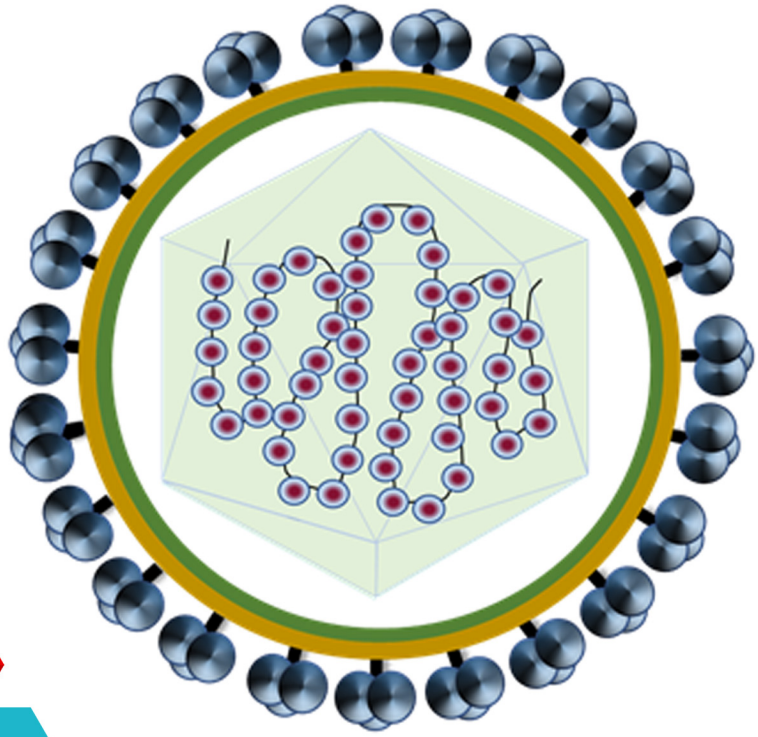
The COVID-19 Pandemic: Epidemiology, Molecular Biology and Therapy

Morphology, genome, proteins & replication

Structural protein genes

Epidemiology & transmission

Risk factors



Management, immune response & pathogenesis

Diagnostics

Therapeutic antivirals & natural compounds

Vaccines

Editor:
Shama Parveen

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The COVID-19 Pandemic: Epidemiology, Molecular Biology and Therapy

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The COVID-19 Pandemic: Epidemiology, Molecular Biology and Therapy

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FOREWORD

Zoonotic viruses are becoming a threat for mankind since they keep on jumping the species. The emergence of some of these viral infections that were seen in the 21st century include Avian Influenza, Ebola, Nipah, Sever Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS). But none of these epidemics reached this magnitude as Coronavirus Disease 2019 (COVID-19). This infection was first reported in December 2019 in Huanan seafood market, in the capital of China's Hubei province, Wuhan. Later, the infection spread to different parts of the globe leading to the pandemic. It has affected millions of people across the globe and has led to death of more than a million affected individuals. It reminds us of the Swine Flu pandemic of 1918-19 when millions of people died, and the morbidity was quite high. COVID-19 is caused by a beta coronavirus that is named as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) because of its similarity with SARS CoV and other bat coronaviruses.

In these unprecedented times of the Coronavirus pandemic which is gripping the whole world, the idea of writing a book on **"The COVID-19 Pandemic: Epidemiology, Molecular Biology and Therapy"** by Dr. Shama Parveen is a very timely effort and is going to provide a comprehensive account for the readers about this topic. The first chapter of the book gives a general introduction to COVID-19 and the topics that are discussed in the book. This book has all the relevant and important topics covered. The next 3 chapters deal with the biology of the SARS-CoV-2 *i.e.,* morphology, genomic organization, proteins and viral replication; molecular characterization of major structural proteins of the pathogen and codon based characterization of S2 subunit of severe acute respiratory syndrome-related coronaviruses. This knowledge about the structure of the virus and its genomics is needed to develop the tools for diagnosis, prevention and treatment. The next chapters are on global epidemiology and transmission of COVID-19; risk factors for severe disease; clinical manifestations, management, pathogenesis and host immune response to COVID-19 and current diagnostic approaches to detect the infection. These chapters are of practical importance and are useful for the public health workers as well as for clinicians and laboratory personnel. The last few chapters are focussed on treatment and prevention and they cover some important topics like natural compounds and drug repurposing candidates as potential therapeutic agents against this infection. Finally, the last chapter focusses on COVID-19 vaccine development: challenges and current scenario.

The efforts put in by Dr. Shama Parveen and her team in compiling all the knowledge in a such a short time is highly commendable. Although there has been an explosion of knowledge on SARS-CoV-2 and COVID-19 and the WHO has named it as 'Infodemic', however a concise and accurate information which covers all the relevant fields in a single platform is lacking. This book will thus be extremely useful for scientists, researchers, doctors, epidemiologists and public health personnel as well students of Science and Virology.

Science and Medicine has made so much progress in the last 100 years and though this pandemic has brought the whole world on its knees, we must also appreciate the efforts of the scientific community in providing diagnostic assays for COVID-19 within 15 days and hopefully a vaccine within a year. With COVID-19, I believe it's God's way of telling us: **"Only Humans can Save Humans with Humanity and Knowledge".**

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PREFACE

COVID-19 pandemic has affected almost every part of the globe with millions of infections and more than a million deaths. According to WHO, the infection has spread to almost all the countries and territories with USA, India, Brazil and Russia being the most affected ones. The infection is caused by a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes Coronavirus Disease 2019 (COVID-19). This pandemic originated as an outbreak in Wuhan, Hubei province in China at the end of 2019. Small animals like pangolin or bat are speculated to be the initial source of human infection in China leading to a local outbreak in Wuhan. Later, the infection spread its tentacles across the world through travellers and it was declared a pandemic by WHO on 11th March 2020. The longer incubation period, aerosol-mediated as well as air-borne spread and pre-symptomatic spread resulted in high transmission rates thereby infecting the humans at an unprecedented scale. The pandemic was fuelled further by the undiagnosed asymptomatic cases. Therefore, WHO recommended "isolation" of the infected patients and quarantining of the individuals who encountered the patients. Most of the countries implemented "lockdown" to take care of the ever-increasing cases and recommended "social distancing" to minimize human interactions. The pandemic has devastated all walks of life but the only saving grace is healing of the nature and nurturing of the environment. Thus, we have less polluted environment, more pristine forest and cleaner water bodies. The pandemic has impacted the social life and global economy as well.

SARS-CoV-2 is a Betacoronavirus like the two other human viruses which includes SARS and MERS of the same group. SARS-CoV-2 is spherical in shape having crown like surface structure formed by the spike (S) protein. Its genome is negative sense single stranded RNA of 30Kb that codes for 4 structural and 16 non-structural proteins. The S, M (membrane), E (envelope) and N (nucleocapsid) forms the structure of the virion. The non-structural proteins play a pivotal role in viral replication within the host cell cytoplasm following the entry of the virus.

The COVID-19 shows diverse clinical manifestations and results in asymptomatic, mild, severe and critical illness. The intense host immune response due to infection of the lower respiratory tract leads to generation of "cytokine storm". Further, complications like respiratory and multi-organ failure and septic shock in many cases cause death. Certain individuals (elderly, males, and type A blood group) and people with co-morbidities (diabetes, hypertension, existing respiratory disease, *etc*) are vulnerable to the severe infection. The most reliable diagnosis is based on the real time PCR assays and antigen detection during first few days of infection. At a later stage, the infection is detected by rapid tests or ELISA that are based on the detection of IgM/IgG serology. The treatment of the patients is symptomatic in the absence of antiviral drugs and prophylactic vaccine. Antipyretics, antivirals and supplements are used for the treatment of the COVID-19 patients. However, clinical trials of several antivirals and other drugs are going on at war footing to contain this infection. Interestingly, several vaccine candidates are also under clinical trials across the globe.

The present book "The COVID-19 Pandemic: Epidemiology, Molecular Biology and Therapy" deals with all the significant aspects of the ongoing COVID-19 pandemic including the pathogen (morphology, genome, proteins, structural protein genes, replication), global epidemiology, transmission, risk factors, clinical manifestation, management, host immune response, pathogenesis, diagnosis, therapeutic agents (antiviral and other drugs, natural compounds) and vaccines.

We hope that this book will provide basic but relevant information about the different aspects of COVID-19 to scientists, academicians and common citizens alike. Eventually, if this book inspires any one in any manner to become more civic and responsible citizen of the globe, we will achieve our goal.

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CHAPTER 1

An Introduction to Coronavirus Disease 2019 (COVID-19)

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Abstract: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the global pandemic of Coronavirus Disease 2019 (COVID-19). More than 24.8 million global cases and 0.84 million deaths have been reported until the 31st August 2020. SARS-CoV-2 is like other human coronaviruses *i.e*. SARS and Middle East respiratory syndrome (MERS) but its transmission rate is much higher, biology is more complicated, and mechanism of action is still elusive. Certain individuals like elderly, males, people with type A blood group and persons with co-morbidities (diabetes, hypertension, obesity, *etc*.) are susceptible to severe infection. One of the major concerns is that the asymptomatic individuals and persons in incubation period may also transmit the pathogen. Treatment of the affected individuals is symptomatic in the absence of antiviral drugs or vaccines. Collaborative clinical, epidemiological, molecular and immunological investigations are needed at war-footing across the globe to identify the evolutionary trajectories, mutational load, host immune response, therapeutics and vaccines against this pathogen. Pandemic has drastically affected the social life, economy, travel and transportation, educational systems, aviation, to name a few. However, it has a positive impact on the environment, wildlife, water bodies and forests. This warrants us to get ready to face aftermath scenario of this pandemic and rebuild the system. This by no means would be a simple task as it involves large scale resource mobilization, unprecedented development of sagging economy and infrastructures, rebooting the society, nations and the world after the default control-alt –delete mode of the pandemic.

Keywords: Clinical symptoms, COVID-19, Diagnosis, Epidemiology, Genome, Management, Natural compounds, Risk factors, SARS-CoV-2, Socio-economic-environmental effects, Therapeutics, Vaccines.

