2019 Novel Coronavirus: A Review on Epidemiology, Pathophysiology, Diagnosis and Current Clinical Trials in Vaccine Development

Megala Jayaraman¹, Sabari Krishnan B.B.¹, Parijat Dutta¹, Jayesh Telang¹, Srestha Adhikari¹ and Arpan Neeraj Pardeshi¹

¹Department of Genetic Engineering, Faculty of Engineering and Technology, SRM Institute of Science and Technology, SRM Nagar, Kattankulathur-603203, Chennai, Tamil Nadu, India

Abstract: Coronavirus disease-2019, a viral disease caused by the novel severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2), was identified by the Centre for Disease Control (CDC), China, on January 7, 2020. This mysterious respiratory epidemic occurred in Wuhan, China, in late December 2019. A month after its outbreak in China, the World Health Organization (WHO) declared it as a public health emergency of international concern (PHEIC) due to its severity and high transmission potential throughout the world, leading many nations to implement multiple lockdown sessions and strict social distancing measures. As of September 21, 2020, 30,675,675 active cases and 954,417 deaths had been reported worldwide. Intensive research is being carried out across the globe to identify precise diagnostic techniques and develop novel, effective vaccines against the virus. Herein, we elaborate on details of epidemiology, genetics, pathophysiology, diagnosis, prevention, and vaccine trials related to this pandemic.

Keywords: COVID-19, global outbreak, novel coronavirus, pathogenesis, SARS-CoV-2, vaccines.

1. INTRODUCTION

Coronavirus was first discovered in the 1930s when an acute respiratory infection of domesticated chickens was reported. Many researchers in the next decade described and successfully isolated the virus, which was called the infectious bronchitis virus [1, 2]. Now, the world has met a crisis in the name of Coronavirus disease-2019, abbreviated as COVID-19, caused by a new strain of Betacoronavirus named SARS-CoV-2. It has been hypothesized that SARS-CoV2 cases were spotted in Wuhan, China. The disease was contagious that it spread worldwide at an alarming rate. In December 2019, mysterious cases of pneumonia were reported in Wuhan, China. The disease was contagious that it spread worldwide at an alarming rate. In December 2019, mysterious cases of pneumonia were reported in Wuhan, China, and most of the reported cases had a contact history with the local seafood market. In January 2020, SARS-CoV-2 was identified by the CDC, China, from a throat swab from one of the patients and named the virus 2019 novel Coronavirus (2019nCoV). The WHO declared the outbreak as a PHEIC on January 30, 2020, and as a pandemic on March 11, 2020. After the outbreak of COVID-19, the Chinese government had initiated a level-1 public health response on January 26, 2020, to prevent the spread of the disease [3]. Scientists are trying tirelessly to reveal the exact cause of the origin, both geographic and microbiological, of this pandemic. Some researchers found that Coronavirus was introduced to the human race from masked palm civet, Paguma larvata, which was carried down to its lineage from viral contamination by horseshoe bats (Rhinolophus genus) [4]. As of September 21, 2020, the disease had spread across more than 215 regions and infected 31,629,302 individuals causing 954,417 deaths globally [5], and is steadily increasing. This might be due to our poor understanding of viral potency and pathology, neglecting the importance of isolation and social-distancing by the public, and due to the unavailability of vaccines. To address these problems, almost all existing data relevant to COVID-19 research, such as COVID-19 epidemiology, genetics, transmission, diagnosis, and clinical trials of vaccines against SARS-CoV-2, had been compiled in this mini-review article.

1.1. Viral Taxonomy

Coronavirus belongs to the family Coronaviridae, subfamily Orthocoronavirinae. The term corona means “crown” or “wreath” in Latin, and was named so due to the unique spikes called peplomer, which contribute to the crown-like morphology of the virus [6]. This is a family of enveloped viruses with long (26-32 kilo (ribo) nucleotides) [7] positive-sense single-stranded RNA (ssRNA(+)) and is subdivided into four genera, namely Alphacoronavirus (α-CoV), Betacoronavirus (β-CoV), Gammacoronavirus (γ-CoV) and Deltacoronavirus (δ-CoV) [8]. Bats are the ideal primary natural reservoir for the viruses in the first two genera, whereas avians are the natural reservoir for the latter two genera [9]. Humans became part of their life-cycle due to our unguarded interaction with avians, bats, and secondary host animals (also known as amplification hosts) like civets, cattle, and camels. Using molecular and phyloge-
nestic studies, it had been found that most of the human coronaviruses (HCoVs) originated from bats and passed down to other mammals [10]. Six species of HCoVs were discovered in the previous years: HCoV-229E (1966), HCoV-OC43 (1967), SARS-CoV (2003), HCoV-NL63 (2004), HCoV-HKU1 (2005), and MERS-CoV (2012). Bats were the primary hosts of HCoV-229E (α-CoV). Cattle were the host of HCoV-OC43 (β-CoV); Himalayan palm civets and bats were the hosts of SARS-CoV (β-CoV) and HCoV-NL63 (α-CoV), whereas dromedary camels and bats were the hosts of MERS-CoV (β-CoV) [4]. Recently, SARS-CoV-2 (a member of the β-CoV genus) - provisional name: 2019nCoV; the virus responsible for this pandemic attack of COVID-19, was discovered.

