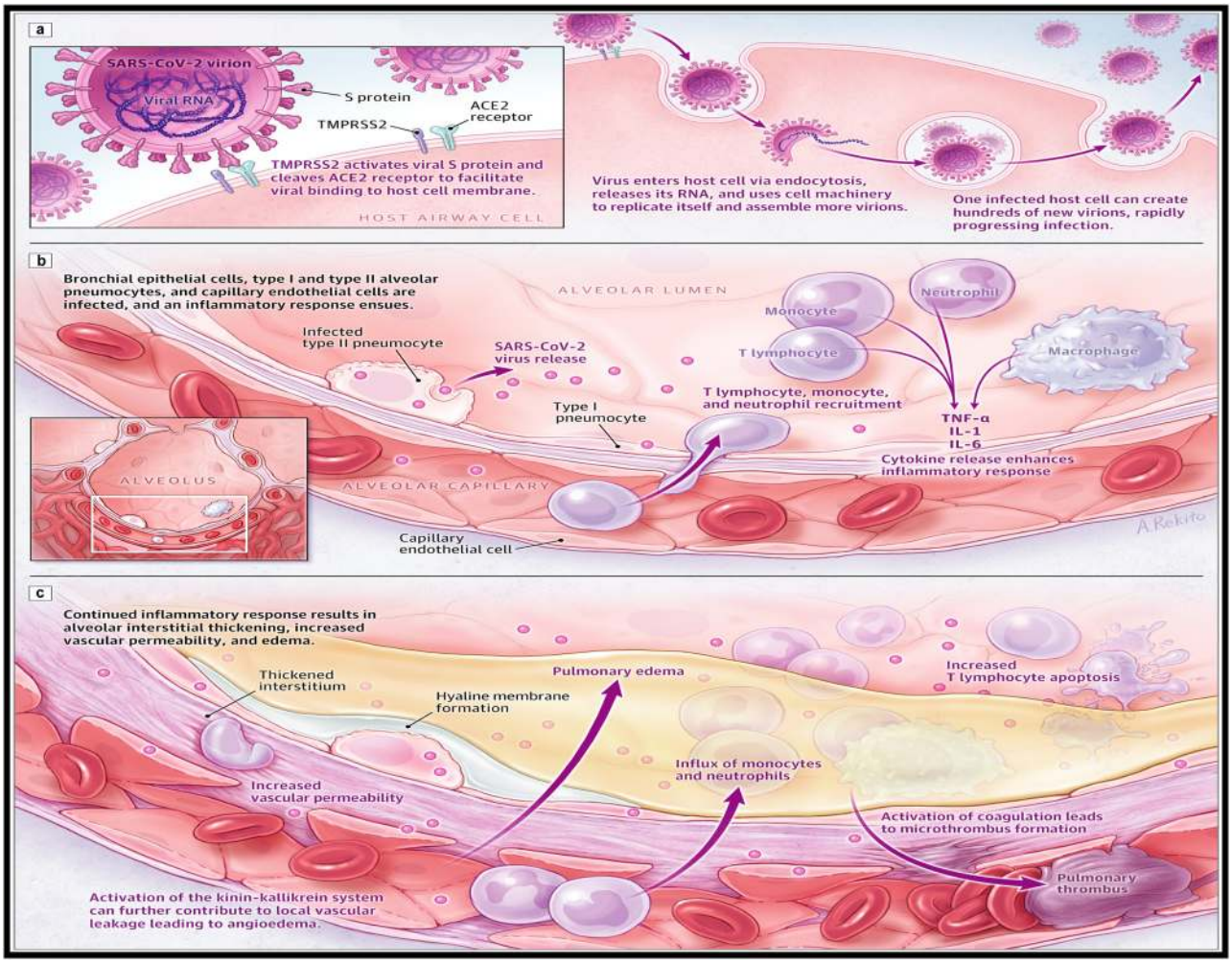


Figure 8: Immunopathogenesis of COVID-19 [93]. (a) SARS-CoV-2 viral infection of host airway cells, (b) early-stage COVID-19, (c) late-stage COVID-19.

9.6 Effects on the kidneys

At least 10 studies have been conducted so far on the effect of COVID-19 on the kidney and its functions and development of acute kidney injury (AKI) in the patients [65,67,82,94–99]. Out of the 10 studies, one study [100] performed in 116 hospitalized patients, among whom five patients had chronic kidney disease (CKD), showed that none of the 111 non-CKD patients developed AKI and no abnormality was observed in any kidney function. The nine out of 10 studies, however, report oppositely with results that AKI occurred in the majority of the studied patients. The dichotomy between the results of the one study with those of the nine other studies may be due to the reason that some of the nine studies were undertaken in older patients only (above 60 years), as in some studies, a proportion of patients had CKD, while some studies were carried out on critically ill patients admitted in ICU, and the majority (6/9) of these didn't meet kidney disease improving global outcome criteria for AKI [100]. Even though a uniform pattern was not followed in these studies and there were limitations, some generalization can be made:

(i) The overall observed AKI range was 0.5 to 23%;
(ii) The incidence and severity of AKI were directly related to mortality rate;
(iii) Proteinuria, hematuria, elevated serum creatinine levels, high blood urea nitrogen, and glomerular filtration rate less than the normal rate were the common observations in almost all these studies [60,82,96–101].

