COVID-19:
DIFFERENT MODELS AND TREATMENT STRATEGIES

Editors:
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Coronavirus Disease-19 (COVID-19): A Perspective of New Scenario

(Volume 2)

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FOREWORD

According to World Health Organization, 122 million cases and over 2.69 million deaths of coronavirus disease-2019 (COVID-19) pandemic have impacted nearly every region of the globe. The pandemic has a huge global economic influence, and successfully fighting against COVID-19 needs efforts. Newly emerging respiratory diseases such as Severe Acute Respiratory Syndrome-coronavirus (SARS-CoV), Middle East Respiratory Syndrome (MERS), and severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) posing a serious threat to the human population and reported in the years 2003, 2012, and 2019, respectively. Furthermore, SARS-CoV-2 new strains shocked the world due to genetic mutation over time.

This book is very relevant and consists of two key modules. The first module provides clear information regarding different animals and cell line models and differentiation from other respiratory diseases. The second part focuses on therapeutic agents (antivirals, natural compounds, ultraviolet radiation, herbal remedies, blood plasma, stem cell therapy, and vaccines. It will give step-by-step awareness to the scholars about SARS-CoV-2. Basic and advanced knowledge about the disease is arranged into clear and easy-to-read chapters for general readers, scholars, and molecular biology teachers, making this book a valuable and thorough guide for all.

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PREFACE

Coronavirus disease-19 (COVID-19) is a complex disease that causes illnesses ranging from mild to severe respiratory problems. It is caused by a novel coronavirus severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), an enveloped, positive-sense, and single-stranded RNA (+ssRNA) virus that belongs to the coronavirus family. It has a fast-spreading potential worldwide, which leads to high death cases regardless of lows death rates. Yet, there are no animal models or specific drugs for disease prevention and/or treatment. Therefore, it is highly demanded to identify the known drugs and test them as a possible therapeutic approach. In this critical situation, one or more of these drugs may represent the only option to treat or reduce the severity of the disease until a specific drug or vaccine is developed and approved. A wide variety of therapeutics have been explored to treat COVID-19, initially suggested for other diseases and already established safety profiles, and approved by the Food and Drug Administration (FDA). Such treatments are referred to by the World Health Organization (WHO) as repurpose medications. Still, there are many ongoing clinical trials regarding the safety and effectiveness of repurposing immune-therapeutics to mitigate the symptoms of COVID-19.

In this book volume-2 proposal, we consolidate the various animal models and treatment strategies widely used for the global emergency of COVID-19. Since SARS-CoV-2 is the closest to SARS-CoV and MERS-CoV, the approaches brought here will be similar and/or varying with a slight degree. It is cleared that in the last 17-18 years, this is the third outbreak of the same coronavirus with a small mutation that shock the whole world. The chapters in this book should be prioritized as up-to-date literature of techniques used in the study SARS-CoV-2 and will act as a suitable reference if any such wary appear soon.

The 2nd volume of the proposed book proposal has been classified into Part IV: Models for SARS-CoV-2 and Part V: Treatment Strategies for SARS-CoV-2. With the emergence of new coronavirus variants, epidemiology, different host tropism permits a thorough analysis of their evolution and acquired adaptability to their host. The 1st volume already discussed the entry, epidemiology, genetic alteration, and diagnostic approaches. In the 2nd volume, part IV, we have planned to describe chapter-wise models used in COVID-19. No studies are complete without animal models closely related to human physiology to replicate the disease and observe the pathology conditions as in human cases. Such animal models play a vital role in virus pathogenesis and prepare a therapeutic immune response. Here describe bio-engineered transgenic mouse model inserting with specific genes, or CRISPR-Case9 gene-editing tool has been used previously for SARS-CoV and MERS-CoV. The chapter will deal with culture techniques or cell lines for COVID-19-also histopathology of COVID-19, essential proteins that up or down-regulate SARS-CoV-2. The last chapter of this part will describe other diseases having similar signs and symptoms and their differentiation. In the last part of the book proposal, part V, chapters will deal with therapeutic approaches to attenuate SARS-CoV-2 as there is no specific treatment available to date, just symptomatic therapy. However, scientists will elucidate effective antiviral drugs in clinical trials, phytochemicals, photomedicine such as ultraviolet A & B, homemade remedies, blood plasma transfusion, stem cell therapy, and computational approaches *in vivo* and *in vitro* trials.

This book will appear as a baseline for academicians, scientists, and health professionals as still, research is going to overcome this outbreak of COVID-19, the novelty of best animal models, and find an effective treatment. However, just a single book proposal like this wouldn't have flourished without enthusiasm and determined publishers' and investigators' strength to take time from their busy schedule and subsidize on time.

We thank the whole investigators who contributed, directly and indirectly, to bring it to reality.

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Part I: Models for COVID-19

CHAPTER 1

Genetically Engineered Mouse Models for COVID-19

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Abstract: Previous severe acute respiratory syndrome-coronavirus (SARS-CoV) outbreaks resulted in a cohort of preclinical studies that utilized various mice models for determining the pathogenesis of the infection, including the viral replication, spread, and mortality of the disease. Such studies have provided a framework upon which new investigations have been launched for understanding the outbreak of new coronavirus disease-19 (COVID-19) causing viral agents and their interaction with the host and its body. Recent investigations showed that the previous SARS-CoV and the recently discovered severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) both require the spike S protein to enter the host cell upon infection the binding with the receptors on the surface of the cells. The viral entry also requires proteases from the host cells. Since there are key similarities between the structure of the viruses and the construct of the viral transmission along with the spread inside the host's body in animal models. They were developed for the previous viral agent. The disease can be emulated or manipulated to bring forth novel investigations leading to key data that can broaden the sphere of COVID-19 studies being conducted. There are several different options to choose the right animal model for the question being raised in the experimental design with the pathogenesis of COVID-19. This chapter focused on the already established animal models for other coronavirus outbreaks and some of the strategies that can be exploited to develop new animal models. For COVID-19, research aimed at targeting the therapy or basic investigations for understanding cellular or organ level mechanisms involved in the disease.

Keywords: COVID-19, Knockout, Mouse models, SARS-CoV-2, Transgenic.

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INTRODUCTION

In December 2019, a novel coronavirus outbreak was reported in Wuhan, China which caused a pandemic and is an ongoing public health concern worldwide [1]. The disease known as coronavirus disease-19 (COVID-19) is caused by the novel severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), which leads to deadly pneumonia and severe health issues related to lung damage [2]. The genomic properties of the viral agent responsible for the local epidemic in China were timely shared across the scientific community. Since then, a valiant effort to combat the disease and understanding the agent responsible for the pandemic has been ongoing. Across the world, millions of people have been affected by the pandemic leading to a change in lifestyle globally [3 - 5]. The diverse scientific community took upon understanding the pandemic and the viral agent responsible for the COVID-19 from the get-go during the earlier days of the pandemic in 2020 [6]. Millions of people have been infected, and hundreds of thousands of people have lost their lives due to the COVID-19 pandemic outbreak [7]. Various attempts have been reported to classify the epidemiological features of the pandemic, including the case fatality rate, reproduction number, and recovery rate. To date, it is being assumed that various factors that affect the rigorous testing and other socio-economic factors hamper any progress in determining the true features of the pandemic [8 - 13].

The SARS-CoV-2 virus belonging to the *Coronaviridae* family of the virus has been studied extensively due to the urgency of pandemics. The vaccine development approaches have been challenging so far, and various mouse models have been utilized for understanding different aspects of the physiology of this novel coronavirus. Previously, the Middle East Respiratory Syndrome (MERS) and SARS candidates for vaccines were evaluated in the different mouse models [14, 15]. The clinical features, including the pathogenesis of the coronavirus, can be studied in great detail upon the development of animal models that can efficiently mimic the properties of the disease. For this purpose, numerous models have been developed in animals such as hamsters, guinea pigs, mice, cats, and rabbits, *etc*., [16 - 20]. The major receptor of SAR-CoV is angiotensin-converting enzyme 2 (ACE2) and is utilized to develop transgenic mouse models containing the human ACE2 gene [21]. *Rhesus macaques* were the first animal model for vaccine development against MERS-CoV, showing the symptoms of the infections as seen in clinical patients [22]. Golden Syrian hamsters were also used as animal models for establishing the vaccine candidate's safety and viral pathogenesis for different strains of SARS-CoV [19]. NSP16 CoV attenuated vaccine development approaches utilized mice as well [23]. Developing animal models, MERS-CoV had several drawbacks due to the inefficiency of the virus being replicated in the respiratory system. Therefore, new approaches like gene

targeting paved the way for demonstrating modified models containing genes of interest or lack thereof for more naturalized infectivity of the virus, *e.g.,* human DPP4 transgenic mice [24]. Clustered regularly interspaced short palindromic repeats and associated protein 9 (CRISPR-Cas9) the system has been efficiently employed for developing mouse models that are engineered to get infected by the virus and show high replication [25, 26]. Small animals like rabbits and mice are used to develop mouse models specific for a particular infection such as SAR-CoV-2 for various reasons, including efficacy, cost issues, and manipulation approaches. There is still much work that is needed to better understand the behavior of COVID-19 and transgenic animal models can efficiently smoothen the process of understanding due to ease in receptor identification, protection, pathogenesis models and immune response studies [15, 27]. Various applications and interference points for different types of mouse models in understanding or combating SARS have been depicted in Fig. (**1**). The aim of this chapter is to summarize the well-known animal models that can be used for coronavirus along with some of the strategies that can be exploited to develop new animal models for COVID-19 as a model to target the therapy or basic investigations the underline molecular mechanisms involved in the disease.

Emerging Role of CRISPR/Cas9

In molecular diagnostics, CRISPR, and the Cas protein-containing CRISPR-Cas systems, have advanced the process of functional research. Since its discovery almost 30 years ago, the field of genome editing using CRISPR systems has been revolutionized [28]. In clinical sciences and modern molecular biology approaches, CRISPR-Cas9 systems are routinely applied for targeting the cells of mammalian origin involving gene editing methodology [29]. Such approaches have been utilized to detect Zika virus and methicillin-resistant *Staphylococcus aureus* along with nucleic acid detection-based diagnosis procedures that use RNA-guided or RNA-targeting CRISPR-Cas systems [30 - 32]. In the wake of the COVID-19 pandemic of 2020, CRISPR-Cas13 approached for diagnostic applications are being considered [33]. The potential for this technology is deemed as tremendous, as described by Xiang *et al.* [34]. A fast and accurate method for detecting SARS-CoV-2 is the need for the hour, and SHERLOCK protocols can provide the scientific community an approach that has huge potential in this area. A recently reported DETECTR assay for the detection of SARS-CoV-2 *via* employing CRISPR leads to a 95% positive prediction along with a 100% negative prediction agreement in a real-time RT-PCR [35]. Genome-wide CRISPR screens were employed to determine the therapeutic targets for COVID-19. Apart from already established protease cathepsin and receptor ACE2, SWI/SNF chromatin complex proteins that comprise the transforming growth factor-beta (TGF-β) signaling network were also identified using the

CHAPTER 2

Different Cell Lines for SARS-CoV-2

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Abstract: The recent emergence of the novel *betacoronavirus*, pathogenic severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) with high nucleotide identity to SARS-CoV represents the causative agent of a potentially deadly disease coronavirus disease-19 (COVID-19) in Wuhan, China, and spreading across several countries globally pose a great global public health concern. Until a vaccine is available, effective therapy must be identified, and many clinical trialshave been executed worldwide. Various *in vitro* investigations are ongoing using different cell cultures to find alternative treatment options, allowing SARS-CoV-2 replications. Various cell lines are susceptible to the SARS-CoV-2 infection. In this chapter, literature regarding SARS-CoV-2 isolated in several cell lines commonly used for diagnostic or research purposes has been summarized. It also shows that SARS-CoV-2 can achieve high titers in various cell cultures derived from different species. In addition, these cell lines are extensively used in the diagnosis, to study pathophysiology, genome studies, and the finding of new targets for drug development and provide new ideas for the discovery of lead compounds with potential therapeutic agents against novel COVID-19.

