Review Article

Uncoating the Global Virus: SARS Coronavirus-2

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ABSTRACT

WHO has declared the COVID-19 as a global pandemic. The genome of 2019-nCoV has 82% similarity with that of human SARS-CoV and the virus is now known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), while the name of the disease it causes is now called COVID-19. Coronaviruses encode five structural proteins in their genomes. With the help of various proteins, it attaches to host cells and enter into the cell and replicate there to release multiple viral assemblies. It causes fever, cough, myalgia, fatigue, shortness of breath, diarrhea etc. Laboratory abnormalities include leukopenia, leukocytosis, lymphopenia and elevated liver enzymes. CT shows ground glass appearance in the chest. A real-time reverse transcription PCR (RT-PCR) assay are being used to detect viral RNA as per protocol. Currently no approved drug is being prescribed for SARS corona virus-2 infection and no antiviral drug licensed by the USFDA. Supportive treatment to manage vitals, oxygenation, maintenance of shock and sepsis is being done to save lives. It is also likely that these viruses will continue to emerge and cause both human and veterinary outbreaks because of their ability to recombine, mutate, and infect multiple species and multiple cell types. Defining the mechanism of pathogenecity of coronaviruses that cause disease and understanding the host immunopoathological response will significantly improve the ability to design vaccines and reduce disease burden.

Key words: 2019-nCoV, SARS-CoV-2, COVID-19, coronaviruses.

INTRODUCTION

In December 2019, an outbreak of pneumonia cases that occured in Wuhan, China, was caused by a betacoronavirus, the 2019 novel coronavirus (2019-nCoV). Presently, 2019-nCoV has spread to many countries around the world. According to the WHO corona virus disease (COVID-19)[1] situation report-50 released on March 10, 2020, more than 1,13,702 cases have been confirmed and at least 4,012 deaths reported so far. It has attracted attention of health professionals and public because of highly contagious nature and morbidity due to the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2) which was initially named as novel coronavirus (2019-nCoV)[1]. Present scenarios suggest that SARS CoV-2 pose a lethal threat to humans. This is a brief review on coronaviruses discussing their epidemiology, pathogenicity, laboratory, clinical characteristics, current prevention and treatment strategies available to manage patients suffering from COVID-19.

CORONA VIRUSES

Coronaviruses (CoVs) are enveloped viruses with a positive sense, non-segmented, single-stranded RNA genome. CoVs have the largest genomes (26.2 to 31.7 kilobases) for RNA viruses and are spherical, pleomorphic viruses of size ranging from 80-120 nm. Based on genetic and antigenic criteria, CoVs have been organised into various groups (Table 1).[2] Taxonomically they belong to order *Nidovirales*, family *Coronaviridae*, subfamily *Coronavirinae*. Coronaviruses usually infect epithelial cells of the gastroenteric or respiratory tracts and cause a variety of diseases in mammals and birds ranging from enteritis in cows and pigs and upper respiratory disease in chickens to potentially lethal human respiratory infections.[3]

Epidemiology: The first member of the coronavirus family was discovered in the 1930s.[4] The world shook in 2002-2003 due to notorious characteristics of the SARS virus. Before 2003, the diseases associated with

Table 1: Coronavirus groups[2]

