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REVIEW PAPER

Virology, Epidemiology and Control of SARS-CoV-2: A Perspective

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ABSTRACT

SARS-CoV-2 is a human-infecting coronavirus, is the causative agent of COVID-19 disease. The World Health Organization declared this disease as a pandemic on 11 March 2020. The genome of SARS-CoV-2 is ~30kb in length which encodes sixteen nonstructural and four main structural proteins. The structural spike protein enables SARS-CoV-2 to bind to the host cell receptor angiotensin-converting enzyme 2 (ACE2), which leads to viral infection. COVID-19 is considered as a respiratory disease that affects just the lungs in most cases. Consequently, the principal approach to prevent this disease is supportive care. Efficient SARS-CoV-2 vaccines and altering treatments such as dexamethasone, tocilizumab and antibody cocktails have been developed in several countries. Due to their potential to increase drug bioavailability, a number of approaches in pharmaceutical nanotechnology are currently being tested against SARS-CoV-2. This includes nano-based products designed for detection, prevention and treatment of COVID-19. Such approaches may help to control this current pandemic and pave the way for prevention and treatment of future coronavirus outbreaks.

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INTRODUCTION

Coronaviruses (CoVs) are classified in the leading group of pathogens that affect the human respiratory system. In 2003 and 2012, SARS-CoV and MERS-CoV outbreaks occurred in Guangdong Province, China, and the Middle East, respectively. Both of these resulted in high pathogenicity in humans after crossing the species barrier from animals to humans. It has been shown that SARS-CoV and MERS-CoV originated from bats and consequently humans, leading to significant public health problems [1]. Several groups of patients with a principal diagnosis of pneumonia from an unknown cause were documented in hospitals in December 2019. Epidemiologically, all of these cases were directly or indirectly connected with seafood and wet animal markets in WuhanChina [2, 3]. After sequencing and analyzing specimens, a novel coronavirus designated 2019-nCoV was identified [4]. Based on the nomenclature of the World Health Organization (WHO), coronavirus disease 2019 (COVID-19) was confirmed as the

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name for this novel coronavirus. In addition, the Coronavirus Study Group of International Committee on Taxonomy of Viruses (ICTV) determined that the virus was related to SARS-CoV and should therefore be designated as SARS-CoV-2 [5]. This virus spread globally and the WHO announced COVID-19 disease as a pandemic on 11 March 2020 [6]. It has now reached more than 173 million cases and caused more than 3.7 million deaths (as of June 5th, 2021). Here, we review the virology, epidemiology, and control of COVID-19 disease. In addition, we describe current attempts to facilitate pharmaceutical and vaccine development through the use of nanocarriers.

Virology of SARS-CoV-2

SARS-CoV-2 is a spherical shaped virus, approximately 125 nm in diameter. At 30kb in length, the SARS-CoV-2 genome possesses at least 6 open reading frames (ORFs). The most significant nonstructural proteins of SARS-CoV-2 are the Nsp12 RNA-dependent RNA polymerase, Nsp13 helicase, main protease (Mpro), and chymotrypsin-like protease (3CLpro). Also, it has 16 structural proteins that play an essential role in viral replication [7-10]. There are four chief structural proteins with different functions, named the envelope (E), membrane (M), a nucleocapsid (N), and spike (S) proteins, which are encoded by ORFs within the region (Fig. 1). The S protein allows binding to host cells, the N protein interacts with the viral RNA to shape the ribonucleoprotein, and the E and M proteins have significant roles in assembly of new virus particles [11].

Direct binding of the SARS-CoV-2 S protein to angiotensin-converting enzyme 2 (ACE2) receptors on host cells, which results in viral infection [10, 12]. ACE2 is expressed predominantly in lung, intestine, kidney and heart tissues, along with many other tissues of the body. With a type I membrane protein structure, it incorporates a C-terminal collectrin-like domain (CLD) and N-terminal peptidase domain (PD). The S protein consists of S1 and S2 subunits which contain different functional elements. The S1 subunit comprises a signal peptide, receptor-binding domain (RBD) and an N-terminal domain (NTD). The S2 subunit has conserved heptad repeats (HR) 1 and 2, a fusion peptide (FP), a cytoplasmic domain (CP), and a transmembrane domain (TM) [13]. For a combination of the host cell and the viral membrane, the S protein has a metastable pre-fusion conformation. Binding to ACE2 results in destabilization of a pre-fusion trimer configuration, shedding of the S1 subunit