Keywords: Anti-viral, Betacoronavirus, Cell lines, Clinical trials, Lead compounds, SARS-CoV-2.

INTRODUCTION

The first outbreak of the novel coronavirus occurred in Wuhan on December 12, 2019, in China, and abruptly spread to several other nations. The World Health Organization (WHO) declared a name for 2019-nCoV infectious disease coronavirus disease-19 (COVID-19) on February 11, 2020. The virus name was renamed Severe Acute Respiratory Syndrome-coronavirus-2 (SARS-CoV-2) by the International Committee of Taxonomy of Viruses, earlier called 2019-nCoV [1].

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During the last 20 years, three infectious diseases, such as SARS and the Middle East Respiratory Syndrome (MERS), have been triggered by coronaviruses.

This suggests that COVID-19 is not the first infection caused by coronavirus [2]. According to the classification of the CoVs, it belongs to the Coronaviridae family having Coronavirinae subfamily of the Nidovirales order, which their subfamily comprises of 4 genera coronavirus, namely α, β, γ and δ [3]. The nucleotide arrangements of the SARS-CoV-2 were associated with SARS-CoV and MERS-CoV that demonstrated greater homology to SARS-CoV, although they were comparably weak with the MERS-CoV [4]. It is not surprising that many researchers have newly verified the genomic similarities associated with the SARS-CoV-2 and bat *betacoronavirus,* which belongs to the sub-genus *Sarbecovirus* [5]. The researchers have also found that the SARS-CoV-2 utilizes the identical receptor known as the Angiotensin-Converting Enzyme 2 (ACE2), similarly to the SARS-CoV [6]. Tyrell and Bynoe first identified the coronaviruses in 1966 and grown the viruses by taking samples of the patients suffering from the common cold [7]. The enclosed, positive single-stranded giant RNA viruses of about 30 kb in size are the characteristics of coronaviruses that infect individuals and the wide-ranging of creatures. Genomic sequencing of the SARS-CoV-2 has already been done. It has been found that the SARS-CoV-2 has originated from the bat that emerged from a photogenetic study [8]. Entry of this virus into the host organism is facilitated with the transmembrane S-glycoprotein (spike), which makes homotrimers protruding from the virus's surface.

Currently, no approved treatment is available for managing COVID-19. There are no specific drugs for the treatment, so there are many supporting measures that are usually given to the patients, including oxygen treatment, antimicrobial agents, antifungal drugs, and Extracorporeal Membrane Oxygenation (ECMO), *etc*., [9]. This chapter aims to comprehensively gather all the medical literature highlighting the various cell lines that are useful in replicating SARS-CoV-2 that can be fruitful for *in vitro* studies of an anti-viral drug effective in managing COVID-19 disease. Also, not only for evaluating the anti-viral drugs but also for these cell lines are useful for various purposes. For instance, to study the pathogenesis, study the genome of this virus, and find the new targets for drug development is important to highlight.

Role of Cell Lines in Scientific Research

Instead of individual cells, immortal cell lines are also used to explore the plethora of biological processes. The benefits include cost-effectiveness, easy-t--use, limitless availability of products, and it avoids the ethical questioning involved with human and animal tissues [10]. In vaccine development, monitoring

of the drug metabolism and cytotoxicity, antibody development, the analysis of genetic material regulation, and artificial tissue creation, in addition to the development of biological substances such as therapeutic proteins and cell lines, have revolutionized medical science [11]. Traditionally, the diagnosis of the viral disease has focused on viral pathogens in cell culture isolation. Even though this technique is always sluggish and needs significant scientific skill. The laboratory diagnosis for the viral disease has been recognized as a "gold standard" for decades. Cell lines are extracted from cells separated from the original tissues after broken down through various methods, including enzymatic, mechanical, or chemical. It has been given a huge quantity of cells appropriate for isolation, allowed the monitoring that helps reduction in the use of laboratory animals, and has contributed to antibiotic and clean air pollution [12]. However, in confirming the causative agent of the disease during high prevalence, the culture of cells has always been essential [13]. In current years, however, technical advances, from monoclonal antibody production to molecular diagnosis, have created powerful methods useful in detecting the various forms of viruses. Molecular identification of viral deoxyribonucleic acid (DNA) and ribonucleic acid (RNAs), molecular multiplication using Polymerase Chain Reaction (PCR), and additional methods in diagnostic laboratories have become increasingly usable. Using an RT-PCR multiplex for SARS-CoV detection, scientists evaluated several human and animal cell lines [14]. The goals of the RT-PCR (real time-PCR) technique are:

1. Glyceraldehyde 3-phosphate dehydrogenase, as an internal RNA integrity regulation and help in the development of cDNA.
2. For the input virus identification process, SARS-CoV genomic RNA is targeted.
3. It also showed the 3' co-terminal specific sub genomic RNAs representative of viral ingestion and appropriate for the viral replication initiation process.

As the SARS-CoV is extremely pathogenic, most laboratories are unwilling to separate this virus in culture. Because of the high pathogenic nature of these viruses, co-cultured cell lines serve as the best alternatives, for instance, R-Mix (Diagnostic Hybrids, Inc.). R-Mix is a blend of the mink pulmonary cells and human adenocarcinoma cells, consisting of the strains Mv1Lu and A549, respectively [15].

Table 1. Summary of the different cell cultures with their species of origin and cell type.

Cell Line	Species of Origin	Cell Type
Caco–2	*Homo sapiens*	Colon carcinoma
Huh-7	*Homo sapiens*	Liver hepatocellular carcinoma

CHAPTER 3

Histopathologic Evaluation and Scoring of SARS-CoV-2 Infection

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Abstract: The recent emergent coronaviruses in the 21st century, such as Severe Acute Respiratory Syndrome-Coronavirus (SARS-CoV), Middle East Respiratory Syndrome-Coronavirus (MERS-CoV), and severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), has caused significant morbidity and mortality around the world. The lung is the most affected organ in the infection of human pathogenic coronaviruses. There is always a scarcity of human signs, symptoms, and modes of transmission. So to study the viral pathogenesis and evaluated interventions of therapies and vaccines, animals need to be used as models, especially at early epidemics. Lesions scoring can be identified from histopathological studies, and it can be helpful to understand the viral pathogenesis and damages to the cells to design effective therapies or vaccines. Histopathology uses the cells to determine viral host receptors and viral host tropism to relate with disease severity and lesions. Moreover, histopathology also plays a role in the qualitative description of affected organs to determine the micro-anatomic location of cells, type of cells, and cellular consequences during and post-infection. Comparatively, this approach has various limitations, but still, it is significant in comparing treatment groups. In comparing various groups, semi-quantitative and quantitative tissue scores are used for statistical analysis to increase the reproducibility of the study. This chapter refers to different features, including the importance of histopathology, principles, technique, scoring methods, and pathological characteristics of COVID-19, which can be valuable to assess the lung infection caused by SARS-CoV-2 and animal models and real situations.

Keywords: COVID-19, Infection, Lung, Pathology, RNA, Scoring.

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INTRODUCTION

Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) belongs to the group of *betacoronaviruses*, enveloped, and genetic material is present in the form of single-stranded RNA which size is approximate ~30 kb. A single-stranded RNA genome codes a variety of structural and non-structural proteins. The non-structural proteins are comprised of various vital enzymes, including protease, helicase, and RNA-dependent RNA polymerase, which play a key role in the pathogenesis and survival of the virus inside the cell. The sequence analysis of pair-wise non-structural proteins indicated that the pathogen belongs to the Severe Acute Respiratory Syndrome-Coronavirus (SARS) coronaviruses group [1]. However, structural proteins comprised of glycoproteins of spikes and other accessory proteins role in the virus binding to the host receptor and its entry into the cell [2]. The structural proteins reported in the outbreak of SARS and the Middle East Respiratory Syndrome-Coronavirus (MERS) can help develop antiviral drugs as a potential target. Therefore, these proteins are documented as potential targets to develop antiviral drugs against SARS and MERS [3], and possibly representing in coronavirus disease-19 (COVID-19) case, according to the genetic analysis of recent SARS-COV-2 isolated from Wuhan [4]. However, new antiviral drugs or vaccines can be developed by using structural proteins as recommended. The phylogenetic analysis of the full-length genome is suggested that SARS-CoV-2 shares 79.5% sequence similarity with SARS-CoV [5 - 7], which indicated that SARS-CoV-2 belongs to subgenus Sarbecovirus [5 - 8]. Various novel coronaviruses have been identified from animals and humans after the emergence of SARS and MERS coronaviruses. It delivers track for the evolutionary origin of incipient coronaviruses from bats source only. Though, camels were also recognized as immediate hosts for the MERS-CoV outbreak. The emergence of SARS-CoV and MERS-CoV from zoonotic source indicate zoonosis in the case of COVID-19 as well [9, 10]. The sputum sample of a patient who suffered from acute pneumonia and kidney failure was found with MERS-CoV. It was isolated for the first time and genetically closed to PiBatCoV HKU5 (*Pipistrellus* bat CoV HKU5) of japan and Ty-BatCoV HKU4 (*Tylonycteris* bat CoV HKU4) in Hong Kong from *Tylonycteris pachypus* (bamboo bat). The lineage C β-CoVs associated to MERS-CoV was detected in bats, together with Hypsugo BatCoV HKU25 and BtVsβCoV/SC2013 from China bats, and coronavirus Bat CoV PREDICT/PDF-2180, BtCoVNeo5038/KZN/RSA/2015, and Neoromicia/PML-PHE1/RSA/2011 (NeoCoV), from African bats [11 - 15]. Additionally, a lineage C β-CoVs, Erinaceus CoV VMC/DEU, afterward well-defined as a novel species, Hedgehog coronavirus 1 was detected in European hedgehogs that are closely related to bats phylogenetically [16]. Likewise, palm civets and raccoon dogs were found with similar coronavirus like the SARS-CoV

isolated from China during the early outbreak, which indicated that these animals have the potential of intermediate host between bats and humans [17].