Genus	Species	Receptor
Alpha coronavirus	Alphacoronavirus 1 comprising:	
	• *Feline Coronavirus (FCoV) serotype 2*	Aminopeptidase N
	• *Canine Coronavirus (CCoV) serotype 2*	Aminopeptidase N
	• *Transmissible gastroenteritis virus (TGEV)*	Aminopeptidase N
	Human coronavirus 229E	Aminopeptidase N
	Human coronavirus NL63	ACE2
	Porcine Epidemic Diarrhea Coronavirus (PEDV)	Aminopeptidase N
	Rhinolophus bat coronavirus HKU2	
	Scotophilus bat coronavirus 512/05	
	Miniopterus bat coronavirus 1	
	Miniopterus bat coronavirus HKU8	
Beta coronavirus	Betacoronavirus 1 comprising:	
	• *Bovine coronavirus (BCoV)*	Neu 5,9 Ac2
	• *Human coronavirus OC43 (HCoV-OC43)*	Neu 5,9 Ac2
	• *Equine coronavirus (ECoV)*	
	• *Human enteric coronavirus (HECoV)*	
	• *Porcine haemagglutinating encephalomyelitis virus (PHEV)*	
	• *Canine respiratory coronavirus (CrCoV)*	
	Murine coronavirus comprising:	
	• *Existing species of mouse hepatitis virus (MHV)*	CEACAM1
	• *Rat coronavirus*	
	• *Puffinosis virus*	
	Human coronavirus HKU9	
	Rousettus bat coronavirus HKU4	
	Tylonycteris bat coronvirus HKU5	
	SARSr-CoV (SARS related Coronavirus) comprising:	
	• *Human SARS-CoV*	ACE2
	• *Rhinolophus bat viruses*	
Gamma coronavirus	Avian coronavirus comprising:	
	• *IBV*	
	• Various coronaviruses infecting turkey, duck, goose, pigeon etc.	
	Beluga Whale coronavirus SW1	
Delta coronavirus	*Bulbul coronavirus HKU11*	
	Thrush coronavirus HKU12	
	Munia coronavirus HKU13	

coronaviruses were mainly of veterinary interest. Coronaviruses causes respiratory and enteric diseases and in some rarer cases, hepatitis and neurologic disease. Infection can be acute or persistent.[5] SARS-CoV infections first appeared in China in 2002 and then quickly spread as a global epidemic in more than 30 countries with 8273 infections and nearly 10% mortality.[6] In 2012, *Middle East respiratory syndrome virus* (*MERS-CoV*) emerged in Saudi Arabia and spread throughout the Middle East. The second pandemic of *MERS-CoV* occurred in South Korea in 2015. The World Health Organization had reported 2494 laboratory-confirmed cases of MERS-CoV infection and about 35% case fatality in 27 countries as reported in November 2019.[7] The remaining common HCoVs, such as 229E, OC43, and NL63, usually infect the human upper respiratory tract and cause the common cold, but they also are involved in severe and fatal diseases in children, elderly, and immunocompromised patients.[8-10] These infections were caused by viruses belonging to the *beta-CoV* group and *alpha-CoVs.*[2] The *mouse hepatitis virus* (*MHV*) is closely related to *SARS-CoV* (*betacorona*) and *MERS-CoV* (*betacorona*), and considered the prototype for the study of both molecular biology and pathogenesis of the members of CoV family.[11,12]

Seven coronaviruses have been shown to infect humans, till date. *Betacoronavirus HCoV-OC43* and *HCoV-HKU1* as well as *Alphacoronavirus HCoV-229E* cause common colds but also severe lower respiratory tract infections in the youngest and oldest age groups; while *Alphacoronavirus HCoV-NL63* is considered to be an important cause of

(pseudo) croup and bronchiolitis in children. Prior to the *SARS-CoV* outbreak, coronaviruses were only thought to cause mild, self-limiting respiratory infections in humans.[3]

SARS Coronavirus-2 (SARS CoV-2)

A cluster of patients with pneumonia of unknown cause was linked to a seafood wholesale market in Wuhan, China in December 2019. A previously unknown betacoronavirus was discovered through the use of unbiased sequencing in samples from patients with pneumonia. A coronavirus was subsequently identified as the causative pathogen, provisionally named 2019-novel coronavirus (2019-nCoV).[1] Human airway epithelial cells were used to isolate a novel coronavirus (2019-nCoV), which is phylogenetically in the SARS-CoV clade within the subgenus *sarbecovirus*, *Orthocoronavirinae* subfamily. 2019-nCoV, the seventh member of the family of coronaviruses is different from both *MERS-CoV* and *SARS-CoV.*[13] Overall, the genome of 2019-nCoV has 82% similarity with that of human SARS-CoV.[14] So, the virus is now known as *severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2), while the name of the disease it causes is now called COVID-19.[1]