While talking about the impact of the infection on the kidneys, two mechanisms can be suggested for the observed AKI. (i) The SIR impairs blood circulation to the kidneys rendering them unable to perform their function;

if the infection continues, it can result in kidney failure. (ii) Due to the excessive expression of ACE-2 and TMPRSS2 in renal tubules [69,82], they could be equally targeted by the virus if it reaches to the kidney. The virus replicates there, infects, and destroys the tubular cells.

9.7 Effects on the liver

At least 12 different studies have reported the impact of COVID-19 on the liver; their findings can be summarized into these points:

(i) The prevalence of liver injury was in the range of 14.3 to 53% of the patients studied;
(ii) In most cases, alanine aminotransferase, and aspartate aminotransferase, levels were found elevated and the abnormal levels were more prevalent in severe and ICU patients than in the mild and non-ICU patients;
(iii) A slight to moderate increase in the serum levels of bilirubin, γ-glutamyl transferase, myoglobin, creatine kinase, and lactate dehydrogenase was also observed in most cases;
(iv) The derangement of the observed parameters and liver injury in almost all studies increased with an increase in the severity of the disease;
(v) Of the studied patients, 2–11% were those who had preexisting liver diseases; and
(vi) The severity and incidence of liver injury were much high (up to 70% reported) in non-survivors than in the survivors [55,65,67,84,88,102–108].

The mechanism of liver injury is not well-established, but different reasons could be proposed.

ACE2 is moderately expressed in liver and highly expressed in the cholangiocytes of the bile duct. Cholangiocytes regenerate the damaged liver cells and have a role in the immune response of the liver. It is safe to assume that more damage to liver happens due to the cytopathic effect of the virus on the cholangiocytes and not on the liver itself. In addition, damage to the liver could also be due to the sepsis and the cytokines storm. In a study, postmortem biopsy of a deceased COVID-19 patient revealed microvascular steatosis along with lobular and portal inflammation – a presentation normally associated with drug-induced injury, but as the data are very limited, it is also possible that the injury was/may be due to the virus infection [84,109,110]. Besides, similar to the situation in SARS, antibiotics, antivirals, and steroids are widely used for the treatment of COVID-19 which are metabolized in the liver; as observed in a study, that was the reason for the liver injury in patients mainly because of the use of lopinavir/ritonavir antiviral in these patients [111].

9.8 Effects on the CVS

As in the kidney, the pathogenic effect on CVS is mediated either due to the SIR, cytokines storm, and hypoxemia or due to the direct cytopathic effect of the virus after the attachment to ACE2 that are abundantly expressed in CVS. Acute myocardial injury, heart damage, and cardiac arrest have been observed in patients. Moreover, the severity of the pathogenic effect is more in the patients with preexisting heart diseases wherein they develop severe symptoms and higher chances of mortality [63].

9.9 Effect on the GIT

The expression of ACE2 and TMPRSS2 in the glandular epithelia of stomach, duodenum, and the enterocytes of ileum and colon makes the GIT equally susceptible to the attack of SARS-CoV-2 as are the lungs [112,113]. The incidence of GIT symptoms like diarrhea, abdominal pain, and vomiting observed in many cases is sufficient to point out the involvement of GIT in the infection [114,115]. The entry and replication of the virus in GIT are also evident from many studies where the viral RNA could be detected in feces even after the respiratory samples were found negative for the presence of the virus, showing the potential leakage of the virus from GIT to feces [79,116,117].

10 Management and treatment

There is no specific recommended treatment for COVID-19; the patients are given supportive care and management based on the stage/severity of their disease.

10.1 Clinical management

The WHO has provided proper guidelines (criteria) on prehospital and clinical management of the disease. Clinical management is further categorized in different categories based on progress of the disease.

(i) Patients with mild symptoms or mild pneumonia do not necessarily require hospitalization, but should be isolated either in hospital, home, or any other setup for containment of the infection and provided with antipyretics and monitored for their symptoms.
(ii) Severe COVID-19 patients who are hypoxemic and have respiratory distress require supplemental oxygen and antimicrobial treatment and close monitoring.
(iii) Critical patients with ARDS who are severely hypoxemic and whose symptoms do not improve require advanced ventilatory support and conservative fluid management.
(iv) Patients with sepsis and septic shock in whom sepsis is recognized require fluid resuscitation and vasopressors administration.