Humans appeared to be susceptible to bat-sourced SARS-CoVs without before variations that indicated the re-emergence of SARS-CoVs or related to it [18, 19]. There is a similarity in the spike proteins of Ty-Bat CoV HKU4 and MERS-CoV, which indicated that the primary host of the ancestor of coronaviruses in bats [20, 21]. Two different highly human pathogenic coronaviruses, such as SARS and MERS emerged in the 21st century in 2002 and 2012 respectively [22, 23]. At the end of December 2019, another coronavirus that was named SARS-CoV-2 has emerged in the Chinese population who had epidemiologically linked to the seafood market in Wuhan, Hubei Province [24]. The SARS-CoV-2 appeared to be transmitted to humans by nasal route, orally and through the membrane of eyes, which indicated that it has broad-scale transmission modes [25]. The recently identified SARS-CoV-2 has 88% sequence similarity with two bat-derived SARS coronaviruses such as bat-SL-CoVZXC21 and bat-SL-CoVZC45 and 79% genetic similarity with SARS-CoV and 50% with MERS-CoV [4]. After phylogenetic analysis, it was revealed that SARS-CoV-2 belongs to the genus of *betacoronavirus*, which comprises SARS/SARS-like-CoV and others isolated from bats and few wild animals [26]. Spikes proteins (also called S proteins) determined the host and tissue tropism of the SARS-CoV-2. S1 and S2 are the two domains of spikes proteins, which have a role in the virus attachment to host cell and membrane fusion, respectively. There is a high sequence similarity found in the S proteins of SARS-CoV-2 and SARS-CoV and both coronaviruses using the same cellular receptor for pathogenesis, which may be due to similarity in the S proteins [27]. Compared with SARS and MERS coronaviruses, the receptor domain of SARS-CoV-2 has a high mutation rate but still uses the ACE2 receptor during pathogenesis. The transmissibility and pathogenesis of SARS-CoV-2 are reported to enhance significantly because of mutation at Furin protease. The lessons learned through experiences result in tremendous advances, and valuable insight near separating the physiognomies of SARS-CoV-2 has been accomplished at an extraordinary speed from coronaviruses' epidemics, occurrence over the last two decades. It is still able to use this binding receptor. Whether mutations in ACE2 affect its binding or change receptor tropism need to explore in detail [4]. This chapter refers to different features, including the importance of histopathology, principles, technique, scoring methods, recent case studies of coronavirus and its types, virological examination, blood cell examination, and counts and pathological characteristic of COVID-19, which can be valuable to assess the lung infection caused by SARS-CoV-2 and animal models and real situation.

CHAPTER 4

Key Proteins and Their Roles in COVID-19

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Abstract: Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) is a novel member of the *Coronaviridae* family that is the causative agent of coronavirus disease-19 (COVID-19), which is now pandemic in the world. Various proteins characteristically responsible for pathogenesis are categorized as structural proteins (S, M, E, and N), non-structural proteins, and accessory proteins (ORF3a, ORF6, ORF7a, ORF7b, ORF8, and ORF10). Substitution of 380 amino acids and mutations in SARS-CoV-2 compared to other SARS-like coronaviruses characteristically plays a key role in entering the development of infection. S1/S2 of S protein has a specific differential role in SARS-CoV-2 stabilization and resultant infection. In addition to these key proteins, the host and virus-related proteins play risk factors in the spread of this disease. The current chapter will describe the structure of key proteins, their role in pathogenesis, starting from viral attachment leading to the infection in the host compared to the viruses belonging to *Betacoronaviruses*.

Keywords: Coronavirus, Open reading frames, Pathogenesis, Proteins, Risk factors.

INTRODUCTION

Severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) is the cause of an outbreak at the world level with the expression of uncommon viral pneumonia

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that firstly occurred in Wuhan, China. The virus belongs to genera *Betacoronavirus,* keeping in view genomic structures and phylogeny interactions with other viruses responsible for severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS). They all have notable differences in their genomic and phenotypic structures [1]. All *Coronaviridae* family viruses are pleomorphic, having RNA as an essential genetic material. The striking feature is the possession of crown-shaped (thus named corona) peplomers or spikes that surround the genetic material. These spike proteins have facilitated the pathways leading to membrane fusion of the virus with the host cells [2]. These peplomers have a special affinity for binding angiotensin-converting enzyme 2 (ACE2), receptors located abundantly on lungs and the heart tissues. S proteins possess highly variant amino acid sequences than those of Open reading frames (ORFs) [3].

SARS-CoV-2 has been identified as a single standard +RNA comprising 2.9kb nucleotides in length encoding 26 proteins and one RNA-dependent RNA polymerase (RdRp). The proteins of this virus contain large polyproteins, *i.e.,* ORF1a and ORF1ab, which proteolytically slash to form 16 non-structural proteins. Besides these, there are 04 structural proteins, sixteen proteins categorized as non-structural, while 06 of those proteins called accessory proteins (Fig. **1**). Though these proteins are not necessary for *in vitro* replicating viruses, it is important for *in vivo* virus-host interactions [4].

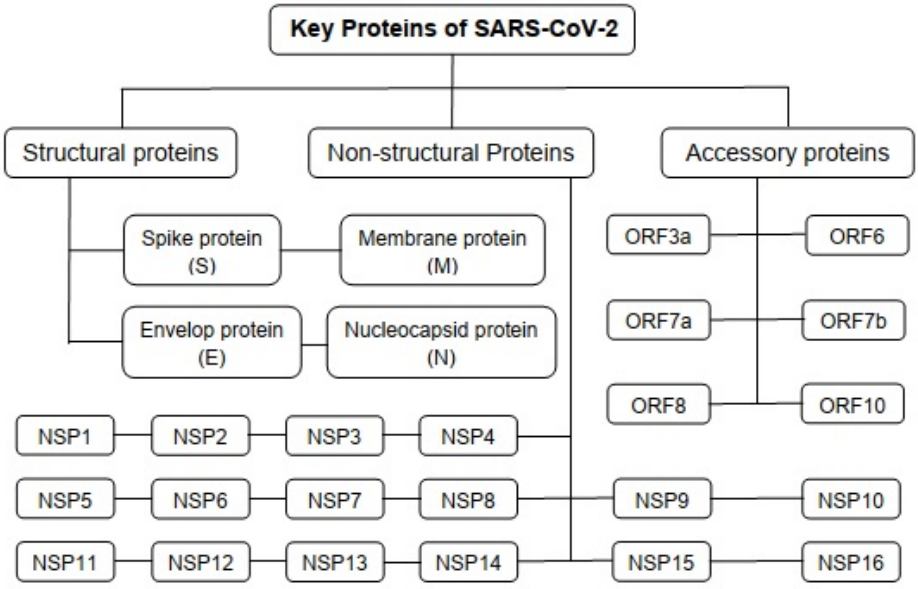

Fig. (1). Structural summary of the proteins expressed by SARS-CoV-2.

Hemagglutinin esterase lacks gene in SARS-CoV-2. Although it is present in a lineage A of *Betacoronavirus* named human coronavirus (hCoV) HKU1 [5]. Spike protein plays a role in binding with receptors and attachment to the membrane, which indicates host tropism and disease spread [6]. In SARS-CoV-2, the S gene is different, having <75% nucleotide sequence resemblance compared to all formerly reported SARS-related coronaviruses [4]. However, the other 03 proteins of the structural category are fairly maintained compared to spike protein and are essential for overall coronavirus function.

The glycosylated spike protein is considered responsible for the induction of an immune response in the host. This spike (S) protein facilitates attack on the host cell by attaching to ACE2 protein receptors found on the host cell membrane. Envelope (E) protein is responsible for performing many functions in the viral replication cycle involving viral assembly, the release of the virion, and viral pathogenesis [7]. Membrane (M) protein is an essential membrane protein of the coronavirus that plays a significant role in viral assembly and induction of apoptosis [5]. The nucleocapsid (N) protein of coronaviruses binds directly to viral RNA, making stability. So it is found to provoke antiviral RNAi in SARS-CoV-2 [8]. These proteins provide a coating to the RNA in protein assemblage (Fig. **2**) responsible for budding, forming an envelope, and pathogenicity of the COVID-19 virus [9].

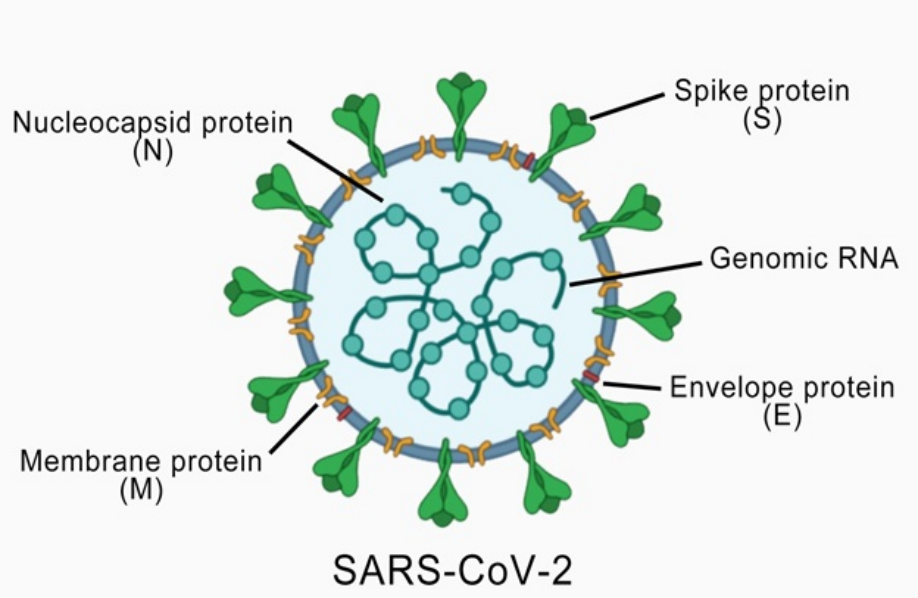

Fig. (2). Structural diagram of the SARS-CoV-2.

CHAPTER 5

Differential Diagnosis of COVID-19

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Abstract: Differential diagnosis is a key step to treat and prevent any disease at current. Differential identification becomes more inevitable in diseases that become pandemic while their signs and symptoms overlap with many diseases. Coronavirus disease-19 shows resemblance in its pneumonic presentation with related coronaviruses (SARS virus, and MERS virus), adenovirus, influenza virus, human Metapneumovirus, parainfluenza, Respiratory Syncytial Virus, rhinovirus, bacterial pneumonia (*Streptococcus pneumonia, Haemophilus influenza pneumonia*, *Moraxella catarrhalis* pneumonia, and *Chlamydia pneumonia*), and *Mycoplasma pneumonia*. Contrary to the discussion of only diagnostic findings, a comprehensive approach of differences in aetiologies, transmission/epidemiology, pathogeneses, clinical signs, and response therapy is necessary to resolve pandemic corona infection. Additionally, mathematical predictive models calculate the reproductive number (R_0) to show the epidemic nature of the disease in comparison to other conditions, thus aids therapeutic and prevention measures. The current chapter differentiates minor and major differences of COVID-19 compared to viral and bacterial diseases that show similar signs and symptoms.

Keywords: Aetiologies, Clinical signs, Coronavirus disease-19, Diagnosis, Differential, Pathogenesis, Response therapy, Transmission/epidemiology.

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INTRODUCTION

Common human coronaviruses are *Alphacoronaviruses* (HCoV-229E, and HCoV-NL63) and *Betacoronaviruses* (HCoV-OC43, and HCoV-HKU1). They are a source of the Common cold and some upper respiratory tract infections in humans. Severe acute respiratory syndrome-coronavirus disease (SARS-CoV), Middle East respiratory syndrome-coronavirus disease (MERS-CoV), and coronavirus disease-19 (COVID-19) belong to the genus *Betacoronavirus*. They are a source of major epidemics and cause severe respiratory and other systemic infections [1]. Differential diagnosis must include the probability of various widespread respiratory diseases. Patients remain asymptomatic while the symptomatic may be categorized as mild, moderate, and severe, seasonal flu symptoms. Few people show more severe symptoms of pneumonia and sometimes acute respiratory distress syndrome (ARDS) and septic shock to be hospitalized immediately [2]. Detection of COVID-19 at the earliest is a necessary step to treat patients on accurate guidelines. For suspected cases, rapid antigen detection and other examinations should be done to evaluate common respiratory pathogens and non-infectious disorders. A COVID-19 diagnostic testing kit has been developed and is available in clinical testing labs.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) is used as a gold standard for testing COVID-19 [3]. A computed tomography (CT) scan has a greater value in initial detection and differential diagnosis of COVID-19 [1]. There are various imaging expressions in COVID-19 at distinct stages primarily associated with pathogenesis. At the initial stages of COVID-19, inflammation of lungs penetrated to the subpleural areas of one or both lungs showing irregular or segmented pure ground-glass opacities (GGOs) along with dilation of vessels. A short number of cases are also noticed with negative CT findings at the initial stages. But in the advanced stage, CT shows chiefly an enhanced amount of pure ground-glass opacities in lungs, consolidations of particular lesions, and GGOs surrounding consolidated lesions, which are the prominent characters of advanced stage COVID-19 infection [4]. Imaging diagnosis for COVID-19 is considered difficult to differentiate for viral pneumonia, including influenza viruses, adenovirus, Respiratory Syncytial Virus, SARS-CoV, MERS-CoV, and bacterial pneumonia [5]. Viral pneumonia, except COVID-19, is chiefly associated with interstitial inflammation of peribronchial and perivascular regions, spreading toward the inner part of the pulmonary interstitium. Their CT expressions are manifold interweaved due to infiltration of the interlobular septa [6].