INTRODUCTION

The pandemic of COVID-19 has caused more than 24 million infections in diffe-

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rent parts of the globe till 31st August 2020. The causative agent is known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The infection started as a pneumonia outbreak in Wuhan, China at the end of 2019 and within a few months the infection spread its tentacles around the globe leading to an unprecedented pandemic. The present chapter illustrates the key features of the pathogen like morphology, genome organization, proteins and replication pattern. The S (spike), M (membrane), E (envelope) and N (nucleocapsid) are the structural proteins that are part of mature virion. We have carried out molecular characterization of these proteins employing phylogenetic, entropy and selection pressure analyses.

In addition, we have also focussed on some crucial aspects of the viral infection such as global epidemiology, transmission, risk factors, clinical manifestations, management of patients, pathogenesis and host immune response. In addition, we have also discussed the current diagnostic approaches for detection of viral infection. The existing and newer therapeutics against COVID-19, including antiviral drugs and natural compounds are highlighted. Finally, the candidates being evaluated for a prophylactic vaccine are shown. All these crucial attributes of the COVID-19 pandemic are discussed in the present book and are shown here diagrammatically (Fig. **1**).

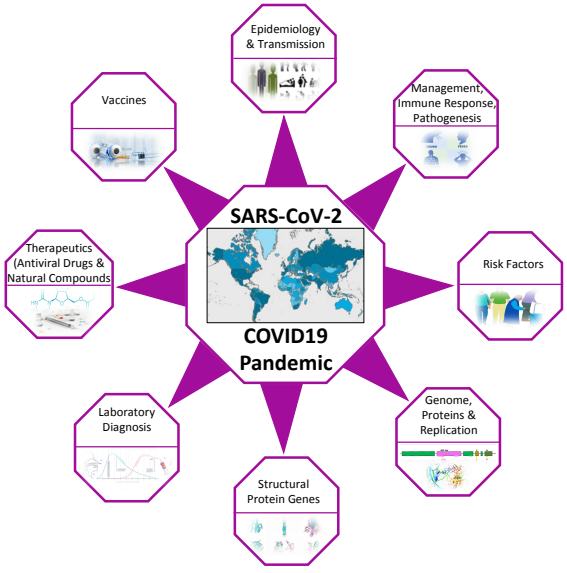

Fig. (1). The Pandemic of COVID-19. Different aspects of SARS-CoV-2 like genome, proteins (including structural proteins) and viral replication are studied. The world map was taken from the WHO website. In addition, the clinical manifestations and their management, pathogenesis and host immune response are taken into consideration for better management of the patients. Different aspects of the pandemic like epidemiology, transmission, risk factors, laboratory diagnosis, therapeutics and vaccine approaches are analysed for containment of the COVID-19 pandemic.

MORPHOLOGY, GENOME, PROTEINS AND VIRAL REPLICATION

SARS-CoV-2 is a spherical pathogen with S protein forming a crown like structure on its surface [1]. SARS-CoV-2 belongs to the family *Coronaviridae*, subfamily *Coronavirinae* and genera Betacoranviruses [2]. Other human pathogens of Betacoronaviruses include SARS and Middle East respiratory syndrome (MERS) that have caused outbreaks in China during 2002-03 and in Saudi Arabia during 2012-13, respectively [3]. The pathogen has showed much higher transmission rate but lower mortality rate as compared to its other two counterparts. SARS-CoV-2 genome shares genetic similarity with bat coronaviruses (88%), SARS (79%) and MERS (50%) [4]. The single stranded negative sense RNA genome of the virus is 30kb in length that codes for 4 structural and 16 non-structural proteins [5]. The structural proteins encompassing S, M, E and N are part of the mature virion. The non-structural proteins (nsp1-16) are present in infected cells and are involved in viral genome replication [5, 6]. The S protein is composed of S1 (binds to receptor) and S2 (host cell membrane fusion) subunits [7]. The receptor binding domain (RBD) of S protein binds to angiotensin converting enzyme 2 (ACE2) receptor on the host cell and determines the host cell tropism for successful infection [1]. The number of pathogen particles (viral load) coming in contact with the host cells is equally important. After the infection, the immune system of the host takes over whereas sometimes, the pathogen succeeds but, in many times, the viral onslaught can be eliminated. Thus, a robust immune system of the host acts as a natural life insurance.

STRUCTURAL PROTEINS

Structural proteins of SARS-CoV-2 have a crucial role in the pathogenicity as it facilitates the assembly and release of the virion [8]. The S, E and M proteins together form an envelope of the virus. The S glycoprotein of SARS-CoV-2 comprises two subunits; S1 which determines the virus host range and cellular tropism and S2 is involved in the virus-cell membrane fusion activity by HR1 [9] and HR2 [10]. The S2 subunit is highly conserved and thus, forms a target for antiviral compounds [10]. The E protein is the smallest structural protein. The M protein is the most abundant and is responsible for the shape of the envelope. The N protein together with the RNA forms the nucleocapsid inside the envelope. The proteins E and N interfere with the host immune response [8]. We have carried out the detailed molecular and genetic characterization of SARS-CoV-2 major structural protein genes using nucleotide composition, codon usage patterns, phylogenetic, entropy and selection pressure analyses. This data is likely to augment the information about the evolution, biology and adaptation of SARS-CoV-2 in the human host.

CHAPTER 2

Morphology, Genomic Organization, Proteins and Replication of SARS-CoV-2

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Abstract: The current pandemic disease (COVID-19) is caused by a highly infectious coronavirus known as severe acute respiratory syndrome coronavirus 2 (SARS-Co--2). It belongs to the family of *Coronaviridae,* subfamily *Orthocoronaa-virinae*, order *Nidovirales*. Coronaviruses (CoVs) are enveloped and spherical shaped pathogens with crown-like protrusions formed by S protein on the surface. The CoVs contain single-stranded positive-sense RNA with genome of 27-32 kb that encodes both structural and non-structural proteins. The Coronavirus genome encodes for at least 6 ORFs, among which two third parts include ORF 1a/1b, which encodes 16 non-structural proteins (nsp 1-16) that play a pivotal role in viral genome replication. The structural proteins are part of mature virion and include spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins. The S protein binds to the receptor (ACE2 or others) on the host cell and determines host tropism. Replication of CoVs is extremely complicated process and includes regulation at various stages including dependency on both the viral and host factors. The present chapter provides updated information about the morphology, genomic structure, properties and function of different proteins and their role in replication mechanism of SARS-CoV-2. The information about the proteins and their role in viral life cycle is likely to assist in the formulation of targeted therapeutic interventions and vaccines against this emerging respiratory pathogen.

Keywords: COVID-19, Genome, Morphology, Proteins, Replication, SARS-CoV-2.

INTRODUCTION

In December 2019, Coronavirus disease 2019 (COVID-19) began in Wuhan city of China and within a span of few months it has spread its tentacles across the

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globe. WHO declared COVID-19 a pandemic on 11thMarch 2020. The disease is caused by newly identified coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In the last two decades, two highly infectious zoonotic pathogenic coronaviruses of the same family *i.e*. SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) have caused widespread epidemics with significant fatality rate in China and Saudi Arabia respectively [1]. Coronaviruses (CoVs) are enveloped pathogens containing single-stranded positive-sense RNA genome of 26-32 kb that encodes both structural and non-structural proteins [2, 3]. The four structural proteins spike (S), envelope (E), membrane (M) and nucleocapsid (N) play a major role in virus entry and replication in the host cell [1]. The spike protein is known to play a major role in viral transmission as it is known to have affinity for angiotensin converting enzyme 2 (ACE-2) receptors on the human host cell [4].

SARS-CoV-2 infection in humans leads to both symptomatic and asymptomatic cases. The incubation period of SARS-CoV-2 is about 14 days [5]. The infected persons usually show symptoms like fever, dry cough, shortness of breath, weakness, body ache and in severe cases, pneumonia [6, 7]. An infected person can transmit COVID-19 to humans through direct contact *via* respiratory droplets [8]. In severe cases, respiratory, hepatic, gastrointestinal and neurological complications are reported which may lead to multi-organ failure and mortality in some cases [6]. Millions of cases and deaths have been reported due to COVID-19 across the globe. Till 31st August 2020, more than 24 million cases and 0.83 million deaths were reported from different parts of the globe. The countries showing maximum infections include USA, Brazil and India [9]. The current situation thus warrants an urgent need of vaccines and therapeutic agents against SARS-CoV-2.

A deep insight into the mechanism of the viral genome replication, role of different proteins in the process and their interaction with host cell system is the need of the hour. These cellular events will provide data on the importance of different proteins in viral replication. These proteins can act as potential target of antiviral drugs and vaccines that will be a step towards management of the current COVID-19 pandemic. In the same context, we have provided a comprehensive overview about the morphology, genomic organization, structural and non-structural proteins of SARS-CoV-2 along with their role in viral life cycle.

VIRAL MORPHOLOGY

The structure of SARS-CoV-2 is quite like SARS-CoV [10]. The characteristic morphology of SARS-CoV-2 was revealed under the electron microscope with the size of the particles ranging between 70-90nm [11]. They are spherical in

shape having the bulb like projections on the surface [12]. These projections on the viral surface is formed by the homotrimer of spike protein [13]. The spike protein is responsible for the initiation of viral infection by binding to the receptor in the host cell membrane [1]. The viral envelope is made up of host derived lipid bilayer [14]. This envelope consists of the major viral structural proteins viz. spike protein, membrane (M) protein and envelope (E) protein. Inside the envelope, a viral nucleocapsid is located. This nucleocapsid is formed by the nucleocapsid (N) protein which binds to RNA genome of the virus in a continuous bead on string type conformation [15, 16].