There is a detailed description of management in each step as provided by WHO with proper guidelines, but still answer is needed to important questions like which type of management is required for which specific patient, the expertise required, and what are the requirements, be it diagnostic, supportive, or for treatment of the symptoms in each step. Specific guidelines are also there for the management of children, pregnant and breastfeeding women, and individuals with chronic conditions [62].

10.2 Medications

Unfortunately, there is no specific vaccine or antiviral drug available for the treatment of COVID-19 yet. Although few companies have launched their vaccines into market, it is still early as they have tested them in hurry and no one is aware about their side effects. Correspondingly, their production on large scale is a challenge to fulfill the need of such huge population living in different countries, especially in third world countries that are thickly populated and are poor. Thus, at present, in terms of patient treatment and management, the main focus is the provision of supportive care for the relief of symptoms and different combinational therapies including administration of systemic corticosteroids and antiviral drugs. The currently in-practice treatments for the cure and management of severely affected COVID-19 patients are summarized in Table 2, while the chemical structures along with molecular formulae of them are given in Table 3 [118].

10.2.1 Corticosteroids

Glucocorticoids have been widely used in syndromes closely related to COVID-19, including SARS, MERS, severe influenza, and community-acquired pneumonia. However, the evidence to support or discourage the use of glucocorticoids under these conditions is weak owing to the lack of data from sufficiently powered randomized, controlled trials. Several studies were conducted to establish the effectiveness of dexamethasone in the COVID-19 patients. It is likely that the beneficial effect of glucocorticoids in severe viral respiratory infections is dependent on a selection of the right dose, at the right time, and in the right patient. High doses may be more harmful (than helpful), as may such treatment have given at a time when control of viral replication is paramount and inflammation is minimal. The greater mortality benefit of dexamethasone in patients with COVID-19 who are receiving respiratory support and among those recruited after the first week of their illness suggests that at this stage the disease may be dominated by immunopathological elements, with active viral replication playing a secondary role [119].

10.2.2 Anti-inflammatory drugs

Glucocorticoids: In the view of the previous knowledge of the severe outcomes by the use of glucocorticoids in viral infection like influenza and MERS and no beneficial result in case of SARS, with several short and long-term

Table 2: Treatment options for COVID-19

Therapeutic class	Treatment options
Antiviral	>85% of patients received antiviral agents, including oseltamivir (75 mg every 12 h orally), ganciclovir (0.25 g every 12 h intravenously), and lopinavir/ritonavir tablets (400/100 mg twice daily). Remdesivir is currently under trials at more than ten medical institutions in Wuhan and has been known to prevent MERS-CoV.
Antimalarial	An antimalarial drug, chloroquine phosphate, has been effective in inhibiting the exacerbation of pneumonia due to its antiviral and anti-inflammatory activities.
Herbal treatments	Traditional Chinese Medicines were used most extensively during the previous outbreak of SARS-CoV in China. They were reported to be used for the treatment of current COVID-19 treatment as well. The herbs used commonly were Glycyrrhizae Radix Et Rhizoma (Gancao), Astragali Radix (Huangqi), Atractylodis Macrocephalae Rhizoma (Baizhu), Saposhnikoviae Radix (Fangfeng), and Lonicerae Japonicae Flo.

Table 3: Chemical structures and molecular formulae of antiviral and antimalarial used as treatment of COVID-19

