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A three months later study of COVID-19 in Pakistan; A systematic review

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Abstract

An ongoing novel highly contagious pneumonia outbreak started in Wuhan, which is the sprawling capital of central china's province, Hubei, in late December 2019. The disease was officially named by the World Health Organization on February 12, 2020, as Coronavirus Disease 2019 (COVID-19) was caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The primary host of SARS-CoV-2 is linked with bat species however; the intermediate host is still not clear. The COVID-19 spread rapidly across the world because of person-person transmission. The SARS-CoV-2 stimulates both the cellular and humoral immunity mediated by viral-specific B and T cells. Cases of COVID-19 infection exhibit several clinical complications for example fever, tiredness and dry cough. Diagnosis is mostly depending on epidemiological characteristics, clinical manifestation, and few through the examination of auxiliary tissues. In this review, we have provided a brief introduction of SARS-CoV-2, pathogenesis, diagnosis, immune responses, and treatments and future perspectives.

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Introduction

Coronaviruses belong to order *Nidovirales,* family *coronaviridae* and subfamily *orthrocoronavirin (*Shereen *et al.*,2020). Coronavirus shows crown-like spikes on their outer surface; therefore, it was named Coronavirus. *Coronavirdae* has the four subgroups Alpha, Beta, Gamma, and Delta (α, β, γ and δ) coronaviruses (Zhong *et al.*, 2003) . The newly emerged SARS-CoV-2 belongs to β-coronaviruses, which is an enveloped, positive (+) sense RNA virus (Su *et al.*, 2016). The SARS-CoV-2 causes a highly contagious disease called coronavirus Disease 2019 (COVID-19)(Adhikari *et al.*, 2020). The phylogenetic studies show that SARS-CoV-2 has a high resemblance with the Severe Acute Respiratory Syndrome virus (SARS-coV) and the relatively poor with the Middle East Respiratory Syndrome virus (MERS-CoV)(Shereen, Khan, Kazmi, Bashir, & Siddique, 2020; Zheng, 2020). This was the 3rd zoonotic coronavirus outbreak in the 1st two decades of the 21st century. However, this time disease is rapidly transmitted from person to person and has raised a global human concern(Xie & Chen, 2020). Human to human transmission through close contact, air droplets and asymptomatic incubation carriers within the families, friends and the community made it extremely contagious(Guo *et al.*, 2020). By following exponential growth up to the end of January 2020, the outbreak reached several countries, grabbing considerable attention in the entire globe. As of March 11, 2020, WHO announced the epidemic to a global pandemic because of 13 folds increase in cases outside China and the number of affected countries also increased 3-folds. A further increase was also expected.

Unfortunately, the Chinese New Year festival “Lunar” holidays, which is celebrated at the beginning of the new year, accord with the outbreak of COVID-19, is the best celebratory event of the year in China during which a huge number of peoples migration occurred to their home towns(Cucinotta & Vanelli, 2020). Approximately 3 billion peoples in China make close trips over the 40 days’ travel period, of “Chunyun”. About 5 million individuals traveled from Wuhan, the epicenter of the COVID-19 outbreak, before the implementations of the travel ban on January 23, 2020(Chen, Yang, Yang, Wang, & Bärnighausen, 2020). Therefore, a rapid expansion and spread of COVID-19 cases transmission observed within China and outside China(Zu *et al.*, 2020). This review article highlights the ongoing challenges of COVID-19, pathogenesis, diagnosis, current and future treatment strategies, immune responses and future perspectives.

Pathogenesis

The coronaviruses' genome structure is best known among all RNA viruses of virosphere. Two-third (2/3) part of their genome (RNA) encodes for the viral polymerases (RdRp), material responsible for RNA synthesis, and two (Ⅱ) big structural polyproteins that are responsible for host immune responses modulation (ORF1a-ORF1b). The remaining one-third (1/3) part of RNA encodes for four (Ⅳ) structural proteins. These structural proteins include spikes (S) proteins, envelop (E) proteins, membrane (M) proteins, and nucleocapsid (N) proteins(Shereen *et al.*, 2020). COVID-19 causes the infection of the lower respiratory tract of humans and results in pneumonia(Zhong *et al.*, 2003). Patients infected with COVID-19 had a higher leukocyte count, respiratory abnormalities, and elevated levels of plasma proinflammatory cytokines(Yang *et al.*, 2020). Few patients can also face headaches or hemoptysis and even relatively asymptomatic. Coronavirus causes approximately 15% of adult common colds while the same strains of coronaviruses can cause debilitation and pneumonia in immunocompromised older adults(Cui, Li, & Shi, 2019). Affected aged individuals with medical findings have a greater chance of respiratory failure due to severe lungs “alveolar” damage(Adhikari *et al.*, 2020). These signs symptoms are analogous with SARS-CoVs and MERS-CoVs infections. The bases of initial infections with SARS-CoV-2 are not completely known until now. Although the pathogenesis mechanism of the COVID-19 is inadequately learned, however, the same mechanism of SARS-CoVs and MERS-CoVs can provide us huge information about