COVID 2019 must also be differentiated from bacterial pneumonia and *Mycoplasma pneumonia (M. pneumonia)* (separated from bacterial pneumonia) is the smallest and atypical bacteria that causes mild respiratory symptoms) [7].

Bacterial pneumonia occurs in the lung parenchyma, and there is bronchial or lobar pneumonia and numerous inflammatory secretions. Its CT expressions exhibit widespread irregular consolidations of the lung parenchyma, while GGOs are rare [8]. *M. pneumonia* usually occurs in children and young people but seldom occurs in adults. The chest CT manifestations of *M. pneumonia* in adults are commonly bronchial wall thickening, an indicator of lobar pneumonia, and centrilobular nodules, which are small airway lesions. In contrast, the CT scan of COVID-19 shows mostly pure GGOs at the initial stage and observable consolidations in the center of the lesions at the advanced stage [9]. The current chapter will describe in detail about pathogenicity, clinical signs, diagnostic procedures, and treatment of COVID-19 as differential indications keeping relevant viral and bacterial diseases of the respiratory system in an intact discussion (Fig. **1**).

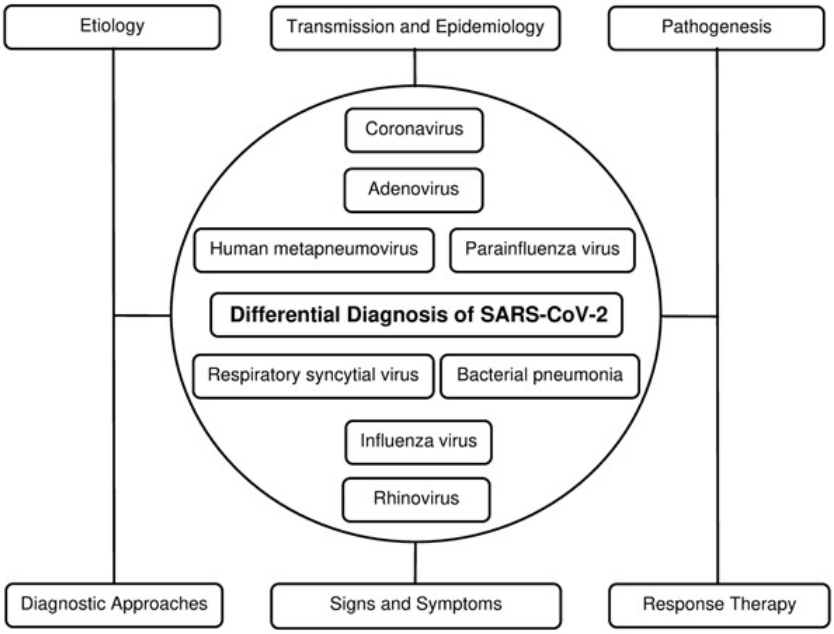

Fig. (1). Differential diagnosis of SARS-CoV-2 with different diseases based on different factors/characteristics.

Differential Diagnosis of COVID-19

Differential diagnosis involves confirmation of a disease condition whose signs and symptoms somehow show similarities with other diseases or disease conditions. To treat or prevent any disease at current and to avoid another illness with similar signs and symptoms, it is imperative to have sound knowledge of the

Part II: Treatment Strategies for COVID-19

CHAPTER 6

Different Antiviral Drugs against COVID-19 and Future Perspective

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Abstract: The outbreak of coronavirus disease-19 (COVID-19) across the world has caused serious health issues in terms of physical and psychological damage to human health. The spread of this virus was rapid and shortly spread to almost every country in the world. Due to the high infection rate and occurrence of complications in the infected individuals, the research and development of anti- COVID-19 drugs became the utmost necessity of time as no specific drug is available to relieve the clinical symptoms of COVID-19. We reviewed reliable information on targeted severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) candidate drugs in the present chapter. Also, we summarized novel insight into the future development of safe, effective, and less-toxic antiviral drugs available and employed for the management of COVID-19. Similarly, we focused on antiviral medications under investigation for this purpose; several medications with different mechanisms of action were noticed for the treatment of COVID-19.

Keywords: COVID-19, Drug repurposing, Hydroxychloroquine, Remdesivir, Umifenovir.

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INTRODUCTION

According to the International Committee on Taxonomy of Viruses, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the virus causing COVID-19 disease. This virus is belonging to the Coronaviridae family of viruses [1]. Acute respiratory distress syndrome is considered the most widespread critical feature of this disease. It is well known that this category among viruses is responsible for severe acute respiratory syndrome with up to 10% mortality rate [2]. In focus, COVID-19 disease is significantly linked with notifiable morbidity and mortality [3]. As a severe and infectious respiratory disease, several suggested clinical guidelines are currently available for treating COVID-19 [4 - 6]. Within these treatment guides, various types of medications are indicated as a part of the treatment strategy. Among the literature, the most commonly investigated treatment choices for COVID-19 disease were antiviral agents, immunomodulatory agents, antibiotics, analgesics, antipyretics, herbal medications, and some other supportive medications [7–9]. Currently, over 38 medications are under investigation for being considered as a treatment for COVID-19 disease [7 - 8].

Several medications with different mechanisms of action were noticed for the treatment of COVID-19 disease. These mechanisms encompass acting as protease inhibitors; RNA polymerase inhibitors, cell membrane fusion inhibitors, ACE2 receptor connection inhibitors, serine protease receptor inhibitors for the TMPRSS2, host cell immunostimulatory agents against virus invasion, mesenchymal stromal cells therapies, monoclonal antibodies, and passive immunization plasma [7 - 10]. One of the most pivotal antiviral medication groups includes protease inhibitors, which affect the 3-chymotrypsin like proteinase and Papain-like protease enzymes that act directly on the processing of the viral polyprotein. The second group of antivirals encompasses RNA polymerase inhibitors which inhibit the viral genome replication through competing natural nucleosides of the virus RNA-dependent RNA polymerase active site and then block the virus RNA synthesis process. A third group works mainly on the inhibition of intracellular viral replication through alkalization of the intracellular medium to inhibit the uncoating process of the virus envelope. Fourthly, the group of medications acting particularly on the avoidance of virus entry and membrane fusion into host cells by several mechanisms, most explicitly, preventing the binding of virus spike protein to host cell wall receptors such as ACE2 and TMPRSS2 receptors. The fifth group including those medications which work mostly extracellularly exerts its effect through binding to interleukin-6 receptor and inhibition of cytokine release to protect from amplified immune response [10].

Many clinical experiments are under investigation to reveal the safety and efficacy of these medications in use for treating COVID-19 infection [7, 11 - 13]. The perspective beyond these trials focuses mainly on repurposing those approved medications that are already existing and utilized for other indications before COVD-19. It is noteworthy that any of the investigated medications have been approved for such indication so far [8, 12, 13]. In this chapter, our main focus will be on the antiviral drugs under study for COVID-19 treatment.

Mechanisms of Different Antivirals Actions in COVID-19 Treatments

Nonstructural proteins (NSPs) are conserved proteins that function in gene expression during virus replication. It is thought that NSPs are fundamentally associated with the replication and function of coronavirus. Structural and accessory proteins are the other two types that are less conserved than the NSPs and only embraced in the virion synthesis. Hence, the antivirals might be pivotal in controlling the proteins during viral transcription and replication as well, then they could be a likely choice to recuperate the emanating COVID-19 infections and clinical consequences [14, 15]. This information sounds crucial especially when talking about the mechanisms of antiviral agents' actions. A brief about these mechanisms of antiviral agents is illustrated in Fig. (**1**).

G-441524 is the active metabolite of remdesivir that veils viral RNA polymerase and shirks proofreading by viral exonuclease, lowering the production of viral RNA. Remdesivir works by slowing up chain discontinuation of nascent viral RNA and positively affects SARS-CoV, MERS-CoV, and bat-CoV strains [16]. Drawing on the repurposing concept, Umifenovir is one of the portentous antiviral drugs with an unrivaled mechanism acting on the S protein/ACE2 interaction and dampening membrane fusion in the viral envelope [17]. It mainly blocks both cell membranes and the fusion of viral endosomes through integration into cell membranes and intercession with the hydrogen bonding network of phospholipids [18]. Favipiravir (FPV) is a guanine analog with a pyrazine carboxamide structure, and competitively decreases purine nucleosides [19]. Through h phosphoribosylation and phosphorylation, FPV as a prodrug is transformed into active FPV ribofuranosyl phosphates as soon as it gets in the infected cells by endocytosis [19].

Oseltamivir (GS4104) is an ester prodrug, which is hydrolyzed by hepatic carboxylesterases to produce the active metabolite (GS4071), a potent, selective inhibitor of influenza virus neuraminidase. Viral neuraminidase disconnects the binding between the cell receptor and the virus during virus spreading from the cellular space to its periphery (budding). Thus, neuraminidase inhibitors (NAIs) inhibit neuraminidase. Renal filtration and tubular secretion are responsible for

CHAPTER 7

Phytochemicals Effective against COVID-19 and Future Perspective

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Abstract: Novel coronavirus disease-19 (COVID-19) is the deadliest form of coronavirus, which has caused pandemics across the world. According to a recent survey of the World Health Organization (WHO), by August 8, 2020, shows around 19187943 cases and 716075 deaths from this virus globally. In addition to the shortage of effective drugs and vaccines, the outbreak of COVID-19 triggered by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) prompted the medical community to scramble for new antiviral formulations. Mankind utilizes the plants' origin medicines from ancient to treat and cure various diseases. They are the best possible tools to tackle the disease as they have the lowest possible side effects compared to other forms of drugs available and in use to treat the diseases. Several phytochemicals extracted from plants could provide a baseline for research into plant extracts in treating and preventing coronavirus. The present chapter aims to summarize phytochemicals' effectiveness against COVID-19.

Keywords: Antiviral, COVID-19, Inhibition mechanism, Phytochemicals, Phytoextracts activity.