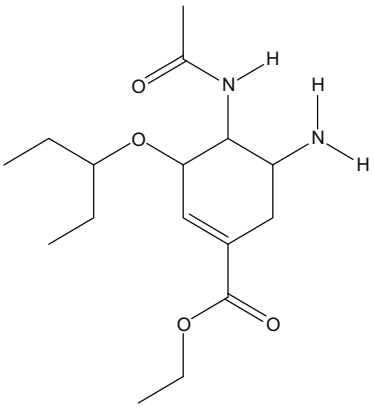
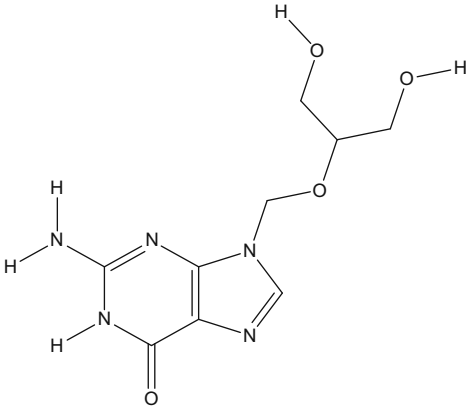
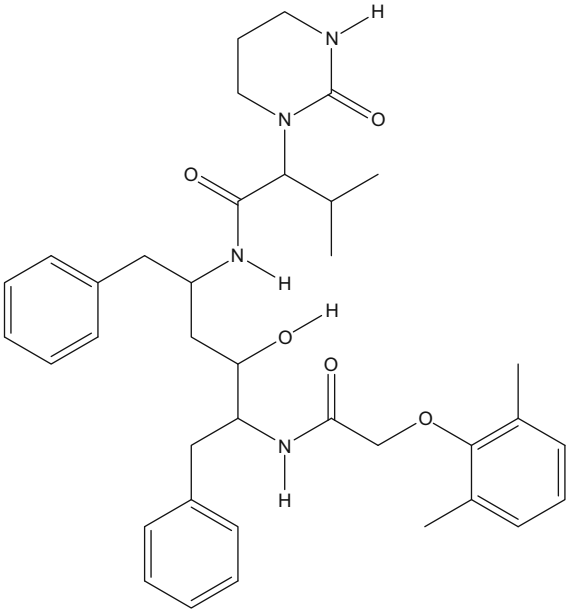
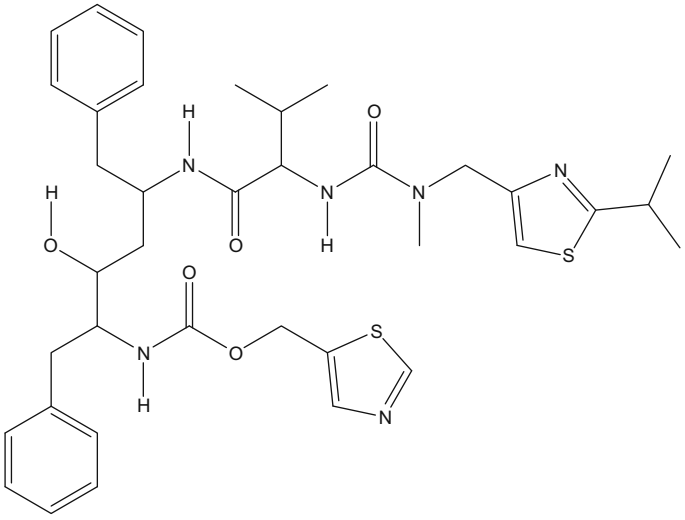
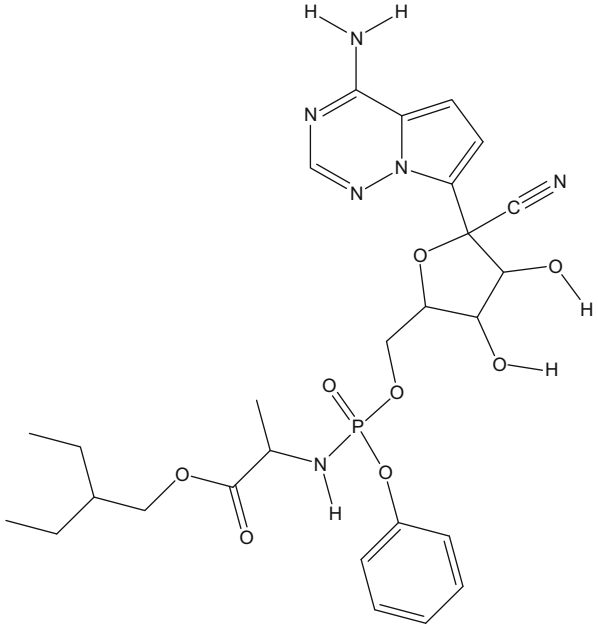
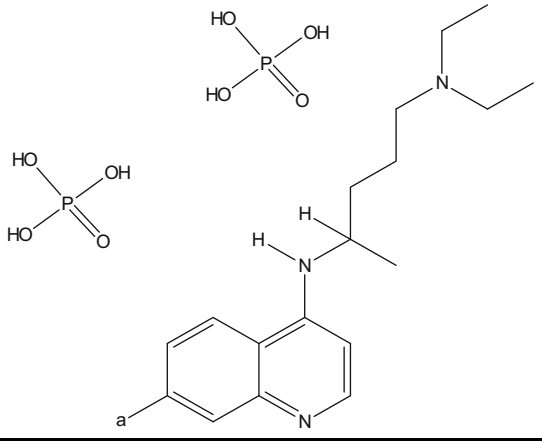
Drug type	Name	Molecular formula	Chemical structure
Antiviral	Oseltamivir	$C_{16}H_{28}N_2O_4$	
Antiviral	Ganciclovir	$C_9H_{13}N_5O_4$	
Antiviral	Lopinavir	$C_{37}H_{48}N_4O_5$	

Table 3: Continued

Drug type	Name	Molecular formula	Chemical structure
Antiviral	Ritonavir	$C_{37}H_{48}N_6O_5S_2$	
Antiviral	Remdesivir	$C_{27}H_{35}N_6O_8P$	
Antimalarial	Chloroquine phosphate	$C_{18}H_{32}ClN_3O_8P_2$	

adverse effects, the use of glucocorticoids is not recommended by WHO and CDC unless in patients with chronic obstructive pulmonary disease [120,121].