the SARS-CoV-2 pathogenesis. Similarly, the attachment of SARS-CoV-2 with lungs cells ACE2 receptors leads to extended production of ACE2, which may catalyze the destruction of host alveolar cells. Injury to human alveolar cells run a group of systemic reactions and even death occured(Nikolich-Zugich *et al.*, 2020). As shown in figure 1. Generally, the coronaviruses infection consists, of attachment, entry, replication, translation, virion assembly and release of virus(Hoffmann *et al.*, 2020; Nikolich-Zugich *et al.*, 2020).

SARS-CoV-2 infection cycle starts from the entry of viral particles into the host cell. SAR-CoV-2 can enter into the host cell in Ⅱ ways either through plasma membrane fusion or either through endosomes(Nikolich-Zugich *et al.*, 2020). When the virions enter into the endosomes, L cathepsin activates the S (spike) protein. Although, the spikes protein can also be activated by cellular serine protease TMPRSS2 close to the ACE2 receptor, which starts with a fusion of viral membrane with the plasma membrane(Nikolich-Zugich *et al.*, 2020). Viral fusion entry less activates the immune system, therefore, more efficient for viral replication (Hoffmann *et al.*, 2020). The S proteins play a vital role in the attachment of virion with the host cell membrane(Shirato, Kawase, & Matsuyama, 2018). It is consisting of two basic subunits S1 and S2. The S1 subunit consists of a signal peptide, which is proceeded through N-terminal domain (NTD), and Receptor Binding domain (RBD), while the S2 subunit consists of the fusion peptide (FP), 1 and 2 heptad repeat (HR), Transmembrane domain (TMD), and a cytoplasmic domain (CPD)(Cucinotta & Vanelli, 2020). Genome encoding is initiated after the entry into human cells (respiratory tract cells) and facilitates the expression of genes (protein synthesis), which run the adaptation of CoVs to their human host(Su *et al.*, 2016).

Translation of SARS-CoV-2 machinery and replication

After the SARS-CoV-2 RNA is released into the host cell, polyproteins are translated. The virus genomic RNA encodes nonstructural proteins (NPs) that have a critical role in viral RNA synthesis, and structural proteins which are important in virion assembly. First polyprotein pp1a and pp1ab are translated to form functional NSPs as helicase or RdRp(Ramanathan *et al.*, 2020).

Translation of virus structural proteins, virion's assembly and release

RdRp is responsible for the replication of structural proteins RNA. Structural proteins S1, S2, (E) and (M) are translated by ribosomes that are bound to the endoplasmic reticulum (ER) and presented on its surface as preparation of virion assembly. The nucleocapsids (N) remain in the cytoplasm and are assembled from genomic RNA. They fuse with the virion precursor which is then transported from the ER through the Golgi Apparatus to the cell surface via small vesicles(Zhong *et al.*, 2003). Virions are then released from the infected cell through exocytosis and search for another host cell.

Immune response

The immune system shows a response against COVID-19 in 2 distinct phases (Ⅰ, Ⅱ). Phase Ⅰ immune responses initiated during the incubation and non-severe stage of SAR-CoV-2, fully functional and specific adaptive immune response are required to eliminate the SAR-CoV-2 and to stop the disease progression to severe stages. At this stage of the disease, anti-sera and IFN α treatments are important strategies for the protective endogenous immune response. The host has generally good health and appropriate immune genetic background that produce excellent antiviral immunity(Shin, Jung, Kim, Baric, & Go, 2018). When the protective immune response failed to elicit the disease, the virus propagates to a severe stage and triggers the phase Ⅱ immune response. Phase Ⅱ immune response includes severe cytokines storms and pro-inflammation(Guo *et al.*, 2020). According to Lancet reports, the prime factor of causalities with COVID-19 is the progress of acute respiratory distress syndrome (ARDS)(Shi *et al.*, 2020). One of the key mechanism of ARDS is the massive release of cytokines named as