INTRODUCTION

Novel coronavirus disease-19 (COVID-19) belongs to the family *Coronaviridae* and genus beta-coronavirus. An enveloped RNA virus has an average size from 60 nm to 140 nm in diameter and 79% identical to severe acute respiratory syndrome-coronavirus (SARS-CoV). Laboratory analysis of this virus showed that it is 88% identical to two SARS-like coronaviruses which are derived from

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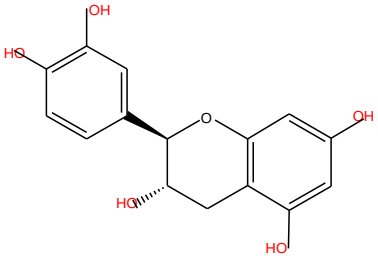
the bat; (a) bat-SLCoVZXC21 and (b) bat-SL-CoVZC45, 50% identical to MERS-CoV (Middle East respiratory syndrome coronavirus) and 'its' similarity index to bat-CoV RaTG13 is 96.2% [1]. The novel coronavirus (nCoV-2019) or severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was first reported in Wuhan (China), followed by a spread to other countries [2]. In December 2019, the first case was reported, then five patients were hospitalized with acute respiratory signs followed by the death of one of these patients [3]. The complete genome of a strain of SARS-CoV-2 (Wuhan-Hu-1-coronavirus WHCV) was first isolated and identified from a pneumonia patient in Wuhan. The size of the genome was 29.9 kb [4]. World Health Organization declared a public health emergency on January 30, 2020, of international concern for COVID-19 [5].

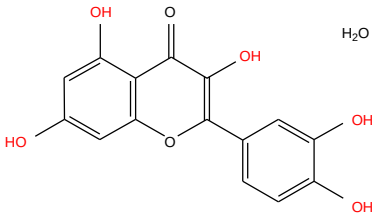
Novel COVID-19 has proved to be a highly deadly form of coronavirus that has special glycoprotein components on its surface that attach to the host body's immune complexes and leads to the arrest of the respiratory system of the host body. So far, there is no approved therapeutic drug or vaccine to combat coronavirus infection. As SARS-CoV-2 has created a global pandemic situation, the urgent discovery of novel antivirals is of utmost importance. Frequent mutations of the virus have made the discovery of vaccines a more challenging job for researchers [6]. Moreover, many vaccines are under trial, and researchers try their best to develop effective vaccines as soon as possible [7].

Globally, medicinal plants are used as alternative sources for the preparation of drugs. Several viral infections have long been treated using traditional medicinal plants that possess strong antiviral properties [8]. Secondary metabolites of medicinal plants (phytochemicals) pose beneficial health effects against viral infections. In many studies, the antiviral properties of phytochemicals such as glycosides, aliphatics, lactones, steroids, and alkaloids have been proved to cause beneficial health effects [9]. Plants have potential antiviral components, as found in flavonoids, coumarins, steroids, lignans, alkaloids, terpenes, and polyketides, *etc*[10]. Researchers have investigated luteolin-7-glucoside, kaempferol, catechin, quercetin, demethoxycurcumin, gingerol, apigenin-7-glucoside, naringenin, oleuropein, epicatechin gallate, zingerol, curcumin and allicin have inhibition activity against COVID-19 Table **1**. These phytochemicals ultimately lead to damage the cell membrane of this COVID-19, thus damaging its DNA components and composition and killing the virus. Another possibility is to develop phytocompounds which can damage the glycoprotein present on this COVID-19 coronavirus. Recent research on phytochemicals revealed that glycosides and flavonoids derived from garlic and Sena or *Salvia officinalis* could help develop specific phytochemicals that can damage the DNA gyrase SARS-CoV-2 [11]. From these all compounds such as catechin, kaempferol, epigallocatechin, quercetin, luteolin-7-glucoside, apigenin-7-glucoside,

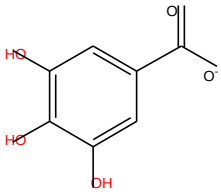
naringenin, demethoxycurcumin, curcumin, and oleuropein are the most suggested biological compounds extracted from medicinal plants that have inhibitory potential against COVID-19 Major protease (Mpro) [12], and their structure has been shown in Fig. (**1**). In the present chapter, the effectiveness of plant phytochemicals and possible antiviral therapies are highlighted, proving to be fruitful and effective to prevent and control this deadly disease.



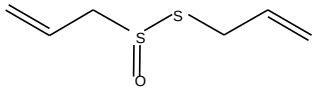
Catechin



Quercetin



Gallate



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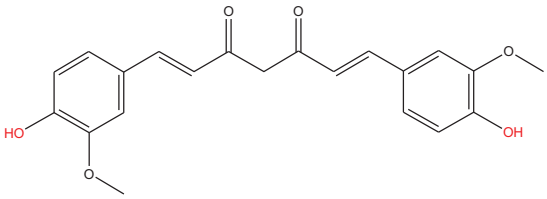

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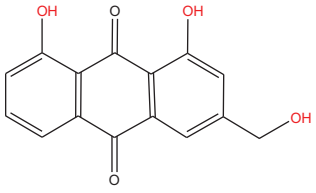

Aloe emodin

CHAPTER 8

Ultraviolet Radiation A (UVA): Modulates the Production of Nitric Oxide (NO) to Combat COVID-19

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Abstract: The ongoing outbreak of coronavirus-19 (COVID-19) has quickly become a daunting challenge to global health. The ultraviolet radiations (UVR) A wavebands fall in the region of 320-400 nm of the solar spectrum and comprise about 95% of overall ultraviolet (UVs) approaching the biosphere. The UVA is extensively used in phototherapy systems, as a disinfectant, and in various skin-related conditions. This chapter aimed to address the reduction of coronavirus replication by direct application of phototherapy (UVA) in lungs and modulation of nitric oxide (NO) in skin cells following UVA exposures. This NO influx inside the bloodstream to deliver into the lungs endothelial cells where it goes to incur cytoprotection to alveolar lung cells. Moreover, it is also proposed for direct application of therapeutic doses of UVA light inside the lungs. It uses a fiberoptic adapter to modulate the production of NO in lung endothelial cells, which will diffuse into the bronchi and lungs to leave bronchodilatory and vasodilatory effects. How NO reduces inflammatory burst and reactive oxygen species (ROS) in the lungs' alveolar cells is also discussed. Moreover, it is also

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Kamal Niaz & Muhammad Farrukh Nisar (Eds.)

proposed that UVA radiation application should be limited to physiological doses and applied every 4-8 h, with at least 24 h of therapy before reassessment. The treating physician should determine discontinuation of this direct UVA treatment into the lungs following observation of the patient's condition and the safety and efficacy of the treatment. This study will highlight and emphasize the importance of utilizing UVA radiation to control this epidemic.

Keywords: Blood, COVID-19, Fiberoptic adapter, Lung endothelial cells, Nitric oxide, ROS, UVA.

INTRODUCTION

The coronavirus disease-2019 (COVID-19) pandemic has caused a momentous economic, healthcare, and social disorder worldwide since early 2020. This coronavirus disease is declared as a pandemic by the World Health Organization (WHO), and it exhibited human to human transmission with rapid spread across the globe [1]. However, it is still unknown to define proper measures to control or treat COVID-19. In December 2019, the COVID-19 epidemic was initially reported in Wuhan, China, responsible for 80904 confirmed cases as of August 31, 2020, China, with 24,854,140 cases reported worldwide 838924 mortalities. Upon the onset of this epidemic, the Chinese Government immediately has taken strict measures to control its transmission to other cities by completely lockdown Wuhan city, and many countries followed this practice. The number of COVID-19 cases is still increasing with few exceptions whatever the measures have been taken. A huge literature has been reported on the weather as an important factor in transmitting infectious diseases, especially influenza and severe acute respiratory syndrome (SARS). A remarked change in ambient temperature is linked with a higher risk of SARS [2], while influenza is linked with dry, hot, or cold air [3]. In Northern Europe, less temperature and low solar ultraviolet radiations (UVR) are examined to be linked with maximum activity and transmission of the influenza virus [4]. Based on the previous observations and reports, it is assumed that the COVID-19 epidemic can be reduced or even completely controlled as soon as the summer approaches. Seasonal variation (Temperature, humidity, and UVR) is important and is key to the spread of several infectious diseases [5 - 7]. Previously, the sunlight-driven nitric oxide (NO) mechanism was described as reducing blood pressure and a lower frequency of myocardial infarctions [8 - 10]. Most outcomes of COVID-19 are linked with dysfunctions of the vascular system, especially in the lungs.

Deadly infections are causing human morbidity and mortalities for centuries of known human history. Novel pathogenic conditions arise by the end of 2019, the novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) [11], and spread globally by early 2020. Certain secure, potent, and effective therapies are a

dire need to minimize this SARS-CoV-2 pandemic unless a verified vaccine came into the market. Many studies indicate the shielding function of UVR in human health and for the disinfection of routine utensils. UVR is further divided into ultraviolet A (UVA) (320-400 nm), ultraviolet B (UVB) (280-320 nm), and ultraviolet C (UVC) (100-280 nm). UVR has long been recognized to pose strong anti-microbial effects. Among UVR, UVC is widely used to decontaminate and disinfect environmental objects and surfaces but is unsafe for human DNAs [12 - 15].

UVA radiation is a major environmental threat, but at the same time, certain therapeutic benefits have also been reported, and recently its potential use to minimize or reduce COVID-19. UVA (365 nm) is well known for inducing cellular signaling when irradiated in the physiological dose range. Solar UVR (UVA and UVB) is FDA-approved for various dermatological conditions such as skin lymphoma, psoriasis, and eczema [12, 16 - 18]. Still, we will elaborate on UVA light in the current draft only due to its least damaging effects for mammalian cells [13, 19]. Recent advancements in light-emitting diodes (LEDs) make it more versatile for the therapeutic application of lights for various internal organs [20]. FDA approves the only external application of the UVA light in therapeutic dosage range, but recently *in vivo* intraluminal UVA irradiations have produced no discernible endoscopic, histologic, or dysplastic changes in mice tissues [12]. Moreover, specific doses of UVA had strong inhibitory effects on coronavirus-229E and have proven a possible, safe, and quite effective treatment regimen for infectious diseases of internal viscera, but still demands clinical studies to define the safety, accuracy, and efficiency of UVA therapy in humans against COVID-19.

The current study aimed to propose the consideration of UVA therapy to control the cure for COVID-19 patients. The reduction of coronavirus replication in the lungs, modulation of NO in skin cells following UVA exposures, and this NO influx inside the bloodstream to deliver into lungs endothelial cells need to be evaluated. It incurs cytoprotection to lung alveolar cells shall be studied. Structural complexity, origin, classification, and molecular structure shall also be elucidated herein. Moreover, it is also proposed for direct application of therapeutic doses of UVA light inside the lungs to modulate the production of NO in lung endothelial cells, which will diffuse into the bronchi and lungs to leave bronchodilatory and vasodilatory effects. Moreover, how NO reduces inflammatory burst and ROS in the lungs' alveolar cells shall also be discussed.

CHAPTER 9

Herbal Remedies Effective against COVID-19

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Abstract: Coronavirus disease-2019 (COVID-19) first originated from China, named Wuhan, and now becomes a pandemic disease. The COVID-19 infections range from asymptomatic to mild and severe conditions. Medicinal plants have potential therapeutic effects for different infectious diseases and have long been used to treat many diseases. Wild plants are well-known for their anti-viral activities and are also used in herbal treatment for COVID-19 infection. Various traditional medicine systems use plants for the treatment of COVID-19. The considerable therapeutic effect was achieved by using traditional Chinese medicine (TCM) during the 2003 outbreak of severe acute respiratory syndrome (SARS), which brought new hope preventing and treating COVID-19. The Ministry of Ayurveda, Yoga & Naturopathy, Unani, Siddha, and Homoeopathy (AYUSH) focuses on prophylactic treatment, dietary management, and simple traditional remedies for the treatment of symptoms of COVID-19 by using medicinal plants. Telemedicine was focused on a new health care model to prevent transmission of COVID-19 by avoiding person-to-person contact. The purpose of this chapter is to identify the effective herbal treatment for COVID-19.