1.2. Risk Factors

Old people, immunocompromised individuals, and people with existing medical complications such as obesity, diabetes mellitus, hypertension, pulmonary diseases like asthma, emphysema, and chronic obstructive pulmonary disease, cardiac, hepatic, and renal diseases, and people under steroid medications are at higher risk of getting COVID-19. Also, the risk of COVID-19 increases with age, and adults above 65 years of age need to take the primary preventive measures such as personal hygiene, quarantine, and less public interaction. This does not mean that the virus can only affect old people; it can infect young adults, teens, or even babies. There are scattered reports of severe COVID-19 in some young, healthy people. Children are very unlikely to become severely ill. US being the top in the list for reported COVID-19 cases and deaths, (2-5)% of deaths in the US were of people who were (65-75) years old, (4-10)% of deaths were of people who were (75-85) years old, and for people of age 85 and above, the death statistics were more than 10%. The American CDC also noted that there could be high-risk situations where some sub-populations were at an increased risk of getting infected by the virus. Dense contact environment, difficulty in contact tracing and identity-revealing, and difficulty to practice successful isolation and quarantine are some of the factors that may increase the risk of COVID-19, sometimes leading to a “super-spreading” event [11].

Of the seven HCoV species discovered, the extremity and risk of the infection depend on several factors like the environment of the patient, age, host and herd immunity, genetics and behavior of the virus species, etc. HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1 were identified to cause mild symptoms, whereas SARS-CoV, MERS-CoV, and SARS-CoV-2 could cause potentially severe symptoms [7]. These symptoms could widely vary, and the clinical manifestation remains obscure. The inter-species transmission of Coronavirus could be understood by observing the ecological contribution made by avians and bats. Due to distant migration and their interaction with other creatures of the biosphere, being predator, prey, or symbiont, the virus was transmitted from one species to another, mutating multiple times to adapt to the new environment. Transmission of bat coronavirus (BCoV) to humans would have happened directly, such as contact with infected bat meat, feces, blood, and other body fluids, or indirectly, such as consuming fruits partially eaten by infectious bats, raw meat of amplification hosts, etc.

2. EPIDEMIOLOGY

2.1. Reproduction Number and Transmission

Basic reproduction number (R₀) is an empirical value to have an idea of how an infectious disease spreads from one person to another if they are susceptible to that infection. It could be considered as a scale to measure the transmission potential and severity of the pathogen but could depend on many factors such as population structure, public health, individual health, herd immunity, etc. [12]. A study of COVID-19 reproduction numbers in India suggested an R₀ of 2.56 units with a herd immunity of 61% [13]. Recent findings from the South Korean population have reported the R₀ (mean reproduction number) of 1.5 [14]. A similar study in China resulted in a higher mean R₀ value reaching up to 3.28 units, suggesting the high transmission potential of SARS-CoV-2 in China and overseas. According to the current collective statistics, the average R₀ of COVID-19 is about 1.4-5.7 units [15, 16]. It should be noted that the R₀ of the severe acute respiratory syndrome (SARS) (2-5 units) and the Middle East respiratory syndrome (MERS) (0.3-0.8 units) are lower than that of COVID-19, indicating that the transmission potential of COVID-19 is higher than its pathological relatives [11].