cytokines storm which leads to abandoned systemic inflammation due to the release of IFN- α, IFN-g, IL-1b, IL-6, IL-12, IL-18, Il-33, TNF- α, and TGFb and many other chemokines CCL2, CCL3, CCL5, CXCL8, and CXCL-10, etc(Goh, Kalimuddin, & Chan, 2020). This leads to multi-organ failures like kidney and lungs(Goh *et al.*, 2020). While the immune system of aged persons bears many age-related consequences, that affect nearly each component of the Immune system collectively termed immune senescence (Nikolich-Žugich, 2018) that changes the faces themselves and enhance the morbidity and mortality rate with infectious diseases especially COVID-19. Which affect both adaptive and innate immune system as well as the cooperation of immune response itself in time and space which works effectively in young adults but deteriorate with age. Globally cytokines signaling, peroxide production, nitric oxide and phagocytic functions of neutrophils are all reduced in older peoples(Ahmed, Shah, Rahim, Flores, & O'Linn, 2020). The macrophages' ability of phagocytoses is also become limited due to defective phosphorylation of activating enzymes to limit and delayed cytokines secretions(Nikolich-Žugich, 2018). Ages related changes disturb the functionality of dendritic cell (DC, s), to encompass reduced uptake of antigen and diminished the maturation, migration and formulation of co-stimulatory molecules and necessary cytokines for T cells stimulations(Ahmed *et al.*, 2020). Furthermore, adaptive immune system is also significantly affected by age related factors. It diminished the both B and T cells functions. Activation of old B cells faces serious issues in the initiation of a vital E47 and AID transcription factor. Improper induction of these important enzymes in case of class switching and somatic cell hyper-mutation head to decreased avidity of antibodies in aged patients(Ahmed *et al.*, 2020). T –cells also affected enormously by age-related changes, the proliferation of T-cells and expression of IL-2 reduced. $CD4^+$ TN cells in old humans whereby diminished T-cells receptors (TCR) signaling and population increment were associated with age-linked destruction of miR-181, an important microRNA 53. MicroRNA commonly suppress the phosphatase that attenuates TCR signaling. T-helper cells and downstream effector molecules like TNF, TNF-γ, granuzyme B cells and others are also reduced(Nikolich-Žugich, 2018). Brief information is beyond this review scope, although, the aggregation of these alterations leaves aged peoples particularly susceptible to emerging infectious disease. This is because, with advancing age, get older, T and B-cells production start dropping and at the age of 40-50 years, only 10% 1of T-cells are left as compared to children and young. This is the reason why elderly people are more prone to infections(Desai, Grolleau-Julius, & Yung, 2010).

Current treatment strategies for covid-19

To date, there is no specific antiviral drug and vaccine recommended for the treatment of COVID-19 right now. The UK and Germany are trailing their vaccines against the COVID-19. The only treatment available is oxygen therapy which constitutes the prime treatment intervention for patients with serious respiratory infections. Moreover, among other therapeutic strategies, several drugs few of them have been used on patients with SARSCoV and MERS-CoV infections, are being tested including remdisivir, baricitnib, hydroxychloroquine and the drugs used against influenza, favipiravir, chloroquine and others are being considered(Ahn *et al.*, 2020).

Wang *et al.* disclose that chloroquine (anti-malaria drug) in combination with remdesivir is highly effective against the COVID-19 because chloroquine shows great in-vitro impacts on the suppression of uncoating of viruses, it does not allow a virus to uncoat and release its genome and it also inhibits alterations of post-translational changes of newly synthesized protein, it also does suppression of glycosylation in several viruses, including human immunodeficiency virus (HIV)(Chinn, Blackburn, Manley, & Sempowski, 2012). The advantage of exploring such drugs is that there is already a large number of information available about the base of their use and safety in humans, and it is important that despite the urgency the introduction of new therapies should not be pressed at the expense of

safety. Moreover, Chinese traditional medicines have gained wide adoption, particularly in curing mild symptoms of COVID-19. A Chinese patent medicine Lianhuaqingwen (LH), which is formed of 13 herbs has played a positive role in the treatment of COVID-19 as it exerts broad-spectrum impacts on a group of influenza viruses by restricting viral propagation(Ahn *et al.*, 2020). Furthermore, immunotherapy by applying IgG in combination with antiviral drugs can be applied to treat and prevent COVID-19 and to make stronger the immune response against this virus. The IgG may be applied to neutralize the virus causing COVID-19 and the efficacy of IgG antibodies would be best if they were isolated from patients recovered from COVID-19(Wang *et al.*, 2020).