WHO released the first situation report of novel corona virus outbreak on 21st January which stated the facts that Chinese authorities identified a new type of coronavirus, which was isolated on 7th January 2020 and on 12th January 2020 China shared the genetic sequence of the novel coronavirus for countries to use in developing specific diagnostic kits, further on 13th January 2020, the Ministry of Public Health, Thailand reported the first imported case of laboratory confirmed novel coronavirus (2019-nCoV) from Wuhan, Hubei Province, China. As per surveillance 282 confirmed cases were reported.[15] Subsequently, daily WHO updates are being given by WHO. It is spreading in various countries by local transmission or via imported cases. In India till date 195 cases have been reported so far.[16]

Structure and genome: The name "coronavirus" is derived from the Latin word *corona*, meaning *crown* or *halo*, which refers to the characteristic appearance of the virus particles (virions). They have a fringe reminiscent of a crown or of a solar corona. Coronaviruses encode five structural proteins in their genomes.[17] These are the spike (S glycoprotein), membrane (M glycoprotein), envelope (E glycoprotein), nucleocapsid (N protein) and hemagglutinin esterase (HE) protein (Figure 1).

S glycoprotein: S glycoprotein is located outside the virion and give the typical shape. This spike protein form homotrimers and allow the formation of sun like morphology or corona appearance.[18]

N protein: N is the only protein (phosphoprotein) that primarily bind to the CoV RNA genome, making up the nucleocapsid.[19]

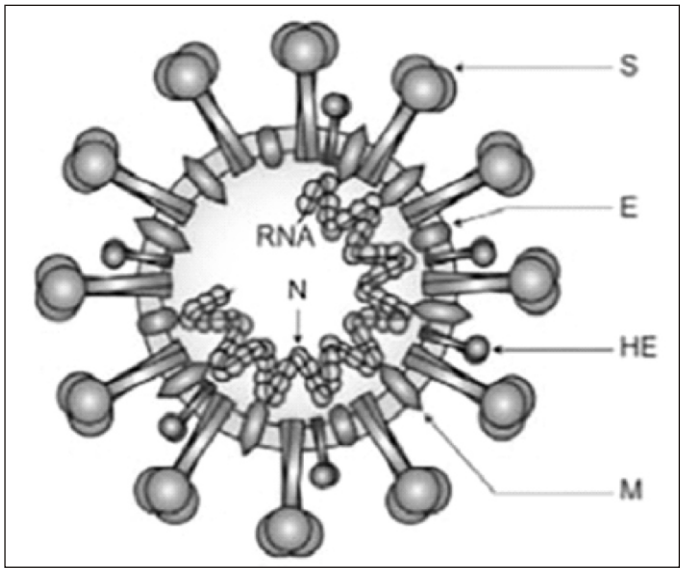

Figure 1: Structure of Coronavirus.[17]

M glycoprotein: The M protein is the most abundant structural protein (2530 kDa)[3] which defines the shape of the viral envelope.[20] These have three transmembrane regions and are glycosylated in the Golgi apparatus[21] which is crucial for the virion to fuse into the cell and to make protein antigenic.[22] N protein forms a complex by binding to genomic RNA and M protein triggers the formation of interacting virions in this endoplasmic reticulum-golgi apparatus intermediate compartment (ERGIC) with this complex.[21,23,24] Binding of M to N stabilises the nucleocapsid (N protein-RNA complex), as well as the internal core of virions. This ultimately, promotes completion of viral assembly.[3,25]

E glycoprotein: The E protein is the smallest of the major structural proteins composed of 76-109 amino acids and 8-12 kDa in size.[36] About 30 amino acids in the N-terminus of E-protein allow attachment to the membrane of virus. During the replication cycle, E is abundantly expressed inside the infected cell, but only a small portion is

incorporated into the virion envelope. The majority of the protein is localized at the site of intracellular trafficking, viz. the ER, Golgi, and ERGIC, where it participates in CoV assembly and budding.[27]

HE protein: HE is only present in some beta coronaviruses.[17] These protein acts as a hemagglutinin, binds sialic acids on surface glycoproteins and contains acetylesterase activity[28] which are thought to enhance S protein-mediated cell entry and virus spread through the mucosa.[29]