According to the WHO, COVID-19 transmission is primarily facilitated through respiratory droplets (>5-10 μm in diameter). Infectious respiratory droplets can be contagious to a healthy person if he/she is in the vicinity (<1 m) of an infected person. A person is clinically recognized as “suspected” if he/she spends 15 minutes or more in the vicinity of an affected individual. The virus can also launch an infection via fomites, which is an indirect mode of disease transmission. Thus, transmission can occur not only through physical or proximal contact but also from a shared environment [17]. This statement clearly explains the importance of wearing personal protective equipment (PPE) such as gloves and masks and using sanitizers, and soaps or detergents. For some part of the population, symptoms may not appear even after disease manifestation, leading to the unnoticed spread of the virus from person-to-person. Other than these airborne modes of viral transmission, there was some evidence for transmission of SARS-CoV-2 through feces, due to the intestinal infection caused in some patients. In a study, the virus had been cultured from an infected stool sample [18], but to this date, no reports on fecal and oral transmission of the virus were made. An investigatory study in Chennai, India, revealed that random samples from five sewage pumping stations tested positive for the viral RNA using reverse-transcriptase PCR (RT-PCR) techniques. Though there is no evidence that the virus can spread through this medium, it is still an important finding to consider under preventive measures [19].
2.2. COVID-19 Demographics

According to the WHO COVID-19 weekly epidemiological update released on September 21, 2020 [5], SARS-CoV-2 had resulted in 31,629,302 COVID-19 cases globally, including international conveyance such as Diamond Princess, from where 741 infection cases were reported and 954,417 patient deaths (13 deaths from Diamond Princess). More than 215 different regions recognized by the WHO were reported to have at least one COVID-19 infection. As of now, the USA is the leading nation having the greatest number of reported COVID-19 cases (6,859,445 cases), contributing to ~21.69% of the total number of reported COVID-19 cases, whereas Anguilla has the least number of reported cases (three cases). India (5,487,371 cases), Brazil (4,630,976 cases), and the Russian Federation (1,122,817 cases) are the nations following the USA, having the total number of reported cases greater than one million. Also, the number of mortalities is the highest in the USA, of 197,442 cases, which accounts for ~20.69% of the total number of patient deaths, followed by Brazil (135,793 deaths), India (86,752 deaths), and Mexico (72,803 deaths) having more than 50,000 deaths. It was calculated that ~91% of the regions were affected by the pandemic wave. Most of the regions which did not report any COVID-19 case to date are the Pacific island nations such as Kiribati, the Marshall Islands, the Federated States of Micronesia, Nauru, Palau, Samoa, Solomon Islands, Tonga, Tuvalu, and Vanuatu [20].

Case fatality rate (CFR) can be a measure of the severity of a disease. It is measured by finding the ratio of the number of fatalities due to the disease to the total number of cases admitted due to symptoms of that disease. The CFR of the nations with a leading number of cases were: the USA (2.88%), India (1.58%), Brazil (2.93%), the Russian Federation (1.73%), Peru (3.97%), Colombia (3.08%), Mexico (9.56%), South Africa (2.36%), Spain (4.55%), France (6.44%), Iran (5.44%), the UK (9.66%), Italy (10.74%), Canada (6.09%), Plurinational State of Bolivia (5.49%), Ecuador (8.11%), Egypt (5.34%), Belgium (8.99%), Netherlands (6.39%), China (4.96%), and Sweden (6.23%). The highest CFR was observed for Yemen (22.40%).

Similar to CFR, the case recovery rate (CRR) of a nation, can be calculated. It can be defined as the ratio between the total number of recoveries to the total number of reported cases in a nation for a particular disease. Many nations such as Anguilla, Holy See, Falkland Islands, Greenland, Saint Kitts & Nevis, Grenada, New Caledonia, Timor-Leste, and Saint Vincent & the Grenadines have gained a 100% CRR by September 21, while Puerto Rico, Netherlands, and the UK have a CRR<1%, based on available data [21]. CRR can depend on many factors such as the age of the patient, their response to drugs, immunity, hospital infrastructure, etc., as Fig. (1) shows a choropleth map of the world with nations indicated based on their approximate CFR:CRR values. The nations having CFR-CRR ratio ≥1 can be considered as regions needing immediate medical attention (hot-spots), without ruling out the severity of the situations in nations having a CFR-CRR ratio below this cut-off. According to this criterion, Puerto Rico, Netherlands (25.06), the UK (14.21), Sweden (2.00), and Kyrgyzstan (1.63) are the major hot-spots where the number of COVID-19 deaths was more than the number of recoveries.