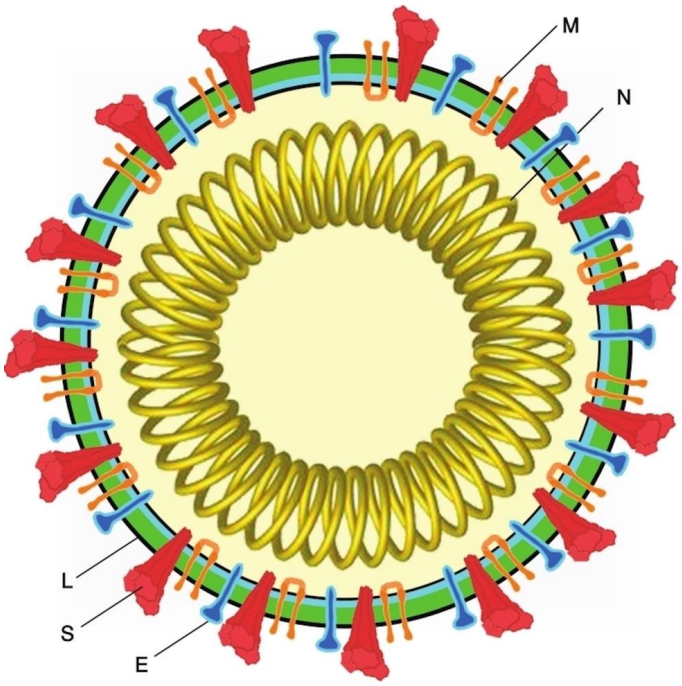

Fig. 1. The structure of SARS-CoV-2 with four main structural proteins: envelope (E); membrane (M); nucleocapsid (N); and spike (S). The viral lipid membrane is denoted by L.

and transition of the S2 subunit post-fusion to a conformation with high stability [14].

Innate Immune Responses and Infection of SARS-CoV-2

In the case of MERS-CoV, the dipeptidyl peptidase-4 (DPP4) was found to be the host cell receptor, although other aspects of viral entry are shared with SARS-CoV and SARS-CoV-2 [15]. Within the lungs, the ACE2 receptor is expressed by the type 2 alveolar cells, the target of both SARS-CoV and SARS-CoV-2. A chief characteristic in SARS-CoV-mediated pathogenesis is that SARS-CoV directly infects T cells and macrophages [16]. However, infection of such immune cells by SARS-CoV-2 is still unknown. Responses of interferon (IFN) type one, and its related downstream cascade, has a crucial effect in active innate immune response against viral infection. The natural immune system detects viral invasion via pathogen-associated molecular patterns (PAMPs), followed by the antiviral responses. In coronaviruses, like other RNA-coding viruses, endosomal RNA receptors known as TLR3 and TLR7, and a cytosolic RNA sensor identify PAMPs in the shape of viral genomic RNA. This triggers induction of the downstream signaling cascades like IFN-regulatory factor 3(IRF3) and nuclear factor NF-κB, accompanied by their nuclear translocation. These transcription factors cause expression of type I IFN and other kinds of pro-inflammatory cytokines in the nucleus of the cell. These quick responses constitute the first defensive line facing viral entry attack [17]. In addition, the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling cascade is triggered through type I IFN via the IFNα/β receptor (IFNAR), resulting in STAT1 and STAT2 phosphorylation by the JAK1 and tyrosine kinase 2 (TYK2) kinases. Then, phosphorylated STAT1 and 2 form a complex with IRF9, enabling nuclear import to induce IFN-stimulated genes (ISGs) transcription, which is controlled by the IFN-stimulated response element (ISRE) containing promoters [17]. An effective type I IFN response can result in suppression of replication and distribution of the virus at an early phase of the infection process. However, both SARS-CoV and MERS-CoV can suppress the type I IFN response in some cases of viral infection. There are several ways that coronaviruses can obstruct the pathways involved in type I IFN production and downstream signaling of IFNARs, which are linked with disease severity [18]. Induction of type I IFN is accomplished by direct interference of SARS-CoV or indirectly via signaling downstream of RNA sensors [19]. Both SARS-CoV and MERS-CoV have mechanisms that block IFN signaling, including a decrease in STAT1 phosphorylation, triggered by releasing type I IFN [17]. These interference effects on the host type I IFN reaction are mediated by the structural and nonstructural proteins viral proteins.

Epidemiology of SARS-CoV-2

By the end of the year in 2019, several incidents of pneumonia of unknown causation were reported by the WHO in Wuhan, China. After performing experiments, it was discerned that these patients were infected by a novel CoV. Dry cough, with fever, malaise and dyspnea were the most notable symptoms of these patients [13]. The area in Wuhan that this infection was initiated is not precisely known, although live animal markets have been implicated. In addition, we still do not know the extent of SARS-CoV-2 pathogenesis. Since this virus generally targets the respiratory tract, it affects only the lungs in most patients. Close contacts are likely to be the main cause of human-to-human transmission, through airborne spread of droplets containing the virus from infected people, via sneezing or coughing. The asymptomatic incubation period of SARS-CoV-2 infections may last from 2-14 days. Within this time period, the virus may be passed on to other people [20-22]. This is why SARS-CoV-2 is a fast distributing virus, with a reproductive number (R_0) of 2.2 to 2.6. More precisely, an infected person can distribute the virus to 2.2-2.6 other people [23, 24]. Fig. 2 is based on Coronavirus disease (COVID-2019) edition 42, published by the WHO on 1 June 2021 [25]. This indicates the number of SARS-CoV-2-postive cases at that time and according to WHO region (Fig. 2).

