Current trends and possible therapeutic options against COVID-19

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ABSTRACT

COVID-19 is a highly contagious disease, caused by SARS-CoV-2, which can be transmitted from person to person. SARS-CoV-2 has thought to be originated in bats and transmitted to humans. SARS-CoV-2 has ssRNA genome that is about 30kb in length and it enters lung epithelial cells using spikes (S) by binding with Angiotensin-converting enzyme 2 (ACE-2). Till date, there is no definitive treatment available for COVID-19 and currently, only symptomatic treatments are available. There is no vaccine developed to combat COVID-19 but scientists are working on developing different types of vaccines including nucleic acid vaccines, subunit vaccines and whole virus vaccines, that are effective and safe to use. Broad spectrum antivirals like ribavirin, lopinavir, ritonavir, arbidol, favipiravir, remdesivir are being test to lessen the viral load. Recently, ivermectin (anti-parasitic drug), has been administered in vitro and showed 99.8% reduction in SARS-CoV-2 in cultured cells in 48 hours. Convalescent plasma (CP) treatments are also being used in order to treat critical cases, nevertheless, no drug has been approved yet. This review focuses on the transmission, clinical features, prevention, current treatments and future possible approaches that can be used to develop therapeutic options against COVID-19. *J Microbiol Infect Dis 2019; 9(3):110-120.*

Keywords: *SARS-CoV-2, clinical features, therapeutic options, antivirals, vaccines*

INTRODUCTION

Coronaviruses (CoVs) family is diverse, positive sense, single stranded group of RNA viruses causing various diseases, including hepatic, neurological and respiratory diseases varying in severity and can be found both in humans and animals [1,2]. In humans CoV has been known to cause mild respiratory infections [3] but in past two decades, Middle East respiratory syndrome CoV (MERS-CoV) and severe acute respiratory syndrome CoV (SARS-CoV-1) two novel coronaviruses emerged which caused severe disease as well as deaths in humans [4, 5]. SARS-CoV-1 death rate was about 10%, which infected 8,000 people with 800 deaths reported and MERS-CoV had a death rate of 35%, while infected 857 people with 334 deaths worldwide [6–8]. Now a new novel CoV known as SARS-CoV-2 has emerged that infect humans and have symptoms like shortness of breath, cough and fever which same as SARS and MERS [9, 10]. The disease caused by SARS-CoV-2 is named as COVID-19 and has become a global threat to health.

COVID-19 outbreak has infected almost 86,361 people in mainland China with about 4,655 confirmed deaths and 15,012,731 people are affected by this infectious disease globally by death toll up to 619,150 according to World Health Organization (WHO) [11] and in Pakistan there are 259,278 confirm cases and death toll is up to 5,763 according to Ministry of National Health Services Regulations and Coordination, Pakistan [12]. Social distancing and lockdowns are the basic ways to stop the spread of this contagious virus as adopted by China but in developing countries like Pakistan, it is difficult to enforce lockdown because it cripples economy and people could die from hunger.

There is no definitive vaccine or treatment available in case of COVID-19 medical staff and doctors are giving only symptomatic and

supportive treatments. The biggest focus nowadays is to prevent the infection from spreading further and home isolation is the first step if mild illness occurs so that its transfer can be minimized. In case of mild symptoms hydration, better nutrition and keeping the fever in check are usual principles. If the patient is tested positive of COVID-19 then use of antibiotics and antivirals should be administered with proper care. If severe condition prevails, then the oxygen should be administered and in critical cases, mechanical ventilators can be used to facilitate oxygen flow in the body [13]. Scientists are working to develop antivirals and vaccines against SARS-CoV-2. Many candidates are under clinical trials but still no vaccine or antiviral against SARS-CoV-2 has been approved yet.

In this concise review, the ways of transmission of COVID-19, its treatment options and possible vaccine options based on current and available evidence will be discussed.