PATHOGENESIS

Attachment and entry: The initial attachment of the virion to the host cell is initiated by interactions between the spike protein (S protein) and its receptor.[3] The envelope spike (S) protein mediates receptor binding and membrane fusion and determines host tropism and transmission capacity.[30] Modification of the spike can alter cell and tissue tropism. The spike protein is a large type I transmembrane fusion[31] protein ranging from 1,160 amino acids for *avian infectious bronchitis virus* (IBV) and up to 1,400 amino acids for *feline coronavirus* (FCoV). The formation of an α-helical coiled-coil structure is characteristic of this class of fusion protein, which contain in their C-terminal part regions predicted to have an α-helical secondary structure and to form coiled-coils.[2] A critical feature of any viral fusion protein is the so-called "fusion peptide", which is a relatively a polar region of 1525 amino acids that interacts with membranes and plays an essential role in the fusion reaction.[32-34]

An important distinction between the spike proteins of different coronaviruses is whether it is cleaved or not during assembly and exocytosis of virions. With few exceptions, in most alphacoronaviruses and the *betacoronavirus SARS-CoV*, the virions have a spike protein that is uncleaved, whereas in some beta- and all gammacoronaviruses the protein is cleaved between the S1 and S2 domains, by furin, a Golgi-resident host protease. The S1 contains two subdomains, a N-terminal domain (NTD) and a C-terminal domain (CTD). Both are able to function as receptor binding domains (RBDs) and bind variety of proteins and sugars.[2]

Additionally, this protein is highly glycosylated as it contains 21 to 35 N-glycosylation sites. Generally, the spike protein of coronaviruses is functionally divided into the S1 domain (especially positions 318-510 of *SARS-CoV*), responsible for receptor binding, and the S2 domain, responsible for cell membrane fusion.[31] The 2019-nCoV S2 protein showed around 93% sequence identity with bat-SL-CoVZC45. Different coronaviruses utilize different receptors to gain entry into human cells.[2,3]

After receptor binding, the virus gain access to the host cell cytosol, generally accomplished by acid-dependent proteolytic cleavage of S protein by a cathepsin, or another protease, followed by fusion of the viral and cellular membranes. Fusion generally occurs in acidified endosomes.[3] Two major conformation changes occur during fusion. Upon endosomal acidification, an unstructured linker becomes helical allowing formation of a long helix in the N-terminal part. In this conformation, called a prehairpin, the fusion peptide is projected towards the target membrane where it is then embedded, connecting the viral and target cell membranes. The second conformational change consists of the inversion of the C-helix that packs into the grooves of the N-terminal trimeric coiled-coils forming a six-helix bundle (6HB). In the resulting conformation, the transmembrane domain and the fusion peptide anchored into the target membrane are brought in close proximity facilitating merging of viral and cell membranes. Over time, coronaviruses have modified their spike proteins, leading to the diversity of triggers used to activate their fusion. These conformational changes can be initiated by receptor binding but may need additional triggers such as pH acidification or proteolytic activation.[2]

Protein expression: After entering the cytoplasm, the virus particle releases the RNA genome. The genome consists of seven genes. It is organized into 5' non-structural protein coding regions comprising the replicase genes (gene 1), which are two-thirds of the genome, and 3' structural and nonessential accessory protein coding regions comprising the gene 2-7.[21] The replicase gene 1 products are encoded by two very large open reading frames ORF1a and 1b, which are translated into two large polypeptides pp1a and pp1b, which are synthesized directly from the 5' two-thirds of the genomic RNA of CoV. After synthesis of these proteins, consisting of 16 units, non-structural protein (nsp1 to nsp16) is converted with the contribution of viral proteases pp1a and pp1b. These 16 proteins form double-membrane vesicles (DMV).[35-36] At

the same time, this DMV is virus 'replication and transcription complex' (RTC).[37] Genes 2 to 7 are translated from sub genomic mRNA. Sub genomics RNAs encode the major viral proteins (S, E, M, N) and the accessory proteins, which are essential for virus-cell receptor binding. The newly structural synthesized proteins are released into the endoplasmic reticulum. All of these proteins, alongwith the N protein, are linked to the viral genomic RNA and localized in the ERGIC region.[38]