GENOMIC ORGANIZATION

SARS-CoV-2 belongs to the family of *Coronaviridae,* subfamily *Orthocoronaavirinae*, order *Nidovirales* which includes four CoV genera that are known as Alpha, Beta, Gamma and Delta-coronavirus. SARS-CoV-2 clusters into the genus Betacoronavirus and subgenus *Sarbecovirus,* which also include the other zoonotic coronaviruses *i.e*. SARS-CoV and MERS-CoV [17]. The phylogenetic analysis showed the similarity of SARS-CoV-2 with two bat derived coronavirus strains bat-SL-CoVZC45 and bat-SL-CoVZXC21 and SARS strain of 2002-2004 epidemic [18, 19]. Further, the studies have shown that SARS-CoV-2 has 54% identity at whole genomic level, 58% identity at non-structural proteins (nsps) coding region and 43% at structural protein coding region among other CoVs [19]. It was also concluded that the receptor binding domain of SARS-CoV and SARS-CoV-2 binds with the same receptor *i.e*., ACE-2 in the host cell [20].

The 30kb single stranded positive sense RNA viral genome encodes both structural and non-structural proteins consisting of a total of 9860 amino acids [19, 21]. The non-structural proteins are encoded by polyprotein 1a/1ab, which is directly translated by genomic RNA and are responsible for the formation of replication-transcription complex in double membrane vesicles [22]. This complex synthesizes sub-genomic RNA through discontinuous transcription [23]. These sub-genomic RNAs are further responsible for the synthesis of sub-genomic mRNAs [24]. The Coronavirus genome encodes for at least 6 ORFs, among which two third parts include ORF 1a/1b that encodes 16 non-structural proteins (Fig. **1**). The gamma coronaviruses lack the nsp1 protein. The production of two polypeptides 1a and 1ab occurs through -1 frameshift between ORF 1a and 1b [25]. The other ORF's near 3'-terminus of the genome encodes at least four structural proteins spike (S), membrane (M), envelope (E) and nucleocapsid (N) protein. The sub genomic RNA also encodes HE protein, 3a/b protein and 4a/b protein [3]. RNA replication and translation are regulated by a highly structured un-translated regions (UTR) located at the 5' and 3' end of the genomic RNA.

CHAPTER 3

Structural Protein Genes of SARS-CoV-2: Comprehensive Molecular Characterization

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Abstract: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the global pandemic of Coronavirus disease 2019 (COVID-19). Limited information is available on evolutionary aspects of the structural proteins: spike (S), envelope (E), membrane (M) and nucleocapsid (N) of the virus. Therefore, we attempted detailed molecular and genetic characterization of SARS-CoV-2 structural protein genes using nucleotide composition, codon usage patterns, phylogenetic, entropy and selection pressure analyses. The RSCU patterns suggested codon biasness due to preference of U/A-ended over C/G-ended codons. Mutational pressure and natural selection influence the synonymous codon usage of structural protein genes in SARS-CoV-2. Phylogenetic analyses of different coronaviruses for all the four structural genes showed that all 2019-nCoV study sequences were clustered under the SARS-CoV-2 clade which was closest to bat coronaviruses. Additional phylogenetic analyses of SARS-CoV-2 structural protein genes showed discordance in the topology, suggesting different patterns of evolutionary relationships among these genes. Few non-synonymous amino acid mutations, low value of entropy and purifying selection suggested limited variations in the studied genes. However, these variations in the SARS-CoV-2 genome are likely to increase in near future since the virus will try to evade the host immune response to enhance its survival in humans. Thus, we evaluated the genetic diversity of the structural protein genes along with the genomic composition and codon usage patterns of SARS-CoV-2. Thus, present data on molecular characterization of structural protein genes is likely to augment the information about the evolution, biology and adaptation of SARS-CoV-2 in the human host.

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Shama Parveen (Ed.)

Keywords: Entropy, Gene ontology, Molecular characterization, Mutational pressure, Natural selection, Nucleotide composition, Phylogenetic analysis, SARS-CoV-2, Structural proteins, Synonymous codon usage.

INTRODUCTION

The newest virus that has captured the world's attention is known as "SARS-CoV-2" that causes Coronavirus disease 2019 (COVID-19). It was first recognized to cause human infection at the end of 2019. The initial cases of the infection were linked to seafood and live-animal market in Wuhan, China [1, 2]. These initial cases led to a local epidemic in this region. The viral infection later spread to other geographical regions by travellers and significant human to human transmission. COVID-19 was declared as the pandemic by the WHO on 12th March 2020 [3]. SARS-CoV-2 belongs to the coronavirus family that has other human pathogens, including severe acute respiratory syndrome (SARS) and Middle East Respiratory Syndrome (MERS). The SARS epidemic of 2002-03 and the MERS outbreak 2012-13 have caused significant disease burden in China and Saudi Arabia, respectively [4] [5].

The SARS-CoV-2 are enveloped, positive single-stranded RNA viruses with a genome of approximately 30 Kb in length [6]. The first open reading frame (ORF-1a/b) encompasses two-third of the viral RNA, encodes 16 non-structural proteins (NSPs). The remaining genome encodes for four essential structure proteins: spike (S), small envelope (E), membrane (M), and nucleocapsid (N) and several accessory proteins. Structural proteins have a crucial role in the pathogenicity of the virus as it facilitates assembly and release of the virion [7]. The S glycoprotein of SARS-CoV-2 comprises two subunits; S1, which determines the virus host range and cellular tropism and S2 is involved in the virus-cell membrane fusion activity by HR1 [8, 9] and HR2 [10]. The S2 unit is highly conserved and thus, forms a target for antiviral compounds [10]. The M protein is responsible for the budding process. The proteins E and N interfere with the host immune response [7].

Previous studies have reported the genotype diversity of gene/genomic characterisation and the epidemiology of SARS-CoV-2 proteins [11]. But the comprehensive genetic information on structural proteins of the virus is limited. It may be noted that the phenomenon of codon usage bias pertains to organisms, where one synonymous codon is preferred over others [12, 13]. The codon usage patterns have been reported in a few viruses [14 - 16] and have been extensively studied. However, in SARS-CoV-2, the RSCU patterns have not been implicated for the structural protein genes. Therefore, we performed the composition and codon usage pattern, phylogenetic, entropy and selection pressure analyses of the

structural protein genes of this novel coronavirus to gain insight into its basis of molecular evolution.

MATERIALS AND METHODS

Sequences

Nucleotide sequences of SARS-CoV-2 were retrieved from the National Center for Biotechnology Information (NCBI). The retrieval of selected sequences was based on the following inclusion criteria: (a) The strain (GenBank Accession number: NC_001434.1) was used as the new coronavirus (SARS-CoV-2) reference strain; (b) Sequences from the same or different countries at varying time intervals were assembled in order to avoid repetition; (c) Sampling dates were clearly mentioned. Accumulated sequences were edited using the Bioedit v.7.2 sequence analysis software (http://bioedit.software.informer.com/7.2/) to obtain structural gene products spike (S), envelope (E), membrane (M), nucleocapsid (N) proteins. Multiple alignments for SARS-CoV-2 sequences were carried out using the Clustal X2 Algorithm (http://www.clustal.org/clustal2/) [17].

Compositional Properties

Nucleotide composition analysis of the four structural protein genes S, E, M and N of SARS-CoV-2 sequences was calculated using CodonW v.1.4.2 software. The overall nucleotides occurrence frequency (A%, C%, T/U%, and G%) and overall occurrence of nucleotide frequency at the third position of codon (A3%, C3% U3% and G3%) were analysed. The overall GC content at different codon positions was also determined. The termination codons do not encode any amino acid, and Met and Trp are only encoded by AUG and UGG codons, respectively, hence these codons do not exhibit usage bias. Therefore, these five codons (UAG, UGA, UAA, AUG and UGG) were not considered for the analysis.

Relative Synonymous Codon Usage (RSCU)

The ratio between the observed and expected usage frequency of a codon is described as the RSCU value if all synonymous codons are used equally for any specific amino acid [18]. The RSCU index was determined as follows:

$$RSCU = \frac{G_{ij}}{\sum_{j}^{n_i} G_{ij}} n_i$$

where *RSCU* is the relative synonymous codon usage value, G_{ij} is the observed number of the i^{th} codon for the j^{th} amino acid that has an *"ni"* type of synonymous codon. The RSCU values of the structural coding genes were calculated using

CHAPTER 4

Codon Based Characterization of S2 Subunit of Severe Acute Respiratory Syndrome-Related Coronaviruses

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Abstract: The emergence of a global pandemic, COVID-19 is caused by the newly identified SARS-CoV-2. The current situation warrants us to understand the molecular basis of the evolution of this emerging pathogen. In this context, we conducted a comparative codon-based characterization of the viruses within the species *Severe acute respiratory syndrome-related coronavirus* (SARSr-CoV). We attempted phylogenetic analysis, and codon-based characterization by employing selection pressure and Shannon entropy analyses in the S2 subunit gene sequences of SARS-CoV, Bat-SL-CoV and SARS-CoV-2. Further, the pattern of N-linked/O-linked glycosylation was analyzed within the SARS-CoV species. The phylogenetic analysis and pairwise distance calculations showed high similarities in the S2 subunit of SARS-CoV-2 with Bat-SL-CoVs. Our findings uncovered the low mean value of dN/dS, suggesting purifying selection, but certain codon positions were found to be under positive selection. The entropy analyses showed 71 codon positions having its high score. Three codon positions (160, 244 and 562) were identified to be positively selected with high entropy value suggesting that they are more prone to mutations. Further, the analysis revealed a conserved pattern in N-linked glycosylation though the discrepancies were found within the O-linked glycosylation pattern. Our findings may help in predicting the signature sequences based on the codon-based model of molecular evolution. Further, this approach may provide information on the evolutionary dynamics of this pathogen, facilitating much-desired control strategies against COVID-19.

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Keywords: Bat-SL-CoV, Coronavirus, N-linked and O-linked glycosylation, SARS-CoV, SARS-CoV-2, Selection pressure analysis, Shannon entropy analysis.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) has resulted in a major pandemic across the world. The disease, caused by a novel coronavirus, is known to be originated from the Wuhan city of China in late 2019 [1]. On the basis of phylogeny and taxonomy, *Coronaviridae* Study Group **(**CGS) recognized this novel identified viral strains forming a sister clade to severe acute respiratory syndrome coronaviruses of the humans and bats (SARS-CoVs), which belongs to the species *Severe acute respiratory syndrome-related coronavirus* (SARSr-CoV). The uncharacterized strains were thus named SARS-CoV-2 [2].