Because recovery occurs in 1-2 weeks in most mild cases of COVID-19, this virus is recognized as a self-limiting infectious disease. Infections with SARS-CoV-2 can be classified with five different outcomes ranging from asymptomatic to death (26). A study in China revealed that among 72,314 people diagnosed with COVID-19, 61.8% were confirmed, 22.4% suspected, 14.6% clinically diagnosed and 1.2% asymptomatic. The features of the confirmed cases are shown in Table 1. Out of these, 1023 deaths occurred giving a fatality rate of 2.3%.

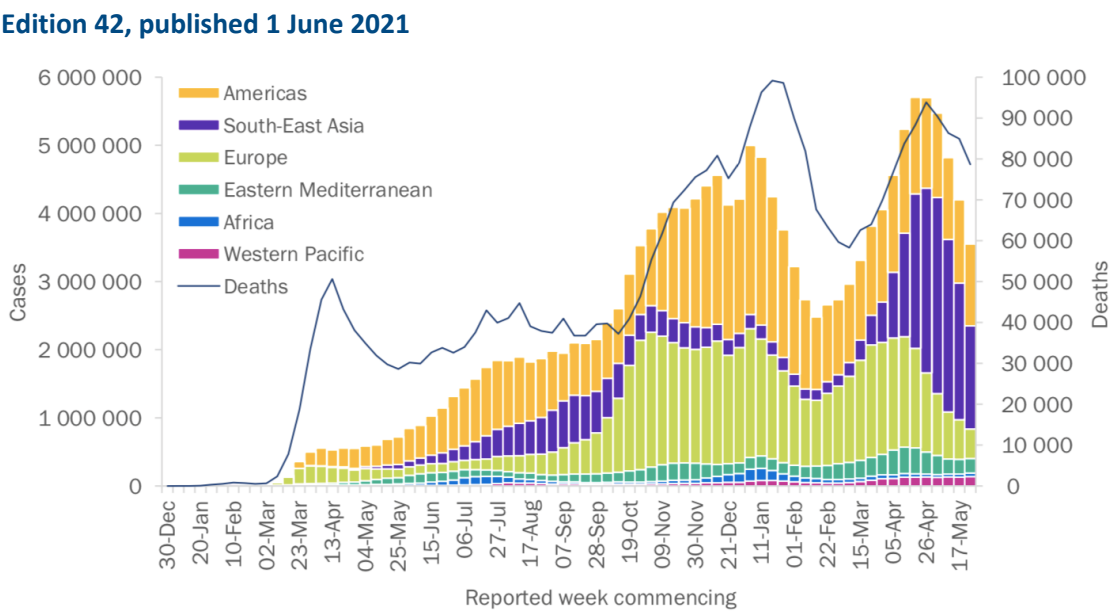

Fig. 2. Epidemic chart of confirmed individuals infected by SARS-CoV-2, the date of report, and WHO region 30 May 2021 [25].

Table 1. Various severities which caused by SARS-CoV-2 infection in 44,672 confirmed cases in China [26].

Severity of cases	Percentage of confirmed cases	Number of confirmed cases
Critical	4.7%	2087
Severe	13.8%	6168
Mild to medium	80.9%	36160
Missing	0.6%	257

It should be noted that the percentage of of children under 10 years-old who are asymptomatic is around 15.8% [27]. As a result, the ratio of asymptomatic infection with this virus needs to be investigated further in future studies.

Clinical Characteristics of SARS-CoV-2

The obtained information from hospitalized patients indicates that around 80 percent of infected cases are thought to be asymptomatic or as having mild symptoms, whereas the other 20 percent represent severe and aggregative symptoms [21, 28]. Dry cough, myalgia, and fever are the most common symptoms of infection by SARS-CoV-2. Infected individuals often showed some symptoms of nausea and diarrhea before the fever. Fever has been proved as the most probable and dominant symptom but not the initial symptom of infection. Rapid aggregation of symptoms might occur at disease onset, which results in dysfunction of multiple organ symptoms, and incube acute respiratory distress syndrome (ARDS), shock, acute kidney and cardiac injury, and even death in the most serious cases [29]. From these data, it appears that the ratio of acute status and fatality in SARS-CoV-2 is not as high as those seen in MERS-CoV and SARS-CoV infections. However, there are some parallel symptoms across these three coronaviruses. Diarrhea has been seen in 20-25% of people infected by SARS-CoV and MERS-CoV, but people infected by SARS-CoV-2 have shown a lower proportion of intestinal symptoms. Fatigue, fever, and respiratory effects including throat soreness, coughing, and shortness of breath are the most common symptoms of SARS-CoV-2. It should be noted that lymphopenia and pneumonia with representative pulmonary ground-glass opacities on chest computed tomography (CT) scans was reported in a significant portion of patients [21, 23, 28]. Loss of viral control and delayed type I IFN response in the initial stages of COVID-19 infections can be induced by changes in total neutrophils and lymphocytes. In a survey in Wuhan which analyzed 99 patients, it was reported

Table 2. Signs and symptoms of SARS-CoV-2, SARS-CoV, and MERS-CoV infections.