STRUCTURE AND TRANSMISSION OF THE VIRUS

SARS-CoV-2 is a single stranded, positive sense RNA virus that is a member of Coronavirus family and was identified in January 2020 [14–16]. Recent studies suggest that SARS-CoV-2 was originated in bats because of its genetic sequence resembles other bat-based coronaviruses but the intermediate host between human and bats is still unknown [17,18].

 The main components of SARS-CoV-2 are single stranded RNA (about 30kb in size), nucleocapsid (N), envelope (E) and envelope spikes (S) as shown in Figure 1. Envelope spikes (S) are very important for the transmission of CoV [19] because it is crucial for receptor binding and membrane fusion [19–21]. Spike protein has 2 functional domains S1 domain in charge of receptor binding and S2 domain in charge for membrane fusion [22]. The cell receptor for SARS-CoV-1 is Angiotensin-converting enzyme 2 (ACE-2) [23–25] and similar is the case of SARS-CoV-2 because of the fact that both may have the same life cycle and use ACE-2 receptor for entry into ACE-2 expressing cells [26]. Recent studies suggest that S protein of SARS-CoV-2 has 10-20-fold (approx.) higher binding affinity with ACE-2 than of SARS-CoV-1 [27].

COVID-19 can be transferred from person to person through direct contact and is proved to be the method for the transfer of COVID-19 infection according to various reports [28, 29]. Human to human transfer COVID-19 occurs when an infected individual cough or sneeze via bio-aerosol production [30] or through direct contact with an infected person.

Viral infection is caused by binding of receptor which is expressed on host cells and later fusion of virus with cell membrane occurs. Various studies suggest that epithelial cells of the lungs are the primary target of viral attachment, therefore as spikes of the virus binds with the ACE-2 receptors (angiotensin-converting enzyme 2) as reported in the case of SARS-CoV-1. There is striking similarity in the sequence of receptor binding domain of SARS-CoV-2 spikes and SARS-CoV-1 spikes, which strongly suggests both use same ACE-2 receptors for their entry into host cells [31,32].
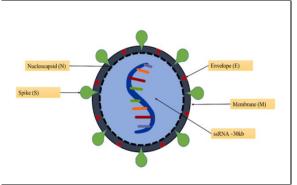

Figure 1. Structure of SARS-CoV-2.

CLINICAL FEATURES

Clinical evidence shows that COVID-19 mostly affects people ranging from age 30 to 79 years [33] and very few cases were reported in younger people having age below than 15 years. Moreover, a study indicates that half of the patients have already co-existing diseases and mostly the fatality rate was increased in patients with pre-existing ailments like cardiovascular diseases, diabetes and hypertension [33–37].

COVID-19 can be asymptomatic in some people and can cause severe respiratory failure in some cases. Fever, dyspnea and dry cough are common symptoms and headache, diarrhea, sputum production and hemoptysis are shown in rare cases, but pneumonia is developed in most cases of COVID-19 infection [33–38].

Symptoms arising in COVID-19 can be divided into three categories: mild symptoms, severe symptoms and critical symptoms. Mild patients had mild pneumonia, but dyspnea, abnormal respiratory frequency, low blood oxygen and pulmonary infiltrates (more than 50%) were observed in severe patients within 48 hours. In patients with critical conditions, septic shock, respiratory failure and in worst cases, multiple organ failure occurred [33,39]. During the progression of disease in mild to critical stage average period to develop dyspnea from mild symptoms is 8 days and in 10-11 days patient needs mechanical ventilation when developing into the critical stage [34]. Various symptoms developed during COVID-19 progression are shown in Figure 2.