The CoV has an antiapoptotic function in infected cells as it suppresses the unfolded protein response (UPR) during infection. UPR are responsible for cell death and suppressed UPR acts as a survival mechanism, to continue viral propagation.[39] Viruses encode proteins that interfere with the immune system to either inhibit a response or enhance one as part of their pathogenicity. Some viral proteins disrupt components of the immune response pathways to disrupt the immune system and promote their viral evasion and pathogenesis.[40,41] Viral proteins can also modulate other cellular factors that could also disrupt the immune response to promote pathogenesis. The expression of S protein at the cell membrane also mediate cell-cell fusion between infected and adjacent, uninfected cells. This results in the formation of giant, multinucleated cells, or syncytia, which has been proposed as a mechanism to allow direct spreading of the virus between cells.[3,42-43] Viral protein also delays the transport of proteins along the secretory pathway by altering the Ca^{2+} and H^{+} concentrations of the golgi and endoplasmic reticulum (ER) compartments and has been proposed to be a mechanism of immune evasion as well.[44] Viral RNA synthesis follows the translation and assembly of the viral replicase complexes. Both genomic and sub-genomic RNAs are produced by RNA synthesis.

Assembly and release: Following replication and sub-genomic RNA synthesis, the viral structural proteins, S, E, and M are translated and inserted into the endoplasmic reticulum (ER). These proteins move along the secretory pathway into the endoplasmic reticulum-golgi intermediate compartment (ERGIC). There, viral genomes encapsidated by N protein bud into membranes of the ERGIC containing viral structural proteins, forming mature virions.[19]

The M protein directs most protein-protein interactions required for assembly of coronaviruses. However, M protein is not sufficient for virion formation, as virus-like particles (VLPs) cannot be formed by M protein expression alone. However, when M protein is expressed along with E protein VLPs are formed. This suggests that these two proteins function together to produce coronavirus envelopes.[45] N protein enhances VLP formation, suggesting that fusion of encapsidated genomes into the ERGIC enhances viral envelopment.[46] The S protein is incorporated into virions at this step, but is not required for assembly. The ability of the S protein to traffic to the ERGIC and interact with the M protein is critical for its incorporation into virions. Being relatively abundant in comparision to E protein, M protein interactions provide the impetus for envelope maturation. The M protein also binds to the nucleocapsid, and this interaction promotes the completion of virion assembly.

It is unclear, how the N protein selectively packages only positive-sense full-length genomes among the many different RNA species produced during infection. Following assembly, virions are transported to the cell surface in vesicles and released by exocytosis. It is not known if the virions use the traditional pathway for transport of large cargo from the Golgi or if the virus has diverted a separate, unique pathway for its own exit.[3]

WHO surveillance guidelines for 2019-nCoV:[47]

Confirmed case: A person with laboratory confirmation of 2019-nCoV infection, irrespective of clinical signs and symptoms.

Suspected case:

A. A patient with acute respiratory illness (that is, fever and at least one sign or symptom of respiratory disease, for example, cough or shortness of breath) AND with no other etiology that fully explains the clinical presentation AND a history of travel to or residence in a country, area or territory that has reported local transmission of COVID-19 disease during the 14 days prior to symptom onset.

OR

B. A patient with any acute respiratory illness AND who has been a contact of a confirmed or probable case of COVID-19 disease during the 14 days prior to the onset of symptoms.

OR

C. A patient with severe acute respiratory infection (that is, fever and at least one sign or symptom of respiratory disease, for example, cough or shortness breath) AND who requires hospitalization AND who has no other etiology that fully explains the clinical presentation.

Probable case: A probable case is a suspected case for whom the report from laboratory testing for the COVID-19 virus is inconclusive.

Clinical symptoms: Incubation period is estimated about 5 days, but may range from 2-14 days. Fever (77-98%), cough (46-82%), myalgia and fatigue (11-52%), shortness of breath are frequently reported signs and symptoms at the onset of COVID-19. Sputum production, headache, hemoptysis, and diarrhea are less common symptoms. Co-morbid conditions (mainly cardiovascular and cerebro-vascular diseases and diabetes) and immunocompromised patients are at increased risk for developing serious respiratory illness. Symptoms can progress to severe cases like acute respiratory distress syndrome (ARDS), septic shock, and multi-organ failure resulting in death. In addition, the gastrointestinal tract can be involved with gastrointestinal symptoms, such as diarrhoea. COVID-19 may progress to severe pneumonia, pulmonary oedema, ARDS, or multiorgan failure.[48] The illness likely to have been caused by this CoV was named "novel coronavirus-infected pneumonia" (NCIP).[13]