10.2.3 Onvalescent plasma

The US Food and Drug Administration (FDA) has approved the use of convalescent plasma in severe or critically ill patients. A limited study on five patients on mechanical ventilator has reported its results. The patients completely recovered after receiving the plasma having SARS-CoV-2-specific IgG from donors. The patients showed improvements in 3 days as indicated by their normal body temperature, the viral load was decreased in 12 days and ARDS was completely resolved in 12–14 days [122].

11 Vaccine

Although the efforts and speed for finding a vaccine against SARS-CoV-2 are unprecedented, a lot of studies are underway, but still there is no approved vaccine available and the journey on the road to an effective vaccine against COVID-19 will take its time. The first phase 1 safety trial started in March 2020, and as of November 2020, 55 vaccines are in clinical trials on humans, out of which 13 have reached to phase three safety trials. An effective vaccine against COVID-19 should not induce undesired enhancement/provoking of immune response and protect the people who are at more risk, i.e., the older individuals and the health care workers and those with underlying health conditions. It is also important that the vaccine should be producible in large scale so that enough amount of it is present at a given time. Although scientists have much learnt from the vaccine development experiences in the previous epidemics, viz., SARS and MERS, none of these studies have gone past the phase I clinical trials [65,123].

The list of the candidate COVID-19 vaccines currently in the process of development, to be produced, or under trial is long; however, majority of these fall under any of the following categories.

11.1 Recombinant protein vaccines

These are highly safe as they are prepared in the lab, so the infectious viruses are not handled in the development process and their efficacy can be increased with the addition of adjuvants. Clover Biopharmaceuticals in partnership with GSK have produced S (spike) protein subunit-trimer, also called S-trimer; the trial is in phase 1 stage. Likewise, University of Queensland in association with Coalition for Epidemic Preparedness Innovations (CEPI) using molecular clamp technology has produced a vaccine in which the prefusion form of the fusion proteins present on the surface of SARS-CoV-2 is stabilized by addition of a polypeptide (the clamp); the project is in phase 1. However, the major drawback normally associated with the type of vaccines is their limited production capacity [123,124].

11.2 mRNA vaccines

The vaccines have mRNA of the virus that is translated in the host cell. They are good for producing strong immune response with no worry of their genome integration and could be produced rapidly and at large scale. Moderna, Inc. in collaboration with National Institute of Allergy and Infectious Diseases produced a vaccine candidate in the form of lipid nanoparticles containing mRNA encoding S protein and started their phase 1 clinical trials in March 2020 with financial support by CEPI. In July 2020, they started their phase 3 clinical trials on 30,000 volunteers, and in November 2020, they declared their vaccine to have 94.5% efficacy for protecting the vaccinated individuals against the development of severe disease. As of 27 Nov, 2020, the company is planning to apply for emergency-use authorization from FDA (, US. CureVac in collaboration with CEPI and Shanghai East Hospital has also developed mRNA vaccines against SARS-CoV-2 which is in phase 2 clinical trials. Pfizer (New York) and BioNTech (Germany) have developed two versions of mRNA vaccine, and after launching their phase 2/3 clinical trial in July, 2020, have announced in Nov 2020 that their vaccine (both version) has shown more than 90% efficacy. The one version BNT162b2 has been specifically effective for producing very fewer side effects. The company has applied for emergency-use authorization by FDA on 20th Nov 2020 [123,125,126].

11.3 DNA vaccines

They are synthesized in the form of plasmid and encoded with SARS-CoV-2 Ag. They have high heat stability,

economical and rapid production, and above all already shown good activity against SARS-CoV in clinical trials. Inovio Pharmaceuticals has synthesized the DNA vaccine and finished their preclinical trials in collaboration with Beijing Advaccine Biotechnology, and now in collaboration with CEPI, has completed their phase I clinical trials in the USA and is looking forward to start their phase 2/3 trials after permission from FDA [127].

11.4 Adenoviral vector-based vaccines

In the type of vaccines, adenoviruses are used as vectors to deliver Ag of SARS-CoV-2, which in most cases is the S protein. The type of vaccines has the advantage of provoking a robust immune response, the viral vector does not replicate in the host, and the vaccines could be produced at large scale. CanSino Biologics in collaboration with Institute of Biotechnology, Academy of Military Medical Sciences has produced Adenovirus Type 5 Vector (Ad5-nCoV) which is under phase 3 clinical trials [128,129]. University of Oxford UK in collaboration with AstraZeneca has produced chimpanzee adenovirus vector (ChAdOx1)-based vaccine candidate which has passed its phase 3 clinical trials and has been shown to have more than 90% efficiency depending upon the dose. Janssen (Johnson & Johnson) with the use of AdVac® and PER.C6® technology has successfully synthesized the adenovirus vector vaccine and the study is in phase 3 clinical trials [65]. Sputnik V developed by the Gamaleya Research Institute in Moscow has shown high efficacy in its phase 3 clinical trials [126,127].