Keywords: AYUSH, COVID-19, TCM, Telemedicine, Wild plants.

INTRODUCTION

Coronaviruses belong to the family of *Coronaviridae*, which include viruses that commonly cause respiratory tract diseases ranging from the common cold to severe conditions. It has Middle East respiratory syndrome-coronavirus (MERS-CoV) or severe acute respiratory syndrome-coronavirus (SARS- CoV) [1]. A new strain of the corona virus was identified in the city of China, named Wuhan, at the end of 2019 [2] and was initially named the 2019-novel Corona Virus (2019-nCoV) [3]. The International Committee on Taxonomy of Viruses (ICTV) named this novel coronavirus as "severe acute respiratory syndrome coronavirus 2" (SARS-CoV2) on February 11, 2020, by picking its name from the causative virus of the 2003 outbreak of severe acute respiratory syndrome (SARS). It is genetically similar to the SARS virus [4]. After that, the World Health Organizat-

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ion (WHO) officially declared the disease name caused by this virus as COVID-19 (coronavirus disease-2019) [5]. After identifying the first case of the novel coronavirus in late December 2019 in China, it rapidly spread to other countries such as Germany, the USA, France, Iran, Spain, Italy, India, and now spread worldwide [6]. COVID-19 has over 1 million cases across the globe, almost 50,000 deaths are caused, and 203 countries are affected by this novel coronavirus decalred by WHO. Emergency Committee of WHO declared the SARS-CoV-2 a global pandemic on March 11, 2020 [7].

The period from exposure of the virus to the appearance of symptoms ranges from 2 to 14 days, but the average incubation period is 5 days [3]. Initial symptoms of COVID-19 are commonly non-specific such as dry cough, fever, and fatigue, *etc*. It may involve multiple systems such as the respiratory system causing sore throat, shortness of breath, cough, chest pain, hemoptysis and rhinorrhea, musculoskeletal causing muscle ache, gastrointestinal causing nausea, vomiting, diarrhea, and neurologic causing confusion and headache [8]. In severe cases, COVID-19 causes multiple organ failure and lead to death [9].

Currently, there is no specific vaccine or treatment available for COVID-19 [10]. Some studies have investigated anti-viral drugs, including protease inhibitors such as Ritonavir/Lopinavir, neuraminidase inhibitors, nucleoside analogs, Lamivudine, Tenofovir Disoproxil, Umifenovir, and Remdesivir for the treatment of patients of COVID-19 [11]. The results of these studies for therapeutic use are clinically not approved for the treatment of infected patients of COVID-19 [12]. Supportive therapies and preventive measures are implemented to avoid further complications of COVID-19 and prevent organ damage [13].

Symptomatic Comparison of SARS-CoV, MERS-CoV, and COVID-19

For the last two decades, the world has suffered from a few of the most devastating outbreaks of viral diseases. It includes the epidemics of coronaviruses like SARS-CoV that emerged in Guangdong, China, MERS-CoV emergence in Saudi Arabia, and newly emerged SARS-CoV-2 in Wuhan, China causing several causalities. HCoV-OC43, HCoV-229E, HCoV-NL63, and HCoV-HKU1 are among the other viruses belonging to the same family causing outbreaks in different regions [14, 15]. The viruses are closely related to each other as several studies reported the sequence homology of SARS-CoV-2 with other viruses belonging to β-*coronaviridae*. The studies conducted by Wu *et al.* [16] and Zhou *et al*. [8] in 2020 reported the sequence homology of 79.5% between SARS-Co--2 and SARS-CoV. However, Chan *et al.* (2020) reported the sequence homology of 89% and 82% with bat SARS-like CoVZXC21 and human SARS-CoV [17]. The previous epidemic of SARS-CoV and MERS-CoV reported being

zoonotically originated from bats and transmitted to humans *via* intermediate hosts of civet cats and camel, respectively. For SARS-CoV-2, bats were considered as native hosts as the virus has 96% similarity with SARS-like coronaviruses, including bat- SL-CoVZX21 and bat-SL-CoVZX45 [18, 19]. The potential route of transmission from person to person is through inhalation of infectious aerosol. The potential of COVID-19 spreading is higher compared to other phylogenetically related viruses. This may be due to mutations like longer 8b segments, shorter 3b segments, absence of 8a, the difference in open reading frame 8 (ORF8), and open reading frame 10 (ORF10), and the difference in nsp2 and nsp3 proteins [20 - 23]. Besides these mutations, the changes in the nsp2 protein of SARS-CoV-2 lead to an increased potential to become more contagious than other related viruses [24].

After incubation of about 5-7 days, SARS-CoV leads to the onset of the disease typically associated with symptoms like fever, headache, myalgia, malaise, dyspnea, and dry cough, in which 25% of the patients develop watery diarrhea. In some severe cases, the disease progresses to severe pneumonia and respiratory distress [25 - 28]. A similar disease goes in the MERS-CoV infected individuals who may develop an asymptomatic or mild infection, including fever, chills, myalgia, and dyspnea, followed by a nonproductive cough. Abdominal pain, diarrhea, and vomiting are reported in 25% of infected individuals. The severity of the progression of the disease may result in acute renal failure along with respiratory distress and pneumonia [29, 30]. The case fatality rate in MERS-CoV infected individuals is 34.4% compared to SARS-CoV with a fatality rate of 9.6%, and SARS-CoV-2, with a mortality rate of >3.4% [31 - 33].

The mean incubation period of SARS-CoV-2 is 5.2 days and 14 days of median duration from onset of symptom to death, and the mortality rate is alarming in the patients with age more than 70 years as they have a median duration of 11.5 days from the initial onset of the symptoms to the death [34]. Human coronaviruses are mostly associated with the upper respiratory tract causing mild to moderate illness. Still, in some moderate to severe cases, the lower respiratory tract can be infected and result in severe complications [35]. The COVID-19 infection ranges from asymptomatic to mild and severe infection as similar to SARS-CoV and MERS-CoV. The symptoms include fever, myalgia, dry cough, fatigue, chest pain, anorexia, nausea, vomiting, dyspnea, arrhythmia, and diarrhea. Complications related to coronaviruses infection involve acute respiratory distress syndrome, multiple organ failure, various degrees of liver infection [36], testicular tissue damage [37], kidney infections, RNAemia, cardiac injury, and metabolic acidosis [38]. Moreover, the infected individual can be observed with ground-glass opacities and peripheral consolidation in the lungs. The bilateral finding seems to be higher in SARS-CoV-2 infected patients [39].

CHAPTER 10

Role of Blood Plasma Transfusion Against COVID-19

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Abstract: Historically, convalescent plasma (CP) has been successfully used in pandemics caused by the influenza A (H1N1) virus, severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and Ebola. As the current pandemic by SARS-CoV-2 poses an unprecedented threat to humanity. The number of infections is increasing exponentially day by day. Identifying, qualifying, collecting, and preparing plasma from convalescents with sufficient SARS-CoV-2 neutralizing antibody titers in this pandemic may be challenging but within the approach of most blood establishments. Careful clinical evaluation may rapidly establish whether CP therapy at the early onset of disease in patients of high risk may improve symptoms, reduce hospital stay and decrease mortality due to COVID-19. Moreover, CP is an excellent therapeutic option due to the lack of specific treatment for COVID-19, as the availability of anti-viral drugs/vaccines may take more time.

Keywords: Adverse effects, Antibody titer, Convalescent plasma, Safety and efficacy, Transfusion.

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INTRODUCTION

Many patients were suffering from chest infections of unknown cause in December 2019 in Wuhan, China. The causative agent was found to be a new coronavirus (CoV), named nCoV2019 (2019 novel coronavirus) by the World Health Organization (WHO) on January 7, 2020 [1]. Subsequently, the virus was retitled severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), and the resulting infection was named coronavirus disease-2019 (COVID-19). This disease spread in many countries within three months, and the WHO declared it a global pandemic on March 11, 2020 [1].

Convalescent plasma (CP) therapy, prime adoptive immunotherapy that has been used in the treatment and prevention of various diseases for more than one century [2]. In the last two decades, CP therapy has been successfully applied with satisfactory efficacy and safety in the treatment and prevention of the Middle East respiratory syndrome (MERS), severe acute respiratory syndrome (SARS), and H1N1 pandemic [3]. In passive immunization therapy, the antibodies are administered to the susceptible person to treat or prevent the infectious disease caused by the same agent

On the contrary, active immunization requires the inductance of an immune response which takes time to develop and varies depending on the recipient. Therefore, for providing prompt immunity to a susceptible person, passive immunization is considered the only way [4]. For therapeutic purposes, passive immunization is the most effective way to administer in a short time after the appearance of symptoms. Passive administration of antibodies works by neutralization of the initial inoculation in which the amount is much smaller than that of established disease [5]. A sufficient amount of antibodies must be administered for passive immunization therapy to be effective. When given to a susceptible individual, the antibodies circulate in the bloodstream for some time. After it reaches the tissues, and provides proper protection against an infectious agent.

According to WHO, the current management and treatment of COVID-19 have been mainly centered on case detection, treatment, monitoring, prevention of infection, and supportive care [6]. Currently, due to the lack of evidence, no specific treatment against nCOV-19 has been recommended [7]. So CP therapy is the only choice for the treatment of severe patients infected with nCOV-19. In the current pandemic of SARS-CoV-2, CP therapy was tested to treat COVID-19 patients in China, as claimed in several studies [8, 9], as shown in Fig. (**1**) . A research study on 10 COVID-19 patients with severe symptoms, CP with neutralizing antibody titers equal to or greater than a 1:640 dilution, was collected

and transfused to all severe COVID-19 patients [10]. No serious side effects were reported in the recipients of CP transfusion. Moreover, the improvement in radiological pulmonary lesions and other symptoms was observed in all 10 patients within 1-3 days of CP transfusion. Studies revealed that CP obtained from COVID-19 recovered patients could be used for the treatment without producing severe adverse effects. Recently, Food and Drug Administration (FDA) has recommended the administration of investigational CP may result in clinical benefits for the management of COVID-19 patients during public health emergencies [11]. The current chapter aimed to summarize all the possible CP transfusion studies that might be beneficial for the treatment and management of individuals infected with SARS-CoV-2.

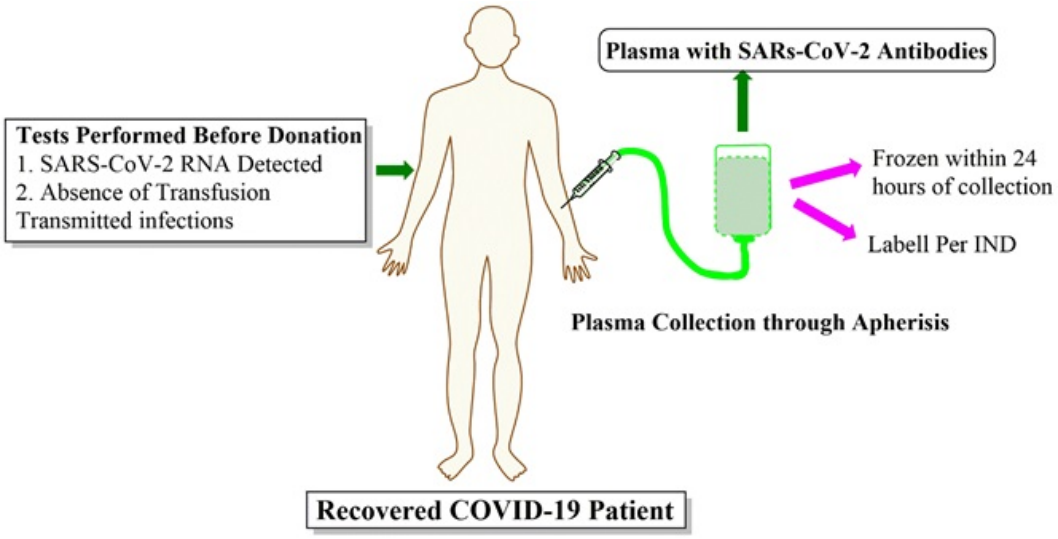

Fig. (1). Flow diagram of blood plasma preparation from infected individuals.