The recent classification of coronaviruses (CoVs) includes 39 species, 27 subgenera, 5 genera and 2 subfamilies. The virus belongs to the family *Coronaviridae* and suborder *Cornidovirineae*. The order and realm are *Nidovirales* and *Riboviria*, respectively [2]. On the basis of serology and phylogenetic clustering, *Coronaviridae* are further categorized into four alpha, beta, gamma and delta CoVs groups [3]. Seven human coronaviruses have been discovered, with the last one being SARS-CoV-2 (https://www.cdc.gov/coronavirus/types.html). The four human CoVs: HCoV-229E, HCoV-NL63, HCoV-OC43 and HCoV-HKU1 have been found to cause mild infections in humans [4]. SARS-CoVs and MERS-CoVs both were identified as the main cause of severe human respiratory illness in China (2002-03) and Saudi Arabia (2012), respectively [3].

The coronavirus (CoV) consists of 4 structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N). The outermost spike protein is classified into class I viral fusion protein. This protein is initially synthesized as a single chain precursor of approximately 1,300 amino acid residues [5]. The protein trimerizes upon folding [5]. The S protein forms a crown on the surface of CoVs and is the key target of neutralizing antibodies upon infection [5]. The amino acid sequence representing S protein of SARS-CoV-2 showed homologies of 76% and 79-80% with SARS-CoV and Bat-SL-CoVs, respectively [6]. The receptor-binding domain (RBD)of the SARS-CoV-2's S protein supports strong interaction with human ACE2 molecules like that of S-protein of SARS-CoVs, despite sequence variation between the two [7]. Thus, it maybe postulated that SARS-CoV-2 transmission poses a risk to human health *via* the S protein–ACE2 interaction pathway [7].

Further, there are two subunits of S glycoprotein. The S1 subunit of S glycoprotein consists of a signal peptide, the N-terminal domain (NTD) and the receptor-binding domain (RBD). The S2 subunit of the spike protein contains fusion peptide (FP), which is conserved in nature and the heptad repeats: HR 1 and HR2, a transmembrane domain, (TM), and the cytoplasmic domain (CP) [8]. A study has reported 99% similarity of the S2 subunit sequences of SARS-CoV-2 to that of Bat-SL-CoVs [8]. Owing to this conservation in the S2 subunit of the variable S protein gene, the present study was conceptualized to attain an understanding of the codon bias phenomena *via* selection pressure and Shannon entropy analysis and to understand its N-linked and O-linked glycosylation pattern. We also analyzed the similarities at the nucleotides and amino acid levels amongst the sequences of the SARSr-CoVs. The codon-based model of molecular evolution of the SARSr-CoVs may be predicted using this data, which is envisaged to assist in designing antiviral peptides against S2 subunit of SARS-CoV-2. This may prove to be a significant preventive measure towards the treatment of COVID-19.

METHODOLOGY

Data Collection and Sequence Alignment

The whole-genome sequences of the SARS-CoV, Bat-SL-CoV and SARS-CoV-2 of the species *Severe acute respiratory syndrome-related coronavirus* were retrieved from the GenBank. Sequences from different isolates like wtic-MB isolate P3pp23, ExoN1 isolate P3pp37, BJ202, *etc.* and from different hosts encompassing bat, civet and humans were included in the present study. The multiple sequence alignment of the full genome of SARS-and SARS-like CoVs was done using ClustalW in Bioedit (v.7.2) software. The S2 subunit gene sequences were extracted from the full genome sequence and used for analyses.

Phylogenetic Analysis

The phylogenetic tree of the S2 subunit sequences was generated by using MEGA6 (v.6.06) software. The evolutionary history of the tree was deduced using the Maximum Likelihood method. This approach was based on the Tamura-Nei model. The numbers on nodes represent bootstrap values. These values were produced by 1000 replications. Genetic distances were calculated using the nucleotide and amino acid alignment of 5 sequences consisting of two sequences of SARS-CoV-2 (MN938384/HKU-SZ-002a 2020 and MN975262/HKU-S--005b 2020), two Bat-SL-CoVs (MG772934/bat-SL-CoVZXC21 and MG772933/bat-SL-CoVZC45) and one SARS-CoV (NC004718/SARS-CoV).This analysis was conducted using the Poisson correction model in MEGA6 software.

CHAPTER 5

Global Epidemiology and Transmission of COVID-19

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Abstract: Coronavirus Disease 2019 (COVID-19) is a viral infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It started as an outbreak in Wuhan, China, at the end of 2019. But within a few months, it took the form of a deadly pandemic affecting millions of people in more than 216 countries. Transmission *via* a large number of asymptomatic cases and international travel played a pivotal role in the spread of infection to different geographical regions. In the present chapter, we have given the chronological details about the spread of the infection across the globe. The countries with the greatest international connectivity showed a large number of cases. Further, the countries that suspended international travelling and sealed their borders are the ones that have managed the spread impressively. Subsequently, we have summarized the emergence of human Coronaviruses (SARS, SARS-CoV-2 and MERS) in humans. We have also described the different modes of human to human transmission of SARS-CoV-2. However, the infectivity is undercounted as many patients with mild or no symptoms are not getting tested. The present chapter summarizes the origin, global epidemiology and modes of transmission of the infection. Comprehensive hospital and community-based surveillance and detailed interpretation of full genomes of SARS-CoV-2 need to be carried out across different geographical regions. This data will assist in the demarcation of mutation rates and resultant evolutionary trajectory of this emerging viral pathogen. Preventive measures like social distancing, wearing masks and good hygiene should be followed religiously to prevent the spread of the contagion.

Keywords: COVID-19, Epidemiology, Origin, Pandemic, SARS-CoV-2, Transmission.

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INTRODUCTION

The world entered the year 2020, witnessing the second pandemic of this century in the form of infectious Coronavirus Disease 2019 (COVID-19). This is a novel infection of the respiratory tract, which may develop into fulminant pneumonia-like disease that is similar to severe acute respiratory syndrome (SARS). This pathogen was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by World Health Organization (WHO) since its genome is similar to SARS. It has affected millions of people across the world in the last one year, and the cases are still increasing exponentially. On January 9, 2020, the WHO issued a statement that the Chinese CDC (Centre for Disease Control and Prevention) has reported the causative agent of this viral infection to be Coronavirus and submitted the whole genome sequence in the GenBank [1].

COVID-19 began with the first report from Wuhan, China, on 31st December 2019. The Municipal Health Commission of Wuhan city reported several pneumonia-like cases with unknown etiology and their common link to Wuhan Hanan Seafood wholesale market. Due to several links with a common seafood market, it was originally believed to have originated from the market [2]. However, there were reports of infection with no direct or indirect interaction with the Hanan seafood market. The virus was probably transferred to humans from a spillover event in a bat virus through an unknown mediator [3]. Various studies in late April 2020 strongly suggested this mediator is probably Pangolins since coronavirus genomes in these animals were found to have a close similarity with the SARS-CoV-2 [4]. However, the convincing source of this viral infection among humans is still not understood.

The virus was initially underappreciated with many asymptomatic infections and a long incubation period. Thus, enormous air travel and the rapid rate of transmission led to its spread worldwide. Eventually, in a span of a few months, the virus moved across the entire globe leading to the current pandemic situation. The spread started with parts of the world connected with China through travelers due to business or tourism purposes. SARS-CoV-2 has many modes of transmission, such as respiratory droplets from the mouth and nose of the infected person, the aerosol transmission, including others [5]. Around 80% of the cases do not develop the severe form of the disease and show only mild signs and symptoms of seasonal flu. These patients recover easily without any major treatment. However, the estimated incidence rate is much higher and is in the range of 20–60% compared to the flu with 8% incidence. A preliminary observation concluded that those with severe disease conditions need hospitalization for a period twice as much as that for acute flu [6].

We have analyzed the sequential events that have led to the spread of the infection across the globe (Fig. **1**). This is followed by a discussion of the probable zoonotic origin of the pathogen along with a comparison of epidemiological features among the three human Coronaviruses (SARS, SARS-CoV-2 and MERS). Later in the chapter, we had discussed different modes of transmission of this viral infection.

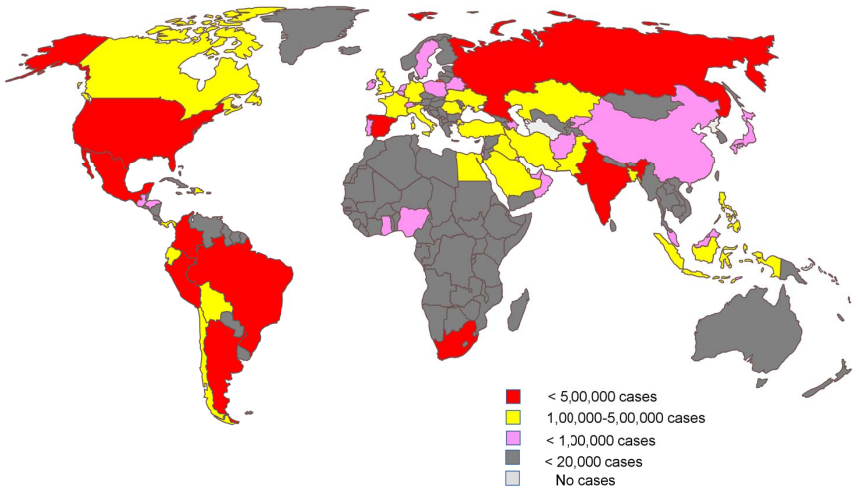

Fig. (1). A map showing distribution of Covid-19 cases in the affected regions of the world till 31st August 2020. The freely editable version of the world map was taken (http://All-free-download.com/ free-vector/simple-world-map-vector.html).