CURRENT TREATMENTS AND PREVENTIONS

There is no definitive vaccine or treatment available in case of COVID-19 medical staff and doctors are giving only symptomatic and supportive treatments. The biggest focus nowadays is to prevent the infection from spreading further and home isolation is the first step if mild illness occurs so that its transfer can be minimized. In case of mild symptoms hydration, better nutrition and keeping the fever in check are usual principles. If the patient is tested positive of COVID-19 then use of antibiotics and antivirals should be administered with proper care. If severe condition prevails, then the oxygen should be administered and in critical cases, mechanical ventilators can be used to facilitate oxygen flow in the body [13]. Vasoactive drugs should be administered if circulation dysfunction occurs. If renal injury occurs in patient blood purification should be administered on time. It is important to monitor brain activity of patients in order to save them from intracranial hypertension [39]. The treatments available are limited and no vaccine or antiviral have been developed against COVID-19 infection. Moreover, the antivirals like ribavirin, lopinavir, ritonavir, remdesivir, oseltamivir has also been used as they are to reduce viral loads and remdesivir showed promising effects in United States when administered to a COVID-19 patient [40, 41]. As of today, no single antiviral or vaccine has been approved for the treatment of COVID-19.

Prevention is crucial for COVID-19 because there is no treatment available currently as discussed earlier. Nonspecific symptoms, longer incubation periods, transmission from asymptomatic patient of the virus make it very difficult to take proper preventive measures. People which have COVID-19 or suspected patients of COVID-19 must be isolated from healthy persons and must wear surgical masks and wash their hands for 15-20 secs with soap. Healthcare workers have the highest risk of getting a COVID-19 infection as in 2002, SARS affected 21% of healthcare workers [42]. N95 masks, goggles and protective suits must be worn by healthcare workers while addressing COVID-19 patients. Crowded areas must be avoided and people should stay home to avoid unnecessary contact to limit the spread of COVID-19. Hands should be washed every 30 mins or sanitized using an alcohol-based hand sanitizer and surfaces should be cleaned regularly. Touching eyes, nose or face must be avoided. Schematic illustration of preventive measures against COVID-19 is shown in Figure 3. In order to combat this epidemic, we must follow these preventive measures to ensure our safety.

POTENTIAL THERAPEUTIC APPROACHES BEING USED AGAINST COVID-19

Inhibition of SARS-COV-2 Entry or Fusion

As discussed above, SARS-CoV-2 enters the host cells using its spikes (S) which binds to ACE-2 receptors of a host cell to gain entry [15, 43] and ACE-2 receptors are recognized more efficiently by SARS-CoV-2 [31]. Therefore, to inhibit the entry of SARS-CoV-2 into host cells, receptor binding domain (RBD) of S protein may be a potential target for neutralizing antibodies. In a study, the neutralizing antibodies of SARS-CoV-1 showed potent binding to RBD of SARS-CoV-2 due to striking similarities in their protein structure [44]. But, SARS-CoV-2 antibody epitopes are proven to show extraordinary variations as compared to SARS-CoV-1 therefore, there is an imminent need to synthesize new monoclonal antibodies to inhibit RBD of SARS-CoV-2 [45]. But in the case of SARS-CoV-2, ACE-2 is not only receptor for entry into host cells, but cellular serine protease (TMPRSS2) is also critical for entry of viruses and use of TMPRSS2 inhibitor camostat mesylate was found out to reduce lung infection

and can be used in future for COVID-19 treatment [46]. Moreover, cell membrane fusion of the virus is also done with the involvement of heptad repeat 1 and heptad repeat 2 (HR1 and HR2) present on SARS-CoV-2 and it was shown in a study that HR2 derived peptides displayed inhibition in the fusion of virus which can be potentially used in the COVID-19 infections [47].

Researchers also identified that pH and receptor dependent endocytosis also plays important role in the entry of Coronaviruses [48, 49] and this approach can also be used in the case of SARS-CoV-2 [50]. Figure 4 show the infection cycle of SARS-CoV-2 and the potential targets for drug/vaccine development.
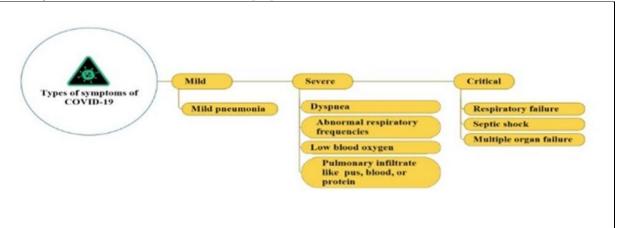

Figure 2. Clinical symptoms of COVID-19.