Therapeutic Agents (Convalescent Plasma and Immune Globulin)

Convalescent plasma, convalescent serum, and hyperimmune immunoglobulin prepared from convalescent plasma are interventions that have been used in the past to treat conditions when no vaccine or pharmacological interventions were available. Diphtheria, pneumococcal pneumonia, mumps, measles, hepatitis A and B, rabies, and polio are conditions where convalescent plasma is effective [12].

CP contains pathogen-specific neutralizing antibodies, which can neutralize viral particles, and treatment with CP or hyperimmune immunoglobulin confers passive immunity to recipients. The duration of granted protection can differ depending on the timing of administration, ranging from weeks to months after treatment [13]. Apheresis is the approved technique to obtain plasma. Blood from

CHAPTER 11

Stem Cell Therapy for COVID-19

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Abstract: Stem cells have long been a topic of interest around the globe. Now and then, stem cells are being studied for revealing their beneficial effects. Countries around the world are in a race in stem cell research. Stem cells possess some unique and considerable qualities that hardly any other cell to date has. The outbreak of novel coronavirus or coronavirus disease-19 (COVID-19) was located in Wuhan, China. After a few months, the episode was declared Pandemic by the World Health Organization (WHO) as it engulfed many countries worldwide. Since then, stem cells have gained more push in clinical as well as pre-clinical stage research studies. COVID-19 shares some molecular properties with other coronaviruses like severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS). Stem cells surprisingly showed some good outcomes in many patients infected with COVID-19. A lot of laboratory evaluation is being carried out to check the feasibility of different stem cells to be used in COVID-19 infected patients. This chapter discusses and highlights the possible interventions in COVID-19 using different lineages and bio-cultured stem cells.

Keywords: Bone marrow, Coronavirus, Cytokines, Hematopoietic, Mesenchymal, Patients, SARS-CoV-2, Stem cells.

INTRODUCTION

Since decades of extensive research, hematopoietic stem cells (HSCs) have been shown to exhibit tissue-specific stem cell characteristics and have been continuously used in many clinical procedures. Upon clinical application, they

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provide a deep understanding of the fundamentals of stem cell sciences. The different fields of HSCs being studied and their clinical applications are believed to benefit significantly. The properties of self-renewal and differentiation have put stem cells at the center of regenerative medicine. The combination of these features is leading to improve ways to treat many medical conditions [1]. The undifferentiated stem cells have their origin in the different stages of life. They can be found in the embryonic, fetal, and adult stages of life. They give rise to different lineages of cells that are necessary for building up various organs and tissues. Many studies have shown the use of stem cells in cellular therapy. In the regeneration of organs and the replacement of damaged cells, these cells are highly valued. Some recent research has shown that stem cell-associated specific cells in some diseases can be transformed to develop drugs [2]. In the jewels of medicine, cellular therapy is considered a favorable and encouraging approach that, instead of generating a new organ, focuses on the regeneration and restoration of various injured tissues [3]. Genetically modified stem cells and the production of engrafts with bio cultures scaffolds have enhanced therapeutic outcomes in animal models.

However, several vigorous pre-clinical studies are required to move forward in the field of stem cell therapy [4]. There are a lot of researches that have shown assuring effects of stem cell therapy. The scope of stem cell therapy can be seen during the developing phase of the embryo in various cardiovascular diseases, including cardiomyopathies, degenerative nervous disorders, metabolic diseases like diabetes, and osteoarthritis [5]. MSCs induce immunomodulatory and anti-inflammatory effects and have ascertained a dynamic role in the cure of COVID-19. The MSCs therapy has been a cost-effective treatment in COVID-19 [6]. This chapter will discuss the fundamentals of stem cells, their origin, and their types. Also, we will discuss the use of stem cells in novel coronavirus (COVID-19), the immune reactions, and the future perspective of HSCs for COVID-19.

Overview of Stem Cells

The term stem cell denotes a type of cell that holds the property to differentiate into several other types of cells while maintaining the self-renewal feature. Stem cells have the following main characteristics: a). The capability of expansive proliferation, *i.e.,* the process of renewing itself b). Potentiality to form different types of cells and c). The ability to grow from a single cell [2]. Going afar and crossing the limitations of cell lineages, hematopoietic stem cells possess a versatile nature to proliferate and give rise to abundant cell types. This process is also called transdifferentiation [7]. Different components in the blood are a reason for the differentiation of the multipotency feature of hematopoietic stem cells. HSCs are solely in charge of forming blood and its elements in all the spans of

life. They develop, maintain, and regenerate a major portion of blood which itself is connective tissue. Multipotent stem cells act as the origin to give rise to the lineage of myeloid and lymphoid cells. Myeloid lineage then differentiates to common myeloid progenitors or CMP, while lymphoid lineage gives rise to common lymphoid progenitors or CLP. CMP and CLL themselves are left with no or negligible transdifferentiating characteristics reaching the stage of these progenitors. They also lose self-renewal ability and show very little division. From the CLP arise the cells that are essential components of cellular immunity. The cells that arise from CLP include T cells, B cells, natural killer cells (NK), lymphocyte subset forming lineages, and antigen-presenting cells like dendritic cells.

CMP differentiates into granulocyte monocyte progenitors (GMP), which can differentiate into granulocytes and monocytes. CMP also gives rise to megakaryocyte erythrocyte progenitors (MEP), which further develops a change to megakaryocytes and erythrocytes [8]. Outbreaking research has been conducted on HSC circulation under different conditions. HSCs don't follow a haphazard path circulating in the blood, but they distribute under proper physiologic rhythmic regulatory signals. The central nervous system has a major part in regulating the circadian clock, which draws the HSCs to the bone marrow [9]. High resistance to viral infections has been found in different stem cells. It has been noticed that stem cells can protect themselves from viruses by inducing interferon stimulating genes (ISGs). Interferon reaction is the first main mechanism of many cells against viral invasion. It has been reported that when stem cells differentiate into other types of cells, as they cannot differentiate any further, the ISGs become lower. Different types of stem cells have a particular way of expressing the ISGs [10]. The stem cells are generated when the embryo cells migrate and specify in multiple sites during its developmental phase. In upper-class vertebrates, various locations can be seen to undergo hematopoiesis, the process of blood formation. Hematopoiesis can be consecutively seen in the yolk sac, aorta gonad mesonephros region that encircles the dorsal aorta, the fetus's liver, and lastly, the bone marrow. Placental tissue has been recently recognized as another spot for the development of HSCs [11]. HSCs can be transplanted in two different ways, *i.e.,* autologous and allogenic. Therapeutically autologous transplantation of stem cells is related to radiotherapy and chemotherapy. This has been regulated to kill the tumor cells and recover the patient later on by Stem cell therapy. Allogenic stem cell transplantation is different from autologous. It uses stem cells of hematopoietic origin from a matched donor to revive the patient without using chemotherapy. The allogeneic transfer can lead to a graft-versus-tumor effect [12]. The hematopoietic stem cell lineages have been illustrated in Fig. (**1**) .

CHAPTER 12

Computational Methods for COVID-19 Treatment

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Abstract: Computational approaches efficiently design the drugs to prevent diseases for which no drug is available. These techniques are also used for the development of new drugs. It involves using a variety of computer software for drug modeling and simulation, hence usually known as computer-aided drug designing (CADD). The computational tools provide crucial drug designing in a short period. These techniques are time and cost-effective as compared to conventional drug development methods. Computational methods can effectively model the suitable drug candidate by optimizing ligand-target interactions and observing the deep insight of cellular processes by its powerful tools. Several studies have applied these modern computational techniques to find out the possible therapy against the pandemic disease of COVID-19. The critical proteins of COVID-19, including 3C-like protease, papain-like protease, and RNA polymerase, are targeted to model the effective drug. CADD approaches suggest anti-viral drugs, anti-coagulant, anti-HIV drugs, and anti-fungal drugs to have little effect against COVID-19. This chapter aims to overview the different CADD approaches to design the possible drug for the treatment of COVID-19.

Keywords: CADD, Computational methods, COVID-19, Treatment.

INTRODUCTION

The development of a novel drug usually takes a long time and is often associated with a high risk of failure and high cost. Typically, the complete drug development procedure, from the drug concept to the market, may take about 14 years [1]. The drug development procedure is not an easy task, and the whole process may involve a very high cost ranges from 0.8 to 1.0 billion USD [2]. However, advanced scientific approaches expedite this time-consuming procedure

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in a short time [3, 4]. In the last few years, heavy investment has been made in the drug discovery procedure, but the output was not up to the expectations because of the low efficiency and high failure rate in the process [3].

Consequently, scientists and researchers searched for alternate tools to increase the success rate with high accuracy in a short research period. Various scientific approaches have been developed for this purpose. Among these techniques, computer-aided drug design (CADD) is one of the most effective methods for drug discovery. CADD is a widely used term that refers to the different computational tools for designing the compounds. It usually includes the analysis, storage, management, and modeling of compounds. CADD covers almost all of the essential aspects of the drug discovery and development process. Different computer-integrated programs are used to design compounds, assess the most appropriate candidate, and develop digital repositories for studying chemical interactions [4]. The application of CADD has altered the pipeline for drug discovery and development. CADD tools potentially identify the drug targets, validate and optimize the procedures, and may be applied in preclinical studies [5, 6].

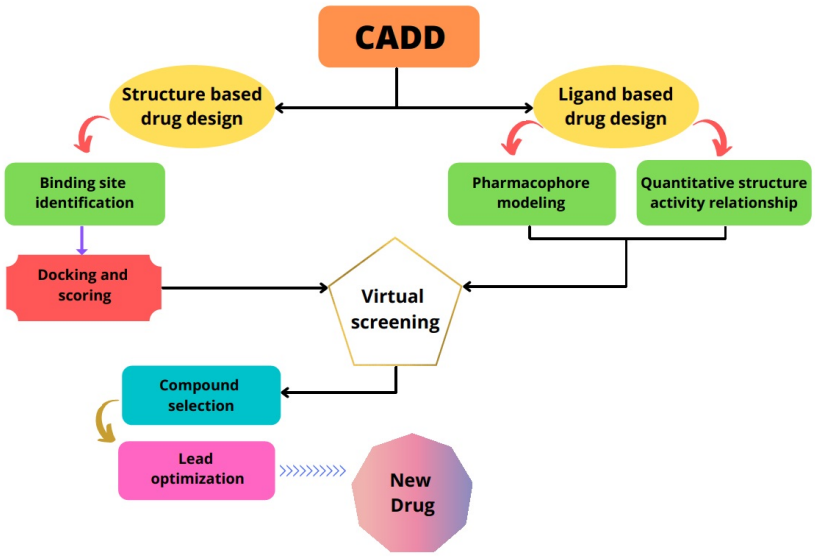

Fig. (1). The major steps involved in the CADD.