**Fig. (1).** A choropleth map showing the CFR-CRR ratio of nations across a color gradient. (A higher resolution / colour version of this figure is available in the electronic copy of the article).
3. PATHOLOGY

3.1. Molecular Structure and Viral Entry

Using advanced microscopic techniques, like cryogenic electron microscopy, in-depth knowledge of the viral structure was obtained. The estimated size (diameter) of a SARS-CoV-2 virion was 50-200 nm [22] and the peplomer size was ~20 nm [23]. A mature SARS-CoV-2 encodes four major types of structural proteins: nucleocapsid phosphoprotein (N), spike or surface glycoprotein (S), envelope small membrane protein (E), and membrane glycoprotein (M) [24]. Another set of proteins is the open-reading frame (ORF) proteins, which help in viral entry, virulence, and host-cell hijacking. All these proteins may serve as antigens to stimulate neutralizing antibodies and increase helper-T (CD4+) and cytotoxic-T (CD8+) cell responses. Like other viruses, Coronavirus also have a particular host-cell receptor or entry point, through which the virus infects the host cells. Molecular studies, such as molecular docking, have revealed the possible entry sites of SARS-CoV-2, such as human angiotensin-converting enzyme 2 (hACE2) and basigin, also known as EMMPRIN: extracellular matrix metalloproteinase inducer (CD147) [25].

More specifically, E and M proteins have important functions in the viral assembly [26]. N protein is important for the packaging and protecting the viral RNA by forming ribonucleoprotein (RNP) complex, RNA synthesis, and transcription, whereas S protein of SARS-CoV-2 is responsible for virus binding and entry. S protein has a sufficient affinity to hACE2 to use them as a point of entry [23, 25]. Unlike the original strain of the SARS virus, SARS-CoV-2 has a higher affinity towards hACE2 [23]. The precursor S protein of SARS-CoV-2 can be cleaved proteolytically into two subunits: S1 (75.4 kDa) and S2 (64.7 kDa). The S1 protein of SARS-CoV-2 shares ~70% similarity with that of SARS-CoV. S1 helps in the attachment of the virion to the cell membrane by interacting with the host ACE2 and/or other receptors like CLEC4M (DC-SIGNR, also known as L-SIGN or CD299). This marks the initial step of the infection and binding to these receptors can cause internalization of the virus into the endosome of the host cell and results in endocytosis. During the endosome formation, the S protein may undergo some conformational changes, which leads to its cleavage by cathepsin L, thus unmasking the S2 fusion peptide. This fusion peptide activates membrane fusion within the endosome. Simultaneously, the S2 subunit facilitates the fusion of the viral membrane and cellular membranes by acting as a class I viral fusion protein. S protein has at least three conformational states viz. pre-fusion native state, pre-hairpin intermediate state, and post-fusion hairpin state. During fusion between the viral membrane and target cell membrane, the coiled-coil regions (heptad repeats) conforms to a trimer-of-hairpins structure, thus positioning the S2 fusion peptide near the C-terminal region of the ectodomain. The formation of this structure helps in the fusion of the viral membrane and the target cell membrane. Another mechanism of viral entry is by S protein-priming by the transmembrane protease, serine 2 (TMPRSS2). After a SARS-CoV-2 virion attaches to a host (target) cell, the cell protease TMPRSS2 cleaves the S protein of the virus, exposing the S2 fusion peptide and mediates membrane fusion. The virion then releases the ssRNA(+) into the cell and hijacks the target cell machinery to synthesize and release numerous copies of the virus to infect more new cells [27, 28] (Fig. 2a).

3.2. Genetics of SARS-CoV-2 and Pathogenesis

Studies showed that SARS-CoV-2 had the highest genetic homology with BCoV RaTG13 [29]. The virus keeps mutating to adapt to the environment. The S, N, orf1ab, orf3a, and orf8 genes were prone to a mutation in which 42% of the variations were non-synonymous [30]. According to the NCBI Reference Sequence Database (RefSeq: NC_045512.2) [31], the entire viral genome is about 29,903 ribonucleotides. As we have already mentioned, the genome is a positive ssRNA and contains mainly 12 genes, that code for about 12 main proteins (NCBI, UniProt), as shown in Table 1.