THE SPREAD

- The first few cases of SARS-CoV-2 infection were speculated to have originated at the end of 2019 from Wuhan, Hubei Province, China. Of all suspected cases from China, 80% were from the Wuhan district, and it was the epicenter of the initial outbreak.
- The first death reported was of a 61-year-old male patient admitted to a hospital with respiratory failure and severe pneumonia and other co-morbidities.
- The first case reported outside China was from Thailand, where a 61-year-old Chinese woman had travelled to Wuhan.
- The virus then briefly entered Japan and marked its 3rd country on January 16th, 2020, where a 30-year-old man with a travel history to Wuhan developed pneumonia-like condition. However, Japan is one of the few countries that recorded the lowest death rate per capita and managed the spread very well.
- On January 15th 2020, the first known travel-related confirmed case was reported from Washington, United States.
- By the end of January, it took the shape of a pandemic affecting Russia, North America, South America, Southeast Asia, Pacific and Middle East countries.

CHAPTER 6

Risk Factors for Severe Covid-19 Disease

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Abstract: Coronavirus disease 2019 (COVID-19) has spread to almost every part of the globe. Numerous risk factors have been identified for predisposition to severe infection. Age is reportedly an incredibly significant risk factor due to high fatality in the elderly population. Further, the infection is more predominant in males as compared to females, probably due to the difference in immunity, hormones, and some specific habits (smoking, drinking) that may influence the viral infection. Correlation of blood group with SARS-CoV-2 infection is also reported as individuals with type A blood group are probably more susceptible to the infection since it is native form. Type O blood group is an evolved form, and thus individuals with this group may be less susceptible to the infection. In addition, existing comorbid conditions like hypertension, diabetes, cardiovascular, endocrine, and chronic respiratory diseases are also associated with an increased risk of severe COVID-19. Obesity has also been reported to have a huge impact on the infection rate and post-infection results. There is also an apprehension of vertical transmission from pregnant females to foetus, but this aspect needs to be analysed in detail in future studies. This review summarizes the effects of different risk factors like age, gender, comorbidities, blood group and prenatal transmission on SARS-CoV-2 infection. The correlation of viral infection with genetic predisposition is another factor that can be explored in future studies. Detailed clinical studies involving large patient groups are required across the globe and on different ethnic populations to clearly define the role of risk factors to COVID-19.

Keywords: Age, Blood group, COVID-19, Diabetes and obesity, Gender, Hypertension, Risk factors.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has created a ravaging situation of Coronavirus disease 2019 (COVID-19) around the world in

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a matter of a few months. Transmission of the virus from an infected to a normal person occurs by means of respiratory droplets *via* the respiratory or gastrointestinal tract. Most of the cases initially are mild, but people with pre-existing diseases and the elderly are more susceptible to severe illness. World Health Organization (WHO) had declared the outbreak of the COVID-19 infection as a pandemic on 11th March 2020 since it had spread to a major part of the globe [1]. The outbreak started from Wuhan, China, which went on to spread in more than 200 countries. Until 31st of August 2020, the number of confirmed cases in the world has crossed 25.6 million [2]. By 31st August 2020, the worst affected country in the world was the USA, which reported more than 6.2 million cases and nearly 190,000 deaths. Brazil has second-most deaths with a tally of 3.9 million cases, and more than 121,000 deaths have occurred due to the novel coronavirus [2]. In the past three months, India has seen a logarithmic increase in COVID-19 cases and has reported more than 3.6 million cases, and more than 65000 people have died [2]. Most countries have undergone complete lockdown to contain the viral outbreak and have imposed various security measures such as restricted international travel *etc*. to make sure that the citizens are in the least possible contact with each other. It is to be noted that the pandemic took 3 months to reach 100,000 confirmed cases and just 12 days to reach the next 100,000 [3]. Within the next 26 days, the numbers increased to 1 million and further crossed the 10 million mark on 27th June 2020, just after 86 days of crossing 1 million [2].

The disease originated in China in late 2019. In mid-December 2019, many patients had reported to hospitals with pneumonia in the Wuhan region of China. Many of these patients had visited the seafood market at least once in the past one or two weeks. Etiological investigation was done on the patients showing similar symptoms in this unexplained viral pneumonia. After investigation of the lavage of the patients from the infected place, it was hypothesised that the novel CoV was transmitted from a zoonotic agent and had further spread to people due to human-to-human interaction [4]. On February 11th, 2020, the novel CoV was named SARS-CoV-2. The symptoms of COVID-19 infection can be seen after an average incubation period of 5-14 days. The time from the onset of the symptoms to fatality ranged from 6 to 41 days with a 14-day median period. This time depends on the patient's age and immune system status. This time was shorter for greater than 70-year-old patients relative to those under 70 years of age. Fever, cough, and fatigue are the most common symptoms at the onset of COVID-19. Other symptoms include sputum development, headaches, haemoptysis, diarrhoea, dyspnoea, and lymphopenia. Clinical characteristics were identified as pneumonia by a chest CT scan, however, there were irregular characteristics such as RNAaemia, acute spiral distress syndrome, acute cardiac injury, and the occurrence of opacity that led to death [5].

On January 20, 2020, it was acknowledged that the novel coronavirus should be classified under subgenus *Sarbecovirus* belonging to the *Betacoronavirus* genus; *Coronavirinae* subfamily, C*oronaviridae* family, and *Nidovirales* order. Thus, this virus was called SARS-CoV-2 since it is the sister of SARS [6]. Coronaviruses are pleomorphic RNA viruses with a diameter ranging from 150nm-160nm with a characteristic crown-shaped appearance. The main structural proteins are spike (S), membrane (M), envelope (E) and nucleocapsid (N). The nucleocapsid protein is bound to the RNA genome in a bead on a string fashion [5].

The genome of coronaviruses is the largest among the RNA viruses; its length is in the range of 27-32 kb. The genome of SARS-CoV-2 shows more similarity to the SARS-CoV (70%) than to the MERS-CoV. High homology was observed in the RNA binding domain of the SARS-CoV-2 and SARS-CoV. The occurrence of this contagious virus is like SARS-CoV in many ways. It was reported that both the viruses have the same mode of entry in the human cells, which is through the angiotensin-converting enzyme-2 (ACE2) receptor; thus it is expected that both the viruses have high clinical similarities [7]. It is also noted that the novel coronavirus Spike glycoprotein has modified itself through homologous recombination. The SARS-CoV-2 spike glycoprotein is a combination of the SARS-CoV bat and an unidentified Beta-CoV [8]. Another ground level similarity is that they both started at the spring festival in China. Billions of people travelled across the country, which also had a high number of tourist visitors from around the globe. This became favourable for the contagious disease to spread so rapidly throughout the world.

Reproduction number (R_0) is used to indicate the transmission capability of a virus. It indicates the number of new infections caused by an infected person in a non-experience population. Earlier WHO had given an estimated R_0 value of 1.4 – 2.5, but studies conducted in China using mathematical models indicated this value is an average of 3.28 [9]. This value is much higher than that of influenza, which has a value below 2 [10]. It is this high value of SARS-CoV-2, which makes it deadlier than many other viruses in terms of spreading. A research paper reported that the R^0 for India was lower, which was 1.82, than many highly infected countries. A few of the reasons could be the testing rate is lower than many countries and the high population of the country [11].

COVID-19 infection may lead to symptomatic and asymptomatic cases in infected individuals. The symptoms may be mild to life-threatening respiratory syndrome and multi-organ failure, some of which may lead to death. Certain predisposing factors are reported for the severe respiratory infection caused by SARS-CoV-2. The present chapter describes some of these risk factors

CHAPTER 7

Clinical Manifestations, Treatment, Immune Response and Pathogenesis of Covid-19

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Abstract: The clinical symptoms of the Coronavirus Disease 2019 (COVID-19) may vary from asymptomatic to mild to severe to critical disease. The clinical symptoms include fever, body ache, severe weakness, cough, breathlessness, nausea/vomiting, diarrhoea, change in taste/smell, *etc*. The management of COVID-19 patients is in its evolutionary phase since the guidelines are being updated regularly due to the gradual addition of unique symptoms to the disease. A large number of asymptomatic cases, longer incubation periods, and high transmission rates are some of the concerns of the pandemic. The treatment is supportive in the absence of a specific therapeutic drug and a prophylactic vaccine. Numerous antipyretics, existing anti-viral drugs, antibiotics supplements, and other approaches are utilized for the management of COVID-19 patients. Though at early-stage (in the upper respiratory tract), the treatment of this infection is easier as compared to its advanced stage when the infection proceeds to the lower respiratory tract. Most of the severe cases may need hospitalization, regular monitoring, and oxygen supplementation. Patients with respiratory failure may need ventilator support. The SARS-CoV-2 mediated immune response has a significant impact on infection severity, which is represented as a cytokine storm and ultimately leads to acute respiratory distress syndrome (ARSD). The present chapter describes clinical manifestations and management of COVID-19 patients. Besides this, we have also discussed the host immune response and pathogenesis of the disease. Further comprehensive patient-based clinical studies will provide insight into the additional clinical manifestations, pathogenesis, and host immune response needed to clear the virus from the human body.

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Shama Parveen (Ed.)

Keywords: Asymptomatic, COVID-19, Clinical manifestations, Critical illness, Cytokine storm, Management, Mild illness, Pathogenesis, SARS-CoV-2, Severe illness, Treatment.

INTRODUCTION

The initial outbreak of novel COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), started in Wuhan, China, in late December 2019, which later took its position as a global pandemic [1]. Millions of confirmed cases and deaths have been reported worldwide due to this contagious infection and its severity. The symptoms of the initial cluster of this viral infection were reported to be like pneumonia, which then turned out to be more critical and severe in patients with comorbidities [1]. Though the virus is mainly transmitted from an infected individual to a healthy one *via* respiratory droplets, its asymptomatic scenario made its transmission more rapid [2]. Additionally, it becomes more challenging to handle the pandemic due to the exponential increase in the cases.