	% cases		
Signs and symptoms	**SARS-CoV-2**	**SARS-CoV**	**MERS-CoV**
Fever	81-91	99-100	81.7-98
Cough	48-68	57-75	56.9-83
Dyspnoea	19-31	40-42	22-72
Sore throat	29	13-25	9.1-14
Dizziness and confusion	22	4-43	5.4
Diarrhea	16	23-70	19.4-26
Nausea and vomiting	6	20-35	14-21
Headache	8.3-18	30-46	11-20
Runny nose	1-14	9-30	4-61

that in infected cases, 38% had an increase in total neutrophils, 35% showed a decrease in total lymphocytes, 52% had increased serum IL-6 and 84% had an increase in C-reactive protein [15]. Another report showed that in 41 patients, ICU vs. non-ICU cases were dissimilar in the amount of total increased neutrophils and decreased lymphocytes. Indeed, a significant increase in neutrophils and a reduction of lymphocytes appears to be connected with disease severity and death outcomes [23]. One study revealed a constant surge in neutrophil and monocyte-macrophages influx in severe and fatal SARS-CoV or MERS-CoV cases [16, 30]. In addition, the decrease in the type I IFN response and inflammatory monocyte-macrophage levels is considered to be the main cause of fatal pneumonia in a mouse model of SARS-CoV infection [18].

Table 2 illustrates the clinical aspects of COVID-19, SARS, and MERS [31,32].

Diagnosis of SARS-CoV-2

Currently, SARS-CoV-2 diagnosis is mostly achieved by real-time reverse-transcription–polymerase-chain-reaction (RT-PCR) tests carried out in diagnostic and medical centers. The main drawback of RT-PCR assay is the high false negatives rate and lengthy time for completion. Many efforts are now underway to develop more rapid viral nucleic acid diagnoses. A nucleic acid test paper has been developed which can detect SARS-CoV-2 in less than 5 minutes with a visual inspection of the outcome [28, 33]. Radiological tests, particularly thin slice chest CT, are widely used methods enabling us to accurately diagnose the early phase of lung infection [34, 35].

Control of SARS-CoV-2 infection

Early approaches thought to be effective in prevention and control of COVID-19 spreading include early detection or diagnosis, along with treatment and quarantining of patients, as these decrease human-to-human transmission and the chances of secondary infections [36]. Supportive care has been the recommended approach. According to WHO guidelines, patients who suffer from coronavirus infection should receive supportive care, consisting of fluid, oxygen and antibiotic therapies for treatment of secondary bacterial infections. In addition, the WHO has indicated that suspected or confirmed cases of COVID-19 disease should be isolated [37]. Prescription of Remdesivir, Lopinavir/ritonavir alone or in combination with interferon-β, convalescent plasma, and mAbs are considered as the primary therapeutic medicines with high impacts on controlling SARS-CoV-2 [38].

It is suggested that in SARS-CoV-2 infected patients with hypoxia agents such as dexamethasone, baricitinib in mixture with remdesivir and tocilizumab are able to decrease mortality [39]. In vitro, antibody cocktails show a promising approach to avoid SARS-CoV-2 neutralization escape [40]. One or more mutations lead to evolution of the viruse and appearance different variants with more transmission, greater infectivity, re-infection, intense disease, and immune escape. These variants are generally considered as Variants of Concern (VOCs) that are are a reason for concern, and are possible to have an impact on the trend of the pandemic. In India, there was an abrupt surge in COVID-19 cases with more transmission and prevalence variants that was correlated with antibody escape and diagnostic test failures. Coronavirus SARS-CoV-2 variants of concern were reported during the sudden surge in COVID-19 cases in the UK (B.1.1.7), South Africa (B.1.351) and Brazil (P.1/B.1.1.28), with subsequent local transmission across the world. It is supposed

that variants of concern spread with journey. SARS-CoV-2 variants related to immune escape have been distributing. Investigation of transmission and observation of changes in SARS-CoV-2 locally are important [41].