SARS-CoV-2 enters the respiratory tract of a person through aerosols expelled by an infected individual and attacks the type-II alveolar (AT2) cells. It enters the cells via the ACE2. SARS-CoV-2 receptor-binding domain (RBD) of the S protein binds to ACE2, promoting the S2 receptor to a more stable state which is necessary for the fusion between membranes for target cell entry. The positive ssRNA of SARS-CoV-2 undergoes translation to produce polyproteins. AT2 cells release inflammatory mediators in response to these polyproteins which involve activation of macrophages and neutrophils to produce cytokines like interleukins (IL-1 and IL-6), and tumor necrosis factors (TNF-α). The elevated level of cytokines causes vasodilatation and increases the permeability of blood capillaries, and as a result, plasma leaks into the alveoli leading to edema; this decreases the number of surfactants in the alveoli and increases the surface tension causing alveolar collapse and decreased gas exchange which eventually end up as difficulty in breathing. This cytokine cascade also causes the release of pro-inflammatory, resetting the thermostat, causing fever and other symptoms like coughing, tachycardia, etc. [32] (Fig. 2b).

3.3. Pathophysiology

SARS-CoV-2 infection can activate innate and adaptive immune responses. In patients with severe COVID-19, lymphopenia was common with a drastic reduction in numbers of T\(_\text{H}\), T\(_\text{C}\), B, and natural killer (NK) cells, monocytes, eosinophils, and basophils. Pro-inflammatory cytokines such as chemokine C-C motif ligand 3 (CCL3), IL-1β, IL-2, IL-6, IL-8, IL-17, and TNF-α were also elevated which can lead to pulmonary, cardiac, hepatic, and renal tissue damage [33]. Exhaustion markers such as the natural killer cell receptor G2A (NKG2A) on cytotoxic lymphocytes (NK and T\(_\text{C}\) cells) are elevated in patients with COVID-19. There is a possibility of antibody-dependent enhancement in SARS-CoV-2 infection which enhances the entry of virus and induction of severe inflammatory response through neutralizing monoclonal antibodies [34]. This is one of the major concerns for anti-viral vaccine development and antibody-based therapies.
Table 1. List of genes in the SARS-CoV-2 genome and the respective encoded proteins with their functions.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Gene ID</th>
<th>Gene</th>
<th>Location (nt)</th>
<th>Size (nt)</th>
<th>Protein Encoded</th>
<th>RefSeq ID</th>
<th>Approx. Size (kDa)</th>
<th>Function(s) of Protein</th>
</tr>
</thead>
</table>
| 1      | 43740578| orf1a (rep1a) | 266-13483 | 13217 | ORF1a polyprotein (replicase polyprotein 1a) | YP_009725295.1 | 485 | • Cysteine-type endopeptidase activity.  
• Omega peptidase activity.  
• RNA-directed 5’-3’ RNAP activity.  
• Zn²⁺ binding.  
• Helps in viral genome replication and protein processing.  
• Induces host autophagy. |
| 2      | 43740578| orf1ab (rep1a-1b) | 266-21555 | 21289 | ORF1ab polyprotein (replicase polyprotein 1ab) | YP_009724389.1 | 781 | • Functions of ORF1a polyprotein.  
• ATP-binding ability  
• Exonuclease activity producing 5’-phosphomonoesters.  
• Methyltransferase activity.  
• RNA helicase activity.  
• Helps in methylation.  
• Helps in DNA-templated transcription of the viral genome. |
| 3      | 43740568| S | 21563-25384 | 3821 | Surface (spike) glycoprotein | YP_009724390.1 | 140 | • Host cell surface receptor-binding.  
• Helps in endocytosis, pathogenesis, and receptor-mediated virion attachment with the host cell. |
| 4      | 43740569| 3a | 25393-26220 | 827 | ORF3a (protein 3a) | YP_009724391.1 | 30 | • Ion-channel activity.  
• Helps in pore formation in the host cell membrane.  
• Protein complex oligomerization.  
• An inhibitory effect on the host IFN receptor and thus the host type-I IFN-mediated signaling pathway is also suppressed. |
| 5      | 43740570| 4 (E) | 26245-26472 | 227 | Envelope small membrane protein | YP_009724392.1 | 8 | • Disruption of the host cell membrane.  
• Viral budding from the Golgi bodies.  
• Induces host cell apoptosis.  
• Activates the host NLRP3 inflammasome leading to an outburst of IL-1β and IL-18 production resulting in pyroptosis. |
| 6      | 43740571| M | 26523-27191 | 668 | Membrane glycoprotein | YP_009724393.1 | 24 | • Structural constituent of the virion.  
• Helps to complete viral life cycle.  
• Reduce the effects of the host immune system. |
| 7      | 43740572| 6 | 27202-27387 | 185 | ORF6 (non-structural protein 6) | YP_009724394.1 | 7 | • Could be a determinant of viral virulence.  
• Might also induce host cellular DNA synthesis in vitro. |
| 8      | 43740573| 7a | 27394-27758 | 365 | ORF7a (protein 7a) | YP_009724395.1 | 13 | • Helps in the modulation of the G/G transition checkpoint of the host cell.  
• Suppresses the host tetherin (an IFN-α inducible cellular factor) activity thus overcoming the natural anti-viral systems of the host cell.  
• Helps the release of viral particles from an infected cell. |
| 9      | 43740574| 7b | 27756-27887 | 131 | ORF7b (non-structural protein 7b) | YP_009725318.1 | 5 | A helical transmembrane protein with non-structural function. |
| 10     | 43740577| 8 | 27894-28259 | 365 | ORF8 (non-structural protein 8) | YP_009724396.1 | 13 | A helical transmembrane protein; an integral component of the viral membrane. |
| 11     | 43740575| N | 28274-29533 | 1289 | Nucleocapsid phosphoprotein (nucleoprotein) | YP_009724397.2 | 46 | • Helps in the packaging of the viral ssRNA(+) genome into a helical RNP.  
• An important role in virion assembly.  
• Enhances the efficiency of sub-genomic viral RNA transcription and viral replication. |
| 12     | 43740576| 10 | 29558-29674 | 116 | ORF10 | YP_009725255.1 | 4 | The function of this protein is not yet confirmed. It has similarities with other non-structural proteins. |