Figure 3. Preventive measures against COVID-19 epidemic.

In recent study use of chloroquine phosphate, an antimalarial drug, showed a tremendous potential against SARS-CoV-2 and in various clinical trials chloroquine phosphate was administered to more than 100 COVID-19 patients that resulted in reduction of pneumonia as compared to control group patients [51,52] In another study hydroxychloroquine showed more inhibition potential than chloroquine phosphate for the COVID-19 [53]. Chloroquine can inhibit virus entry via 2 potential mechanisms: a) They might block the production of inflammatory cytokines like IL6, whose abnormal production during COVID-19 leads to acute respiratory syndrome [54]; b) It accumulates in endosomes thus raised the pH of endosome which helps in can interfere with entry or fusion of virus [55, 56]. Nevertheless, there is still a dire need to test these potential approaches in the lab as well

as in clinical trials to develop treatment for COVID-19 infection.

Disruption of Replication of SARS-CoV-2

To fight against RNA viruses, a great number of antivirals has been developed which disrupts viral replication via inhibition of viral polymerases. As discussed above, antivirals like ribavirin, lopinavir, ritonavir, arbidol, favipiravir, remdesivir are being employed against SARS-CoV-2 and remdesivir showed the most promising results against COVID-19 [51, 57] which was also used through intravenous injection in United States, thus improving the condition of the COVID-19 patient [40]. It is a monophosphoramidate prodrug of an adenosine analog which causes RNA synthesis arrest by incorporation into newly synthesized viral RNA with the help of RNA-dependent RNA

polymerases [58]. Ribavirin and favipiravir are monophosphoramidate prodrugs of guanine analogues are being used for other RNA viruses treatments but there is still no data supporting their antiviral effects in patients with COVID-19 [59]. Protease inhibitors like ritonavir and lopinavir targets coronavirus proteinase 3CLpro (3C-like protease), which is responsible for processing of translation products of RNA viruses, can be effectively used against SARS-CoV-2[60]. Tegobuvir, prulifloxacin, nelfinavir and bictegravir are small molecules that showed reasonable bindings to viral protease in result inhibiting it and are screened using high-throughput screening methods in clinical laboratories [61]. A recent study, showed that ivermectin, an FDA approved drug having anti-parasitic activity, has shown promising results in reduction of SARS-CoV-2. In the experiment, 5µM of ivermectin was administered in Vero/hSLAM cells infected by SARS-CoV-2 and the viral RNA was found out to be reduced by 99.8%, in vitro [62].

Another approach that can be used to inhibit SARS-CoV-2 replication is to use the novel CRISPR/Cas13 system to cleave RNA genome of the virus which can be delivered to the infected lung via adeno associated virus vectors. It contains guide RNAs, containing spacer sequences explicitly complementary to the virus RNA genome and Cas13d protein [63].

Inflammatory Response Suppression

An optimal level of cytokines is essential for immune system to work properly, but it is reported that during COVID-19 infection the concentration of cytokines was increased in critical patients as compared to mild patients which shows higher cytokine concentrations are directly linked to disease severity [34]. Therefore, inhibition of elevated inflammatory response can be a potential approach to decrease the severity of COVID-19 and corticosteroid can be used to alleviate the inflammatory response caused by abnormal concentrations of cytokines [57]. There is no evidence that corticosteroid administration improved the inflammatory response in the case of SARS and MERS so, currently it is recommended by National Health Commission, China (NHC) not to use corticosteroids to treat COVID-19 [64]. A recent study showed the probable benefits of corticosteroids if

administered in low dosage in person with COVID-19 [65] but more clinical trial and studies are needed to prove the effectiveness of corticosteroids against COVID-19.

During SARS-CoV-2 infection, CD14+, CD16+, monocytes were activated along with high expression of IL6 which was induced by activation of GM-CSF (Granulocyte-macrophage colony-stimulating factor) and other inflammatory cytokines at cellular level [66]. Thus, IL6 and GM-CSF could be a potential target to reduce overexpressed inflammatory response in COVID-19 patients. Activation of Fc receptors (FcR) mediated inflammatory response could contribute to the abnormal inflammatory response in SARS-CoV-2 infection thus, FcR inhibitors can be a potential option to reduce inflammatory responses induced by SARS-CoV-2 infection [67].