Laboratory and radiographic findings: Most common laboratory abnormalities include leukopenia, leukocytosis, lymphopenia, and elevated liver enzymes (alanine amino-transferase, aspartate aminotransferase). Chest CT shows bilateral or unilateral lung involvement in most patients with multiple areas of consolidation and ground glass opacities.[49] MuLBSTA score system predicts mortality in viral pneumonia. This score system include 6 indexes: multilobular infiltration, lymphopenia, bacterial co-infection, smoking history, hypertension, and age.[49]

Viral diagnostic methods: Lower respiratory tract samples including bronchoalveolar-lavage fluid collected from patients with pneumonia of unknown cause who are suspected. Extraction of nucleic acids from clinical samples (including uninfected cultures that served as negative controls) performed with a viral nucleic acid kit, as described by the manufacturer. Extracted nucleic acid samples being tested for viruses and bacteria by polymerase chain reaction (PCR), using the kit and the real-time PCR system, in accordance with manufacturer instructions. A real-time reverse transcription PCR (RT-PCR) assay are being used to detect viral RNA as per protocol.[50]

Treatment strategies: In animal studies, it was found that two HIV drugs block enzymes that viruses need to replicate and they have reduced levels of the coronaviruses that cause severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS).[51] Remdesivir, a nucleotide analogue (Gilead in Foster City, California) has had some success against coronaviruses in animals, too.[52,53] It gets incorporated into nascent viral chains and results in premature termination.[54] During the first week of February 2020, China launched two placebo-controlled trials of remdesivir, slated to include 760 people with COVID-19. The studies should be completed by the end of April, and remdesivir could be approved by Chinese authorities as early as May 2020. China has launched a few trials that test chloroquine, that killed off the new coronavirus (recently named *SARS-CoV-2*) in cell culture.[55] Chloroquine, a widely-used anti-malarial and autoimmune disease drug, has recently been reported as a potential broadspectrum antiviral drug.[56,57] Chloroquine is known to block virus infection by increasing endosomal pH required for virus/cell fusion, as well as interfering with the glycosylation of cellular receptors of *SARS-CoV*.[58] Besides its antiviral activity, chloroquine has an immune-modulating activity, which may synergistically enhance its antiviral effect in vivo. Chloroquine is widely distributed in the whole body, including lung, after oral administration.[56] It is also under study whether steroids diminish inflammation in people with severe COVID-19, or cause harm. Another study will test serum from COVID-19 survivors. It is based on the idea that the antibodies one person steadily builds up to fight a virus can rapidly help someone freshly infected to fight it off.[59] Two stem-cell trials are also registered in China. A spike-shaped protein on the surface of the viruses underlying SARS, MERS, and COVID-19 provides a tantalizing target. Already, Jiang and other research groups have found compounds and antibodies that glom onto that spike,[60] which could prevent coronaviruses

from invading human cells.

Currently no approved drug is being prescribed for *SARS corona virus*-2 infection and no antiviral drug has been licensed by the USFDA. Supportive treatment to manage vitals, oxygenation, shock, and sepsis is being done to save lives. Strategic objectives to prevent crises, preparedness and response strategies, recommendation and advice for the public is given by the WHO.[61]

CONCLUSION

Over the past 80 years, the emergence of so many different coronaviruses has caused a wide variety of human and veterinary diseases. It is also likely that these viruses will continue to emerge and cause both human and veterinary outbreaks because of their ability to recombine, mutate, and infect multiple species and multiple cell types. Although the disease caused by *SARS CoV-2* is highly contagious, mortality reported in China and other countries is on an increasing trend. Inspite of many clinical trials and study of drugs for corona virus disease (COVID-2019), no definitive treatment is available so far. Defining the mechanism of pathogenecity of coronaviruses that cause disease and understanding the host immunopoathological response will significantly improve the ability to design vaccines and reduce disease burden. Currently, disease can be managed by supportive medical treatment only. Universal precaution measures should be taken to prevent the infection.

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