Clinically, COVID-19 patients have been found to show symptoms like fever, dry cough, breathing difficulties, arthralgia, diarrhea, *etc*. The immune response triggered through SARS-CoV-2 infection has an essential influence on the individuals' overall health condition. Some clinical findings suggested an abrupt rise in the activated cytokines in an advanced stage of its infection, termed as cytokine storm [3]. Interestingly, individuals with strong immunity were able to escape the harsh effect caused by this virus. Moreover, in multiple critical cases, patients were also shown to develop serious pneumonia-like symptoms together with acute respiratory distress syndrome (ARSD) and multi-organ collapse, which ultimately led to death [4]. In this chapter, we will elaborate mainly about COVID-19 patients' clinical manifestations and their management. An overview of the host immune response and viral pathogenesis will also be given in the latter part of the chapter.

CLINICAL MANIFESTATIONS

COVID-19 infection in humans may lead to symptomatic or asymptomatic cases. Individuals with COVID-19 infection show diverse symptoms ranging from mild to severe disease [5]. Commonly encountered initial symptoms mimic that of any other viral infection. Usually, the first symptoms to appear are the loss of smell (anosmia) and taste (ageusia: the tongue is not able to taste sweetness, saltiness, bitterness, and sourness), fever, cough, extreme weakness, and some experience shortness of breath. Other minor symptoms are headache, dizziness, runny nose, congestion, and nausea. Some patients may also develop gastrointestinal disturbances like diarrhea (Fig. **1**) [6 - 8].

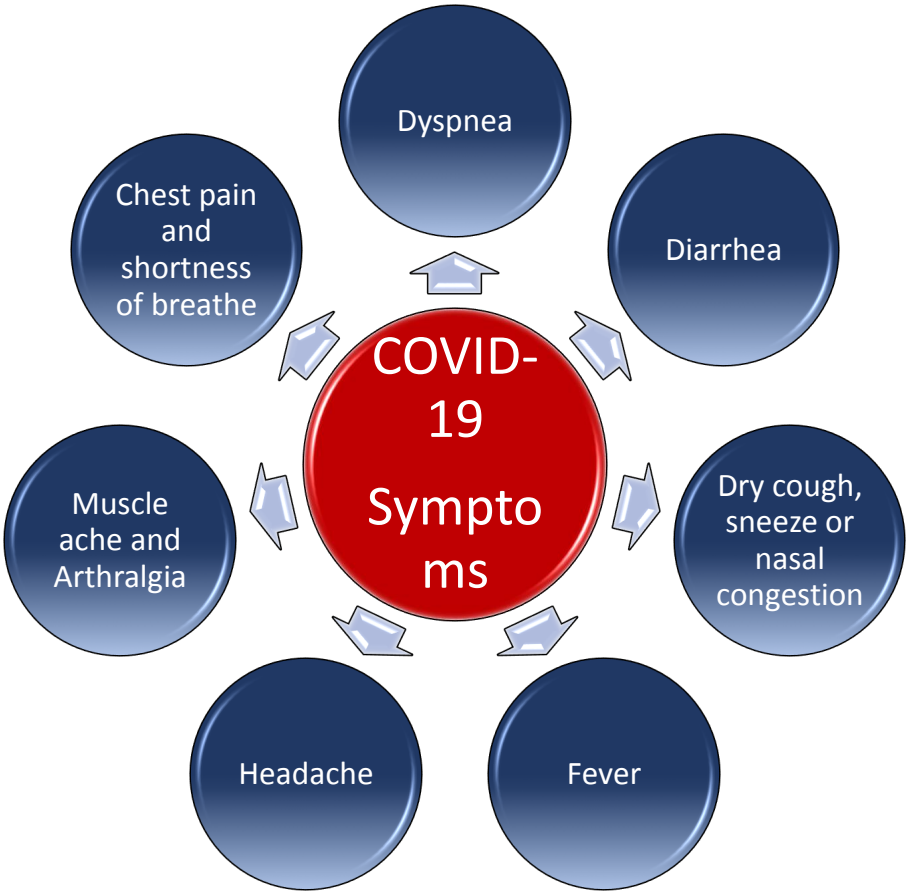

Fig. (1). Clinical manifestations of COVID-19.

Infection in most of the patients is mild or asymptomatic (80-90%). It is estimated that more than 40% people are wondering as asymptomatic COVID-19 individuals in society. It may also be noted that pre- and asymptomatic people are the most frequent source (almost 70-80%) of COVID-19 spread. It is estimated that around 10% of the cases are severe and around 5% of the cases develop a critical illness with respiratory failure, pneumonia, shock, ARSD, multi-organ failure, and ultimately death. Examination of CT scans of patients admitted to ICU revealed bilateral multiple lobular and subsegmental areas of consolidation. In addition, non-ICU patients bilateral ground-glass opacity and subsegmental areas of consolidation have also been documented. Laboratory investigations suggested that COVID-19 positive patients showed lymphocytopenia, leukocytopenia, an increase in lactic dehydrogenase enzyme, and an increase in C-reactive protein. Few immunocompromised or mid to older aged people may

CHAPTER 8

COVID-19: Current Diagnostic Approaches

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Abstract: Coronavirus Disease 2019 (COVID-19) has spread rapidly across the world, leading to a pandemic. The diagnostic methods for COVID-19 are still in the evolutionary phase since scientists are continuously trying to implement the latest technologies to achieve this objective. The infection is difficult to diagnose in the early stage as patients can stay asymptomatic from 2 to 14 days or more. A rapid, sensitive, and specific reverse transcription real-time PCR (rRT-PCR) detects viral RNA and is used to detect early infections to prevent disease spread. Although the gold standard in diagnosis, this method cannot be implemented in remote areas because of the requirements of expensive setup and trained staff. Therefore, a relatively economical method known as loop-mediated isothermal amplification (LAMP), which also detects viral RNA, is designed for use at point-of-care and remote settings. The non-nucleic acid based rapid antigen detection method detects viral antigen on nasal/pharyngeal specimen and implies current viral infection. The serological diagnostic methods detect early serological markers (IgM/IgG) in the serum of patients after a week of infection. Antigen detection and serological diagnostic methods are rapid, specific, and sensitive, with the potential to screen a large number of people during a pandemic. Thus, the genome and antigen-based diagnostic assay can detect the virus in the early stages of infection, while serological methods can be used to diagnose infections at later stages. The combination of nucleic acid and non-nucleic acid laboratory detection methods can assist in a timely and accurate diagnosis of COVID-19 that will be a step towards better patient management and containment of the pandemic.

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Keywords: Asymptomatic, Antigen detection, COVID-19, Diagnosis, ELISA, SARS-CoV-2, LAMP, Rapid diagnostic test, Real-time PCR, Serology.

INTRODUCTION

China encountered cases of pneumonia of unknown etiology in Wuhan City of Hubei Province during December 2019. The causative agent was found to be a previously unknown coronavirus and was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1, 2], and the disease was called COVID-19. The SARS-CoV-2 threat emerged in China and later rapidly spread to other countries and was declared a pandemic on 11th March, 2020 by the WHO [3]. Rapid spreading patterns of SARS-CoV-2 suggested that its human-to-human transmission was quite efficient as compared to SARS-CoV [4, 5]. More than 24 million global cases and 0.83 million deaths till 31st August 2020, have been attributed to the virus.

Clinical characteristics alone cannot establish COVID-19 in patients as the early symptoms often overlap with that of flu, including fever, cough, or shortness of breath, weakness, body ache, *etc*. The infection spreads rapidly and is difficult to diagnose clinically in the early stages as patients can stay asymptomatic from 2 to 14 days or more. The initial diagnosis of COVID-19 was based on imaging, but this was not feasible for the diagnosis of a large part of the population and has health hazards associated with it [6, 7].

Since early laboratory diagnosis of COVID-19 is crucial for the prevention and control of this pandemic, the rapid, reliable, and quick nucleic acid-based detection approaches like rRT-PCR are currently used in the diagnosis. It is a robust, sensitive, and accurate diagnostic method and was developed to identify patients in the early stages of infection [8]. Although successfully implemented, rRT-PCR is expensive and requires costly instrumentation and trained staff; therefore, it cannot be implemented at point-of-care and remote settings. Therefore, another relatively cheap method of target DNA or RNA sequence amplification, known as loop-mediated isothermal amplification (LAMP), has been designed. LAMP has some advantages of being cheap, sensitive, and specific and can be applied at point-of-care and remote testing [9, 10]. Various LAMP assays have been developed for SARS-CoV-2 detection [11, 15].

RNA viruses (SARS-CoV-2) have an error-prone replication system that may affect the binding potential of the oligos in rRT-PCR and LAMP and result in misdiagnosis or false negatives. Therefore, non-nucleic acid based serological diagnostic tools are used wherein the earliest serological marker (IgM or IgG) in the serum of the person are detected after 5 or 6 days of infection to know the person's exposure to the particular pathogen. Various serological diagnostic (SD)

methods like Rapid diagnostic tests (RDTs), Enzyme-linked immunosorbent assay (ELISA), Neutralization assay (NA), and Chemiluminescent immunoassay (CLIA) detect serum antibodies against highly immunogenic spike (S) or nucleocapsid (N) protein. Serological diagnostic tests are rapid and can be used for point-of-care testing to detect qualitatively the presence or absence of antibodies against a pathogen. Another non-nucleic acid based antigen detection test detects viral antigen in nasal/pharyngeal specimens of the patient. It is rapid, specific, and implies current infection. The present review summarizes all the molecular, serological, and antigen detection tests that are available for the diagnosis of SARS-CoV-2 infection. Early and accurate diagnosis of the infection will assist in timely management of clinical cases that will be a step towards containment of the ongoing pandemic.

SAMPLE COLLECTION

The preferred samples include nasopharyngeal (NP) swabs/aspirates or other upper respiratory tract specimens, such as throat swabs and saliva, for initial screening for SARS-CoV-2. Samples are taken with a flocked swab, preferably with aluminum or plastic shaft, to collect maximum quantity with minimum PCR inhibitors. The NP swabs are preferred due to the ease of collection by the healthcare worker and are comfortable for the patient as well. The samples (swabs) should be collected in a viral transport medium and transported to the laboratory immediately in an icebox to prevent degradation of viral nucleic acid. Improper sample collection and inadequate processing may lead to false results.