Research which includes Ⅳ rhesus monkeys introduces that formulating SARS-coronavirus-2 saved against future reoccurring infections. When scientists re-infected Ⅱ of the Ⅳ monkeys by this virus again after the 28 days of initial infection, a total of 96 anal swabs and nasopharyngeal swabs resulted in the negative (–ve) after the re-exposure of SARS-CoV-2. The euthanasia and necropsy findings of Ⅰ of the Ⅱ monkeys confirmed these results. These results suggested that the immune response raised by Ⅱ animals have saved them from future infection of SARS-coronavirus-2. Hoffmann *et al*; studied, whether antibodies produce by patients who have been previously diagnosed positive (+) for SARS would prevent SARS-CoV-2 entry into the cell. They also analyzed that antibodies against the SARS-CoV S protein limits how well in the in-vitro model virus with a SARS-CoV-2 S protein could infect cells (Xia *et al.*, 2020). They also saw similar findings with antibodies against S proteins produced in rabbits.

These findings showed that neutralizing antibody responses formed against SARS-CoVs could offer some safety against SARS-CoV-2 infection, which can be used for the prevention of COVID-19 infection (Runfeng *et al.*, 2020). Moreover, the passive immunization with convalescent sera having (Ab) antibodies from individuals who have recovered from COVID-19, could prevent COVID-19 infection as argued by Casadevall and Pirofski (Rahman, 2020). Some investigational treatments for COVID-19 are given in Table 1.

Table 1. Investigational treatments of COVID-19.

Viral drugs under clinical trials	Possibilities other than antiviral drugs
Leponavir and ritonavir(Anderson, Heesterbeek, Klinkenberg, & Hollingsworth, 2020)	Monoclonal antibodies isolation from patients recovered from COVID-19(Barnard *et al.*, 2006)
Remedisvir (Ko *et al*,. 2020)	Blood plasma transfers(Vincent *et al.*, 2005)
Favipiravir(Dong, Hu, & Gao, 2020)	Stem cells(Zhang, Wang, Qi, Shen, & Li, 2020)
Chloroquine (Gao *et al* 2020)	
Hydroxychloroquine (Chowdhury *et al.*, 2020)	
Herbal treatments: 4 most commonly used herbs in China under trial are	
Astragali Radix (Wang, 2009)	Saposhnikoviae Radix (Du, 2013)
Glycyrrhizae Radix Et Rhizoma (Luo, 2020)	Lonicerae Japonicae Flo (Bittmann, 2020)

To reduce the damage linked to COVID-19, global public health and infection control programs are exigently needed to bind the worldwide transmission of the virus. In COVID-19 infections travel history has great significance for early discovery and isolation of SARS-Cov2 pneumonia cases. It is necessary to reduce the person to person transmission to limit secondary infections(Jawhara, 2020). Currently, prevention is the only strategy that can limit the spread of COVID-19 (Zhang *et al*,. 2020).

Chloroquine as a potential inhibitor among all other antivirals

Chloroquine (CQ) is an anisotropic amine form of quinine. For decades CQ is a medication for the treatment and prevention of malaria and a variety of viruses such as Human immune deficiency virus (HIV) Zika virus, Marburg virus, dengue virus and SARS-Cov-1 (Abdel-Aty *et al*,. 2020). The reason behind this frequent use is the anti-inflammatory and immunomodulatory effects of this drug, which can be

beneficial in treating COVID-19. The China National Centre for Biotechnology Development found that CQ is Ⅰ of Ⅲ drugs with an encouraging profile against SARS-Cov-2 caused by COVID-19 (X. Z. Zhang *et al*,. 2020) . Chloroquine has multiple mechanisms of action depending on the type of pathogen interaction. Chloroquine can limit the pre-entry step of the viral replication cycle by interfering with viral particles binding to their intracellular receptors, and it can also inhibit pH-dependent endosome mediated entry of enveloped viruses (Karuppannan *et al*., 2012). This feature can be attributed to treating COVID-19 as SARS-Cov-2 entry is also reported to be endosome mediated(Chinn *et al*., 2012).