11.5 Whole virion vaccines

It is the traditional strategy of vaccination; the whole virion either live attenuated or inactivated are used. It would be helpful in the present scenario because of the already available facilities, infrastructure, and technology for developing this type of vaccines. The farthest development in the category is by Wuhan Institute of biological products which has developed an inactivated whole virion vaccine that has entered phase 3 clinical trials in UAE and in China. The vaccine has been given an emergency approval in the said countries to be injected to health care professionals, government officials, and other selected groups. Although the phase 3 trials are in progress, Sinopharm has applied for marketing of the vaccine [128].

11.6 Nanoparticle vaccines

Novavax has synthesized a nanoparticle vaccine that carries Ags from the S protein through its recombinant protein nanoparticle technology and aims to use its Matrix-M™ adjuvant for optimizing its immunogenicity. Their development has entered in phase 3 clinical trials [65,124].

11.7 Other vaccines

Other vaccines are also in the development stages, like Genexine Inc. (phase 1/2 trials) is using hyleukin-7 platform technology for development of vaccine that, on one hand, would enhance the immune response, and on the other, would prevent ADCC and CDC (complement-dependent cytotoxicity) to avoid adverse immunogenicity [124,128]. Generex Biotechnology is aiming to synthesize peptides using NuGenerex Immuno-Oncology Ii-Key technology; the synthetic peptides mimic important protein regions in the virus and are chemically linked to its proprietary Ii-Key immune activation; the combination would lead to the activation of a strong and safe immune response [124,126]. Likewise, University of Pittsburgh, School of Medicine, US, has synthesized a patch of microneedles that would deliver Ags derived from the S protein through the skin and are in the preclinical stage. The list goes on, with many others like Vaxart, Medicago, Altimmune, Takis Biotech and Applied DNA Sciences, etc., with their own technologies and strategies, participating in the race [126].

In summary, as of 27 Nov 2020, Moderna, Pfizer, University of Oxford/AstraZeneca, Wuhan Institute of Biological Products/Sinopharm, and Gamaleya Research Institute are leading the race and have completed their phase 3 clinical trials announcing the efficacy rate of their vaccines to be more than 90% and they are looking forward for obtaining emergency-use authorization [126]. Several others like Novavax and CanSino Biologics Inc are in their phase 3 clinical trials and the results would take some more time.

12 Prevention and mitigation Of COVID-19

The key approach in the current circumstances is the prevention, protection, and taking mitigation measures.

The WHO and CDC have provided complete guidelines on how to protect oneself and prevent and mitigate the infection. Protection and prevention consist in knowing how the infection is spread and transmitted, followed by adopting measures to avoid the same. The first preventive measure therefore is to isolate the infected persons from the healthy ones so that the chain of transmission is broken. The other effective preventive steps include social or physical distancing (as each one of us could be a potential transmitter because there are reports of transmission from asymptomatic individuals and that the virus can stay in aerosols for up to 3 h), practicing good hygiene habits such as washing hands regularly with soap, cleaning and disinfecting regularly the exposed surfaces like in kitchens, bathrooms, and floors, avoiding touching face, eyes, nose, and mouth with unwashed hands, covering face with face cover and using personal protective equipment, and covering mouth and nose with tissue paper or bent elbow while sneezing or coughing. The WHO and CDC have provided a complete guidance on disinfection and cleaning, social distancing, and staying at home [76,120].

Apart from the above-mentioned precautions adopted at individual level, efforts are being made on governmental level by states to minimize the spread of the virus: prevention of SSEs by large-scale lockdowns, ban on public gatherings, restriction on nonessential travels, public holiday, and special biosafety measures for those who are more at risk of being exposed to the virus, i.e., health care workers, law enforcement agencies personnel, and essential services providers; surveillance, diagnosis, and isolation of the suspects having symptoms and/or travel history and quarantine of their primary contacts. To contain and mitigate the pandemic, strategies on national, international, and local levels are called for. Health facilities should be strengthened and medical supplies be upgraded to the best possible level [130,131].

13 Immunity to COVID-19 and the possibility of reinfection

13.1 Who is immune to COVID-19?

The question coming from everywhere in the present pandemic is whether immunity has been developed against the infection in individuals after being infected or not, and if developed, then up to what extent? To answer, experts take help from the previous knowledge of immunity against infections caused by CoVs like seasonal flu and the two previous epidemics, i.e., SARS and MERS. According to studies carried out on evaluating the degree and duration of immunity against seasonal flu causing COVs, the immunity lasts only for a year that was highly specific for the given specific viral strain. However, in the majority of the reinfection cases the symptoms were still developed, but to a lesser extent [132,133]. Immunity against SARS and MERS was recorded to be lasted for 2 and 3 years, respectively [134,135]. Thus, it is assumed that most of the affected people will develop an immune response and perhaps some would develop a strong immune response than the others. How long the immune response will remain is yet to be known. Very limited data are available on the Ab response in COVID-19 patients and only few studies have been published so far that report the production, types, and the presence of Abs in infected and recovered individuals [136,137]. Scientists urge for more blood tests to be carried out for the presence of Abs against COVID-19 in people. The WHO is going to launch the same in several countries, while some countries on their own have planned to start Ab tests nationwide [138,139].