SARS-CoV-2 Vaccines

Vaccines can be recognized as an effective method for controlling viral infections, including SARS-CoV-2. There are different kinds of vaccines consisting of epitopes, mRNA, and S protein-RBD structure-based candidate approaches [42, 43]. In addition, a synthetic genomics platform has been used for rapid reconstruction of SARS-CoV-2, which is applicable for vaccine development [44]. For developing testing SARS-CoV-2 vaccines, human ACE2 transgenic mouse and rhesus monkey models have been constructed as animal models [45] . Currently, several SARS-CoV-2 vaccines are under clinical examinations throughout the world, most of which target the S protein [12, 42]. In order to end the pandemic, vaccination is considered as the main strategy, while appearance of several SARS-CoV-2 variants by decreased vaccine-induced immunity and susceptibility to disease is an alarm for progress. There are several SARS-CoV-2 vaccines that have been assessed by different groups. These vaccines are designed by three main approaches; inactivated and protein subunit vaccines, viral vector vaccines, and mRNA vaccines. One of the most common method to product vaccines is using chemically inactivated virus that are grown in culture, which results in native antigenic epitopes. Sinopharm and Sinovac are vaccine that use this strategy to obtain immunity. Another way is to obtain the vaccine is using the S protein. This method is being used by Novavax to produce efficient vaccines. Viral vector vaccines are a group of vaccines that use viruses that express the genetic sequence of viral antigen in host cells. Johnson & Johnson and AstraZeneca vaccines are prepared based on viral vector vaccines strategy. For mRNA vaccines which are produced against Covid-19 infection, lipid nanoparticles are utilized to keep the prefusion-stabilized S protein–encoding mRNA within the intracellular space. The mRNA is used by host to produce the target protein (S protein in this case), that results in the proper immune response. Pfizer-BioNTech and Moderna vaccines are produced based on mRNA-based vaccines strategy [46].

Nanocarriers in the Development of SARS-CoV-2 Vaccines

A number of attempts are now underway to facilitate vaccine development through the use of nanocarriers. This approach aims to deliver vaccines, drugs and other payloads to their intended sites with increased bioavailability. Nanomedicine has now been applied in many different medical areas, such as infectious diseases like COVID-19 [47, 52]. In this objective, nanocarriers can be used to encapsulate vaccines to facilitate delivery to antigen presenting cells. In addition, they can be used to encapsulate pharmaceuticals to aid in bioavailability and direct targeting of infected organs, tissues and cells. Nanoparticles can also be used to create virus-like particles (VLPs) as a delivery system and to enable study of the infection and replication mechanisms [53, 54]. There are now hundreds of clinical trials underway around the world focussed on treatment of prevention of COVID-19 disease. Approximately 300 of these are listed by the European Union Clinical Trials Register focusing on vaccines, therapeutics targeting the SARS-CoV-2 life-cycle, targeting the immune response, as well as other prophylactic treatments, such as the prevention of organ damage [55]. The advantages of using nanocarriers for drug delivery include increased solubility, controlled release for long-term effects and increased exposure, safe delivery to avert too rapid degradation or clearance by the body, and decreased side-effects due to more accurate targeting [54, 56]. For delivery of pharmaceuticals, nanomaterials could be used to target the interaction between (ACE2 receptors and the SRAS-CoV-2 S protein or to counteract the cytokine storm. In addition nanotechnology may have a role in aiding diagnosis. In the case of vaccines, the potential of lipid- based and virus-like nanoparticles is under intensive investigation due to similarity of these systems with viruses, such as SARS-CoV-2 [54]. In particular, the role of the S protein in the SARS-CoV-2 infection mechanism makes it a logical target of different forms for vaccine development. At present, many clinical trials are underway to explore the efficacy of different forms of vaccines targeting this protein [54, 55]. Most relevant to this review, the use COVID-19 vaccines involving nanocarriers has come under increasing scrutiny to overcome some of the limitations of current approaches. Nanocarriers can be used for delivery to avoid premature degradation of the

immunogens, for a more lasting immune response by the host [57]. Antigens can also be attached to the surface of nanocarriers so that the entire parcel more closely resembles the virus itself [58]. This is particularly important in the face of the new SARS-CoV-2 variants erupting in hotspots around the world, as described above. This is because the system must be adaptable to incorporate targeting of these new viral sequences. Currently a Canadian company called Medicago is investigating a virus-like particle (VLP) vaccine against SARS-CoV-2 in a phase 1 clinical trial of 180 patients [59]. Another promising approach involves the encapsulation of mRNA encoding the SARS-CoV-2 S protein in a nanocarrier system by the company Moderna [60]. Other companies are exploring similar approaches [61].