CADD approach is cost-effective and could reduce costs by half than conventional drug discovery and development [7]. The standard approaches of CADD can be divided into structure-based drug design and ligand-based drug design techniques.

The important steps involved in the CADD approach are shown in Fig. (**1**) . The CADD technique is generally divided into two important categories. The first category is the structure-based drug design, while the other refers to the ligand-based drug design. Structure-based design is usually applied to the availability of the 3D structure of a molecule. In this technique, the *de novo* approach provides valuable information. If the 3D structure of the target is unknown, then a ligand-based drug design can be applied. In this approach, pharmacophore modeling and quantitative structure-activity relationship (QSAR) can provide a deep insight into ligand-target interactions, which may be further processed by Virtual screening, selection of suitable compound, lead discovery, and ultimately the modeling of the new drug [8]. The purpose of this chapter is to highlight the importance of CADD approaches and the possible use of these tools in exploring the novel treatment for COVID-19.

Integrated Pipelines of COVID-19

Computational methods are more convenient in terms of less time-consuming and more cost-effective. These modern techniques have advantages over the historical practices of drug development in the pharmaceutical industry. In conventional methods, a series of experiments must be performed to find the suitable compound with desired properties. These experiments are usually time-consuming. With the advancement of technology, CADD tools efficiently analyze several compounds and quickly sort out the compound of interest to be evaluated for further biochemical reactions. Modern technology enables us to search for the drug target by the visual image of the 3D structure of the compounds.

In the current scenario of COVID-19, it is crucial to find an effective and safe drug very quickly; however, the potential drug candidate for the treatment of COVID-19 could be identified by using the modern computational screening of drug libraries. Virtual screening (VS) of ligand databases plays a vital role in exploring suitable molecules and has accelerated the initial stages of drug discovery [9, 10]. The purpose of *vs* is to rapidly recognize the potential hit molecules and can be tested experimentally and clinically. The crystal structure of molecules provides essential information in the identification process of potential drug molecules. *vs* is used by docking drug fragment libraries where the crystal structure of molecules is available. If the crystal structure is not available, then homology models are used. *vs* is used to identify the inhibitors in this scenario [11].

Virtual Screening of COVID-19

Two important proteases are coded by SARS-CoV-2 polyprotein. These proteases play an essential role in the process of non-structural protein (NSPs) and their

CHAPTER 13

Vaccine Development, Advantages, and Disadvantages of Group Immunity and Future Perspective for COVID-19

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Abstract: Since its outbreak in late 2019, SARS-CoV-2 has infected millions of people and caused 432, 204 deaths across the globe up to June 14, 2020 (WHO website). SARS-CoV-2 has a high transmission, prolonged incubation time, and an increased prevalence of asymptomatic infection. All over the world, the authorities are insisting on non-pharmaceutical interventions such as social distancing, imposing lockdowns, social isolation, and provision of personal protective equipment (PPEs) to the hospital and other essential departments. However, these measures have only slowed down the virus and cannot prevent rebounds on easing the restrictions. Moreover, the hype about the off-label use of drugs is on the rise. However, the efficacy and the risk of adverse effects have yet to be explored in clinical trials. Therefore, the only way to confer immunity and control the pandemic, the development of vaccines, is currently the research focus. The genetic sequencing of SARS-Cov-2 was done quickly, only in one month. To develop vaccines at pandemic speed, the scientific community faces the challenge of proof of clinical safety and efficacy. Moreover, the previous work is done, and scientists are utilizing the experience of SAR-CoV and MERS and the technologies thereof for COVID-19. Several manufacturers have announced their program and progress on vaccine development. Some vaccines have cleared phase I, and phase II clinical trials, while few are in phase III.

Keywords: Clinical trials, Coronavirus, COVID-19 vaccines, Group immunity, Herd immunity, Vaccine candidate.

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INTRODUCTION

Historically, vaccination is the most efficient countermeasure against infections, as the administration of pathogen-resembling agents increases the immune response against the invader. Vaccine technology continuously progressed in emerging viral threats to promote immune response [1, 2]. The development of vaccines is more time-consuming and complicated, which is different from the conventional medicine development process. Vaccine development usually takes 12-15 years [3]. Conventional medicines are used to treat disease whose symptoms have appeared, while vaccines are being used for a disease, the symptoms of which are yet to occur [4]. Clinical trials evaluate the effectiveness of vaccines and determine their ability to prevent disease with minimum adverse effects [5].

COVID-19 vaccines are initially tested in mice/rabbits for assessment of safety. Testing in humans is initiated after the animals do not exhibit signs of disease after administering the vaccine. The number of subjects is gradually increased with advancing stages of clinical trials to detect rare adverse effects. Before the approval of general-purpose vaccines, thousands of vaccinated people are followed for several months [6]. If the vaccine results in malfunctioning of the immune system or causes severe inflammation, the vaccine may not be suitable for widespread use. Delay in vaccine development is inevitable when such adverse effects are detected [7].

The term “herd immunity” was introduced almost a century ago and is now widely used after increasing vaccines for the eradication of disease [8]. It is also known as group immunity or community immunity. Herd immunity is achieved when a large portion of the population is immune to a specific disease, protecting the susceptible individuals indirectly. The higher the portion of the population that is immune to a disease, the lower the chance for a susceptible individual to contact an infectious person. If many people (herd) are immune, the circulation of infection in the community will stop. Herd immunity should be in more than 80% of individuals to confer protection upon susceptible individuals [9]. This percentage is different for different pathogens depending on the contagiousness of pathogens. Higher the contagiousness of pathogen, a more significant portion of the population must be immune to provide herd immunity. For example, in SARS-CoV-2, the percentage of the people needed for herd immunity is 70% [8]. If the fraction of susceptible individuals in a population is too few, then the pathogen cannot successfully spread, and its prevalence will decline. Herd immunity threshold is the point at which the proportion of susceptible individuals falls below the threshold required for transmission [10]. Sensitive individuals take benefit from indirect protection from infection above the herd immunity

threshold. The benefits of herd immunity imply various segments of the population. For example, it is useful to protect immuno compromised individuals who cannot be vaccinated. It includes elders who cannot exhibit an optimal immune response to vaccines, children too young to be vaccinated, individuals having no access to mass immunization, and people who do not get vaccinated [10].

For COVID-19 vaccine efficacy, immune deficiency is a risk factor, especially in elders whose immune system has already been weakened by many factors. Moreover, obesity also results in the weakening of the immune system. It occurs due to lower IgG levels and a higher level of IL-6. Respiratory infections and infections caused by parasites also affect the immune response to COVID-19 vaccines [11]. Research on SARS-CoV and MERS has helped scientists to unveil the mechanism of protection offered by the immune system against the disease and the human body's response to coronaviruses. In pandemic situations, hundreds of millions of vaccine doses are required, which, if already production lines are available, are manufactured in about six months. A new vaccine needs several quality control checks because the unique production process is involved [12]. This chapter aims to summarize the development of vaccines for COVID-19 and the advantages and disadvantages of group immunity.

Clinical-Phase Vaccine Candidates for COVID-19

Several strategies are adopted for COVID-19 vaccine development; most of these target S proteins as the primary inducer of neutralizing antibodies [13]. The S protein molecule has S1 and S2 subunits. Subunit S1 contains a receptor-binding domain (RBD) which interacts with the host cell receptor (ACE2). The S protein plays a crucial role in inducing protective immunity during infection with SARS-CoV by eliciting T-cell responses and neutralizing antibodies. In contrast, the subunit S2 facilitates the fusion of the virus with the host cell membrane to release the viral RNA into the cytoplasm for replication [13]. Hence, vaccines based on S-protein should induce antibodies that block virus genome uncoating as well asnd viral receptor binding. Thus, the whole S glycoprotein or its appropriate parts are considered the key candidates for the vaccine composition of CoVID-19 [14].

Up till now, no vaccine is licensed to prevent human respiratory infection. About 10 COVID-19 vaccine candidates are in different stages of development, as shown in Table **1** [15]. The main criterion for a vaccine is safety, efficacy, and duration of immunity. The pandemic vaccine needs high production capacity and rapid development. This limits their rapid deployment during pandemics. Furthermore, live attenuated coronavirus vaccines are produced by reverse

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Dr. Kamal Niaz (KN) obtained a Doctor of Veterinary Medicine (DVM) degree from The University of Agriculture Peshawar-Pakistan under Registration number 2009- Agr-U-19578 and got CGPA 3.21/4.00. After that, KN completed Doctor of Philosophy (PhD) in Toxicology and Pharmacology at Tehran University of Medical Sciences (TUMS), Tehran-Iran, in 2017. He pursued Early-Stage Researcher (ESR) in 2017-18 from the University of Teramo- Italy. KN is working as an Assistant Professor in the Department of Pharmacology & Toxicology, Faculty of Bio-Sciences, Cholistan University of Veterinary & Animal Sciences (CUVAS), Bahawalpur, since 20 November 2018 till date. Here, KN has developed excellent research and communication skills. KN has expertise in the domain of environmental toxicants, diabetes, endocrine disruptor, and genomic disruption. He is committed to further continue in the area of expertise that includes phytomedicine and ecotoxicity but is not limited to toxicants, and also includes epigenetic alteration due to toxicants and the role of these chemicals in diabetes. KN has attended several national and international conferences to present talks on phytochemical, cell signaling, cytokines, milk as an antioxidant, and oxidative stress responses of cells to environmental toxicants. Currently, KN has published almost 80 plus articles in prestigious national and international journals within a short period with h-index-19, 150+ impact factors, and 40 book chapters in various publications such as Elsevier, Springer, and Bentham Science. He is working as a volunteer reviewer in different prestigious journals Frontier of Veterinary Sciences, Food and Chemical Toxicology, Scientific Report, Science of the Total Environment, and Current Protein & Peptide Science, etc.

He has recently completed the submission of a book as editor entitled “Coronavirus Disease-2019 (COVID-19): Epidemiology, Biochemistry, Diagnostics, and Treatment” at Bentham Science Publishing while other projects are monograph books: “Herb Around You” and “Genetic Diversity of Coronaviruses: From SARS to SARS-CoV-2” are under process.

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Dr. Muhammad Farrukh Nisar completed doctorate from the college of Bioengineering, Chongqing University, Chongqing, China in December 2016, and has been working as an Assistant Professor in the Department of Physiology & Biochemistry, Cholistan University of Veterinary and Animal Sciences (CUVAS), Bahawalpur, since, December 2018. Dr. Farrukh is mainly involved in research areas including photobiochemistry, biochemistry, biochemical pathways, oxidative stress, and cellular defense mechanisms, cell biology, cell signaling and pathways biology, screening of natural drugs as potential anti-cancer(skin) and skincare drugs, phytomedicine, photo- and chemo-therapies to treat skin cancer/conditions, natural drugs, and cell signaling, photomedicine and free radicals biology (ROS/RNS).

Dr. Farrukh has attended several national and international conferences to present talks on photomedicine, photo-induced drug delivery, cell signaling, and oxidative stress responses of cancer cells. Over 70 research articles have been published so far on multifarious aspects of biomedical/life sciences with a cumulative impact factor of over 120. Dr. Farrukh has Many national awards to his credit particularly the Chinese Government Scholarship during 2013 for Ph.D., a silver medal for securing 2 nd position in the M.Sc. exam from IUB, Bahawalpur, and several others. Currently, Dr. Farrukh is actively involved in exploring novel biomedical materials for drug delivery, photosensitive drug release, and plant isolations to find novel anticancer, antioxidant, and antiaging drugs.