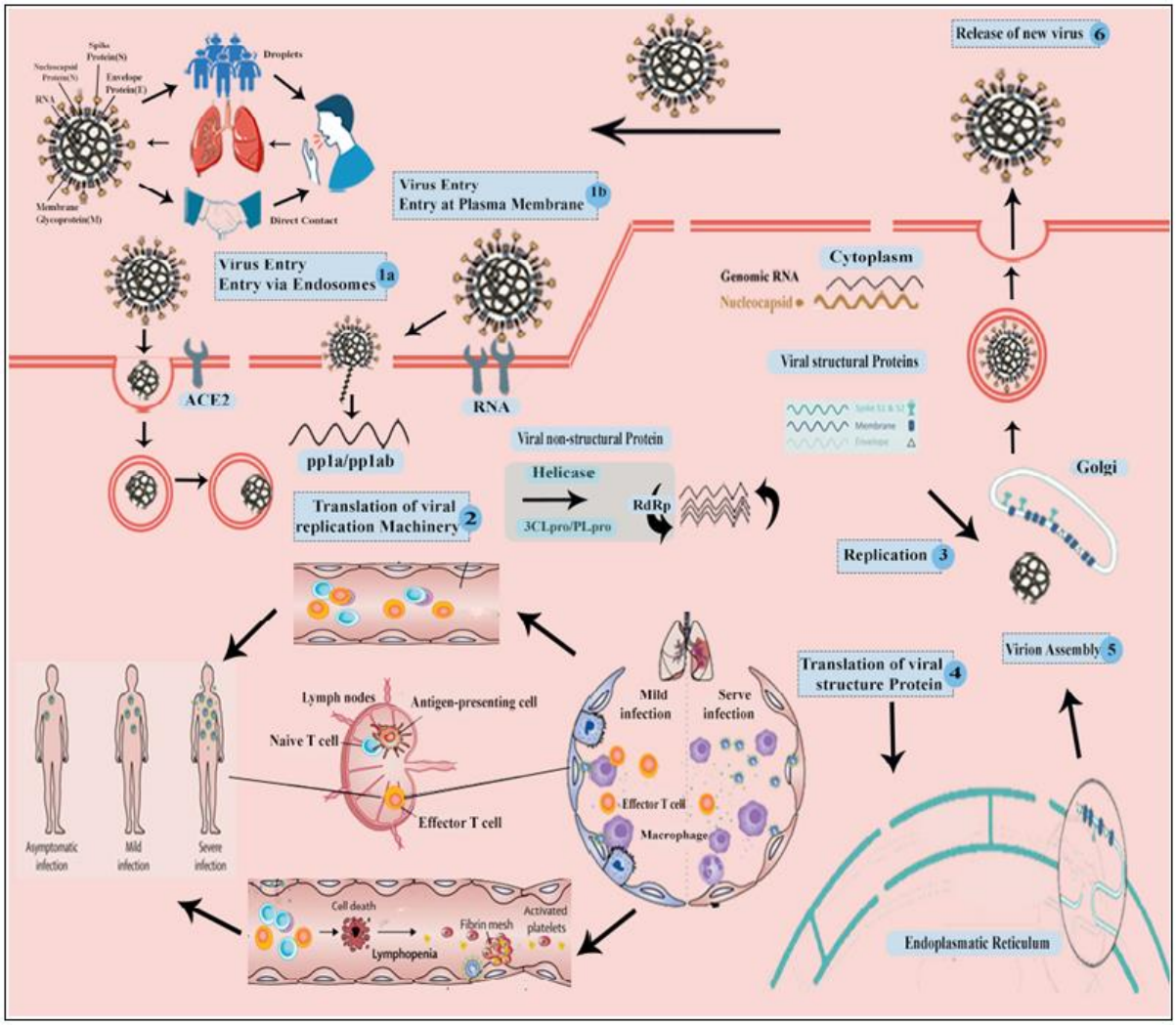

Fig. 1. Person to person transmission of SAR-CoV-2 mainly depends upon air droplets and direct contact with an infected person. After the transmission the viral particles undergo six-step viral replication in the human body such as 1) Viral entry through endosomes or Via plasma membrane 2) translation of viral replicating machinery 3) replication of viral particles 4) translation of viral structure protein 5) virion assembly 6) release of the viruses. Furthermore, the fig also explains the immune responses and conditions developed after the infection. There should be three types of infections asymptomatic, mild and severe.