CONCLUSIONS

COVID-19 is one of the most threatening pandemic diseases considered a dangerous threat to global public health and the human way of life. The primary way of human-to-human transmission of SARS-CoV-2 is through spraying of droplets by coughing or sneezing from an infected person. It is projected that infection has an average incubation period of around 6 days and a R_0 of 2.2-2.6. Despite a number of recent advances, there are still no effective treatments or vaccines to control symptoms and prevent spread of this virus. A number of approaches in pharmaceutical nanotechnology are currently being tested as a means of increasing bioavailability and effectiveness of these approaches. These nano-based approaches may help to control this current pandemic and pave the way for prevention and treatment of future coronavirus outbreaks. As an added note, the first five months of 2021 have seen the rollout of effective SARS-CoV-2 vaccines in multiple countries as well as the implementation of disease course altering treatments such as dexamethasone, tocilizumab and antibody cocktails [62]. However, this rollout will take many months to years before the positive effects are felt across the world. It has now become critical to stop the spread of the virus, especially since the emergence of new and more virulent SARS-CoV-2 variants arising in countries such as the UK, Brazil, South Africa and India [63]. Meanwhile, the world is again scrambling to reduce these eruptions of new variants by development of "updated" vaccination booster programs. This ongoing endeavour requires an adaptable system, such as the nanocarriers, such as those described in this review [64-66]. These can help in updating the world wide vaccination programs and thereby accelerate our ability to eradicate the devastating effects of SARS-CoV-2 on human health and global economies.

In conclusion, the understanding of the various epidemiological aspects of SARS-CoV-2 will help us to implement suitable plans for proper diagnosis and management of COVID-19 infection and the reduction of its mortality rates. Indeed more comprehensive studies are needed for better understanding of the virus nature to find more effective strategies to control the COVID-19 infection (67-68). Moreover the integration of new emerging sciences is an important issue to fight COVID-19 disease more efficiently in the future (69-70).

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COMPETING INTERESTS

The author declares there are no conflicts of notice in preparing the manuscript.

REFERENCES

1. Cui J, Li F, Shi Z-L. Origin and evolution of pathogenic coronaviruses. Nature Reviews Microbiology. 2018;17(3):181-92.
2. Lu H, Stratton CW, Tang YW. Outbreak of pneumonia of unknown etiology in Wuhan, China: The mystery and the miracle. Journal of Medical Virology. 2020;92(4):401-2.
3. Halaji M, Farahani A, Ranjbar R, Heiat M, Dehkordi FS. Emerging coronaviruses: first SARS, second MERS and third SARS-CoV-2: epidemiological updates of COVID-19. Infez Med. 2020; 28 (suppl 1):6-17.
4. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. New England Journal of Medicine. 2020;382(8):727-33.
5. Gorbalenya AE. Severe acute respiratory syndrome-related coronavirus–The species and its viruses, a statement of the Coronavirus Study Group. BioRxiv. 2020.
6. Agrawal S, Goel AD, Gupta N. Emerging prophylaxis strategies against COVID-19. Monaldi Archives for Chest Disease. 2020;90(1).
7. Yang H, Xie W, Xue X, Yang K, Ma J, Liang W, et al. Design of Wide-Spectrum Inhibitors Targeting Coronavirus Main Proteases. PLoS Biology. 2005;3(10):e324.
8. Bacha U, Barrila J, Velazquez-Campoy A, Leavitt SA, Freire E. Identification of Novel Inhibitors of the SARS Coronavirus Main Protease 3CLpro. Biochemistry. 2004;43(17):4906-12.
9. Jia Z, Yan L, Ren Z, Wu L, Wang J, Guo J, et al. Delicate structural coordination of the Severe Acute Respiratory Syndrome coronavirus Nsp13 upon ATP hydrolysis. Nucleic