While finding a satisfactory treatment and developing a perfect vaccine (the developed one has still to be tested for side effects, etc.) will take time, everyone is hoping that build-up of herd immunity will ultimately end the pandemic. Though the herd immunity does not seem possible to happen in such a limited time as most people have not developed the immunity against the virus yet, studies have suggested that there are a greater number of individuals than the reported ones who have been infected, developed mild symptoms, and recovered, but because they are never tested that's why they go unreported. A study proposed that the number of non-reported COVID-19 patients may be 10–100 or even much more times higher than the reported cases [140]. If this is really the case, it is expected that they would have developed immunity and if they have passed the infection to others and they also went asymptomatic/mildly symptomatic, then consequently they would also have developed immunity. If this continues, more and more people within a given population would become immune leading to the development of herd immunity in which rate of transmission and number of infected cases will drop in a year or so [43]. This is at least an ideal case scenario, but there are two factors that could prevent the scenario from happening.

(i) First factor is that not everyone infected with COVID-19 necessarily produces immunity against it. In a Chinese study (preprint) carried out in 175 infected

individuals with mild symptoms to assess their Abs response against the disease, only 70% were observed to have developed strong immune response, 25% developed it only to a lesser degree, and 5% didn't develop any immune response at all [29].

(ii) Second factor is the possibility of reinfection as reported in several studies where the patients, who previously tested negative, tested positive again for the presence of the virus when examined several days after the negative test. One study that was performed in a smaller cohort of four patients, all medical professionals, reported that the patients who were moderately symptomatic, upon recovery, were discharged for the mandatory five days home quarantine after qualifying the criteria necessary for declaring the patient "recovered" from COVID-19. Along with the other conditions, the criteria included two negative RT-PCR tests for viral presence and satisfactory chest CT scans and improvements in signs and symptoms. The patients were tested again after 5–13 days and they were tested positive in three RT-PCR tests. Their CT scans were fine and they were having no symptoms; their primary contacts also tested negative [141]. Likewise, according to another study (preprint), 14.5% of the studied hospitalized patients after testing negative (both on the nasopharyngeal and anal swabs) and declared recovered again tested positive when followed up for 14 days. All of the re detectable positive patients in the study were under 14 years, exhibiting mild symptoms on their first admission in the hospital and they remained asymptomatic on readmission with no signs of disease progression and also had not passed the infection to their primary contacts [142]. Recently, South Korea CDC (KCDC) declared that 124 patients previously tested negative for the viral presence and declared recovered, when tested again, tested positive [142,143].

13.2 Is it really reinfection? If not, what could it be?

Experts around the world are of the opinion that instead of declaring it reinfection, the positive test after the negative ones can be due to any of these several reasons: (i) False negatives – which may be due to declining viral that have load result in negative test, which after few days of multiplication become detectable again in the test or due to less sensitivity of the test kit, (ii) due to the presence of residuals of the viral genome in the cells – which does not necessarily show the presence of live virus, and/or (iii) due to viral shedding in convalescent individuals. The KCDC's experts say that instead of false negative, there is the possibility that the virus may have "relapsed and reactivated." The WHO, however, has announced that it will be investigated further to decide whether the emergence is reinfection or not [43,142,144].

A study was performed in 4 Rhesus macaque monkeys; the monkeys were infected with the virus. They developed moderate symptoms, recovered, and tested negative for the viral presence. One of the monkeys was rechallenged after 5 days of negative test and two of them after 28 days with the infection; they didn't develop symptoms and tested negative. The fourth monkey which was not rechallenged didn't show the recurrence of the infection either. The study clearly indicates the development of immunity against the infection and no possibility of reinfection within the observed timeline [145].

However, there are studies (although a limited number of) that are suggestive of potential reactivation of the virus. A study conducted by Dao et al [146] demonstrated that reinfection in the patient recovered from COVID-19 seems very probable, although the exact cause is unknown. According to Ye et al. [147], out of 55 studied hospitalized patients, five were observed to have their virus reactivated shortly after they were discharged from the hospital. Likewise, Ravioli et al. [148] observed reactivation in two patients and the same was observed by Coppola et al. in one patient after 43 days of initial onset of COVID-19 [149]. Additionally, according to a study, the observed IgG and Nab against SARS-CoV-2 may decline steadily in 2–3 months after the onset of COVID-19.