RNAP: RNA polymerase; IFN: interferon; NLRP3: Nucleotide-binding oligomerization domain (NOD)-like receptor (NLK) family pyrin domain containing 3
4. DIAGNOSIS

Most of the signs and symptoms of COVID-19 such as fever, headache, fatigue, chills, and myalgia, are non-specific, i.e., these symptoms can be due to other viral infections too. One of the specific symptoms observed was the early loss of taste and smell [35]. Therefore it is very difficult to differentiate COVID-19 from other respiratory diseases based on the symptoms hence a distinct diagnosis procedure is required to confirm a patient as positive for this particular disease. Besides the physical examination, the diagnosis of COVID-19 is made using biochemical assays (molecular, immunological, and biomarkers) and/or radiological tests.

4.1. Molecular Assays

The molecular assays for detecting COVID-19 mainly included high-throughput sequencing and real-time RT-PCR (rRT-PCR). The high-throughput sequencing, despite being a reliable identification method, was not preferred mostly because of its high price. Compared to sequencing, rRT-PCR was found to be a simpler and cheaper way to detect the vi-
4.1. Personal Hygiene and Healthcare

Maintaining personal hygiene is necessary to avoid getting infected, and this involves regular use of soaps and sanitizers from time to time. Repeatedly touching the mouth, eyes, and nose without hand sanitization should be avoided because these are the sites for viral entry into the body. Washing hands with good quality soap in the ten recommended steps for at least 20 seconds or applying high-grade sanitizer (70% alcohol) whenever touching a surface, wearing face masks (N95, PPE) and gloves while going out, and abiding by social-distancing policies are essential for preventing COVID-19 transmission. Practicing these healthcare interventions strictly would help reduce $R_0$, thus reducing the number of people getting affected [56].

Treatment for COVID-19 mainly involved symptom-based management and oxygen therapy, with mechanical ventilation for patients suffering from respiratory failure. Even though most of the existing medical procedures could be used to treat COVID-19 patients, the use of non-invasive positive-pressure ventilation and high-flow nasal cannula should be avoided to prevent aerosolizing of the virus [57].

5.2. Convalescent Plasma Therapy (CPT)

A more effective and beneficial therapeutic strategy for COVID-19 is now CPT. It involves the use of blood plasma from people who have recovered from the infection to help other patients in their recovery, and it has been authorized
by the U.S. Food and Drug Administration (USFDA). Blood donated by recovered people has neutralizing antibodies (nAbs) against SARS-CoV-2, and it is extracted by a simple plasma separation. These antibodies are predominantly IgG and IgM (classified as VH3-53 human nAbs with short CDRH3s and ACE2-blocking nAbs) which were demonstrated to target the SARS-CoV-2 spike RBD, S₁ subunit or the S₁/S₂ proteolytic cleavage site [58]. Once the plasma is extracted and has reached the optimum nAb titer, the nAb-rich plasma is transfused into infected COVID-19 patients as post-exposure prophylaxis [59]. From various clinical studies conducted in China, South Korea, and Taiwan, it was validated that CPT has the potential to reduce mortality [60].