Acids Research. 2019;47(12):6538-50.
10. Mohammadpour S, Torshizi Esfahani A, Halaji M, Lak M, Ranjbar R. An updated review of the association of host genetic factors with susceptibility and resistance to COVID-19. J Cell Physiol. 2021 ;236(1):49-54.
11. Mirzaei R, Karampoor S, Sholeh M, Moradi P, Ranjbar R, Ghasemi F. A contemporary review on pathogenesis and immunity of COVID-19 infection. Molecular Biology Reports. 2020;47(7):5365-76.
12. Sheikhshahrokh A, Ranjbar R, Saeidi E, Safarpoor Dehkordi F, Heiat M, Ghasemi-Dehkordi P, et al. Frontier Therapeutics and Vaccine Strategies for SARS-CoV-2 (COVID-19): A Review. Iranian Journal of Public Health. 2020.
13. Chan JF-W, Kok K-H, Zhu Z, Chu H, To KK-W, Yuan S, et al. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerging Microbes & Infections. 2020;9(1):221-36.
14. Tripp RA, Tompkins SM. Roles of Host Gene and Non-coding RNA Expression in Virus Infection: Springer; 2018.
15. Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak
associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-3.
16. Dandekar AA, Perlman S. Immunopathogenesis of coronavirus infections: implications for SARS. Nature Reviews Immunology. 2005;5(12):917-27.
17. de Wit E, van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: recent insights into emerging coronaviruses. Nature Reviews Microbiology. 2016;14(8):523-34.
18. Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. Seminars in Immunopathology. 2017;39(5):529-39.
19. Kindler E, Thiel V, Weber F. Interaction of SARS and MERS Coronaviruses with the Antiviral Interferon Response. Coronaviruses: Elsevier; 2016. p. 219-43.
20. Mahase E. Coronavirus: UK screens direct flights from Wuhan after US case. BMJ. 2020:m265.
21. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet. 2020;395(10223):497-506.
22. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The Lancet. 2020;395(10224):565-74.
23. Wu F, Zhao S, Yu B, Chen Y-M, Wang W, Song Z-G, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020;579(7798):265-9.
24. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The Lancet. 2020;395(10223):507-13.
25. Organization WH. Coronavirus disease (COVID-19) Situation Report 2020;190.
26. Novel CPERE. The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China. Zhonghua liu xing bing xue za zhi= Zhonghua liuxingbingxue zazhi. 2020;41(2):145.
27. Lu X, Zhang L, Du H, Zhang J, Li YY, Qu J, et al. SARS-CoV-2 Infection in Children. New England Journal of Medicine. 2020;382(17):1663-5.
28. Chan JF-W, Yuan S, Kok K-H, To KK-W, Chu H, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. The Lancet. 2020;395(10223):514-23.
29. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus–Infected Pneumonia in Wuhan, China. JAMA. 2020;323(11):1061.
30. Zumla A, Hui DS, Perlman S. Middle East respiratory syndrome. The Lancet. 2015;386(9997):995-1007.
31. Pormohammad A, Ghorbani S, Khatami A, Farzi R, Baradaran B, Turner DL, et al. Comparison of Confirmed COVID-19 with SARS and MERS Cases - Clinical Characteristics, Laboratory Findings, Radiographic Signs and Outcomes: A Systematic Review and Meta-Analysis. SSRN Electronic Journal. 2020.
32. Petrosillo N, Viceconte G, Ergonul O, Ippolito G, Petersen E. COVID-19, SARS and MERS: are they closely related? Clinical Microbiology and Infection. 2020;26(6):729-34.
33. Pan Y, Guan H. Imaging changes in patients with 2019-nCov. European Radiology. 2020;30(7):3612-3.
34. Kanne JP. Chest CT Findings in 2019 Novel Coronavirus (2019-nCoV) Infections from Wuhan, China: Key Points for the Radiologist. Radiology. 2020;295(1):16-7.
35. Zu ZY, Jiang MD, Xu PP, Chen W, Ni QQ, Lu GM, et al. Coronavirus Disease 2019 (COVID-19): A Perspective from China. Radiology. 2020;296(2):E15-E25.
36. World Health O. Clinical management of severe acute respiratory infection (SARI) when COVID-19 disease is suspected. Interim guidance. Pediatria i Medycyna Rodzinna. 2020;16(1):9-26.
37. Organization WH. Clinical management of severe acute respiratory infection when novel coronavirus ('nCoV)' infection is suspected: interim guidance, 25 January 2020. World Health Organization; 2020.
38. Li H, Wang Y, Xu J, Cao B. Potential antiviral therapeutics for 2019 Novel Coronavirus. Zhonghua jie he he hu xi za zhi= Zhonghua jiehe he huxi zazhi= Chinese journal of tuberculosis and respiratory diseases. 2020;43:E002-E.
39. Ku Z, Xie X, Davidson E, Ye X, Su H, Menachery VD, et al. Author Correction: Molecular determinants and mechanism for antibody cocktail preventing SARS-CoV-2 escape. Nature Communications. 2021;12(1).
40. Pattabiraman C, Prasad P, George AK, Sreenivas D, Rasheed R, Reddy NVK, et al. Importation, circulation, and emergence of variants of SARS-CoV-2 in the South Indian state of Karnataka. Wellcome Open Research. 2021;6:110.
41. Abdelmageed MI, Abdelmoneim AH, Mustafa MI, Elfadol NM, Murshed NS, Shantier SW, et al. Design of multi epitope-based peptide vaccine against E protein of human COVID-19: An immunoinformatics approach. Cold Spring Harbor Laboratory; 2020.
42. Sun C, Chen L, Yang J, Luo C, Zhang Y, Li J, et al. SARS-CoV-2 and SARS-CoV Spike-RBD Structure and Receptor Binding Comparison and Potential Implications on Neutralizing Antibody and Vaccine Development. Cold Spring Harbor Laboratory; 2020.
43. Nhu Thao TT, Labroussaa F, Ebert N, V'kovski P, Stalder H, Portmann J, et al. Rapid reconstruction of SARS-CoV-2 using a synthetic genomics platform. Cold Spring Harbor Laboratory; 2020.
44. Bao L, Deng W, Huang B, Gao H, Liu J, Ren L, et al. The Pathogenicity of SARS-CoV-2 in hACE2 Transgenic Mice.