Treatment with Convalescent Plasma (CP)

Convalescent plasma (CP) is the plasma taken from a patient that survived any infection by developing antibody-mediated immunity against that specific pathogen. When there is no cure or treatment available for a disease, CP can be used effectively to help lessen the severity of disease. Regardless of the fact that efficiency of CP therapy is proved by many studies, good random trials and clinical studies are needed to properly use CP treatment as a treatment option [68].

During the novel corona virus outbreaks convalescent plasma (CP) can be used as a treatment option if there is no proper treatment available [69] which is done by taking CP from person recovered from COVID-19 by developing immunity against viruses [70]. In a recovered person, specific antibody has been developed against infection, which give CP therapeutic attributes [68] but clinical trials and studies must be done to evaluate the efficiency of CP treatment. Patients in critical condition should be administered with CP of the patients recovered form COVID-19 [71]. However, many trials are being conducted in order to test the safety and efficiency of CP for treatment of COVID-19 patients.

Vaccines Development

In the current global epidemic of COVID-19, the most efficient way is to vaccinate people in order

to prevent the spread of disease [72]. Currently, many teams are working hard to develop a vaccine against COVID-19 and S protein is a potential target for development of vaccines. Recently the cryo-EM structure of SARS-CoV-2 S trimer has been published which will help in the timely development of new vaccine [27]. In other research, human and viral proteomes were compared and found 107 human peptides that were embedded in the S protein of SARS-CoV-2 that paved way toward the development of a vaccine by yielding 66 candidate epitopes from S protein pentapeptides [73]. Currently, more than 15 vaccine candidates are being developed for COVID-19 as shown in Table 1. These include DNA/RNA based vaccines, adenoviral vector-based vaccines, inactivated vaccines etc. [26, 74]. Johnson & Johnson and Codagenix/Serum Institute of India are working on whole virus vaccines, Novavax, University of Queensland/CEPI, Clover Biopharmaceuticals are working on subunit vaccines and CureVac/CEPI, Inovio/Beijing Advaccine Biotechnology Co./CEPI, Moderna/NIH/CEPI are working of nucleic acid-based vaccine. There is still too little data on clinical trial of vaccines at this time and vaccines must be made safe and accessible for the whole world to fight this epidemic.

Whole Virus Vaccines

Inactive whole virus or live-attenuated virus can be used as vaccine option and has been used since the discovery of vaccination. Janssen (Johnson & Johnson) has been working to develop adenovirus-vectored vaccine [75] and Codagenix has been working on live-attenuated vaccine by using codon deoptimization technology [76]. Whole virus vaccines exhibit major advantages like their ability to stimulate toll-like receptors (TLRs) and their intrinsic immunogenicity but live virus vaccines require rigorous testing to ensure their safety. Moreover, in case of coronaviruses, administration of killed or live virus can increase the infection of that coronavirus [84]. So, clinicals trials and research should be done to test the safety and efficiency of whole virus vaccines prior to their use.

Subunit vaccines

A vaccine that contains subunits of virus that helps in stimulating protective immune response for a long period of time is called subunit vaccine

[85]. In the case of coronaviruses S protein is used as primary target of subunits vaccines to prevent its docking to ACE-2 by provoking immune response against S protein [84]. The University of Queensland is developing protein-based vaccine using Molecular Clamp platform [79], Clover Biopharmaceuticals is developing S-trimer recombinant protein using Trimer-Tag technology [80] and Novavax are trying to develop subunit vaccine using recombinant nanoparticle technology [78]. These subunit vaccines may prove to be effective in the activating the hosts immune response to develop antibodies that inhibits the binding of S protein of SARS-CoV-2 to ACE-2, thus inhibiting the entry of virus into lungs cell.