5.3. Drugs in Use Against COVID-19

Antiviral drugs like nucleotide-analog Remdesivir (RDV) and Favipiravir (FPV) are being tested, but none has been specifically approved. These drugs work by inhibiting RdRP and stop the replication of viral RNA [61, 62]. Some drugs act as inhibitors to ACE2 and/or TMPRSS2, such as Resochin™ chloroquine phosphate, hydroxychloroquine (HCQ) and cepharanthine–selamectin–mefloquine hydrochloride, synthetic peptide inhibitors like DX600, MLN-4760 and TAPI-2, phytochemicals like nicotiamine, aegigenin, wogonoside, emodin, and resveratrol, recombinant hACE2 protein (ACE2 inhibitors), FOIPAN® camostat mesilate and Buipel™ nafamostat mesilate (TMPRSS2 inhibitors) [63].

---

**Fig. (3).** An illustration of different steps involved in the development of vaccines using Adenovirus vector (ChAdOx1 nCoV-19) and its possible mechanisms of action in an immunized individual. (A higher resolution / colour version of this figure is available in the electronic copy of the article).
Another clinical trial (ChiCTR2000029765) using human IL-6 receptor-targeted monoclonal antibody produced from mice, ACTEMRA® Tocilizumab (TmAb) has reported quick control over the fever and respiratory function by reducing vasodilation of alveolar capillaries by targeting and inhibiting IL-6 receptor [64, 65].

The use of HCQ has shown efficiency in nasopharyngeal viral clearance within 3–6 days in most of the patients and inhibits SARS-CoV-2 in vitro [66]. The efficiency of this drug was found to be better than chloroquine. HCQ can be used along with azithromycin to produce a synergistic effect against SARS-CoV-2. A “mechanism of action” was developed for HCQ used in combination with azithromycin, which involves the assembly and delivery of HCQ by azithromycin and two ionic coupling elements to adenine-uracil RNA pairings of the SARS-CoV-2 virus to inhibit replication and translation of the viral RNA [67].

Recently, dexamethasone (DXM), a corticosteroid medication, was tested in COVID-19 patients of the UK. Though it is not recommended by the WHO due to its side-effects, it is turning to be a lifesaving drug. DXM acts by mimicking the function of the anti-inflammatory hormones of the body [48, 68, 69]. Advanced treatment using stem cells (SCs) like mesenchymal SC-based therapy in patients can have anti-inflammatory and anti-apoptotic effects that can repair the pulmonary epithelial cell damage caused by viral replication and enhance alveolar fluid clearance. Currently, this work is in progress in China and other nations [70].

5.4. Availability of Vaccines

An attenuated viral vaccine can be prepared by following the established protocols involving UV radiation, methanal, and β-propiolactone. Screening of serially propagated COVID-19 virus with reduced pathogenesis such as minimal lung injury, the limited neutrophil influx, and increased anti-inflammatory cytokine expressions, when compared to the wild-type SARS-CoV-2 strain, can be used for attenuated-viral vaccine production.