Cold Spring Harbor Laboratory; 2020.
45. Creech CB, Walker SC, Samuels RJ. SARS-CoV-2 Vaccines. JAMA. 2021;325(13):1318.
46. Henderson C, Madison A, Shah A. Size Matters - Nanotechnology and Therapeutics in Rheumatology and Immunology. Current Rheumatology Reviews. 2014;10(1):11-21.
47. Klinman DM, Sato T, Shimosato T. Use of nanoparticles to deliver immunomodulatory oligonucleotides. WIREs Nanomedicine and Nanobiotechnology. 2015;8(4):631-7.
48. Bartneck M. Immunomodulatory Nanomedicine. Macromolecular Bioscience. 2017;17(10):1700021.
49. Zahin N, Anwar R, Tewari D, Kabir MT, Sajid A, Mathew B, et al. Nanoparticles and its biomedical applications in health and diseases: special focus on drug delivery. Environmental Science and Pollution Research. 2019;27(16):19151-68.
50. El-Sayed A, Kamel M. Advances in nanomedical applications: diagnostic, therapeutic, immunization, and vaccine production. Environmental Science and Pollution Research. 2019;27(16):19200-13.
51. Itani R, Tobaiqy M, Al Faraj A. Optimizing use of theranostic nanoparticles as a life-saving strategy for treating COVID-19 patients. Theranostics. 2020;10(13):5932-42.
52. Weiss C, Carriere M, Fusco L, Capua I, Regla-Nava JA, Pasquali M, et al. Toward Nanotechnology-Enabled Approaches against the COVID-19 Pandemic. ACS Nano. 2020;14(6):6383-406.
53. Heinrich MA, Martina B, Prakash J. Nanomedicine strategies to target coronavirus. Nano Today. 2020;35:100961.
54. EU Clinical Trials Register. Crossref; 2015.
55. Clinical Trials for covid-19, EU Clinical Trials Register 2020. https://www.clinicaltrialsregister.eu/.
56. Pati R, Shevtsov M, Sonawane A. Nanoparticle Vaccines Against Infectious Diseases. Frontiers in Immunology. 2018;9.
57. Al-Halifa S, Gauthier L, Arpin D, Bourgault S, Archambault D. Nanoparticle-Based Vaccines Against Respiratory Viruses. Frontiers in Immunology. 2019;10.
58. Megha S. Medicago, GSK announce positive data for tobacco-grown COVID-19 vaccine. Chemical & Engineering News. 2021:13-.
59. Medicago Begins Phase I Clinical Trials for Its COVID-19 Vaccine Candidate.www.medicago.com
60. Erasmus JH, Khandhar AP, Walls AC, Hemann EA, O'Connor MA, Murapa P, et al. Single-dose replicating RNA vaccine induces neutralizing antibodies against SARS-CoV-2 in nonhuman primates. Cold Spring Harbor Laboratory; 2020.
61. Erasmus JH, Khandhar AP, O'Connor MA, Walls AC, Hemann EA, Murapa P, et al. An Alphavirus-derived replicon RNA vaccine induces SARS-CoV-2 neutralizing antibody and T cell responses in mice and nonhuman primates. Sci Transl Med. 2020; 12(555):eabc9396.
62. Cao Y, Yisimayi A, Bai Y, Huang W, Li X, Zhang Z, et al. Humoral immune response to circulating SARS-CoV-2 variants elicited by inactivated and RBD-subunit vaccines. Cell Research. 2021.
63. Nel AE, Miller JF. Nano-Enabled COVID-19 Vaccines: Meeting the Challenges of Durable Antibody Plus Cellular Immunity and Immune Escape. ACS Nano. 2021;15(4):5793-818.
64. Shapiro RS. COVID-19 vaccines and nanomedicine. International Journal of Dermatology. 2021.
65. Varahachalam SP, Lahooti B, Chamaneh M, Bagchi S, Chhibber T, Morris K, et al. Nanomedicine for the SARS-CoV-2: State-of-the-Art and Future Prospects. International Journal of Nanomedicine. 2021;Volume 16:539-60.
66. Ranjbar R, Mahmoodzadeh Hosseini H, Safarpoor Dehkordi F. A Review on Biochemical and Immunological Biomarkers used for Laboratory Diagnosis of SARS-CoV-2 (COVID -19). The Open Microbiology Journal. 2020;14(1):290-6.
67. Torabi R, Ranjbar R, Halaji M, Heiat M. Aptamers, the bivalent agents as probes and therapies for coronavirus infections: A systematic review. Molecular and Cellular Probes. 2020;53:101636.
68. Heiat M, Hashemi-Aghdam M-R, Heiat F, Rastegar Shariat Panahi M, Aghamollaei H, Moosazadeh Moghaddam M, et al. Integrative role of traditional and modern technologies to combat COVID-19. Expert Review of Anti-infective Therapy. 2020;19(1):23-33.
69. Mirzaie A, Halaji M, Dehkordi FS, Ranjbar R, Noorbazargan H. A narrative literature review on traditional medicine options for treatment of corona virus disease 2019 (COVID-19). Complementary Therapies in Clinical Practice. 2020;40:101214.