Nucleic Acid Vaccines

A vaccine in which DNA or RNA construct is made using viral gene and injected into host cell thus developing innate immunity against that specific viral protein are called nucleic acid-based vaccines [86]. CureVac [81] and Moderna [83] are working of mRNA-based vaccines against SARS-CoV-2, whereas DNA based vaccine is in development by Inovio Pharmaceuticals [82]. The concept of nucleic acid vaccine was initiated in 1993 in mice against influenza but it hasn't shown promising results in humans [26]. Nowadays nucleic acid vaccine has developed further but more studies and clinical trials are needed to ensure the safety and efficiency of nucleic acid-based vaccines.

Vaccines in clinical trials

As of now many vaccine candidates have entered phase 3 of clinical trials and showing promising results in developing antibodies against SARS-CoV-2.SINOPHARM and SINOVAC have entered phase 3 of clinical trials, after the administration of vaccine neutralizing antibodies were observed in phase 1 and 2 clinical trials without any adverse side effects [87, 88]. The vaccines by University of Oxford, Moderna and CanSino Biologics have completed phase 2 clinical trials and observed the production of neutralizing antibodies within 14 days of administration with no side effects [77, 89]. The vaccine from CanSino Biologics developed humoral immunity and reduced the symptoms of COVID drastically [90].
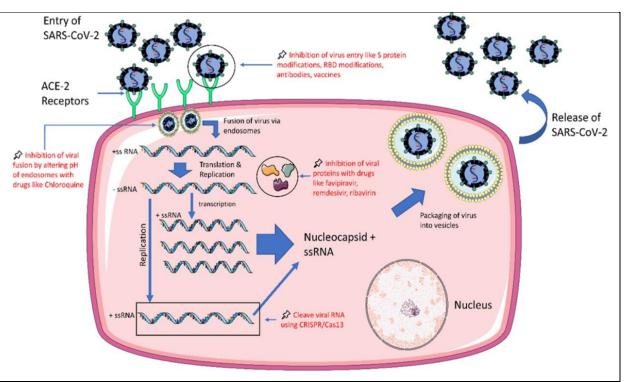
Figure 4. SARS-CoV-2 infection cycle and potential therapeutic targets for drug/vaccine development (highlighted in red color).

Table 1. Different vaccines being developed against SARS-CoV-2.

Type of Vaccine	Vaccine candidates	Companies Working on Vaccine	References
Whole virus	Adenovirus-vectored vaccine	Janssen (Johnson & Johnson)	75
vaccines	Live-attenuated vaccine	Codagenix/Serum Institute of India	76
	Adenovirus-vectored vaccine	University of Oxford	77
Subunit	Recombinant nanoparticle technology	Novavax	78
vaccines	Protein-based vaccine using Molecular Clamp platform	University of Queensland/CEPI	79
	S-trimer recombinant protein using Trimer-Tag technology	Clover Biopharmaceuticals	80
Nucleic acid	mRNA vaccine	CureVac/CEPI	81
vaccines	DNA vaccine	Inovio/Beijing Advaccine Biotechnology Co./CEPI	82
	mRNA vaccine	Moderna/NIH/CEPI	83

CONCLUSION AND FUTURE DIRECTIONS

The SARS-CoV-2 emerged in Wuhan, China and has been declared as a global pandemic by the WHO. It is of zoonotic origin and thought to originate and mutated from bats. SARS-CoV-2 has a single stranded RNA genome and enters host cells through ACE receptors using spike protein. Although SARS-CoV-2 has much similarities with the SARS-CoV-1 but still no

treatments or vaccine has been developed against COVID-19 infection. Antivirals with broad spectrum antibiotics are being used to treat COVID-19 in order to find a cure for it. Remdesivir has found out to be effective against SARS-CoV-2 during in vitro studies. Many companies are working on different type of vaccines, but not a single one is approved for use till date. Therefore, prevention is the only effective measure against this virus so far and

there is a dire need to develop an effective vaccine and other treatment options to deal with COVID-19.

ACKNOWLEDGMENTS

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