To sum up, we can carefully conclude that although studies on reactivation are available, they are limited and lack details about description of the observations made, duration and severity of symptom upon potential reinfection, as well as the mode of sampling. There is a possibility of sampling error, as already discussed in Section 7.2 of the article; there could be false negative result depending upon the sample source (nasal, pharyngeal, fecal, blood, etc.). Likewise, a false positive result is also expected if there are any remains of the dead virus as the PCR detects viral RNA that could possibly come from an inactivated virus which has remained in the body after the infection.

In the end, as we are still in the pandemic, a lot of questions remain to be answered, many of which will be resolved with time through the conduction of more tests and studies. Monitoring the Abs response in the infected individuals will help to evaluate the immunity against

the infection and then it will become easy to decide whether reinfection is possible or not. The Abs response in unreported asymptomatic individuals to be evaluated is also important to ascertain the possibility of reinfection and control transmission of disease further.

14 Conclusion

COVID-19 caused by SARS-CoV-2 has spread globally in no time; like SARS and MERS, the disease is the result of zoonotic transfer of SARS-CoV-2 from an animal to human. The genome of SARS-CoV-2 is very similar to the genome of CoVs found in bats and those found in pangolins, but it has some very unique genome features that are not present in either bat or pangolin CoVs. Scientists are of the view that the virus is not genetically manipulated. COVID-19 is highly contagious infection and although it has quite low CFR and mortality rate than SARS and MERS, it has caused a greater number of deaths across the globe. The infection is transferred via respiratory droplets from the infected person, contaminated surfaces, feces, and other contaminated sources.

People from the older age group, having underlying health conditions and poor immunity, are more at risk to be infected and develop severe symptoms. The gold standard for diagnosis of the disease is RT-PCR test for the viral presence. The target receptor of the virus is ACE-2 that is expressed in mucous membrane of mouth, pharynx, lungs, GIT, CVS, liver, and kidneys. The virus enters the receptor through its spike S and start replication using host cell machinery, and if not cleared in time, can lead to an exuberant immune response and cytokines storm which is the main reason for acute respiratory distress, septic shock, and multiorgan failure. Specific treatment is not available presently, although clinical trials and development of several anti-SARS-CoV-2 medications are underway. Symptomatic treatment with antipyretics and anti-inflammatory drugs and antiviral treatment with the already available antivirals is used against other viruses which are still tried in most countries. Few companies have claimed their vaccines to be 95% effective and expected to get their emergency-use authorization very soon. However, it is still too early to claim such success in general populations at this stage. Furthermore, the vaccine may not be available to a considerable proportion of the world's population as a big percentage of the densely populated countries in the world has poor economy; these countries may not be able to afford the vaccine in such a huge bulk at present. Prevention and mitigation are the two core strategies to cope with the pandemic. Reinfection with COVID-19 is very much unlikely, however, due to lack of sufficient data on the immune response of the individuals infected with the disease; it is still early to conclude anything. Whether there would be any secondary complications with the use of the developed vaccine/s is still obscure as these vaccines have not been passed through extensive trials.

Abbreviations

Ab	antibody
ACE2	angiotensin-converting enzyme 2
ACS	acute coronary syndrome
ADE	antibody-dependent enhanced immunity
Ag	antigen
AKI	acute kidney injury
APC	antigen-presenting cells
ARDS	acute respiratory distress syndrome
BB coronaviruses	B subgroup β-CoVs
CDC	complement-dependent cytotoxicity
CFR	case fatality rate
ChAdOx1	chimpanzee adenovirus vector
CKD	chronic kidney disease
COPD	chronic obstructive pulmonary disease
COVID-19	coronavirus disease 2019
CoVs	coronaviruses (corona-viridae)
CVD	cardiovascular diseases
CVS	cardiovascular system
E	a small membrane
EMA	European medicines agency
EPA	environmental protection agency
FcR	Fc receptors
FPV	favipiravir
GGOs	ground-glass opacities
hACE2	human ACE2
HT	hypertension
I	internal protein
ICU	intensive care unit
IFN	IL-10; interferon
IL	interleukins
IP-10	γ inducible protein-10
KDIGO	kidney disease improving global outcome
LDH	lactate dehydrogenase
LPV	lopinavir
M	membrane

MERS	middle east respiratory syndrome
MODS	multiorgan dysfunction syndrome
MOF	multiple organ failure
Nab	neutralizing antibodies
NIAID	National Institute of Allergy and Infectious Diseases
NSAIDs	nonsteroidal anti-inflammatory drugs
PPE	personal protective equipment
PRRA	polybasic sequence with a leading proline
PRRs	pattern-recognition receptors
RAS	renin-angiotensin system
RBD	receptor-binding domain
RP	Re detectable positive
rRT-PCR	real-time polymerase chain reaction
RTV	ritonavir
S	spike
SARS	severe acute respiratory syndrome
SARSr-CoVs	SARS related coronaviruses
SSEs	super spreading events
TNF	tumor necrosis factor

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