The pH intonation by CQ can diminish the proper maturation of viral protein and recognition of viral antigens by receptors. CQ can also inhibit post-translational modifications of viral proteins such as glycosylation which require low pH i.e HIV and CQ increase the pH (Savarino *et al*., 2001). During in-vitro studies, CQ has shown to the deficit the glycosylation of angiotensin-converting enzyme 2(ACE2), a virus cell surface receptor (Li, 2003). Chloroquine worked at both entries and after entry

stages of COVID-19 infection. Its immune-modulating functions synergistically increase its antiviral effects on animals as it is mostly distributed in the entire body as well as lungs after oral administration. Recently CQ has been shown by several studies to reduce SARS-Cov-2 viral load and duration of viremia (Garcia-Cremades *et al*,. 2020). Till now 15 clinical trials have been conducted in China each trial containing 100 patients, to define the safety and efficacy of CQ in treating COVID-19 but whether their immune-modulatory effects also play a role in treating COVID-19 require further investigation (Scavone *et al*,. 2020).

Vaccines

Currently, there is no FDA approved vaccine is available for COVID-19. But several groups have been started their work on preparing vaccines shortly after Chinese scientists have shared virus genetic material(Vincent *et al*., 2005). These include MODERNA, Inovio, Curevac Biotech Company and University of Queensland in Australia.

Moderna

At the end of March 2020 this company started testing its mRNA-1273 vaccine against COVID-19 in Washington on 45 healthy volunteers' ages between 18-55, this mRNA vaccine when injected into human body cells, then the lymphocytes (B and T cells) in lymph nodules can operate that mRNA or initiate the formulation of protein in just the right way for other immune cells to mark and identify them for destruction(Dong *et al*., 2020).

Inovio

This Company has quickly developed a vaccine against COVID-19 as they are working since December on DNA vaccine for MERS, caused by the same coronavirus. The company is expected to start a clinical trial of COVID-19 vaccine in May 2020(Dong *et al*., 2020).

University of Queensland in Australia

Researchers are developing vaccines by growing viral proteins in cell culture. They expect to start clinical trials at the end of May 2020(Zhang *et al*., 2020).

Curevac biotech

This company in Turbingen, Germany will begin a human trial of the mRNA-based vaccine in June 2020 (Huggett, 2016).

Another trial at the Kaiser Permanente Washington Health Research Institute in Seattle, USA, is under consideration. In this trial, the vaccine is injected into 45 healthy volunteers contain a part of genetic material duplicated from SARS-CoV-2. Because this vaccine does not have the actual SARS-CoV-2, the individuals will not develop COVID-19 (Boulos, 2020).

In UK Sarah Gilbert and her colleague at Oxford University begin imminently trials on humans and animals of ChAdOx1 vaccine and predicting that the vaccine will be accessible in late 2020 (Purushotham, 2019).

In China CanSino Biologics in participation with the Academy of Military Medical sciences are also working to formulate the recombinant vaccine. A clinical trial has begun among 108 volunteers in Wuhan aged between 18-60 years (Ali *et al*., 2020)
According to the director of the National Institute of Allergy and Infectious Diseases, a vaccine won't be available for widespread use for at least another 12-18 months (Mortimer, 1990).

Conclusion

There is no proper treatment available for COVID-19 right now. Therefore, it is spreading rapidly across the globe and has already paralyzed life in several countries. Its spread can be limited only by having a strict implementation of preventive strategies developed by WHO. Now a day it is too early to predict any practical situation, but it will strongly threaten the entire world. Low-income countries like Pakistan are facing worst-case scenarios, by having very low economic support to fight against this lethal virus. This is an interesting and hot topic for which to develop guidelines, not only for the instant time of the

pandemic, it is very important to keep an eye on the future in case there are upcoming waves as looking forward. There is a need of unveiling the detailed entry processes of SARS-CoV-2 into human cells. Besides, research work is required to fill the gaps associated with the transmission of the virus from zoonotic sources to humans. Without knowing the intermediate zoonotic source that had received the virus from the source of origin and caused the infection in humans, it is almost impossible to eradicate the infection. Proper strategies should be applied in healthcare settings with restriction of people to their homes to mitigate the spread.

Conflicts of Interest

No.

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