The lower respiratory tract sample collection includes bronchoalveolar lavage from the patients showing severe pneumonia. Further, stool samples were also positive for some patients showing severe pneumonia. The healthcare worker collecting the sample should take appropriate precautions, including the use of full Personal Protective Equipment (PPE) kit. The processing of the samples in the laboratory should be done in BSL2/3 facilities using the WHO guidelines and appropriate precautions.

REAL-TIME REVERSE TRANSCRIPTASE-PCR (RT-PCR)

The complete genome sequencing of the SARS-CoV-2 was a fundamental requirement for designing and development of nucleic acid based diagnostic tests. The genome sequence of the SARS-CoV-2 (WH-Human_1) was revealed and it had 14 ORFs that encode 27 proteins like the SARS-like bat coronaviruses [16 - 18]. The genome sequence analysis also identified some specific sequences associated with only the SARS-CoV-2 genome [19]. The primers and probes are designed from these specific sequences and are the backbone of the polymerase chain reaction (PCR) based diagnostics. The PCR based detection methods are

CHAPTER 9

Natural Compounds as Potential Therapeutic Agents against COVID-19

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Abstract: Research scientists across the globe are attempting to unearth the possible therapeutic agents against Coronavirus disease 2019 (COVID-19). The natural compounds from plant sources constitute a rich source of potential antiviral, antibacterial, antioxidant, anticancer, immune enhancer and other activities with minimal side effects. Approximately 25% of the European Medical Agency (EMA) or Food and Drug Administration (FDA) approved drugs are based on plant products highlighting their importance in the medical field. In recent years, *in-silico* methods have provided fast and cost-efficient approaches for designing potential inhibitors. Several virtual screening, molecular simulation, pharmacokinetics and druggability studies have been carried out to identify potential inhibitors against structural (spike, envelope, and membrane) and non-structural (Protease, RdRp, endoribonucleoase) proteins of SARS-CoV-2. In the present chapter, we have reviewed all such studies that recommended naturally occurring bioactive compounds (flavonoids, terpenes, curcuminoids, tannins, essential oil *etc*.) of plant origin as potential inhibitors of COVID-19. We have listed 100 such potential compounds and have analyzed significance of some of these (Myricitrin, Baicalin, Hesperidin, Theaflavin, Apigenin, Isothymol, Saikosaponin U, Curcumin, Tannin *etc*.) in detail based on computational studies. Furthermore, we have also studied several medicinal plants (*Curcuma longa, Vitis vinifera, Glycyrrhiza glabra, Malus domestica, Azadirachta indica, Camellia sinensis and Nigella sativa*). These plants are part of normal human diet and can also be considered as potential herbs with immune system enhancing effects. In addition, these phytoconstituents should be further analyzed in detail for toxicity, pharmacokinetics, antiviral and therapeutic potential in cell culture and animal models against SARS-CoV-2.

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Keywords: Curcuminoids, Flavonoids, Natural compounds, Plants, SARS-CoV-2, Terpenes, Therapeutics.

INTRODUCTION

Plants have been used by the human beings as health resource for millions of years before the evolution of science. During the course of history, the medicinal plants have played a significant role in the therapeutic approach for different diseases. The ancient civilizations utilized the medicinal plants by consuming them in raw form. After learning that many diseases can be cured using plants, the ancient civilizations started to collect evidence to assess their therapeutic values. Initially, it was mainly dependent on the trial and error method; however, after extensive exploration and discovery of important effects of specific plants for treating the diseases, it became clearer. Eventually, they developed the understanding that harvesting the herbs at the right time, followed by their preservation in specific preparations, may increase their efficacy and availability for a long duration.

The Egyptians described several plant-based preparations around 1500 BC [1, 2]. Later on, the Romans and Greeks further developed and refined these plant-based extracts and preparations [3]. By the early 17th century, several plants were documented and majority of them can be found in the first official drug book from Great Britain, the *Pharmacopeia Londonensis* [1, 4]. The herbal preparations still receive universal attention, with a considerable increase in their use in the developed countries with its market expansion in the United States and European countries [5]. Several ointments, tea, alcoholic extracts and salves were often recommended for treating many diseases ranging from wounds of war to menstrual complications [1, 2]. Furthermore, several other countries have their own traditional system of medicine with official transcripts and books such as India, China, Korea, Japan, Africa and many others. The traditional Chinese medicine (TCM) and Indian traditional medicinal system are one of the oldest systems, presently under the jurisdiction of the National Health Commission and Ministry of AYUSH, respectively. The Indian traditional medicinal system has unique characteristics with different well-acknowledged traditional systems of medicine, such as Unani, Ayurveda, Siddha, Naturopathy and with some classical transcripts including Unani Pharmacopeia of India, Rigveda, and Sushruta Samhita.

SIGNIFICANCE OF PLANTS DERIVED COMPOUNDS AS ANTIVIRALS

Nature is a silent companion to human world which provides a reliable source of different phytochemicals. In recent times, a lot of efforts have been made to identify antiviral compounds from various plants and other natural resources as

they are a rich source of phytochemicals like flavonoids, polyphenols, alkaloids, saponins, anthocyanins, tannins, including others. These purified natural compounds offer a rich supply for novel antiviral drug development, as evident by several ancient practices and recent research works. These phytochemicals also exhibit several therapeutic properties and novel scaffolds for designing new medications. Around 40% of the available drugs in the market are directly or indirectly derived from the plants [6]. Notably, while mono or poly-herbal preparations are complex products containing multiple pharmacologically active secondary metabolites, isolated bioactive compounds frequently perform better and in a specific way. Furthermore, even for a plant species, the nature and relative quantities of the active phytoconstituents may vary due to its geographical origin, cultivar, environmental conditions, plant parts used, storage condition, preparation method, contamination, and adulteration.

Nonetheless, herbal compounds, by the virtue of their structural diversity, provide a great opportunity to screen compounds against SARS-CoV-2 with a distinct mechanism of action and novel structure. Identifying the antiviral mechanisms of the natural compounds helps in exploring their activities and exactly at which stage (entry, replication, assembly, release, and virus–host interactions) they may interrupt the life cycle of the virus. Improved understanding of the mode of action of natural antiviral compounds may prove to be helpful in providing a new insight for developing novel antiviral drugs for efficacious viral control.

Considering the present scenario of COVID-19, this chapter summarizes some notable natural compounds studied against the SARS-CoV-2 since its outbreak. Several *in-silico* but very few *in vitro* studies have been done to identify the antiviral and immuno-modulatory properties of these compounds against SARS-CoV-2. Several of these have been suggested for the prevention and treatment of mild COVID-19 symptoms in addition to the routine therapeutic approaches against this infection. It may be noted that scientific evidence of their recommendations against SARS-CoV-2 is still currently under study. Thus, the option to use these natural compounds as accepted antivirals is yet to be established.

ANTIVIRAL ACTIVITY OF SELECTED NATURAL COMPOUNDS AGAINST SARS-COV-2

The plant produces a wide range of secondary metabolites or other phytochemicals such as lignans, flavonoids, tannins, phytoalexins, saponins, terpenes and several other polyphenols which plays a vital role in plant's growth, metabolism and development. The flavonoids comprise a major group of secondary metabolites found in seeds, stem, leaves, fruits, vegetables, nuts, spices,

CHAPTER 10

Drug Repurposing Candidates and Therapeutic Approaches towards the Treatment of COVID-19

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Abstract: The current outbreak of the novel coronavirus disease known as COVID-19 caused by the newly identified coronavirus strain SARS-CoV-2 has become a prominent health problem worldwide. Therefore, there is an urgent requirement to uncover the specified preventive measures to control the spread of the disease. Different therapeutic approaches such as administration of corticosteroids, vitamins, trace elements, immune enhancers and convalescent plasma recovery can be good alternatives, but the current emergent situation demands specified treatment. The development of vaccines will require longer durations; therefore, deployment of existing drugs as a repurposing approach remains a great option to combat this pathogenic virus. WHO includes different categories of drug treatment options such as antivirals, antimalarial, antiparasitic, antifungal anti-inflammatory, immunosuppressants, inhibitors of kinase and protease monoclonal antibodies immunomodulators, ACE inhibitors and others. Antiviral drugs such as remdesivir, lopinavir/ritonavir, favipiravir, umifenovir and antimalarial drugs such as chloroquine/hydroxychloroquine, and several combinations of these drugs are being utilized in different clinical trials and have shown efficacy in the treatment of COVID-19. We have reviewed a general outline about these drugs in the present chapter along with the strategies that may be deployed in the identification of further antiviral agents. However, the side effects associated with their administration and their minimal and maximum dosage norms require special attention. Therefore, the safety and efficacy criteria for the available drugs need confirmation *via* further clinical trials.

Keywords: Arbidol, Chloroquine, COVID-19, Favipiravir, Hydroxy-chloroquine, Lopinavir, Remdesivir, Ritonavir, Therapeutics.

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INTRODUCTION

In late December 2019, an illness of respiratory infections of unknown cause emerged in Wuhan, Hubei province of China. The genome sequence analysis revealed a novel severe acute respiratory syndrome-related coronavirus SARS-CoV-2, formerly known as 2019-nCoV, as the cause of this emerging respiratory illness [1]. The respiratory infection caused by SARS-CoV-2 is named coronavirus disease 2019 or COVID-19. The earlier identified strains of human coronavirus *i.e*, SARS-CoV and MERS-CoV were found zoonotic in origin and reported as the causative agent of severe respiratory infections outbreak from China in 2003 and Saudi Arabia in 2012, respectively [2]. At present, COVID-19 has spread its tentacles worldwide and was later declared as pandemic [3]. Therefore, the present situation of the COVID-19 emergency demands the critical need of potential control strategies for the protection of the people who are at high risk of infection.