The S protein of SARS-CoV-2 consists of the RBD, which facilitates viral entry into the sensitive AT2 cells through hACE2. Thus, the generation of antibodies targeting the S subunit of SARS-CoV-2 would be an important preventive and treatment strategy. A detailed illustration of the possible development of an antibody vaccine, particularly the ChAdOx1 nCoV-19 vaccine is shown in Fig. (3). Subunit vaccines should be combined with adjuvants such as alum, GlaxoSmithKline adjuvant system (GSK-AS) for a faster and safer way of vaccination [71]. Table 2 shows the upcoming and on-going vaccine trials against COVID-19.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Vaccine Name &amp; Characteristics</th>
<th>Status</th>
<th>Clinical Trial Details</th>
<th>Companies/Institutes in Collaboration</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AZD1222-ChAdOx1 nCoV-19 chimpanzee adenovirus vector.</td>
<td>Phase III (NCT04516746)</td>
<td>No. of subjects: 30,000 (≥18 y.); intramuscular injection; double-blind, placebo-controlled; ECD: Dec 2, 2020</td>
<td>The University of Oxford, AstraZeneca</td>
<td>UK</td>
</tr>
<tr>
<td>2</td>
<td>mRNA-1273 LNP-encapsulated mRNA expressing S protein.</td>
<td>Phase III (NCT04470427)</td>
<td>No. of subjects: 30,000 (≥18 y.); intramuscular injection; randomized, observer-blind, placebo-controlled; ECD: Oct 27, 2022</td>
<td>Moderna Therapeutics, Inc., National Institute of Allergy and Infectious Diseases (NIAID), Biomedical Advanced Research and Development Authority (BARDA)</td>
<td>US</td>
</tr>
<tr>
<td>3</td>
<td>PiCoVace inactivated adsorbed SARS-CoV-2 virus vaccine.</td>
<td>Phase III (NCT04456595)</td>
<td>No. of subjects: 8,870 (18 y.); intramuscular injection; randomized, double-blinded, placebo-controlled; ECD: Sep, 2021</td>
<td>Sinovac Life Sciences Co., Ltd., Instituto Butantan</td>
<td>China, Brazil</td>
</tr>
<tr>
<td>4</td>
<td>BBV152 whole-virion inactivated SARS-CoV-2 vaccine.</td>
<td>Phase I/II (NCT04471519)</td>
<td>No. of subjects: 1,125 (12-65 y.o.); intramuscular injection; randomized, double-blinded, placebo-controlled; ECD: Jun 30, 2021</td>
<td>Bharat Biotech International Limited, Indian Council of Medical Research (ICMR)</td>
<td>India</td>
</tr>
<tr>
<td>5</td>
<td>Ad5-nCoV adenovirus type 5 vector expressing S protein.</td>
<td>Phase II (ChiCTR2000031781)</td>
<td>No. of subjects: 1,125 (≥18 y.); intramuscular injection; randomized, double-blinded, placebo-controlled; ECD: Jan 31, 2021</td>
<td>CanSino Biologics Inc., Institute of Biotechnology, Academy of Military Medical Sciences</td>
<td>China</td>
</tr>
<tr>
<td>6</td>
<td>BNT162 (b1 and b2) RNA vaccine.</td>
<td>Phase II/III (NCT04368728)</td>
<td>No. of subjects: 29,481 (18-85 y.o.); intramuscular injection; randomized, observer-blinded, placebo-controlled; ECD: Nov 14, 2021</td>
<td>BioNTech, Fosun Pharma, Pfizer</td>
<td>Germany, China, US</td>
</tr>
<tr>
<td>7</td>
<td>ZyCoV-D</td>
<td>Phase I/II (ChiCTR200007026352)</td>
<td>18-55 y.o.; intradermal injection; randomized, observer-blinded, placebo-controlled; ECD: Jul, 2021</td>
<td>Zydas Cadila</td>
<td>India</td>
</tr>
</tbody>
</table>

(Table 2 contd....)
**CONCLUSION**

COVID-19 is a global emergency due to the spread of *SARS-CoV-2*. It is a crisis because we have very limited knowledge of the secondary symptoms and transmission of the virus, and its preventive measures. A vast amount of resources is spent in the medical, pharmaceutical, and biotechnological fields for life-sustaining drug and vaccine development. By the end of 2020, we would be accessing a COVID-19 vaccine, but still, the side-effects and immune profile of the vaccine is yet to be learned thoroughly in humans. As always, the most effective cure is prevention. Avoiding physical interaction with the public is highly recommended to prevent the upcoming of a super-spreading pandemic event. Wearing masks, gloves, usage of sanitizers (70% alcohol), soaps, maintaining a minimum social-distance of 1 m, and using natural immune foods are some of the preventive measures which we could practice in daily life. Drugs like RDV, FPV, TmAb, HCQ, and DXM are being tested for their efficiency in the treatment of the disease. There are no approved vaccines for COVID-19, but many are undergoing clinical trials. One of them being ChAdOx1 nCoV-19, maybe a successful vaccine against COVID-19 soon. As of now, CPT is a promising therapeutic strategy with minimal side-effects and has the potential to immunize people until an effective and more specific vaccine is out in the market.

**CONSENT FOR PUBLICATION**

Not applicable.
FUNDING
None.

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

ACKNOWLEDGEMENTS
The authors thank Ms. Ayushi Telang, MapChart.net, and Biorender.com for their kind support in digitalizing the illustrations for this review. The authors also thank the Department of Genetic Engineering, SRM Institute of Science and Technology, Kattankulathur Campus, Chennai for their valuable support and favors for completing this mini-review article.

REFERENCES