The Available Therapeutic Options

Currently, the emerging viral disease, COVID-19 embarks global health with no specific treatments available. However, general treatments, which include maintenance of nutritional elements and administration of immune enhancers, become an efficient way of providing supportive care to the affected individuals [4]. Prior to administration, the nutritional status of the affected individuals should be monitored against nutrition or trace elements to determine its appropriate dosage and minimal risk of side effects associated with it [4]. Many medicinal plants that are part of the Unani and Ayurvedic medicine system under the AYUSH Ministry, Government of India have reported antiviral, anti-inflammatory, antioxidant and immune-modulatory properties. These are plant-derived natural compounds or their combination that can thus be used as nutritional supplements to enhance the sagging immune system in the context of the ongoing COVID-19 pandemic. Alternatively, Traditional Chinese Medicines (TCM) is also based on the plant system. These can also be an effective treatment for COVID-19 since they also possess efficient antiviral activity and provide symptomatic relief as well [5]. In addition, convalescent plasma or passive immune therapy can be given for the treatment of severe and critical cases [4]. Convalescent plasma usually becomes a great option when no specific vaccine or drug is available for the emerging infection [6]. These kinds of general treatment may be extremely helpful in the maintenance and recovery of the patients. However, the current pandemic scenario of COVID-19 warrants an urgent need for vaccines and specified antiviral drugs.

One cannot rule out the existence of generic antiviral treatments that include corticosteroids. Corticosteroids were efficiently used for the treatment of SARS in China and Hong Kong. The reason for their use in SARS illness is that in acute respiratory viral infections, the early response cytokines such as IFN-γ, TNF, IL-1, and IL-6 contribute to tissue damage [7, 8] and corticosteroid treatment may repress this cytokine storm [9]. One such corticosteroid named dexamethasone is widely used and has anti-inflammatory and immunosuppressant effects. It was tested in patients with COVID-19 in the United Kingdom's national clinical trial RECOVERY and was found to have benefits for seriously ill patients [10].

The formulation of vaccines against COVID-19 will take some time; therefore rapid deployment of anti-COVID-19 drugs can be proved as a great therapy to combat the severity of the disease. However, the ongoing pandemic and global emergency of COVID-19 makes the drug therapy development pathway unacceptable as it owes higher costs and longer durations [11]. Therefore, the repurposing of the existing drugs such as those for influenza, hepatitis B (HBV), hepatitis C (HCV), malaria, HIV and other pathogens with an expedition in antiviral treatments can result as an efficient therapeutic option against COVID-19 emergency [12].

Strategies for the Development of an Antiviral Drug

There are three strategies that can be envisaged to discover a potential antiviral drug against this emerging viral pathogen [13]. The first method involves the testing of broad-spectrum antivirals using standard assays [14]. The productive advantage of using these drugs lies in their known pharmacokinetics and pharmacodynamics properties such as, metabolic characteristics, dosages used, potential efficacy, side effects and drug regimens. However, being broad-spectrum and hence non-specific, they may possess adverse side reactions, which should not be underestimated. The second approach utilizes existing compounds for antiviral therapy. The identification of these antiviral compounds is done *via* high throughput screening of chemical libraries or molecular databases [15]. The selected compounds are further evaluated by antiviral assays. The third strategy is based on the development of new specific or targeted drugs [16]. However, this approach will require comprehensive information on genome, biophysical properties and pathology of SARS-CoV-2, which further poses a challenge for the scientific community. A general outline in Fig. (**1**) briefly describes the types of antiviral treatments and drugs available at present against COVID-19 and the strategies that can be envisaged for the development of antiviral drugs.

CHAPTER 11

COVID-19 Vaccine Development: Challenges and Current Scenarios

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Abstract: The ongoing situation of COVID-19 pandemic entails us towards the development of a prophylactic vaccine as a public health priority. The emergence of SARS-CoV-2 in Wuhan, China during December 2019 marked the third introduction of a highly pathogenic Coronavirus into human population in the twenty-first century. Knowledge from the former vaccine candidates of SARS-CoV and MERS-CoV has unlocked the door for the developers to accelerate the global vaccine development pathway for ongoing COVID-19 pandemic, soon after the online publication of SARS-CoV-2 genomic sequence. The vaccine development pipeline for COVID-19 shows a promising result by utilizing various platforms (nucleic acid, viral vector, recombinant protein, live attenuated viruses, inactivated viruses and virus like particles) with different strategies. Surprisingly till now, we have about 190 vaccine candidates in the clinical and pre-clinical pipeline till 31st August 2020. Approximately, 39 of these vaccine candidates are impending into the human clinical trials after showing significant safety data in preclinical studies of which, 8 vaccine candidates are running in final phase3 stage. Three of them have got an emergency approval for limited or early use. At least 8 candidate vaccines have been developed from India, from which 2 of them have entered phase2 trials. Already existing tuberculosis vaccines are also being tested in clinical trials bridging the gap before a potential COVID-19 vaccine is developed. This chapter highlights the obstacles for implementation of vaccine development for SARS-CoV-2. One of the impediments is identification of high-risk population including frontline health care workers, elderly individuals and persons with pre-existing chronic diseases. We have also provided a comprehensive overview about

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the COVID-19 vaccine candidates that are in preclinical and clinical stages of development. Thus, fast track clinical trials of many candidates are implemented in different geographical regions promising a prophylactic vaccine against SARS-CoV-2.

Keywords: COVID-19, Clinical trial, Clinical pipeline, Challenges, SARS CoV-2, Pre-clinical trial, Vaccine development.

INTRODUCTION

Coronaviruses are one of the critical pathogens, affecting humans (mainly respiratory system) and vertebrates. The world witnessed a rapid outbreak of Coronavirus infection that became pandemic, putting global public health in peril from the very beginning of twenty-first century. This century had already experienced the emergence of two formerly identified Coronaviruses. The commencement of Coronavirus emergence as a severe acute respiratory syndrome (SARS-CoV) occurred in China during 2002-2003 [1, 2]. After 10 years, in 2012 an outbreak of Middle East Respiratory Syndrome (MERS-CoV) took place in Saudi Arabia. At the end of 2019, another mysterious ongoing outbreak of Coronavirus was reported in the city of Wuhan, China, (epicentre of COVID-19) that manifests respiratory like ailments [3]. WHO on 11th February 2020 named the disease as "the Corona Virus Disease-2019; **COVID-19**" and the pathogen was designated as a 2019 novel Coronavirus (**2019-nCoV**) that was later named as **SARS CoV-2**. On 11th March 2020, WHO declared COVID-19 as a pandemic [2].

Most of the affected individuals manifest mild respiratory illness (fever, cough, difficulty in breathing) but some of the individuals show severe manifestations like respiratory bilateral interstitial pneumonia which advance to respiratory failure [4]. Moreover, some patients have also been reported with hypo/dysgeusia (loss of sense of smell), hypo/anosmia (loss of sense of taste) and gastrointestinal problems as early symptoms of the infection. However, more severe illness has been demonstrated with health care workers and in the individuals of older age, immunocompromised and those with chronic health conditions. This pathogen is extremely contagious, transmitted from either infected asymptomatic or symptomatic individuals through the droplets of saliva or discharge from the nose [5].

Coronavirus belongs to the family Coronaviridae and the genus beta-Coronavirus (β-CoVs). β-CoVs are enveloped viruses with positive sense single stranded RNA genome (+mRNA). They show eminently higher mutation rate due to the constant transcription errors as well as RNA Dependent RNA Polymerase (RdRP) jump.

The genome consists of functional and structural proteins (surface glycoprotein or spike (S), envelope (E), matrix (M), and nucleocapsid (N)) [6]. Of these structural proteins, the S protein imparts a vital role in the host cell attachment [7, 8]. Receptor binding domain (RBD), a key part of S protein helps in initial attachment of virus to the host cell surface. SARS-CoV-2 uses the same ACE2 (angiotensin converting enzyme 2) human receptor, which is abundantly present on the mucosal epithelial cells of respiratory tract and lung parenchyma [9 - 12]. A recent analysis suggested that SARS-CoV-2 has lower pathogenicity rate (about 3%) than SARS-CoV (10%) and MERS-CoV (40%) [13]. Furthermore, it has higher transmissibility rate (R0:1.4–5.5) than both SARS-CoV (R0:2–5) and MERS-CoV (R0: <1) [13].

Till 31st August 2020, WHO reported over 29,107,970 confirmed cases of COVID-19 with 926,910 deaths in 216 countries and territories across the world [14]. By the mid of April 2020, several short approaches like good hygiene practices, social distancing and quarantine methods have been implemented to lessen the transmission of SARS-CoV-2. Other approaches include the implementation of therapeutic drugs (remdesivir, favipiravir, hydroxychloroquine, combination of lopinavir and ritonavir and many more) to reduce the effect of viral attack. The most promising approach being followed to minimize the impact of SARS-CoV-2 is the development of a prophylactic vaccine. Due to the current pandemic situation, COVID-19 vaccine is concurrently needed all over the world. In this review, we describe some of the major vaccine candidates in the clinical pipeline and the obstacles behind the implementation of vaccine.

VACCINES

There was a rapid acceleration in the expansion of SARS-CoV-2 globally and the announcement of outbreak as a public health emergency of international concern by the WHO. This prompted the research scientists to accelerate the development process for safe and efficacious vaccine. Development of a vaccine is a complex, cost-effective and a time-consuming process. Traditionally it takes about a period of 12-15 years to produce a licensed vaccine [15]. Due to the risk of failure and high expenses, developers should pursue a paradigm (sequential steps) for vaccine development with various halts to check its safety and efficiency. The safety of the vaccine is first evaluated in the laboratories with animal models (rats or monkeys). If the vaccine shows no disease in animal models, then the testing is progressed towards humans in different phases with gradually rise in number of subjects (Fig. **1**). But during this Covid-19 pandemic, a fast-tracked paradigm with 12-18 months of timeline was suggested with a quick start and many shortened steps. Moreover, few steps will be carried out in parallel before safety data will be obtained from a previous step, thus leading to a high financial risk to

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